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(54)【発明の名称】延長した半減期を有する分子ならびにその組成物および用途

(57)【要約】
本発明は、FcRnに対するIgG定常ドメインまたはそのFcRn結合性フラグメントの親和性を増加させる1以上のアミノ酸修飾を有するIgG定常ドメインまたはそのFcRn結合性部分の存在のために増加したin vivo半減期を有する、IgG、非IgG免疫グロブリン、タンパク質および非タンパク質物質を含めた分子を提供する。増加した半減期を有するそのようなタンパク質および分子は、かかる分子の治療、予防または診断用途において、より少量および／またはより低頻度の投与しか必要としないという利点を有する。

【特許請求の範囲】
【請求項１】
野生型IgG定常ドメインに対して1以上のアミノ酸修飾を含むIgG定常ドメインを含んでなる修飾されたIgGであって、該修飾IgGが、野生型IgG定常ドメインを有するIgGの半減期と比較して増加した半減期を有し、前記1以上のアミノ酸修飾が251、253、255、285-290、308-314、385-389および428-435位の1以上に存在することを特徴とする、前記修飾IgG。
【請求項２】
野生型非ヒトIgG定常ドメインに対して1以上のアミノ酸修飾を含む非ヒトIgG定常ドメインを含んでなる修飾された非ヒトIgGであって、該修飾IgGが、野生型非ヒトIgG定常ドメインを有するIgGの半減期と比較して増加した半減期を有し、前記1以上のアミノ酸修飾が251-256、285-290、308-314、385-389および428-436位の1以上に存在するが、前記1以上のアミノ酸修飾は、252位におけるロイシン、254位におけるセリンおよび256位におけるフェニルアラニンによる置換を含まないことを特徴とする、前記修飾非ヒトIgG。
【請求項３】
野生型ヒトIgG定常ドメインに対して1以上のアミノ酸修飾を含むヒトIgG定常ドメインを含んでなる修飾されたヒトまたはヒト化IgGであって、該修飾ヒトまたはヒト化IgGが、野生型ヒトIgG定常ドメインを有するヒトまたはヒト化IgGの半減期と比較して増加した半減期を有し、前記1以上のアミノ酸修飾が251-256、285-290、308-314、385-389および428-436位の1以上に存在することを特徴とする、前記修飾ヒトまたはヒト化IgG。
【請求項４】
前記アミノ酸修飾の少なくとも1つがアミノ酸の置換である、請求項1、2または3記載の修飾IgG。
【請求項５】
前記アミノ酸修飾の少なくとも1つがアミノ酸の欠失である、請求項1、2または3記載の修飾IgG。
【請求項６】
前記アミノ酸修飾の少なくとも1つがアミノ酸の挿入である、請求項1、2または3記載の修飾IgG。
【請求項７】
FcRnに対して、野生型IgG定常ドメインを有するIgGより高い親和性を有する、請求項1、2または3記載の修飾IgG。
【請求項８】
FcRnに対して、pH 7.4におけるより高い親和性をpH 6.0において有する、請求項7記載の修飾IgG。
【請求項９】
前記1以上のアミノ酸修飾が251、255、308、309、311、312、314、385、386、387、389、428、433、434または436位の1以上におけるアミノ酸の置換である、請求項1記載の修飾IgG。
【請求項１０】
前記1以上のアミノ酸修飾が251、252、254、255、256、308、309、311、312、314、385、386、387、389、428、433、434または336位の1以上におけるアミノ酸の置換である、請求項2または3記載の修飾IgG。
【請求項１１】
前記1以上のアミノ酸修飾が、251位におけるロイシンによる置換、252位におけるチロシン、トリプトファンまたはフェニルアラニンによる置換、254位におけるトレオニンまたはセリンによる置換、255位におけるアルギニンによる置換、256位におけるグルタミン、アルギニン、セリン、トレオニンまたはグルタミン酸による置換、308位におけるトレオニンによる置換、309位におけるプロリンによる置換、311位におけるセリンによる置換、312位におけるアスパラギン酸による置換、314位におけるロイシンによる置換、385位におけるアルギニン、アスパラギン酸またはセリンによる置換、386位におけるトレオニン

またはプロリンによる置換、387位におけるアルギニンまたはプロリンによる置換、389位におけるプロリン、アスパラギンまたはセリンによる置換、428位におけるメチオニンまたはトレオニンによる置換、434位におけるチロシンまたはフェニルアラニンによる置換、433位におけるヒスチジン、アルギニン、リシンまたはセリンによる置換、あるいは436位におけるヒスチジン、チロシン、アルギニンまたはトレオニンによる置換である、請求項1または3記載の修飾IgG。

【請求項１２】

前記1以上のアミノ酸置換が、252位におけるチロシン、254位におけるトレオニンおよび256位におけるグルタミン酸による置換である、請求項11記載の修飾IgG。

【請求項１３】

前記1以上のアミノ酸置換が、433位におけるリシン、434位におけるフェニルアラニンおよび436位におけるヒスチジンによる置換である、請求項11記載の修飾IgG。

【請求項１４】

前記アミノ酸置換が、252位におけるチロシンまたはトリプトファンによる置換である、請求項11記載の修飾IgG。

【請求項１５】

前記1以上のアミノ酸修飾が、251位におけるロイシンによる置換、252位におけるチロシン、トリプトファンまたはフェニルアラニンによる置換、254位におけるトレオニンによる置換、255位におけるアルギニンによる置換、256位におけるグルタミン、アルギニン、セリン、トレオニンまたはグルタミン酸による置換、308位におけるトレオニンによる置換、309位におけるプロリンによる置換、311位におけるセリンによる置換、312位におけるアスパラギン酸による置換、314位におけるロイシンによる置換、385位におけるアルギニン、アスパラギン酸またはセリンによる置換、386位におけるトレオニンまたはプロリンによる置換、387位におけるアルギニンまたはプロリンによる置換、389位におけるプロリン、アスパラギンまたはセリンによる置換、428位におけるメチオニンまたはトレオニンによる置換、434位におけるチロシンまたはフェニルアラニンによる置換、433位におけるヒスチジン、アルギニン、リシンまたはセリンによる置換、あるいは436位におけるヒスチジン、チロシン、アルギニンまたはトレオニンによる置換である、請求項2記載の修飾IgG。

【請求項１６】

前記1以上のアミノ酸置換が、252位におけるチロシン、254位におけるトレオニンおよび256位におけるグルタミン酸による置換である、請求項15記載の修飾IgG。

【請求項１７】

前記1以上のアミノ酸置換が、433位におけるリシン、434位におけるフェニルアラニンおよび436位におけるヒスチジンによる置換である、請求項15記載の修飾IgG。

【請求項１８】

前記アミノ酸置換が、252位におけるチロシンまたはトリプトファンによる置換である、請求項15記載の修飾IgG。

【請求項１９】

SYNAGIS（登録商標）の重鎖可変ドメインおよび軽鎖可変ドメインを有する、請求項3または15記載の修飾IgG。

【請求項２０】

AFFF、p12f2、p12f4、p11d4、Ale109、A12a6、A13c4、A17d4、A4B4、A8C7、1X-493L1FR、H3-3F4、M3H9、Y10H6、DG、AFFF(1)、6H8、L1-7E5、L215B10、A13A11、A1H5、A4B4(1)、A4B4L1FR-S28RまたはA4B4-F52Sの重鎖可変ドメインおよび軽鎖可変ドメインを有する、請求項3または15記載の修飾IgG。

【請求項２１】

修飾されたIgG定常ドメインまたはそのFcRn結合性フラグメントに共有結合した非IgGポリペプチドを含んでなる融合タンパク質であって、該修飾IgG定常ドメインまたはフラグメントが野生型IgG定常ドメインに対して1以上のアミノ酸修飾を含み、前記1以上の修飾が2

51、253、255、285-290、308-314、385-389および428-436位の1以上に存在し、該融合タンパク質が、該非IgGポリペプチドのみの場合より長い半減期を有することを特徴とする、前記融合タンパク質。

【請求項２２】

修飾された非ヒトIgG定常ドメインまたはそのFcRn結合性フラグメントに共有結合した非IgGポリペプチドを含んでなる融合タンパク質であって、該修飾非ヒトIgG定常ドメインまたはフラグメントが野生型非ヒトIgG定常ドメインに対して1以上のアミノ酸修飾を含み、前記1以上の修飾が251-256、285-290、308-314、385-389および428-436位の1以上に存在するが、該修飾アミノ酸配列は、252位にロイシン、254位にセリンおよび256位にフェニルアラニンをもたず、該融合タンパク質が、該非IgGポリペプチドのみの場合より長い半減期を有することを特徴とする、前記融合タンパク質。

【請求項２３】

修飾されたヒトIgG定常ドメインまたはそのFcRn結合性フラグメントに共有結合した非IgGポリペプチドを含んでなる融合タンパク質であって、該修飾ヒトIgG定常ドメインまたはフラグメントが野生型ヒトIgG定常ドメインに対して1以上のアミノ酸修飾を含み、前記1以上の修飾が251-256、285-290、308-314、385-389および428-436位の1以上に存在し、該融合タンパク質が、該非IgGポリペプチドのみの場合より長い半減期を有することを特徴とする、前記融合タンパク質。

【請求項２４】

前記アミノ酸修飾の少なくとも1つがアミノ酸の置換である、請求項21、22または23記載の融合タンパク質。

【請求項２５】

前記アミノ酸修飾の少なくとも1つがアミノ酸の欠失である、請求項21、22または23記載の融合タンパク質。

【請求項２６】

前記アミノ酸修飾の少なくとも1つがアミノ酸の挿入である、請求項21、22または23記載の融合タンパク質。

【請求項２７】

前記修飾IgG定常ドメインまたはフラグメントが、FcRnに対して、野生型IgG定常ドメインと比較して増加した親和性を有する、請求項21、22または23記載の融合タンパク質。

【請求項２８】

前記修飾IgG定常ドメインまたはフラグメントが、FcRnに対して、pH 7.4におけるより高い親和性をpH 6.0において有する、請求項27記載の融合タンパク質。

【請求項２９】

前記非IgGポリペプチドが免疫グロブリンである、請求項21、22または23記載の融合タンパク質。

【請求項３０】

前記1以上のアミノ酸修飾が251、255、308、309、311、312、314、385、386、387、389、428、433、434または436位の1以上におけるアミノ酸の置換である、請求項21記載の融合タンパク質。

【請求項３１】

前記1以上のアミノ酸修飾が251、252、254、255、256、308、309、311、312、314、385、386、387、389、428、433、434または336位の1以上におけるアミノ酸の置換である、請求項22または23記載の融合タンパク質。

【請求項３２】

前記1以上のアミノ酸修飾が、251位におけるロイシンによる置換、252位におけるチロシン、トリプトファンまたはフェニルアラニンによる置換、254位におけるトレオニンまたはセリンによる置換、255位におけるアルギニンによる置換、256位におけるグルタミン、アルギニン、セリン、トレオニンまたはグルタミン酸による置換、308位におけるトレオニンによる置換、309位におけるプロリンによる置換、311位におけるセリンによる置換、

312位におけるアスパラギン酸による置換、314位におけるロイシンによる置換、385位におけるアルギニン、アスパラギン酸またはセリンによる置換、386位におけるトレオニンまたはプロリンによる置換、387位におけるアルギニンまたはプロリンによる置換、389位におけるプロリン、アスパラギンまたはセリンによる置換、428位におけるメチオニンまたはトレオニンによる置換、434位におけるチロシンまたはフェニルアラニンによる置換、433位におけるヒスチジン、アルギニン、リシンまたはセリンによる置換、あるいは436位におけるヒスチジン、チロシン、アルギニンまたはトレオニンによる置換である、請求項21または23記載の融合タンパク質。

【請求項３３】

前記1以上のアミノ酸置換が、252位におけるチロシン、254位におけるトレオニンおよび256位におけるグルタミン酸による置換である、請求項32記載の融合タンパク質。

【請求項３４】

前記1以上のアミノ酸置換が、433位におけるリシン、434位におけるフェニルアラニンおよび436位におけるヒスチジンによる置換である、請求項32記載の融合タンパク質。

【請求項３５】

前記アミノ酸置換が、252位におけるチロシンまたはトリプトファンによる置換である、請求項32記載の融合タンパク質。

【請求項３６】

前記1以上のアミノ酸修飾が、251位におけるロイシンによる置換、252位におけるチロシン、トリプトファンまたはフェニルアラニンによる置換、254位におけるトレオニンによる置換、255位におけるアルギニンによる置換、256位におけるグルタミン、アルギニン、セリン、トレオニンまたはグルタミン酸による置換、308位におけるトレオニンによる置換、309位におけるプロリンによる置換、311位におけるセリンによる置換、312位におけるアスパラギン酸による置換、314位におけるロイシンによる置換、385位におけるアルギニン、アスパラギン酸またはセリンによる置換、386位におけるトレオニンまたはプロリンによる置換、387位におけるアルギニンまたはプロリンによる置換、389位におけるプロリン、アスパラギンまたはセリンによる置換、428位におけるメチオニンまたはトレオニンによる置換、434位におけるチロシンまたはフェニルアラニンによる置換、433位におけるヒスチジン、アルギニン、リシンまたはセリンによる置換、あるいは436位におけるヒスチジン、チロシン、アルギニンまたはトレオニンによる置換である、請求項32記載の融合タンパク質。

【請求項３７】

前記1以上のアミノ酸置換が、252位におけるチロシン、254位におけるトレオニンおよび256位におけるグルタミン酸による置換である、請求項36記載の融合タンパク質。

【請求項３８】

前記1以上のアミノ酸置換が、433位におけるリシン、434位におけるフェニルアラニンおよび436位におけるヒスチジンによる置換である、請求項36記載の融合タンパク質。

【請求項３９】

前記アミノ酸置換が、252位におけるチロシンまたはトリプトファンによる置換である、請求項36記載の融合タンパク質。

【請求項４０】

修飾されたIgG定常ドメインまたはそのFcRn結合性フラグメントに結合した非タンパク質物質を含んでなる分子であって、該修飾IgG定常ドメインまたはフラグメントが野生型IgG定常ドメインに対して1以上のアミノ酸修飾を含み、前記1以上の修飾が251、253、255、285-290、308-314、385-389および428-436位の1以上に存在し、該分子が、該非タンパク質物質のみの場合より長い半減期を有することを特徴とする、前記分子。

【請求項４１】

修飾された非ヒトIgG定常ドメインまたはそのFcRn結合性フラグメントに結合した非タンパク質物質を含んでなる分子であって、該修飾非ヒトIgG定常ドメインまたはフラグメントが野生型非ヒトIgG定常ドメインに対して1以上のアミノ酸修飾を含み、前記1以上の修

飾が251-256、285-290、308-314、385-389および428-436位の1以上に存在するが、該修飾アミノ酸配列は、252位にロイシン、254位にセリンおよび256位にフェニルアラニンをもたず、該分子が、該非タンパク質物質のみの場合より長い半減期を有することを特徴とする、前記分子。

【請求項４２】

修飾されたヒトIgG定常ドメインまたはそのFcRn結合性フラグメントに結合した非タンパク質物質を含んでなる分子であって、該修飾ヒトIgG定常ドメインまたはフラグメントが野生型ヒトIgG定常ドメインに対して1以上のアミノ酸修飾を含み、前記1以上の修飾が251-256、285-290、308-314、385-389および428-436位の1以上に存在し、該分子が、該非タンパク質物質のみの場合より長い半減期を有することを特徴とする、前記分子。

【請求項４３】

前記アミノ酸修飾の少なくとも1つがアミノ酸の置換である、請求項40、41または42記載の分子。

【請求項４４】

前記アミノ酸修飾の少なくとも1つがアミノ酸の欠失である、請求項40、41または42記載の分子。

【請求項４５】

前記アミノ酸修飾の少なくとも1つがアミノ酸の挿入である、請求項40、41または42記載の分子。

【請求項４６】

前記修飾IgG定常ドメインまたはフラグメントが、FcRnに対して、野生型IgG定常ドメインと比較して増加した親和性を有する、請求項40、41または42記載の分子。

【請求項４７】

前記修飾IgG定常ドメインまたはフラグメントが、FcRnに対して、pH 7.4におけるより高い親和性をpH 6.0において有する、請求項46記載の分子。

【請求項４８】

前記1以上のアミノ酸修飾が251、255、308、309、311、312、314、385、386、387、389、428、433、434または436位の1以上におけるアミノ酸の置換である、請求項40記載の分子。

【請求項４９】

前記1以上のアミノ酸修飾が251、252、254、255、256、308、309、311、312、314、385、386、387、389、428、433、434または336位の1以上における置換である、請求項41または42記載の分子。

【請求項５０】

前記1以上のアミノ酸修飾が、251位におけるロイシンによる置換、252位におけるチロシン、トリプトファンまたはフェニルアラニンによる置換、254位におけるトレオニンまたはセリンによる置換、255位におけるアルギニンによる置換、256位におけるグルタミン、アルギニン、セリン、トレオニンまたはグルタミン酸による置換、308位におけるトレオニンによる置換、309位におけるプロリンによる置換、311位におけるセリンによる置換、312位におけるアスパラギン酸による置換、314位におけるロイシンによる置換、385位におけるアルギニン、アスパラギン酸またはセリンによる置換、386位におけるトレオニンまたはプロリンによる置換、387位におけるアルギニンまたはプロリンによる置換、389位におけるプロリン、アスパラギンまたはセリンによる置換、428位におけるメチオニンまたはトレオニンによる置換、434位におけるチロシンまたはフェニルアラニンによる置換、433位におけるヒスチジン、アルギニン、リシンまたはセリンによる置換、あるいは436位におけるヒスチジン、チロシン、アルギニンまたはトレオニンによる置換である、請求項40または42記載の分子。

【請求項５１】

前記1以上のアミノ酸置換が、252位におけるチロシン、254位におけるトレオニンおよび256位におけるグルタミン酸による置換である、請求項50記載の分子。

【請求項５２】
前記1以上のアミノ酸置換が、433位におけるリシン、434位におけるフェニルアラニンおよび436位におけるヒスチジンによる置換である、請求項50記載の分子。
【請求項５３】
前記アミノ酸置換が、252位におけるチロシンまたはトリプトファンによる置換である、請求項50記載の分子。
【請求項５４】
前記1以上のアミノ酸修飾が、251位におけるロイシンによる置換、252位におけるチロシン、トリプトファンまたはフェニルアラニンによる置換、254位におけるトレオニンによる置換、255位におけるアルギニンによる置換、256位におけるグルタミン、アルギニン、セリン、トレオニンまたはグルタミン酸による置換、308位におけるトレオニンによる置換、309位におけるプロリンによる置換、311位におけるセリンによる置換、312位におけるアスパラギン酸による置換、314位におけるロイシンによる置換、385位におけるアルギニン、アスパラギン酸またはセリンによる置換、386位におけるトレオニンまたはプロリンによる置換、387位におけるアルギニンまたはプロリンによる置換、389位におけるプロリン、アスパラギンまたはセリンによる置換、428位におけるメチオニンまたはトレオニンによる置換、434位におけるチロシンまたはフェニルアラニンによる置換、433位におけるヒスチジン、アルギニン、リシンまたはセリンによる置換、あるいは436位におけるヒスチジン、チロシン、アルギニンまたはトレオニンによる置換である、請求項41記載の分子。
【請求項５５】
前記1以上のアミノ酸置換が、252位におけるチロシン、254位におけるトレオニンおよび256位におけるグルタミン酸による置換である、請求項54記載の分子。
【請求項５６】
前記1以上のアミノ酸置換が、433位におけるリシン、434位におけるフェニルアラニンおよび436位におけるヒスチジンによる置換である、請求項54記載の分子。
【請求項５７】
前記アミノ酸置換が、252位におけるチロシンまたはトリプトファンによる置換である、請求項54記載の分子。
【請求項５８】
請求項3記載の修飾ヒトまたはヒト化IgGと製薬上許容される担体とを含んでなる医薬組成物。
【請求項５９】
請求項23記載の融合タンパク質と製薬上許容される担体とを含んでなる医薬組成物。
【請求項６０】
請求項42記載の分子と製薬上許容される担体とを含んでなる医薬組成物。
【請求項６１】
疾患または障害の治療を要する患者に、請求項3記載の修飾ヒトまたはヒト化IgGの治療的有効量を投与することを含んでなる、疾患または障害の治療方法。
【請求項６２】
受動免疫療法を含む、請求項61記載の方法。
【請求項６３】
疾患または障害の治療を要する患者に、請求項23記載の融合タンパク質の治療的有効量を投与することを含んでなる、疾患または障害の治療方法。
【請求項６４】
疾患または障害の治療を要する患者に、請求項42記載の分子の治療的有効量を投与することを含んでなる、疾患または障害の治療方法。
【請求項６５】
請求項1、2または3記載の修飾IgG定常ドメインまたはそのFcRn結合性フラグメントをコードするヌクレオチド配列を含んでなる核酸。

【請求項６６】
請求項21、22または23記載の融合タンパク質をコードするヌクレオチド配列を含んでなる核酸。
【請求項６７】
請求項65記載の核酸を含んでなる宿主細胞。
【請求項６８】
請求項66記載の核酸を含んでなる宿主細胞。
【請求項６９】
請求項3記載の修飾ヒトまたはヒト化IgGを含んでなるキット。
【請求項７０】
請求項23記載の融合タンパク質を含んでなるキット。
【請求項７１】
請求項42記載の分子を含んでなるキット。
【請求項７２】
請求項3記載の修飾ヒトまたはヒト化IgGの予防的有効量を被験者に投与することを含んでなる、疾患または障害の予防方法。
【請求項７３】
受動免疫療法である、請求項72記載の方法。
【請求項７４】
前記疾患がRSV感染症である、請求項72記載の方法。
【請求項７５】
前記修飾ヒトまたはヒト化IgGが、修飾されたSYNAGIS（登録商標）抗体である、請求項74記載の方法。
【請求項７６】
前記修飾ヒトまたはヒト化IgGが、修飾されたAFFF、p12f2、p12f4、p11d4、Ale109、A12a6、A13c4、A17d4、A4B4、A8C7、1X-493L1FR、H3-3F4、M3H9、Y10H6、DG、AFFF(1)、6H8、L1-7E5、L215B10、A13A11、A1H5、A4B4(1)、A4B4L1FR-S28RまたはA4B4-F52S抗体である、請求項74記載の方法。
【請求項７７】
免疫応答を惹起するのに有効な量の請求項3記載の修飾ヒトまたはヒト化IgGを被験者に投与することを含んでなる、被験者へのワクチン接種方法。
【請求項７８】
免疫応答を惹起するのに有効な量の請求項23記載の融合タンパク質を被験者に投与することを含んでなる、被験者へのワクチン接種方法。
【請求項７９】
前記1以上のアミノ酸修飾を欠く以外は前記修飾ヒトまたはヒト化IgGと同一である第2 IgGの最低の治療的有効量より低い用量の該修飾ヒトまたはヒト化IgGを投与することを含む、請求項61記載の方法。
【請求項８０】
第2 IgGの治療的有効量の投与より少ない又は重篤でない副作用を与える、請求項79記載の方法。
【請求項８１】
前記1以上のアミノ酸修飾を欠く以外は前記修飾ヒトまたはヒト化IgGと同一である第2 IgGの最低頻度の投与を伴う治療的に有効な投与計画より低い頻度の該修飾ヒトまたはヒト化IgGの投与を伴う治療的に有効な投与計画で投与することを含む、請求項61記載の方法。
【請求項８２】
前記1以上のアミノ酸修飾を欠く以外は前記修飾ヒトまたはヒト化IgGと同一である第2 IgGの最低の予防的有効量より低い予防的有効量の該修飾ヒトまたはヒト化IgGを投与することを含む、請求項72記載の方法。

【請求項８３】
第2 IgGの予防的有効量の投与より少ない又は重篤でない副作用を与える、請求項82記載の方法。
【請求項８４】
前記1以上のアミノ酸修飾を欠く以外は前記ヒトまたはヒト化IgGと同一である第2 IgGの最低頻度の投与を伴う予防的に有効な投与計画より低い頻度の該修飾ヒトまたはヒト化IgGの投与を伴う予防的に有効な投与計画で投与することを含む、請求項72記載の方法。
【請求項８５】
（a）検出可能なマーカーで標識された請求項3記載の修飾ヒトまたはヒト化IgGの有効量を被験者に投与すること、ただし、該修飾ヒトまたはヒト化IgGは、疾患または障害に関連した抗原と特異的に結合すること、
（b）該抗原が見出される被験者の部位に該修飾ヒトまたはヒト化IgGを集中させること、
（c）該検出可能なマーカーを検出すること、
を含んでなり、その際、バックグラウンドまたは標準レベルを超える検出可能なマーカーの検出が、該被験者が疾患または障害を有することを示すことを特徴とする、被験者におけるin vivo診断方法。
【請求項８６】
RSV抗原に免疫特異的に結合する、請求項3記載の修飾ヒトまたはヒト化IgG。
【発明の詳細な説明】
【技術分野】
【０００１】
１．緒言
本発明は、IgG定常ドメインまたはそのFcRn（Fc Receptor-neonate）結合性ドメインの修飾によりin vivo半減期が増加した分子に関する。より詳しくは、これらの分子は、FcRnに対する該定常ドメインまたはそのフラグメント（断片）の親和性を増加させるアミノ酸修飾を有する。本発明を用いた治療用および診断用のIgGおよび他の生物活性分子の半減期の増加は、例えばワクチン、受動免疫療法ならびに他の治療および予防方法におけるこれらの分子の投与量および／または投与頻度の減少を含む多数の利点を有する。本発明は更に、これらのアミノ酸修飾の1以上を有するIgG定常ドメインの全部または一部（FcRn結合性部分）と、そのような修飾IgG定常ドメインに結合した非IgGタンパク質または非タンパク質分子とを含有する融合タンパク質に関する。ここで、修飾IgG定常ドメインの存在は非IgGタンパク質または分子のin vivo半減期を増加させる。
【背景技術】
【０００２】
２．発明の背景
治療剤としての免疫グロブリンの使用は近年劇的に増加しており、医学的治療の種々の領域に拡張されている。そのような使用は、無γグロブリン血症および低γグロブリン血症の治療、自己免疫疾患および移植片対宿主病を治療するための免疫抑制剤としての使用、リンパ性悪性疾患の治療、ならびに種々の全身性および感染性疾患を治療するための受動免疫療法を包含する。免疫グロブリンは、例えば診断イメージング法におけるin vivo診断ツールとしても有用である。
【０００３】
これらの療法における1つの重要な問題は、循環中の免疫グロブリンの持続性である。免疫グロブリンの消失速度は免疫グロブリンの投与量および投与頻度に直接的に影響を及ぼす。投与量および投与頻度の増加は患者に悪影響を及ぼすことがあり、医療コストを増加させうる。
【０００４】
IgGは、ヒトおよび他の哺乳動物における最も一般的な免疫グロブリンクラスであり、種々のタイプの免疫療法および診断方法において利用される。循環中のIgGの異化のメカニズムは、胎盤もしくは卵黄嚢を介した又はげっ歯類における初乳を介した（IgGのトラン

スサイトーシスによる母児移行）母親から胎児／新生児への受動免疫の移行に関連した研究により解明されている（Brambell, Lancet, ii: 1087-1093,1966; Rodewald, J. Cell Biol., 71: 666-670,1976; Morrisら, In: Antigen Absorption by the Gut, pp. 3-22,1978, University Park Press, Baltimore; Jonesら, J. Clin. Invest., 51: 2916-2927,1972）。

【０００５】

母親IgGの母児移行における或る受容体の関与が、Brambellのグループにより、新生児ラットにおける摂取乳からの母親抗体の腸吸収に関する彼らの研究において最初に示唆された（Halliday, Proc. R. Soc. B., 143: 408-413,1955; Halliday, Proc. R. Soc. B., 144: 427-430,1955; Halliday, Proc. R. Soc. B., 148: 92-103,1957; Morris, Proc. R. Soc. B., 148: 84-91,1957; Brambellら, Proc. R. Soc. B., 149: 1-11,1958; Morris, Proc. R. Soc. B., 160: 276-292,1964）。Brambellらは、異種IgGが特定の抗体の移行を妨げたという観察に基づき、種々の生物種からのIgG分子が、共通受容体に結合するのに十分な程度に類似した構造または配列を有しうることを示唆した（Brambellら, Proc. R. Soc. B., 149: 1-11,1958）。

【０００６】

この移行メカニズムには高親和性Fc受容体FcRnが関与している。FcRn受容体は乳飲みラットの十二指腸上皮刷子縁から単離されており（Rodewaldら, J. Cell Biol., 99: 154s-164s, 1984 ; Simisterら, Eur. J. Immunol., 15: 733-738,1985）、対応する遺伝子がクローニングされている（Simisterら, Nature, 337: 184,1989およびCold Spring Harbor Symp. Quant. Biol., LIV, 571-580,1989）。後にマウス（Ahouseら, J. Immunol., 151: 6076-6088,1993）およびヒト（Storyら, J. Exp. Med., 180: 2377-2381,1994）からFcRnコード化遺伝子がクローニングされ、これにより、ラットFcRnに対するこれらの配列の高い相同性が示された。このことは、FcRnが関与するIgGの母児移行のメカニズムが、これらの生物種において類似していることを示唆している。

【０００７】

一方、IgG異化のメカニズムもBrambellのグループにより提示されている（Brambellら, Nature, 203: 1352-1355,1964; Brambell, Lancet, ii : 1087-1093,1966）。飽和しうる或る細胞受容体が循環中のIgG分子の一部に結合し、それにより該IgGは分解から防御され最終的に循環中にリサイクルされ、一方、該受容体が結合していないIgGは分解される、と彼らは提示した。その提示されたメカニズムは、高γグロブリン血症または低γグロブリン血症の患者において観察されるIgG異化と符合した。さらに、彼の研究および他の研究者の研究（例えば、Spiegelbergら, J. Exp. Med., 121: 323-338,1965; Edelmanら, Proc. Natl. Acad. Sci. USA, 63: 78-85,1969）に基づき、Brambellはまた、IgGの母児移行に関与するメカニズムとIgGの異化に関与するメカニズムとが同じであるか又は少なくとも非常に密接に関連している可能性があることを示唆した（Brambell, Lancet, ii : 1087-1093,1966）。実際のところ、後になって、Fc-ヒンジフラグメント中の突然変異が異化、母児移行、新生児トランスサイトーシス、そして特にFcRnへの結合における付随的変化を引きこすことが報告された（Ghetieら, Immunology Today, 18 (12): 592-598,1997）。

【０００８】

これらの観察は、IgG定常ドメインの一部が、FcRnとの相互作用により血清中のIgG分解の速度を含むIgG代謝を制御することを示唆した。実際のところ、FcRnへの結合親和性の増加は該分子の血清半減期を増加させた（Kimら, Eur. J. Immunol., 24: 2429-2434,1994; Popovら, Mol. Immunol., 33: 493-502,1996; Ghetieら, Eur. J. Immunol., 26: 690-696,1996; Junghansら, Proc. Natl. Acad. Sci. USA, 93: 5512-5516,1996; Israelら, Immunol., 89:573-578,1996）。

【０００９】

マウスIgGのFc領域における種々の部位特異的突然変異誘発実験は、IgGとFcRnとの相互作用に関与する或る重要なアミノ酸残基の同定をもたらした（Kimら, Eur. J. Immunol., 2

4: 2429-2434,1994; Medesanら, Eur. J. Immunol., 26: 2533,1996; Medesanら, J. Immunol., 158: 2211- 2217,1997）。これらの研究および配列比較研究は、253位のイソロイシン、310位のヒスチジンおよび435位のヒスチジン（Kabatの番号付けに基づく: Kabatら, In: Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, 1991（その全体を参照により本明細書に組み入れることとする））がヒトおよびげっ歯類IgGにおいて高度に保存されていることを見出した。これは、IgG-FcRn結合におけるそれらの重要性を示唆している。

【００１０】

また、種々の刊行物が、半減期が修飾された生理活性分子を入手するための方法を記載しており、これらの方法は、FcRn結合性ポリペプチドを該分子中に導入することにより（WO 97/43316; 米国特許第5,869,046号; 米国特許第5,747,035号; WO 96/32478; WO 91/14438）、またはFcRn結合親和性は維持されているが他のFc受容体に対する親和性は著しく減少した抗体に該分子を融合することにより（WO 99/43713）、または抗体のFcRn結合性ドメインに融合することにより行われた（WO 00/09560; 米国特許第4,703,039号）。しかし、これらの刊行物のいずれにおいても、半減期に影響を及ぼすIgG定常ドメイン中の特定の突然変異は開示されていない。

【００１１】

従来の研究は、或る定常ドメイン突然変異が実際にFcRnへの結合を減弱してIgGのin vivo半減期を減少させることを示している。PCT公開WO 93/22332（Wardら）は、およそ残基253と残基434との間の突然変異によりin vivo半減期が減少した種々の組換えマウスIgGを開示している。特に、253位のイソロイシン、310位のヒスチジン、311位のグルタミン、433位のHisおよび434位のアスパラギンの置換がIgGの半減期を減少させることが判明した。

【００１２】

また、FcRnに対する親和性を増加または減少させるアミノ酸の置換、付加または欠失によるIgG分子のモジュレーションがWO 98/23289に開示されているが、該刊行物は、より長い又はより短いin vivo半減期を示す特定の突然変異体を何ら記載していない。

【００１３】

実は、半減期の増加を実際に示したマウスIgG１の突然変異体（Thr252からAla、Thr254からSerおよびThr256からPheの三重突然変異）の1つが同定されているに過ぎない（WO 97/34631）。

【００１４】

免疫グロブリンおよび他の生物活性分子のin vivo半減期の増加の医薬的重要性を考慮すると、免疫グロブリンおよび他の生物活性分子にin vivo半減期の増加をもたらす修飾IgGおよびそのFcRn結合性フラグメント（特に修飾ヒトIgG）を開発することが必要とされている。

【発明の開示】

【００１５】

３．発明の概要

本発明は、FcRnに対するIgG分子の親和性を増加させるヒトIgG分子の定常ドメイン中のいくつかの突然変異の本発明者らによる同定に基づく。特に、本発明者らは、該定常ドメインの特定の領域中に導入されたランダムなアミノ酸突然変異を有するヒトIgG1定常ドメインのライブラリーを、FcRnに対する親和性の増加に関してスクリーニングした。そのようなランダム突然変異は、ヒトIgG1ヒンジ-Fc領域の残基251-256、285-290および308-314（これらのすべてはCH2ドメイン中に存在する）ならびに385-389および428-436（これらはCH3ドメイン中に存在する）の領域中に施された（残基は図2（配列番号83）に示すとおりであり、あるいは他のIgG分子のヒンジ-Fc領域中の類似残基は配列アライメントにより決定した）。本発明で用いるIgG定常ドメインの全残基はKabatら（Sequences of Proteins of Immunological Interest, U. S. Department of Health and Human Services, 1991（その全体を参照により本明細書に組み入れることとする））に従い番号づけされており、図2（配列番号83）に記載されているとおりであり、配列アライメントにより決定された

他のIgG定常ドメイン中の対応残基を含む。抗体および他の治療剤および他の生物活性分子のin vivo半減期または被験者の血清もしくは他の組織中での維持は、抗体（または任意の他の医薬分子）の投与量および投与頻度を決定する重要な臨床的パラメーターである。したがって、増加した半減期を有するそのような分子（抗体を含む）は医薬上極めて重要である。

【００１６】

したがって、本発明は、FcRnに対する親和性を増加するようIgG定常ドメインまたはそのフラグメントが（例えば、アミノ酸の置換、欠失または挿入により）修飾された修飾IgG定常ドメインまたはそのFcRn結合性部分（好ましくはFcまたはヒンジ-Fcドメイン）（好ましくはヒトIgG由来）の存在により増加したin vivo半減期を有する修飾分子（好ましくはタンパク質であるが、非タンパク質物質であってもよい）に関する。特定の実施形態においては、本発明は、FcRn受容体とのヒンジ-Fcドメインの相互作用に関与することが確認されたアミノ酸残基の修飾によりin vivo半減期が延長された修飾IgGに関する。好ましくは、該定常ドメインまたはそのフラグメントは、pH 6.0においては、pH 7.4におけるより高い親和性をFcRnに対して有する。また、そのような修飾は該分子のバイオアベイラビリティ（例えば、粘膜表面または他の標的組織への輸送）を改変（すなわち、増強または減弱）しうる。本発明はまた、1以上のそのようなアミノ酸修飾を有するIgG定常ドメインまたはそのFcRn結合性フラグメントに融合または結合した或いはそれを含有するよう操作された他の型の免疫グロブリンまたはそのフラグメント（すなわち、非IgG免疫グロブリン）、非免疫グロブリンタンパク質および非タンパク質物質に関する。

【００１７】

好ましい実施形態においては、本発明は、FcRnに対する定常ドメインまたはそのフラグメントの親和性を増加させるアミノ酸残基251-256、285-290、308-314、385-389および428-436の1以上の修飾を有するIgG定常ドメインまたはそのFcRn結合性フラグメント（好ましくはFcまたはヒンジ-Fcドメイン）の存在によりin vivo半減期が延長された分子、特に免疫グロブリンを提供する。ある実施形態においては、好ましくは、これらの修飾は、IgG定常ドメインまたはそのフラグメントがマウスのものである場合には特に、残基252、254および256以外のものである。特定の実施形態においては、該修飾は1以上の表面露出残基におけるものであり、該修飾は、置換される残基に類似した電荷、極性または疎水性の残基による置換である。好ましい実施形態においては、該修飾IgG定常ドメインまたはそのフラグメントは、pH 6.0においては、pH 7.4におけるより高い親和性でFcRnに結合する。好ましい実施形態においては、該定常ドメインまたはそのフラグメントは、FcRnに対する定常ドメインまたはそのFcRn結合性フラグメントの親和性を増加させるアミノ酸残基251-256、285-290、308-314、385-389および428-436の1以上の置換により修飾される。ある実施形態においては、定常ドメインまたはそのフラグメントがマウスIgGに由来する場合には特に、ロイシンによる残基252の置換、セリンによる残基254の置換、および／またはフェニルアラニンによる残基256の置換は除かれる。

【００１８】

特定の実施形態においては、本発明は、308、309、311、312および314位の1以上におけるアミノ酸修飾を有する、より詳しくは、それぞれトレオニン、プロリン、セリン、アスパラギン酸およびロイシンによる308、309、311、312および314位の1以上における置換を有するIgG1定常ドメインまたはそのFcRn結合性フラグメント（好ましくはFcまたはヒンジ-Fcドメイン）（好ましくはヒトのもの）を含有する免疫グロブリンおよび他の生物活性分子を提供する。もう1つの実施形態においては、308、309および311位の1以上における残基は、それぞれイソロイシン、プロリンおよびグルタミン酸により置換される。さらにもう1つの実施形態においては、308、309、311、312および314位の1以上における残基は、それぞれトレオニン、プロリン、セリン、アスパラギン酸およびロイシンにより置換される。本発明は更に、これらのアミノ酸置換の組合せに関する。

【００１９】

さらに、本発明は、251、252、254、255および256位の1以上におけるアミノ酸修飾を有す

る、より詳しくは、これらの位置の1以上における置換を有するIgG1定常ドメインまたはそのFcRn結合性フラグメント（好ましくはFcまたはヒンジ-Fcドメイン）（好ましくはヒトのもの）を含有する免疫グロブリンおよび他の生物活性分子を提供する。特定の実施形態においては、残基251はロイシンまたはアルギニンにより置換され、残基252はチロシン、フェニルアラニン、セリン、トリプトファンまたはトレオニンにより置換され、残基254はトレオニンまたはセリンにより置換され、残基255はロイシン、グリシン、イソロイシンまたはアルギニンにより置換され、および／または、残基256はセリン、アルギニン、グルタミン、グルタミン酸、アスパラギン酸、アラニン、アスパラギンまたはトレオニンにより置換される。より具体的な実施形態においては、残基251はロイシンにより置換され、残基252はチロシンにより置換され、残基254はトレオニンまたはセリンにより置換され、および／または残基255はアルギニンにより置換される。さらにもう1つの特定の実施形態においては、残基252はフェニルアラニンにより置換され、および／または、残基256はアスパラギン酸により置換される。好ましい実施形態においては、残基251はロイシンにより置換され、残基252はチロシンにより置換され、残基254はトレオニンまたはセリンにより置換され、および／または、残基255はアルギニンにより置換される。本発明は更に、これらの置換の任意の組合せに関する。

【００２０】

さらに、本発明は、428、433、434および436位の1以上におけるアミノ酸修飾を有する、より詳しくは、これらの位置の1以上における置換を有するIgG1定常ドメインまたはそのFcRn結合性フラグメント（好ましくはFcまたはヒンジ-Fcドメイン）（好ましくはヒトのもの）を含有する免疫グロブリンおよび他の生物活性分子を提供する。特定の実施形態においては、残基428はメチオニン、トレオニン、ロイシン、フェニルアラニンまたはセリンにより置換され、残基433はリシン、アルギニン、セリン、イソロイシン、プロリン、グルタミンまたはヒスチジンにより置換され、残基434はフェニルアラニン、チロシンまたはヒスチジンにより置換され、および／または、残基436はヒスチジン、アスパラギン、アルギニン、トレオニン、リシン、メチオニンまたはトレオニンにより置換される。より具体的な実施形態においては、433、434および436位の1以上における残基が、それぞれリシン、フェニルアラニンおよびヒスチジンにより置換される。好ましい実施形態においては、残基428はメチオニンにより置換され、および／または、残基434はチロシンにより置換される。

【００２１】

さらに、本発明は、385、386、387および389位の1以上におけるアミノ酸修飾を有する、より詳しくは、これらの位置の1以上における置換を有するIgG1定常ドメインまたはそのFcRn結合性フラグメント（好ましくはFcまたはヒンジ-Fcドメイン）（好ましくはヒトのもの）を含有する免疫グロブリンおよび他の生物活性分子を提供する。特定の実施形態においては、残基385はアルギニン、アスパラギン酸、セリン、トレオニン、ヒスチジン、リシンまたはアラニンにより置換され、残基386はトレオニン、プロリン、アスパラギン酸、セリン、リシン、アルギニン、イソロイシンまたはメチオニンにより置換され、残基387はアルギニン、ヒスチジン、セリン、トレオニン、アラニンまたはプロリンにより置換され、および／または、残基389はプロリンまたはセリンにより置換される。より具体的な実施形態においては、385、386、387および389位の1以上における残基は、それぞれアルギニン、トレオニン、アルギニンおよびプロリンにより置換される。さらにもう1つの特定の実施形態においては、385、386および389位の1以上における残基は、それぞれアスパラギン酸、プロリンおよびセリンにより置換される。

【００２２】

本発明の分子は、残基251、252、254、255、256、308、309、311、312、385、386、387、389、428、433、434および／または436の1以上における前記置換の任意の組合せを含む。好ましい実施形態においては、本発明の分子は、以下の置換の1以上を有するFc領域またはそのFcRn結合性ドメインを含有する：残基251のロイシン、残基252のチロシン、残基254のトレオニンまたはセリン、残基255のアルギニン、残基308のトレオニン、残基309のプ

ロリン、残基311のセリン、残基312のアスパラギン酸、残基314のロイシン、残基385のアルギニン、残基386のトレオニン、残基387のアルギニン、残基389のプロリン、残基428のメチオニン、および／または残基434のチロシン。

【００２３】

本発明には、延長された半減期を有する本発明の修飾免疫グロブリン、タンパク質および他の生物活性分子を使用する医薬組成物ならびに予防および治療方法が含まれる。また、延長された半減期を有する本発明の修飾免疫グロブリン、タンパク質および他の生物活性分子を使用する診断方法が含まれる。特定の実施形態においては、本発明は、FcRnに対する抗体の親和性を増加させる定常ドメイン中の1以上のアミノ酸修飾を有する及び増加したin vivo半減期を有する、SYNAGIS（登録商標）（米国特許第5,824,307号およびJohnsonら，J. Infectious Disease 176: 1215-1224,1997を参照されたい（それらの両方の全体を参照により本明細書に組み入れることとする））のような呼吸器合胞体ウイルス（RSV）感染を治療または予防するのに有用な抗RSV抗体、およびSYNAGIS（登録商標）の変異体（2000年11月28日付け出願の米国特許出願第09/724,396号，2000年11月28日付け出願の米国特許出願第09/724,531号，2001年11月28日付け出願の米国特許出願第____号(代理人整理番号10271-047)および2001年11月28日付け出願の米国特許出願第____号(代理人整理番号10271-048)（発明の名称はすべて「予防および治療のための抗RSV抗体の投与／投薬方法(Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment)」であり、すべてYoungらによるものであり、それらの全体、特にそれに開示されている抗RSV抗体の重および軽鎖可変ドメインならびにCDRの配列を参照により本明細書に組み入れることとする）を参照されたい）を含む他の抗RSV抗体を提供する（例えば、後記第5.1節も参照されたい）。

【００２４】

3.1 定義

本明細書で用いる「IgG Fc領域」なる語は、IgG分子のパパイン消化により得られる結晶性フラグメントと関連するIgG分子の部分を意味する。該Fc領域は、ジスルフィド結合により連結された2つのIgG分子重鎖のC末端半分よりなる。それは抗原結合活性を有しないが、FcRn受容体を含むFc受容体および補体に対する結合部位および炭水化物部分を含有する（後記を参照されたい）。Fcフラグメントは、全第2定常ドメインCH2（Kabatの番号付け系によれば、ヒトIgG1の残基231-340）（例えば、配列番号80）および第3定常ドメインCH3（残基341-447）（例えば、配列番号81）を含有する。

【００２５】

本明細書で用いる「IgGヒンジ-Fc領域」または「ヒンジ-Fcフラグメント」なる語は、Fc領域（残基231-447）と、Fc領域のN末端から伸長するヒンジ領域（残基216-230、例えば配列番号82）とよりなるIgG分子の領域を意味する。ヒトIgG1ヒンジ-Fc領域のアミノ酸配列の一例として、配列番号83が挙げられる。

【００２６】

「定常ドメイン」なる語は、抗原結合部位を含有する免疫グロブリンの他の部分（すなわち、可変ドメイン）と比べて、より保存されたアミノ酸配列を有する免疫グロブリン分子の部分を意味する。定常ドメインは、重鎖のCH1、CH2およびCH3ドメインと軽鎖のCHLドメインとを含有する。

【００２７】

本明細書で用いる「FcRn受容体」または「FcRn」なる語は、ヒトもしくは霊長類の胎盤または卵黄嚢（ウサギ）を介した胎児への或いは小腸を介した初乳から新生児への母親IgGの移行に関与することが知られているFc受容体（「n」は新生児を示す）を意味する。また、FcRnは、該IgG分子に結合しそれを血清中にリサイクルすることにより、一定の血清IgGレベルの維持に関与することが公知である。IgG分子とFcRnとの結合は厳密なpH依存性であり、最適な結合はpH 6.0で達成される。FcRnは、分子量がそれぞれ約50kDおよび15kDである2つのポリペプチドのヘテロ二量体からなる。50kDポリペプチドの細胞外ドメインは主要組織適合性複合体（MHC）クラスI α鎖に関連しており、15kDポリペプチドは非多

形性β_2-ミクログロブリン（β_2-m）であることが示された。FcRnは、胎盤および新生児腸に加えて、種を越えた種々の組織および種々の型の内皮細胞系においても発現される。それはヒト成人血管内皮、筋肉脈管系および肝シヌソイドにおいても発現され、内皮細胞はヒトおよびマウスの血清IgGレベルの維持において最も大きな役割を果たしうると示唆されている。ヒトFcRnおよびマウスFcRnのアミノ酸配列は、それぞれ配列番号84および配列番号85により示される。FcRn活性を有するこれらの配列の類似体も含まれる。

【００２８】

本明細書で用いる「in vivo半減期」なる語は、所与の動物の循環系中の特定の型のIgG分子またはそのFcRn結合部位含有フラグメントの生物学的半減期を意味し、該動物において投与された量の半分が該動物における循環系および／または他の組織から消失するのに要する時間により表される。所定のIgGの消失（クリアランス）曲線を時間の関数として作成すると、該曲線は、通常、二相性であり、血管腔の内外の注入IgG分子の平衡を表し、一部には分子のサイズにより決定される迅速なα相と、血管腔内のIgG分子の異化を表す、より長いβ相とを有する。「in vivo半減期」なる語は、実際には、該β相におけるIgG分子の半減期に相当する。

【００２９】

「単離（された）」または「精製（された）」抗体または融合タンパク質は、該タンパク質が由来する細胞または組織源からの細胞物質または他の混入タンパク質を実質的に含有せず、あるいは化学合成された場合には、化学的前駆体または他の化学物質を実質的に含有しない。「細胞物質を実質的に含有しない」なる表現は、抗体または融合タンパク質の調製物であって、該抗体または該融合タンパク質が、それが単離または組換え的に製造された細胞の細胞成分から分離されている調製物を包含する。したがって、細胞物質を実質的に含有しない抗体または融合タンパク質は、約30%、20%、10%または5%（乾燥重量比）未満の混入タンパク質を含有する抗体または融合タンパク質の調製物を包含する。また、該抗体または該融合タンパク質を組換え的に製造した場合には、それは、好ましくは、培地を実質的に含有しない。すなわち、培地は、該タンパク質調製物の体積の約20%、10%または5%未満に相当する。該抗体または該融合タンパク質を化学合成により製造した場合には、それは、好ましくは、化学的前駆体または他の化学物質を実質的に含有しない。すなわち、それは、該タンパク質の合成に関与する化学的前駆体または他の化学物質から分離されている。したがって、該抗体または該融合タンパク質のそのような調製物は、約30%、20%、10%または5%（乾燥重量比）未満の、関心のある抗体または抗体フラグメント以外の化学的前駆体または化合物を含有する。本発明の好ましい実施形態においては、抗体は単離または精製されている。本発明のもう1つの好ましい実施形態においては、融合タンパク質は単離または精製されている。

【００３０】

「単離（された）」核酸分子は、該核酸分子の天然源に存在する他の核酸分子から分離された核酸分子である。さらに、cDNA分子のような「単離（された）」核酸分子は、他の細胞物質を、または組換え技術により製造された場合には培地を実質的に含有しないもの、あるいは化学合成された場合には化学的前駆体または他の化学物質を実質的に含有しないものでありうる。「単離（された）」核酸分子は、DNAライブラリー中のcDNA分子を含まない。本発明の好ましい実施形態においては、抗体をコードする核酸分子は単離または精製されている。本発明のもう1つの好ましい実施形態においては、融合タンパク質をコードする核酸分子は単離または精製されている。

【００３１】

本明細書で用いる「宿主細胞」なる語は、核酸分子でトランスフェクトされる或いはファージミドまたはバクテリオファージに感染する特定の対象細胞およびそのような細胞の後代または潜在的後代を意味する。そのような細胞の後代は、後の世代において生じうる突然変異もしくは環境的影響または該宿主細胞ゲノム中への該核酸分子の組込みにより、該核酸分子でトランスフェクトされた親細胞と同一でなくてもよい。

【００３２】

本明細書で言及するアミノ酸の名称は三文字表記または一文字表記で表される。

【００３３】

2つのアミノ酸配列または2つの核酸配列の同一性（％）を測定するためには、それらの配列を最適な比較のために整列させる（例えば、第2アミノ酸または核酸配列との最適なアライメントのために、第1アミノ酸または核酸配列の配列中にギャップを導入することができる）。ついで、対応するアミノ酸位置またはヌクレオチド位置におけるアミノ酸残基またはヌクレオチドを比較する。第1配列中の位置が、第2配列中の対応位置と同じアミノ酸残基またはヌクレオチドにより占められた場合には、それらの分子はその位置において同一である。それらの2つの配列間の同一性（％）は、それらの配列により共有される同一位置の数の関数である（すなわち、同一性（％）＝同一重複位置の数／位置の総数×100%）。1つの実施形態においては、それらの2つの配列は同じ長さである。

【００３４】

2つの配列間の同一性（％）の測定は、数学的アルゴリズムを使用しても達成することができる。2つの配列の比較に利用される数学的アルゴリズムの好ましい非限定的な一例としては、KarlinおよびAltschul, 1993, Proc. Natl. Acad. Sci. U. S. A. 90: 5873-5877に記載のとおりに修飾されたKarlinおよびAltschul, 1990, Proc. Natl. Acad. Sci. U. S. A. 87: 2264-2268のアルゴリズムが挙げられる。そのようなアルゴリズムは、Altschulら, 1990, J. Mol. Biol. 215: 403のNBLASTおよびXBLASTプログラム中に組込まれている。BLASTヌクレオチド検索は、本発明の核酸分子に相同なヌクレオチド配列を得るために例えばスコア＝100、ワード長＝12に設定されたNBLASTヌクレオチドプログラムパラメーターで行うことができる。BLASTタンパク質検索は、本発明のタンパク質分子に相同なアミノ酸配列を得るために例えばスコア＝5、ワード長＝3に設定されたXBLASTプログラムパラメーターで行うことができる。比較のためのギャップ付きアライメントを得るためには、Altschulら, 1997, Nucleic Acids Res. 25: 3389-3402に記載のとおりに、Gapped BLASTを利用することができる。あるいは、分子間の遠縁関係を検出する反復検索を行うために、PSI-BLASTを使用することができる（同上）。BLAST、Gapped BLASTおよびPSI-Blastプログラムを使用する場合には、それぞれのプログラム（例えば、XBLASTおよびNBLAST）のデフォルトパラメーターを使用することができる（例えば、.nlm.nih.govを参照されたい）。配列の比較に利用される数学的アルゴリズムのもう1つの好ましい非限定的な一例としては、MyersおよびMiller, 1988, CABIOS 4: 11-17のアルゴリズムが挙げられる。そのようなアルゴリズムは、GCG配列アライメントソフトウェアパッケージの一部であるALIGNプログラム（バージョン2.0）中に組込まれている。アミノ酸配列を比較するためにALIGNプログラムを利用する場合には、PAM120重み残基表、12のギャップ長ペナルティおよび4のギャップペナルティを使用することができる。

【００３５】

2つの配列間の同一性（％）は、ギャップを許容して又は許容することなく前記と同様の技術を用いて測定することができる。同一性（％）を計算する際には、典型的には、厳密なマッチのみを計数する。

【００３６】

４．図面の簡単な記載

図1は、FcRn受容体との相互作用に関与することが確認された残基の位置を示すIgGヒンジ-Fc領域の構造を示す（Ghetieら, Immunology Today 18(12): 592-598,1997）。

【００３７】

図2は、ヒンジ領域（配列番号82）、CH2ドメイン（配列番号80）およびCH3ドメイン（配列番号81）を含有するヒトIgG1ヒンジ-Fc領域のアミノ酸配列（配列番号83）を示す。

【００３８】

図3（AおよびB）は、それぞれ（A）ヒトFcRn（配列番号84）および（B）マウスFcRn（配列番号85）のアミノ酸配列を示す。

【００３９】

図4は、ヒトIgG1ヒンジ-Fc領域（配列番号83）のアミノ酸配列を示す。ここで、アミノ酸

置換により突然変異した野生型残基は下線付き太字で示されている。

【００４０】

図5は、ファージにより提示された修飾ヒンジ-Fcライブラリーに関するパニング法の概略図である。

【００４１】

図6は、スクリーニングしたライブラリー中の変異体位置における選択された突然変異残基の出現の概要を示す。

【００４２】

図7（A～D）。（A）は、M252Y/S254T/T256E置換を有する固定化IgG1へのマウスFcRnの結合を示す。4000共鳴単位（RU）のIgG1が結合した表面上に、マウスFcRnを1nM～556nMの範囲の10の異なる濃度で注入した。平衡に達した後、残留結合タンパク質をPBS（pH 7.4）のパルスで溶出した。（B）は、固定化IgG1/M252Y/S254T/T256Eへのヒト FcRnの結合を示す。1000RUのIgG1が結合した表面上に、マウスFcRnを71nM～2.86μMの範囲の8つの異なる濃度で注入した。平衡に達した後、残留結合タンパク質をPBS（pH 7.4）のパルスで溶出した。（C）および（D）は、非特異的結合に関する補正後のそれぞれ（A）および（B）におけるデータのスキャッチャード解析を示す。R_{eq}は、与えられた濃度Cにおける補正平衡応答である。それらのプロットは直線状であり、相関係数はそれぞれ0.97および0.998である。見掛けK_dは、それぞれ24nMおよび225nMである。

【００４３】

図8（A～H）。（A）～（D）は、非特異的結合に関する補正後のそれぞれ（A）野生型ヒトIgG1、（B）M252Y/S254T/T256E、（C）H433K/N434F/Y436H、および（D）G385D/G386P/N389SへのpH 6.0およびpH 7.4におけるマウスFcRnの結合のBIAcore分析からの結果を示す。1000RUの野生型IgG1、1000RUのM252Y/S254T/T256E、955RUのH433K/N434F/Y436H、および939RUのG385D/Q386P/N389Sが結合した表面上に、マウスFcRnを1.1μmの濃度で注入した。（E）～（H）は、非特異的結合に関する補正後のそれぞれ（E）野生型ヒトIgG1、（F）M252Y/S254T/T256E、（G）H433K/N434F/Y436H、および（H）G385D/Q386P/N389SへのpH 6.0およびpH 7.4におけるヒトFcRnの結合のBIAcore分析からの結果を示す。1000RUの野生型IgG1、1000RUのM252Y/S254T/T256E、955RUのH433K/N434F/Y436H、および939RUのG385D/Q386P/N389Sが結合した表面上に、ヒトFcRnを1.4μmの濃度で注入した。

【００４４】

図9は、Deisenhofer, 1981, Biochemistry 20: 2361-2370のヒトIgG1構造に基づくヒトIgG1のFcフラグメントの表面の空間充填型模型を示す。残基は、Fc-FcRn複合体の安定化の自由エネルギーの獲得に応じて色付けされている：赤，これらの位置における置換は親和性をFc/ヒトFcRn相互作用においては少なくとも2.5倍およびFc/マウスFcRn相互作用においては少なくとも5倍増加させることが判明した；青，それらの位置における置換は親和性をFc-ヒトFcRnおよびFc-マウスFcRnの両方の相互作用において2倍未満の倍率で増加させることが判明した。この図は、Swiss pdb viewer (GuexおよびPeitsch, 1997, Electrophoresis 18: 2714-2723)を使用して作成した。

【００４５】

図10は、野生型定常ドメインを有する抗体（SYNAGIS（登録商標））（白正方形）、または突然変異M252Y/S254T/T256E（白丸）、G385D/Q386P/N389S（黒正方形）およびH433K/N434F/Y436H（黒丸）を含有する定常ドメインを有する抗体の、時間（日単位）経過に対する血清濃度（[Mab]ng/ml）における変化を示す。抗体濃度は、抗ヒトIgG ELISAを用いて測定した。

【００４６】

５．発明の詳細な記載

本発明は、野生型IgG定常ドメインに対して1以上のアミノ酸修飾（該修飾はFcRnに対する該IgG定常ドメインまたはそのフラグメントの親和性を増加させる）を含有するIgG定常ドメインまたはそのFcRn結合性フラグメント（すなわち、FcRnに結合するそのフラグメント）（好ましくはFcまたはヒンジ-Fcドメイン）を含む増加したin vivo半減期を有する分子

、特にタンパク質、より詳しくは免疫グロブリンに関する。好ましい実施形態においては、本発明は、特に、ヒトにおける治療、予防および診断において特に有用である、ヒトまたはヒト化IgGおよびヒトIgGのFcRn結合性部分を含有する他の生物活性分子の修飾に関する。

【００４７】

5.1 増加したin vivo半減期を有する分子

本発明は、FcRnと相互作用するIgG定常ドメインの特定の部分におけるアミノ酸修飾（該修飾はFcRnに対するIgGまたはそのフラグメントの親和性を増加させる）の同定に基づくものである。したがって、本発明は、FcRnと相互作用する1以上の領域中に1以上のアミノ酸修飾（すなわち、置換、挿入または欠失）（該修飾はFcRnに対するIgGまたはそのフラグメントの親和性を増加させ、該分子のin vivo半減期をも増加させる）を含有するIgG定常ドメインまたはそのFcRn結合性フラグメント（好ましくはFcまたはヒンジ-Fcドメインフラグメント）を含む分子、好ましくはタンパク質、より好ましくは免疫グロブリンに関する。好ましい実施形態においては、前記の1以上のアミノ酸修飾は、IgG1ヒンジ-Fc領域の残基251-256、285-290、308-314、385-389および428-436（例えば、図4、配列番号83に記載のヒトIgG1ヒンジ-Fc領域におけるとおり）、または他のIgGヒンジ-Fc領域中の、アミノ酸配列アライメントにより決定されたそれらの類似残基の1以上において施される。好ましい実施形態においては、該アミノ酸修飾は、ヒトIgG定常ドメインまたはそのFcRn結合性ドメイン中に施される。ある実施形態においては、該修飾は、IgG定常ドメインの残基252、254または256においては施されない（すなわち、すべては、残基251、253、255、285-290、308-314、385-389、または428-436の1以上において施される）。より好ましい実施形態においては、該アミノ酸修飾は、残基252におけるロイシンによる置換、残基254におけるセリンによる置換、および／または256位におけるフェニルアラニンによる置換ではない。特に、好ましい実施形態においては、IgG定常ドメイン、ヒンジ-Fcドメイン、ヒンジ-Fcドメインまたは他のそのFcRn結合性フラグメントがマウスに由来する場合には、そのような修飾は施されない。

【００４８】

前記のアミノ酸修飾は、IgG定常ドメインまたはそのFcRn結合性フラグメント（例えば、Fcまたはヒンジ-Fcドメイン）およびそれに結合した任意の分子のin vivo半減期を増加させFcRnに対する該IgGまたはそのフラグメントの親和性を増加させる任意の修飾（好ましくは、残基251-256、285-290、308-314、385-389および428-436の1以上におけるもの）でありうる。好ましくは、前記の1以上の修飾はまた、pH 6.0において、pH 7.4におけるより高い、FcRnに対する該定常ドメインまたはそのFcRn結合性フラグメントの結合親和性を与える。他の実施形態においては、該修飾は、該分子のバイオアベイラビリティを改変（すなわち、増強または減弱）し、特に、（例えば肺の）粘膜表面または標的組織の他の部分への該分子の輸送（または濃度または半減期）を改変する（すなわち、増加または減少させる）。好ましい実施形態においては、該アミノ酸修飾は、肺への該分子の輸送または濃度または半減期を改変する（好ましくは増加させる）。他の実施形態においては、該アミノ酸修飾は、心臓、膵臓、肝臓、腎臓、膀胱、胃、大腸、小腸、気道、リンパ節、神経組織（中枢および／または末梢神経組織）、筋肉、表皮、骨、軟骨、関節、血管、骨髄、前立腺、卵巣、子宮、腫瘍または癌組織などへの該分子の輸送（または濃度または半減期）を改変する（好ましくは増加させる）。好ましい実施形態においては、該アミノ酸修飾は、該定常ドメインの他の免疫エフェクターまたは受容体結合機能（例えば、当技術分野において良く知られた常套的な方法により測定されうる補体結合、ADCC、およびFcγRI、FcγRIIおよびFcγRIIIへの結合が挙げられるが、これらに限定されるものではない）を阻止せず、より好ましくは、改変しない。もう1つの好ましい実施形態においては、該定常ドメインの修飾FcRn結合フラグメントは、免疫エフェクター機能または他の受容体結合を媒介する配列を含有しない。そのようなフラグメントは、非IgGまたは非免疫グロブリン分子に結合させてそのin vivo半減期を増加させるのに特に有用でありうる。さらにもう1つの実施形態においては、該エフェクター機能が選択的に改変される（例えば、エフ

ェクター機能が減弱または増強される）。

【００４９】

好ましい実施形態においては、該アミノ酸修飾は、残基308、309、311、312および314の1以上における置換、特に、308位におけるトレオニン、309位におけるプロリン、311位におけるセリン、312位におけるアスパラギン酸および／または314位におけるロイシンによる置換である。あるいは、該修飾は、308位におけるイソロイシン、309位におけるプロリン、および／または311位におけるグルタミン酸による置換である。さらにもう1つの実施形態においては、308、309、311、312および314位の1以上における残基は、それぞれトレオニン、プロリン、ロイシン、アラニンおよびアラニンにより置換される。したがって、ある実施形態においては、308位の残基はトレオニンまたはイソロイシンにより置換され、309位の残基はプロリンにより置換され、311位の残基はセリン、グルタミン酸またはロイシンにより置換され、312位の残基はアラニンにより置換され、および／または、314位の残基はロイシンまたはアラニンにより置換される。好ましい実施形態においては、該置換は308位のトレオニン、309位のプロリン、311位のセリン、312位のアスパラギン酸および／または314位のロイシンである。

【００５０】

好ましい実施形態においては、該アミノ酸修飾は、残基251、252、254、255および256の1以上における置換である。特定の実施形態においては、残基251はロイシンまたはアルギニンにより置換され、残基252はチロシン、フェニルアラニン、セリン、トリプトファンまたはトレオニンにより置換され、残基254はトレオニンまたはセリンにより置換され、残基255はアルギニン、ロイシン、グリシンまたはイソロイシンにより置換され、および／または、残基256はセリン、アルギニン、グルタミン、グルタミン酸、アスパラギン酸、アラニン、アスパラギンまたはトレオニンにより置換される。より具体的な実施形態においては、残基251はロイシンにより置換され、残基252はチロシンにより置換され、残基254はトレオニンまたはセリンにより置換され、残基255はアルギニンにより置換され、および／または、残基256はグルタミン酸により置換される。

【００５１】

好ましい実施形態においては、該アミノ酸修飾は残基428、433、434および436の1以上における置換である。特定の実施形態においては、残基428はトレオニン、メチオニン、ロイシン、フェニルアラニンまたはセリンにより置換され、残基433はリシン、アルギニン、セリン、イソロイシン、プロリン、グルタミンまたはヒスチジンにより置換され、残基434はフェニルアラニン、チロシンまたはヒスチジンにより置換され、および／または、残基436はヒスチジン、アスパラギン、アルギニン、トレオニン、リシンまたはメチオニンにより置換される。より具体的な実施形態においては、428および／または434位の残基は、それぞれメチオニンおよび／またはヒスチジンにより置換される。

【００５２】

好ましい実施形態においては、該アミノ酸修飾は残基385、386、387および389の1以上における置換であり、より詳しくは、これらの位置の1以上における置換を有する。特定の実施形態においては、残基385はアルギニン、アスパラギン酸、セリン、トレオニン、ヒスチジン、リシン、アラニンまたはグリシンにより置換され、残基386はトレオニン、プロリン、アスパラギン酸、セリン、リシン、アルギニン、イソロイシンまたはメチオニンにより置換され、残基387はアルギニン、プロリン、ヒスチジン、セリン、トレオニンまたはアラニンにより置換され、および／または、残基389はプロリン、セリンまたはアスパラギンにより置換される。より具体的な実施形態においては、385、386、387および389位の1以上における残基は、それぞれアルギニン、トレオニン、アルギニンおよびプロリンにより置換される。さらにもう1つの特定の実施形態においては、385、386および389位の1以上における残基は、それぞれアスパラギン酸、プロリンおよびセリンにより置換される。

【００５３】

特定の実施形態においては、アミノ酸修飾は残基251、252、254、255、256、308、309、3

11、312、314、385、386、387、389、428、433、434および／または436の1つ又はそれらの組合せにおいて施され、該修飾がこれらの残基に関して直前に記載したアミノ酸置換の1以上である場合には特にそうである。

【００５４】

好ましい実施形態においては、本発明の分子は以下の置換の1以上を有するFc領域またはそのFcRn結合性ドメインを含有する：残基251のロイシン、残基252のチロシン、残基254のトレオニンまたはセリン、残基255のアルギニン、残基308のトレオニン、残基309のプロリン、残基311のセリン、残基312のアスパラギン酸、残基314のロイシン、残基385のアルギニン、残基386のトレオニン、残基387のアルギニン、残基389のプロリン、残基428のメチオニン、および／または残基434のチロシン。

【００５５】

好ましい実施形態においては、該FcRn結合性ドメインは、1、2、3、4、5、6、7、8、9、10、12、14、16、または残基251、252、254、255、256、308、309、311、312、314、385、386、387、389、428、433、434および／または436の全18残基における置換を有する。

【００５６】

アミノ酸修飾は、当技術分野で公知の任意の方法により行うことが可能であり、多数のそのような方法が当業者に良く知られており常套的なものである。例えば、限定的なものではないが、アミノ酸の置換、欠失および挿入は、任意の良く知られたPCRに基づく技術を用いて達成することができる。アミノ酸の置換は、部位特異的突然変異誘発（例えば、ZollerおよびSmith, Nucl. Acids Res. 10: 6487-6500,1982; Kunkel, Proc. Natl. Acad. Sci USA 82: 488,1985（その全体を参照により本明細書に組み入れることとする）を参照されたい）。FcRnに対する親和性の増加およびin vivo半減期の増加をもたらす突然変異体は、後記5.11節に記載されているような良く知られた常套的アッセイを用いて容易にスクリーニングすることができる。好ましい実施形態においては、アミノ酸の置換を、IgG定常ドメインまたはそのFcRn結合性フラグメント中の1以上の残基において導入し、該突然変異定常ドメインまたはフラグメントをバクテリオファージの表面上で発現させ、ついでそれを、増加したFcRn結合親和性に関してスクリーニングする（特に、後記第5.2節および第5.11節を参照されたい）。

【００５７】

好ましくは、修飾されるアミノ酸残基は表面露出残基である。また、アミノ酸置換を行う場合には、好ましくは、置換されるアミノ酸残基は同類アミノ酸置換であり、例えば、極性残基は極性残基により置換され、親水性残基は親水性残基により置換され、疎水性残基は疎水性残基により置換され、正に荷電した残基は正に荷電した残基により置換され、あるいは負に荷電した残基は負に荷電した残基により置換される。さらに、好ましくは、修飾されるアミノ酸残基は、種間で高度には又は完全には保存されておらず、および／または、該定常ドメイン三次構造の維持またはFcRn結合に決定的に重要なものである。例えば、限定的なものではないが、310位におけるヒスチジンの修飾は好ましくない。

【００５８】

FcRnに対して増加した親和性を有するFcドメインの特定の突然変異体を、残基308-314（310位のヒスチジンおよび313位のトリプトファンは固定されている）に突然変異を有する突然変異ヒトIgG1分子のライブラリーからの第3ラウンドのパニング（第6節に記載のとおり）の後に単離した。残基251-256（253位のイソロイシンは固定されている）に関するライブラリーの第5ラウンドのパニングの後に単離したもの、残基428-436（429位のヒスチジン、430位のグルタミン酸、431位のアラニン、432位のロイシンおよび435位のヒスチジンは固定されている）に関するライブラリーの第4ラウンドのパニングの後に単離したもの、および残基385-389（388位のグルタミン酸は固定されている）に関するライブラリーの第6ラウンドのパニングの後に単離したものを、表Iに示す。野生型ヒトIgG1は、308-314位に配列Val-Leu-His-Gln-Asp-Trp-Leu（配列番号（SEQ ID NO:）86）、251-256位にLeu-Met-Ile-Ser-Arg-Thr（配列番号87）、428-436位にMet-His-Glu-Ala-Leu-His-Asn-His-Tyr（配列番号88）、385-389位にGly-Gln-Pro-Glu-Asn（配列番号89）を有する。

【表１】

表 I

パニング により単離された突然変異体

ライブラリー	突然変異体*
251-256	Leu Tyr Ile Thr Arg Glu (SEQ ID NO:90)
	Leu Tyr Ile Ser Arg Thr (SEQ ID NO:91)
	Leu Tyr Ile Ser Arg Ser (SEQ ID NO:92)
	Leu Tyr Ile Ser Arg Arg (SEQ ID NO:93)
	Leu Tyr Ile Ser Arg Gln (SEQ ID NO:94)
	Leu Trp Ile Ser Arg Thr (SEQ ID NO:95)
	Leu Tyr Ile Ser Leu Gln (SEQ ID NO:96)
	Leu Phe Ile Ser Arg Asp (SEQ ID NO:97)
	Leu Phe Ile Ser Arg Thr (SEQ ID NO:98)
	Leu Phe Ile Ser Arg Arg (SEQ ID NO:99)
	Leu Phe Ile Thr Gly Ala (SEQ ID NO:100)
	Leu Ser Ile Ser Arg Glu (SEQ ID NO:101)
	Arg Thr Ile Ser Ile Ser (SEQ ID NO:102)
308-314	Thr Pro His Ser Asp Trp Leu (SEQ ID NO:103)
	Ile Pro His Glu Asp Trp Leu (SEQ ID NO:104)
385-389	Arg Thr Arg Glu Pro (SEQ ID NO:105)
	Asp Pro Pro Glu Ser (SEQ ID NO:106)
	Ser Asp Pro Glu Pro (SEQ ID NO:107)
	Thr Ser His Glu Asn (SEQ ID NO:108)
	Ser Lys Ser Glu Asn (SEQ ID NO:109)
	His Arg Ser Glu Asn (SEQ ID NO:110)
	Lys Ile Arg Glu Asn (SEQ ID NO:111)

ライブラリー	突然変異体*
	Gly **Ile Thr** Glu **Ser** (SEQ ID NO:112)
	Ser Met Ala Glu **Pro** (SEQ ID NO:113)
428-436	*Met His Glu Ala Leu Arg Tyr His His* (SEQ ID NO:114)
	Met His Glu Ala Leu His **Phe** His **His** (SEQ ID NO:115)
	Met His Glu Ala Leu **Lys Phe** His **His** (SEQ ID NO:116)
	Met His Glu Ala Leu **Ser Tyr** His **Arg** (SEQ ID NO:117)
	Thr His Glu Ala Leu His **Tyr** His **Thr** (SEQ ID NO:118)
	Met His Glu Ala Leu His **Tyr** His **Tyr** (SEQ ID NO:119)

* 置換残基は太字で示されている

【００５９】
表I中の下線付き配列は、最終ラウンドのパニングにおいて10～20回出現した配列に対応し、イタリック体の配列は、最終ラウンドのパニングにおいて2～5回出現した配列に対応する。下線も付されておらずイタリック体でもない配列は、最終ラウンドのパニングにおいて1回出現したものである。

【００６０】
1つの好ましい実施形態においては、本発明は、未修飾分子と比較して増加したin vivo半減期およびFcRnに対する親和性（そして、好ましい実施形態においては、改変されたバイオアベイラビリティ、例えば、粘膜表面または他の標的組織への増加または減少した輸送）を有する修飾された免疫グロブリン分子（例えば、種々の抗体）を提供する。そのような免疫グロブリン分子は、FcRn結合性ドメインを天然で含有するIgG分子、およびFcRn結合性フラグメントを含有するように操作された他の非IgG免疫グロブリン（例えば、IgE、IgM、IgD、IgAおよびIgY）または免疫グロブリンのフラグメント（すなわち、非IgG免疫グロブリンまたはその一部とFcRn結合性ドメインとを含む融合タンパク質）を包含する。どちらの場合も、該FcRn結合性ドメインは、FcRnに対する該定常ドメインフラグメントの親和性を増加させる1以上のアミノ酸修飾を有する。

【００６１】
該修飾免疫グロブリンは、（特異的抗原-抗体結合をアッセイするための当技術分野で良く知られたイムノアッセイにより測定した場合に、好ましくは免疫特異的に、すなわち、非特異的結合に優先して）抗原に結合する任意の免疫グロブリン分子を包含し、FcRn結合性フラグメントを含有する。そのような抗体は、ポリクローナル抗体、モノクローナル抗体、二重特異性抗体、多特異性抗体、ヒト抗体、ヒト化抗体、キメラ抗体、一本鎖抗体、Fabフラグメント、$F(ab')_2$フラグメント、ジスルフィド結合Fvs、および抗原に特異的に結合する、VLもしくはVHドメインまたは更には相補性決定領域（CDR）を含有するフラグメントのうち、ある場合には、FcRn結合性ドメインを含有するよう又はそれに融合するよう操作されたものを包含するが、これらに限定されるものではない。

【００６２】
本発明のIgG分子およびそのFcRn結合性フラグメントは、好ましくは、IgGのIgG1サブクラスであるが、与えられた動物の任意の他のIgGサブクラスであることも可能である。例えば、ヒトにおいては、該IgGクラスはIgG1、IgG2、IgG3およびIgG4を包含し、マウスIgGはIgG1、IgG2a、IgG2b、IgG2cおよびIgG3を包含する。あるIgGサブクラス、例えばマウスIgG2bおよびIgG2cは、例えばIgG1より高い消失（クリアランス）速度を有することが公知である（Medesanら, Eur. J. Immunol., 28: 2092-2100,1998）。したがって、IgG1以外のI

gGサブクラスを使用する場合には、IgG1配列とは異なる残基（特に、CH2およびCH3ドメイン中のもの）の1以上をIgG1の残基により置換してそのIgG1以外のタイプのIgGのin vivo半減期を増加させることが有利かもしれない。

【００６３】

該免疫グロブリン（および本発明で使用する他のタンパク質）は、鳥類および哺乳類を含む任意の動物に由来しうる。好ましくは、該抗体はヒト、げっ歯類（例えば、マウスおよびラット）、ロバ、ヒツジ、ウサギ、ヤギ、モルモット、ラクダ、ウマまたはニワトリの抗体でありうる。本発明で用いる「ヒト」抗体は、ヒト免疫グロブリンのアミノ酸配列を有する抗体を包含し、ヒト免疫グロブリンライブラリーから又は1以上のヒト免疫グロブリンに関してトランスジェニックであり内因性免疫グロブリンを発現しない動物から単離された抗体（後記のとおり、および例えば、Kucherlapatiらの米国特許第5,939,598号）を包含する。

【００６４】

本発明の抗体は、単一特異性、二重特異性、三重特異性またはそれ以上の多特異性のものでありうる。多特異性抗体はポリペプチドの種々のエピトープに特異的でありうる。あるいは多特異性抗体は、異種エピトープ、例えば異種ポリペプチドまたは固体支持体物質に特異的でありうる。例えば、PCT公開WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tuttら, J. Immunol. 147: 60-69,1991; 米国特許第4,474,893号; 第4,714,681号; 第4,925,648号; 第5,573,920号; 第5,601,819号; Kostelnyら, J. Immunol., 148: 1547-1553,1992を参照されたい。

【００６５】

本発明の抗体は、他のやり方で修飾された誘導体、すなわち、抗原への該抗体の結合および／または抗イディオタイプ応答の生成を共有結合が妨げないよう該抗体に任意のタイプの分子が共有結合することにより修飾された誘導体を包含する。例えば、抗体誘導体は、例えばグリコシル化、アセチル化、PEG化、リン酸化、アミド化、公知の保護基／遮断基による誘導体化、タンパク質分解切断、細胞性リガンドまたは他のタンパク質への結合などにより修飾された抗体を包含するが、これらに限定されるものではない。多数の化学修飾はいずれも、特異的化学的切断、アセチル化、ホルミル化、ツニカマイシンの代謝合成を含む公知技術により行うことができる。また、該誘導体は1以上の非古典的アミノ酸を含有しうる。

【００６６】

モノクローナル抗体は、ハイブリドーマ、組換え体およびファージディスプレイ技術またはそれらの組合せの使用を含む当技術分野で公知の多種多様な技術を用いて製造することができる。例えば、モノクローナル抗体は、当技術分野で公知であり例えばHarlowら, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerlingら,: Monoclonal Antibodies and T-Cell Hybridomas, pp. 563-681 (Elsevier, N. Y., 1981)（それらの両方の全体を参照により本明細書に組み入れることとする）に教示されているものを含むハイブリドーマ技術を使用して製造することができる。本発明で用いる「モノクローナル抗体」なる語は、ハイブリドーマ技術により製造された抗体には限定されない。「モノクローナル抗体」なる語は、任意の真核性、原核性またはファージクローンを含む単クローンに由来する抗体を意味し、それが製造された方法によらない。

【００６７】

ハイブリドーマ技術を用いて特異的抗体を製造しそれに関してスクリーニングするための方法は、常套的なものであり当技術分野において良く知られている。非限定的な一例においては、関心のある抗原またはそのような抗原を発現する細胞でマウスを免疫することができる。免疫応答を検出したら、例えば、該抗原に特異的な抗体が該マウス血清中で検出されたら、該マウス脾臓を集め、脾細胞を単離する。ついで該脾細胞を、良く知られた技術により、任意の適当な骨髄腫細胞に融合させる。ハイブリドーマを選択して限界希釈法によりクローニングする。ついで該ハイブリドーマを、該抗原に結合しうる抗体を分泌す

る細胞に関して、当技術分野で公知の方法によりアッセイする。高レベルの抗体を一般には含有する腹水は、陽性ハイブリドーマクローンをマウスの腹腔内に接種することにより生成させることができる。

【００６８】

特異的エピトープを認識する抗体フラグメントは、公知技術により製造することができる。例えば、FabおよびF(ab')$_2$フラグメントは、（Fabフラグメントを製造するための）パパインまたは（F(ab')$_2$フラグメントを製造するための）ペプシンのような酵素を使用する免疫グロブリン分子のタンパク質分解切断により製造することができる。F(ab')$_2$フラグメントは完全な軽鎖ならびに重鎖の可変領域、CH1領域およびヒンジ領域を含有する。

【００６９】

また、抗体は、例えば、当技術分野で公知の種々のファージディスプレイ法を用いて製造することも可能である。ファージディスプレイ法においては、機能的抗体ドメインが、それをコードするポリヌクレオチド配列を保持するファージ粒子の表面上に提示される。特定の実施形態においては、レパートリーまたは組合せ（combinatorial）抗体ライブラリー（例えばヒトまたはマウスのもの）から発現されたFabおよびFvまたはジスルフィド結合安定化Fvのような抗原結合ドメインを提示するために、そのようなファージを使用することができる。関心のある抗原に結合する抗原結合性ドメインを発現するファージは、抗原を使用して選択または同定することができる。すなわち、該ファージは、標識された抗原、または固体表面もしくはビーズに結合もしくは捕捉された抗原を使用して選択または同定することができる。これらの方法において使用するファージは、典型的には、fdおよびM13を含む繊維状ファージである。該抗原結合性ドメインは、ファージ遺伝子IIIまたは遺伝子VIIIタンパク質に組換え的に融合したタンパク質として発現される。あるいは、本発明の免疫グロブリンの修飾FcRn結合性部分もファージディスプレイ系において発現されうる。本発明の免疫グロブリンまたはそのフラグメントを製造するために使用しうるファージディスプレイ法の具体例は、Brinkmanら，J. Immunol. Methods, 182: 41-50,1995; Amesら，J. Immunol. Methods, 184: 177-186, 1995; Kettleboroughら，Eur. J. Immunol., 24: 952-958,1994; Persicら，, Gene, 187: 9-18,1997; Burtonら，Advances in Immunology, 57: 191-280,1994; PCT出願番号PCT/GB91/01134 ; PCT公開WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/1 1236; WO 95/15982; WO 95/20401; ならびに米国特許第5,698,426号；第5,223,409号；第5,403,484号；第5,580,717号；第5,427,908号；第5,750,753号；第5,821,047号；第5,571,698号；第5,427,908号；第5,516,637号；第5,780,225号；第5,658,727号；第5,733,743号および第5,969,108号（それらのそれぞれの全体を参照により本明細書に組み入れることとする）に開示されているものを包含する。

【００７０】

前記参考文献に記載されているとおり、ファージ選択の後、該ファージからの抗体コード領域を単離し、それを使用して、ヒト抗体を含む全抗体または任意の他の所望のフラグメントを得、例えば後記で詳しく説明するとおり哺乳類細胞、昆虫細胞、植物細胞、酵母および細菌を含む任意の所望の宿主中で発現させることができる。例えば、Fab、Fab'およびF(ab')$_2$フラグメントを組換え的に製造するための技術も、PCT公開WO 92/22324; Mullinaxら，BioTechniques, 12 (6): 864-869,1992; およびSawaiら，AJRI, 34: 26-34,1995; およびBetterら，Science, 240: 1041-1043,1988（それらのそれぞれの全体を参照により本明細書に組み入れることとする）に開示されているような当技術分野で公知の方法により用いることができる。一本鎖Fvおよび抗体を製造するために使用しうる技術の具体例は、米国特許第4,946,778号および第5,258,498号；Hustonら，Methods in Enzymology, 203: 46-88,1991; Shuら，PNAS 90: 7995-7999,1993; ならびにSkerraら，Science, 240: 1038-1040,1988に記載されているものを包含する。

【００７１】

ヒトにおける抗体のin vivoでの使用およびin vitro検出アッセイを含むいくつかの用途には、キメラ抗体、ヒト化抗体またはヒト抗体を使用するのが好ましいことがある。キメ

ラ抗体は、該抗体の種々の部分が種々の動物種に由来する分子、例えば、マウスモノクローナル抗体由来の可変領域とヒト免疫グロブリン由来の定常領域とを有する抗体である。キメラ抗体の製造方法は当技術分野において公知である。例えば、Morrison, Science, 229: 1202,1985; Oiら, BioTechniques, 4: 214 1986; Gilliesら, J. Immunol. Methods, 125: 191-202,1989; 米国特許第5,807,715号; 第4,816,567号; および第4,816,397号（それらの全体を参照により本明細書に組み入れることとする）を参照されたい。ヒト化抗体は、非ヒト種由来の1以上の相補性決定領域（CDR）とヒト免疫グロブリン分子由来のフレームワーク領域とを有する、所望の抗原に結合する非ヒト種由来の抗体分子である。しばしば、ヒトフレームワーク領域中のフレームワーク残基は、抗原結合を改変（好ましくは改善）するためにCDR供与抗体由来の対応残基で置換される。これらのフレームワーク置換は、当技術分野で良く知られた方法、例えば、抗原結合に重要なフレームワーク残基を同定するための該CDRおよびフレームワーク残基の相互作用のモデル化、ならびに特定の位置における異常なフレームワークを同定するための配列比較により同定される。例えば、Queenら, 米国特許第5,585,089号; Riechmannら, Nature, 332: 323,1988（それらの全体を参照により本明細書に組み入れることとする）を参照されたい。抗体は、例えば、CDRグラフティング（EP 239,400; PCT公開WO 91/09967; 米国特許第5,225,539号; 第5,530,101号および第5,585,089号）、ベニアリング（veneering）またはリサーフェシング（resurfacing）（EP 592,106; EP 519,596; Padlan, Molecular Immunology, 28 (4/5): 489-498,1991; Studnickaら, Protein Engineering, 7 (6): 805-814, 1994; Roguskaら, Proc Natl. Acad. Sci. USA, 91: 969-973,1994）、および鎖シャフリング（chain shuffling）（米国特許第5,565,332号）（それらのすべての全体を参照により本明細書に組み入れることとする）を含む当技術分野で公知の種々の技術を用いてヒト化することができる。

【００７２】

ヒト患者の治療には完全ヒト抗体が特に望ましい。ヒト抗体は、ヒト免疫グロブリン配列由来の抗体ライブラリーを使用する前記のファージディスプレイ法を含む当技術分野で公知の種々の方法により製造することができる。米国特許第4,444,887号および第4,716,111号; ならびにPCT公開WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; および WO 91/10741（それらのそれぞれの全体を参照により本明細書に組み入れることとする）を参照されたい。

【００７３】

また、ヒト抗体は、機能的内因性免疫グロブリンを発現し得ないがヒト免疫グロブリン遺伝子を発現しうるトランスジェニックマウスを使用することによっても製造することができる。ヒト抗体を製造するためのこの技術の総説としては、LonbergおよびHuszar, Int. Rev. Immunol., 13: 65-93,1995を参照されたい。ヒト抗体およびヒトモノクローナル抗体を製造するためのこの技術ならびにそのような抗体を製造するためのプロトコールの詳細な考察については、例えば、PCT公開WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; 欧州特許第0 598 877号; 米国特許第5,413,923号; 第5,625,126号; 第5,633,425号; 第5,569,825号; 第5,661,016号; 第5,545,806号; 第5,814,318号; 第5,885,793号; 第5,916,771号; および第5,939,598号（それらの全体を参照により本明細書に組み入れることとする）を参照されたい。また、Abgenix, Inc. (Freemont, CA)、Medarex (NJ)およびGenpharm (San Jose, CA)のような企業が、前記と同様の技術を用いて、選択された抗原に対するヒト抗体を提供することに関わることができる。

【００７４】

選択されたエピトープを認識する完全ヒト抗体は、「ガイディッド・セレクション（guided selection）」と称される技術を用いて製造することができる。このアプローチにおいては、選択された非ヒトモノクローナル抗体、例えばマウス抗体を使用して、同じエピトープを認識する完全ヒト抗体の選択を導く（Jespersら, Bio/technology, 12:899-903,1988）。

【００７５】

特定の実施形態においては、該修飾抗体はin vivoでの治療および／または予防用途を有する。そのように修飾されうる治療用および予防用抗体の具体例は、呼吸器合胞体ウイルス（RSV）感染の患者の治療用のヒト化RSVモノクローナル抗体であるSYNAGIS（登録商標）（MedImmune, MD）、転移性乳癌の患者の治療用のヒト化抗HER2モノクローナル抗体であるHERCEPTIN（登録商標）（Trastuzumab）（Genentech, CA）、クローン病の患者の治療のためのキメラ抗TNFαモノクローナル抗体であるREMICADE（登録商標）(インフリキシマブ（infliximab））(Centocor, PA）、血餅形成の予防のための血小板上の抗糖タンパク質IIb/IIIaであるREOPRO（登録商標）（アブシキマブ（abciximab））（Centocor）、急性腎同種移植拒絶の予防のための免疫抑制性ヒト化抗CD25モノクローナル抗体であるZENAPAX（登録商標）（ダクリズマブ（daclizumab））（Roche Pharmaceuticals, Switzerland）を包含する。他の具体例としては以下のものが挙げられる：ヒト化抗CD18 $F(ab')_2$（Genentech）、ヒト化抗CD18 $F(ab')_2$（Celltech, UK）であるCDP860、CD4に融合した抗HIV gp120抗体であるPRO542（Progenics/Genzyme Transgenics）、ヒト抗B型肝炎ウイルス抗体であるOstavir（Protein Design Lab/Novartis）、ヒト化抗CMV IgG1抗体であるPROTOVIR（商標）（Protein Design Lab/Novartis）、マウス抗TNFα $F(ab')_2$であるMAK-195（SEGARD）（Knoll Pharma/BASF）、抗CD14抗体であるIC14（ICOS Pharm）、ヒト化抗VEGF IgG1抗体（Genentech）、マウス抗CA 125抗体であるOVAREX（商標）（Altarex）、マウス抗17-IA細胞表面抗原IgG2a抗体であるPANOREX（商標）（Glaxo Wellcome/Centocor）、マウス抗イディオタイプ（GD3エピトープ）IgG抗体であるBEC2（ImClone System）、キメラ抗EGFR IgG抗体であるIMC-C225（ImClone System）、ヒト化抗α-Vβ3インテグリン抗体であるVITAXIN（商標）（Applied Molecular Evolution/MedImmune）、ヒト化抗CD52 IgG1抗体であるCampath 1H/LDP-03（Leukosite）、ヒト化抗CD33 IgG抗体であるSmart M195（Protein Design Lab/Kanebo）、キメラ抗CD20 IgG1抗体であるRITUXAN（商標）（IDEC Pharm/Genentech, Roche/Zettyaku）、ヒト化抗CD22 IgG抗体であるLYMPHOCIDE（商標）（Immunomedics）、ヒト化抗HLA抗体であるSmart ID 10（Protein Design Lab）；ONCOLYM（商標）（Lym-1）は放射能標識マウス抗HLA DIAGNOSTIC REAGENT抗体である（Technicclone）；ABX-IL8はヒト抗IL8抗体である（Abgenix）；抗CD11aはヒト化IgG1抗体である（Genetech/Xoma）；ICM3はヒト化抗ICAM3抗体である（ICOS Pharm）；IDEC-114は霊長類化抗CD80抗体である（IDEC Pharm/Mitsubishi）；ZEVALIN（商標）は放射能標識マウス抗CD20抗体である（IDEC/Schering AG）；IDEC-131はヒト化抗CD40L抗体である（IDEC/Eisai）；IDEC-151は霊長類化抗CD4抗体である（IDEC）；IDEC-152は霊長類化抗CD23抗体である（IDEC/Seikagaku）；SMART抗CD3はヒト化抗CD3 IgGである（Protein Design Lab）；5G1.1はヒト化抗補体因子5（C5）抗体である（Alexion Pharm）；D2E7はヒト化抗TNF-α抗体である（CAT/BASF）；CDP870はヒト化抗TNF-α Fabフラグメントである（Celltech）；IDEC-151は霊長類化抗CD4 IgG1抗体である（IDEC Pharm/SmithKline Beecham）；MDX-CD4はヒト化抗CD4 IgG抗体である（Medarex/Eisai/Genmab）；CDP571はヒト化抗TNF-α IgG4抗体である（Celltech）；LDP-02はヒト化抗α4β7抗体である（LeukoSite/Genentech）；OrthoClone OKT4Aはヒト化抗CD4 IgG抗体である（Ortho Biotech）；ANTOVA（商標）はヒト化抗CD40L IgG抗体である（Biogen）；ANTEGREN（商標）はヒト化抗VLA-4 IgG抗体である（Elan）；MDX-33はヒト抗CD64（FcγR）抗体である（Medarex/Centeon）；SCH55700はヒト化抗IL-5 IgG4抗体である（Celltech/Schering）；SB-240563およびSB-240683はそれぞれヒト化抗IL-5およびIL-4抗体である（SmithKline Beecham）；rhuMab-E25はヒト化抗IgE IgG1抗体である（Genentech/Norvartis/Tanox Biosystems）；IDEC-152は霊長類化抗CD23抗体である（IDEC Pharm）；ABX-CBLはマウス抗CD-147 IgM抗体である（Abgenix）；BTI-322はラット抗CD2 IgG抗体である（Medimmune/Bio Transplant）；Orthoclone/OKT3はマウス抗CD3 IgG2a抗体である（ortho Biotech）；SIMULECT（商標）はキメラ抗CD25 IgG1抗体である（Novartis Pharm）；LDP-01はヒト化抗β2インテグリンIgG抗体である（LeukoSite）；抗LFA-1はマウス抗CD18 F $(ab')_2$である（Pasteur-Merieux/Immunotech）；CAT-152はヒト抗TGF-β_2抗体である（Cambridge Ab Tech）；およびCorsevin Mはキメラ抗VII因子抗体である（Centocor）。

【００７６】
特定の実施形態においては、本発明は、本明細書に記載の突然変異の1以上を有しRSVに免疫特異的に結合する修飾された抗体（例えば、SYNAGIS（登録商標））を提供する。本発明はまた、表IIIに記載の任意の可変重鎖（VH）および／または可変軽鎖（VL）ドメインのアミノ酸配列を有するVHおよび／またはVLドメインを含み本明細書に記載の突然変異の1以上を有する修飾された抗体を提供する。本発明は更に、表IIに記載の相補性決定領域（CDR）の1以上または表IIIに記載の1以上のVH CDRおよび／またはVL CDRのアミノ酸配列を有する1以上のVH CDRおよび／または1以上のVL CDRを含む抗RSV抗体を含む（ここで、太字または下線で示した残基の1以上は、好ましくは、RSVに対する該抗体の親和性を増加させるアミノ酸置換を有する）。特定の実施形態においては、修飾される抗体は、AFFF、p12f2、p12f4、p11d4、A1e109、A12a6、A13c4、A17d4、A4B4、A8C7、1X-493L1FR、H3-3F4、M3H9、Y10H6、DG、AFFF(1)、6H8、L1-7E5、L215B10、A13A11、A1H5、A4B4(1)、A4B4L1FR-S28R、A4B4-F52Sである。

【表２】

表 II

SYNAGIS(登録商標)の CDR 配列

CDR	配列	配列番号(SEQ ID NO):
VH1	T**S**GMSVG	1
VH2	DIWWD**D**K**KD**YNPSLK**S**	2
VH3	**S**MI**T**N**W**YFDV	3
VL1	**KCQLS**VGYMH	4
VL2	DT**SKLA**S	5
VL3	FQGS**G**YP**F**T	6

【００７７】
【表３】

表 III

抗 RSV 抗体

抗体名称	VH ドメイン	VH CDR1	VH CDR2	VH CDR3	VL ドメイン	VL CDR1	VL CDR2	VL CDR3
SYNAGIS	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNWYFDV (SEQ ID NO:3)	SEQ ID NO:8	KCQLSVGYMH (SEQ ID NO:4)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
AFFF	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNFYFDV (SEQ ID NO:12)	SEQ ID NO:13	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQFSGYPFT (SEQ ID NO:16)
p12f2	SEQ ID NO:17	TPGMSVG (SEQ ID NO:18)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:21	SLSSRVGYMH (SEQ ID NO:22)	DTFYLSS (SEQ ID NO:23)	FQGSGYPFT (SEQ ID NO:6)
p12f4	SEQ ID NO:24	TPGMSVG (SEQ ID NO:18)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:26	SLSSRVGYMH (SEQ ID NO:22)	DTRGLPS (SEQ ID NO:27)	FQGSGYPFT (SEQ ID NO:6)
p11d4	SEQ ID NO:28	TPGMSVG (SEQ ID NO:18)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:30	SPSSRVGYMH (SEQ ID NO:31)	DTMRLAS (SEQ ID NO:32)	FQGSGYPFT (SEQ ID NO:6)
Ale109	SEQ ID NO:33	TAGMSVG (SEQ ID NO:10)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:34	SLSSRVGYMH (SEQ ID NO:22)	DTFKLSS (SEQ ID NO:35)	FQGSGYPFT (SEQ ID NO:6)
A12a6	SEQ ID NO:36	TAGMSVG (SEQ ID NO:10)	DIWWDGKKDYNPSLKD (SEQ ID NO:37)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:38	SASSRVGYMH (SEQ ID NO:39)	DTFKLSS (SEQ ID NO:35)	FQGSGYPFT (SEQ ID NO:6)
A13c4	SEQ ID NO:40	TAGMSVG (SEQ ID NO:10)	DIWWDGKKSYNPSLKD (SEQ ID NO:41)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:42	SLSSRVGYMH (SEQ ID NO:22)	DTMYQSS (SEQ ID NO:43)	FQGSGYPFT (SEQ ID NO:6)
A17d4	SEQ ID NO:44	TAGMSVG (SEQ ID NO:10)	DIWWDDKKSYNPSLKD (SEQ ID NO:45)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:46	LPSSRVGYMH (SEQ ID NO:47)	DTMYQSS (SEQ ID NO:43)	FQGSGYPFT (SEQ ID NO:6)

抗体名称	VH ドメイン	VH CDR1	VH CDR2	VH CDR3	VL ドメイン	VL CDR1	VL CDR2	VL CDR3
A4B4	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:49	SASSRVGYMH (SEQ ID NO:39)	DTFFLDS (SEQ ID NO:50)	FQGSGYPFT (SEQ ID NO:6)
A8C7	SEQ ID NO:51	TAGMSVG (SEQ ID NO:10)	DIWWDDKKSYNPSLKD (SEQ ID NO:45)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:52	SPSSRVGYMH (SEQ ID NO:31)	DTRYQSS (SEQ ID NO:53)	FQGSGYPFT (SEQ ID NO:6)
1X-493LJFR	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNWYFDV (SEQ ID NO:3)	SEQ ID NO:54	SASSSVGYMH (SEQ ID NO:14)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
H3-3F4	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
M3H9	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTYKQTS (SEQ ID NO:57)	FQGSGYPFT (SEQ ID NO:6)
Y10H6	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:58	SASSSVGYMH (SEQ ID NO:14)	DTRYLSS (SEQ ID NO:59)	FQGSGYPFT (SEQ ID NO:6)
DG	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
AFFF(1)	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNFYFDV (SEQ ID NO:12)	SEQ ID NO:60	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSFYPFT (SEQ ID NO:61)
6H8	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:62	SASSSVGYMH (SEQ ID NO:14)	DTFKLTS (SEQ ID NO:63)	FQGSGYPFT (SEQ ID NO:6)
L1-7E5	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:64	SASSRVGYMH (SEQ ID NO:39)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
L215B10	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:65	SASSSVGYMH (SEQ ID NO:14)	DTFRLAS (SEQ ID NO:66)	FQGSGYPFT (SEQ ID NO:6)
A13A11	SEQ ID NO:67	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:68	SPSSRVGYMH (SEQ ID NO:31)	DTYRHSS (SEQ ID NO:69)	FQGSGYPFT (SEQ ID NO:6)

抗体名称	VH ドメイン	VH CDR1	VH CDR2	VH CDR3	VL ドメイン	VL CDR1	VL CDR2	VL CDR3
A1H5	SEQ ID NO:70	TAGMSVG (SEQ ID NO:10)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:71	SLSSSVGYMH (SEQ ID NO:72)	DTFFHRS (SEQ ID NO:73)	FQGSGYPFT (SEQ ID NO:6)
A4B4(1)	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:74	SASSRVGYMH (SEQ ID NO:39)	DTLLLDS (SEQ ID NO:75)	FQGSGYPFT (SEQ ID NO:6)
A4B4L1F R-S28R	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:11	SASSRVGYMH (SEQ ID NO:39)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
A4B4-F52S	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:76	SASSRVGYMH (SEQ ID NO:39)	DTSFLDS (SEQ ID NO:77)	FQGSGYPFT (SEQ ID NO:6)

【００７８】

他の実施形態においては、該抗体は、修飾された抗$\alpha_v\beta_3$抗体、好ましくは、Vitaxin抗体（HuseらのPCT公開WO 98/33919およびWO 00/78815（それらの両方の全体を参照により本明細書に組み入れることとする）の両者を参照されたい）である。

【００７９】

野生型より長い半減期を有する本発明の修飾IgGはまた、抗原結合部位、Fc-受容体結合部位または補体結合部位のような生物活性部位が、野生型と比較してそのような活性を増加または減少させるよう遺伝子工学により修飾されたIgGを包含しうる。

【００８０】

in vivo半減期を増加させるためのこれら及び他の治療用抗体の修飾は、該治療用抗体の、より低い有効量および／またはより少ない頻度の投与を可能にする。in vivo半減期を増加させるためのそのような修飾は、診断用免疫グロブリンの改良に有用であり、十分な診断感度を達成する、より低い用量の投与を可能にしうる。

【００８１】

本発明はまた、ヒンジ-Fc領域とFcRn受容体との相互作用に関与することが確認されたアミノ酸残基中の1以上の修飾（すなわち、置換、欠失または挿入）を有するヒンジ-Fc領域またはそのフラグメント（好ましくはヒトのもの）と生物活性分子とを含む融合タンパク質を提供する。特に、本発明は、アミノ酸残基251-256、285-290および／またはアミノ酸残基308-314中の1以上の修飾を有するCH2ドメインに及び／又はアミノ酸残基385-389および／または428-436中の1以上の修飾（特に、前記のアミノ酸置換の1以上）を有するCH3ドメインに組換え的に融合した又は化学的に結合（共有または非共有結合の両方）した生物活性分子を含む融合タンパク質を提供する。そのような修飾の1以上を有する定常ドメインまたはそのフラグメントへの生物活性分子の融合は、該生物活性分子のin vivo半減期を増加させる。

【００８２】

好ましい実施形態においては、本発明の融合タンパク質は、アミノ酸残基251-256、285-290および／またはアミノ酸残基308-314中の1以上のアミノ酸残基の置換を有するCH2ドメインに及び／又はアミノ酸残基385-389および／または428-436中の1以上の修飾を有するCH3ドメインに組換え的に融合した又は化学的に結合した生物活性分子を含む。ある実施形態においては、融合タンパク質は、アミノ酸残基253、310および313が修飾されていないIgG分子のCH2ドメインを含む。もう1つの実施形態においては、融合タンパク質は、アミノ酸残基388、429、430、431、432および435が修飾されていないIgG分子のCH3ドメインを含む。

【００８３】

生物活性分子は、当業者に公知の任意のポリペプチドまたは合成薬物でありうる。好ましくは、生物活性分子は、少なくとも5、好ましくは少なくとも10、少なくとも20、少なくとも30、少なくとも40、少なくとも50、少なくとも60、少なくとも70、少なくとも80、少なくとも90または少なくとも100アミノ酸残基よりなるポリペプチドである。生物活性ポリペプチドの具体例は、種々の型の抗体、サイトカイン（例えば、IL-2、IL-3、IL-4、IL-5、IL-6、IL-7、IL-10、IL-12、IL-15、IFN-γ、IFN-αおよびIFN-β）、細胞接着分子（例えば、CTLA4、CD2およびCD28）、リガンド（例えば、TNF-α、TNF-βおよび抗血管新生因子、例えばエンドスタチン）、受容体、抗体および増殖因子（例えば、PDGF、EGF、NGFおよびKGF）を包含するが、これらに限定されるものではない。

【００８４】

生物活性分子はまた、細胞毒素（例えば、静細胞剤または殺細胞剤）、治療剤または放射性元素（例えば、α放射体、γ放射体など）のような治療用部分でありうる。静細胞剤または殺細胞剤の具体例は、パクリタクソール、サイトカラシンB、グラミシジンD、臭化エチジウム、エメチン、マイトマイシン、エトポシド、テノポシド、ビンクリスチン、ビンブラスチン、コルヒチン、ドキソルビシン、ダウノルビシン、ジヒドロキシアントラシンジオン、ミトザントロン、ミトラマイシン、アクチノマイシンD、1-デヒドロテストステロン、グルココルチコイド、プロカイン、テトラカイン、リドカイン、プロパノロールお

よびピューロマイシンならびにそれらの類似体またはホモログを包含するが、これらに限定されるものではない。治療剤は、代謝拮抗剤（例えば、メトトレキセート、6-メルカプトプリン、6-チオグアニン、シタラビン、5-フルオロウラシルデカルバジン）、アルキル化剤（例えば、メクロルエタミン、チオエパクロラムブシル、メルファラン、カルムスチン（BSNU）およびロムスチン（CCNU）、シクロトスファミド、ブスルファン、ジブロモマンニトール、ストレプトゾトシン、マイトマイシンCおよびシスジクロロジアミン白金（II）（DDP）シスプラチン）、アントラサイクリン（例えば、ダウノルビシン（旧称ダウノマイシン）およびドキソルビシン）、抗生物質（例えば、ダクチノマイシン（旧称アクチノマイシン））、ブレオマイシン、ミトラマイシンおよびアントラマイシン（AMC）および抗有糸分裂剤（例えば、ビンクリスチンおよびビンブラスチン）を包含するが、これらに限定されるものではない。

【００８５】

本発明はまた、増加した親和性を有する修飾されたFcRn結合部位を含有する本発明の修飾IgGまたはそのフラグメントをコードするヌクレオチド配列を含むポリヌクレオチド、および該ポリヌクレオチドを含むベクターを提供する。さらに、本発明は、本発明の修飾IgGをコードするポリヌクレオチドに、ストリンジェントな又はより低いストリンジェントの条件下でハイブリダイズするポリヌクレオチドを含む。

【００８６】

修飾IgGのヌクレオチド配列およびそれをコードするポリヌクレオチドは、一般的なDNA配列決定法、例えばそれぞれジデオキシチェインターミネーション法（Sanger配列決定法）およびオリゴヌクレオチドプライミングとPCRとの組合せを含む当技術分野で公知の任意の方法により得ることができる。

【００８７】

5.2 免疫グロブリン分子のヒンジ-Fc領域中の突然変異の同定

定常ドメインのアミノ酸残基251-256、285-290、308-314、385-389および428-436中の1以上の修飾は、当業者に公知の任意の技術を用いて導入することができる。アミノ酸残基251-256、285-290、308-314、385-389および428-436中の1以上の修飾を有する定常ドメインまたはそのフラグメントは、例えば、FcRn受容体に対する増加した親和性を有する定常ドメインまたはそのフラグメントを同定するための結合アッセイによりスクリーニングすることができる（例えば、後記第5.11節に記載のとおり）。FcRn受容体に対する定常ドメインまたはそのフラグメントの親和性を増加させる該ヒンジ-Fcドメインまたはそのフラグメント中の修飾を抗体中に導入して、該抗体のin vivo半減期を増加させることが可能である。さらに、FcRnに対する定常ドメインまたはそのフラグメントの親和性を増加させる定常ドメインまたはそのフラグメント中の修飾体を生物活性分子に融合して、該生物活性分子のin vivo半減期を増加させ、好ましくは、該分子のバイオアベイラビリティを改変（増強または減弱）する（例えば、粘膜表面（または他の標的組織）（例えば、肺）への輸送を増加または減少させる）ことができる。

【００８８】

5.2.1. 突然変異誘発

突然変異誘発は、修飾される抗体の定常ドメインまたはそのフラグメント（例えば、CH2またはCH3ドメイン）の配列中の1以上の修飾を有するオリゴヌクレオチドの安定化を含む（それらに限定されるものではない）当技術分野で公知の技術のいずれかに従い行うことができる。部位特異的突然変異誘発は、所望の突然変異のDNA配列と、横断する欠失結合部の両側で安定な二本鎖を形成するのに十分なサイズおよび配列複雑度のプライマー配列を与えるのに十分な数の隣接ヌクレオチドとをコードする特異的オリゴヌクレオチド配列の使用により、突然変異体の産生を可能にする。典型的には、改変される配列の結合部の両側に約10～約25以上の残基を有する約17～約75ヌクレオチド以上の長さのプライマーが好ましい。突然変異体のライブラリーを作製するために、1以上の位置に種々の異なる突然変異を導入する多数のそのようなプライマーを使用することができる。

【００８９】

部位特異的突然変異誘発の技術は、種々の刊行物（Kunkelら，Methods Enzymol., 154: 367-82,1987（その全体を参照により本明細書に組み入れることとする））に例示されているとおり当技術分野で良く知られている。一般には、部位特異的突然変異誘発は、まず、所望のペプチドをコードするDNA配列を配列中に含む一本鎖ベクターを得るか又は該配列を含む二本鎖ベクターの2つの鎖を融解解離させることにより行う。所望の突然変異配列を保持するオリゴヌクレオチドプライマーを、一般には合成的に製造する。ついでこのプライマーを一本鎖ベクターでアニーリングし、T7 DNAポリメラーゼのようなDNA重合酵素に付して、該突然変異保持鎖の合成を完成させる。このようにしてヘテロ二本鎖が形成され、ここで、一方の鎖は元の非突然変異配列をコードしており、もう一方の鎖は所望の突然変異を保持する。ついでこのヘテロ二本鎖ベクターを使用して大腸菌（E. coli）細胞のような適当な細胞を形質転換またはトランスフェクトし、該突然変異配列配置を保持する組換えベクターを含むクローンを選択する。理解されるとおり、該技術は、典型的には、一本鎖および二本鎖の両方の形態で存在するファージベクターを用いる。部位特異的突然変異誘発に有用な典型的なベクターは、M13ファージのようなベクターを包含する。これらのベクターは容易に商業的に入手可能であり、それらの使用は概ね当業者に良く知られている。また、関心のある遺伝子をプラスミドからファージへ導入する工程を省く部位特異的突然変異誘発においては、二本鎖プラスミドが常套的に使用される。

【００９０】

あるいは、Taq DNAポリメラーゼのような商業的に入手可能な熱安定酵素と共にPCR（商標）を用いて、突然変異誘発性オリゴヌクレオチドプライマーを増幅DNA断片中に組込むことが可能であり、ついでそれを適当なクローニングまたは発現ベクター中にクローニングすることができる。例えば、PCR（商標）媒介突然変異誘発法については、Tomicら，Nucleic Acids Res., 18 (6):1656,1987およびUpenderら，Biotechniques, 18 (1): 29-30,32,1995（それらの全体を参照により本明細書に組み入れることとする）を参照されたい。また、熱安定ポリメラーゼに加えて熱安定リガーゼを使用するPCR（商標）を用いてリン酸化突然変異誘発性オリゴヌクレオチドを増幅DNA断片中に組込み、ついでそれを適当なクローニングまたは発現ベクター中にクローニングすることができる（例えば、Michael, Biotechniques, 16 (3): 410-2,1994（その全体を参照により本明細書に組み入れることとする）を参照されたい）。

【００９１】

抗体のFcドメインまたはそのフラグメントの配列変異体を製造するための当業者に公知の他の方法を用いることが可能である。例えば、抗体の定常ドメインまたはそのフラグメントのアミノ酸配列をコードする組換えベクターをヒドロキシルアミンのような突然変異誘発剤で処理して、配列変異体を得ることができる。

【００９２】

5.2.2. パニング

アミノ酸残基251-256、285-290、308-314、385-389および／または428-436中に1以上の修飾を有する定常ドメインまたはそのフラグメントを発現するベクター（特にファージ）をスクリーニングして、FcRnに対する増加した親和性を有する定常ドメインまたはそのフラグメントを同定して、ファージの集団から最高親和性結合体を選び出すことが可能である。アミノ酸残基251-256、285-290、308-314、385-389および／または428-436中に1以上の修飾を有する定常ドメインまたはそのフラグメントの、FcRnへの結合を分析するために使用しうるイムノアッセイは、ラジオイムノアッセイ、ELISA（酵素免疫検定法）、「サンドイッチ」イムノアッセイおよび蛍光イムノアッセイを包含するが、それらに限定されるものではない。そのようなアッセイは常套的なものであり、当技術分野で良く知られている（例えば、Ausubelら編，1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York（その全体を参照により本明細書に組み入れることとする）を参照されたい）。典型的なイムノアッセイは後記で簡潔に説明される（がそれらに限定されるものではない）。また、アミノ酸残基251-256、285-290、308-314、385-389および／または428-436中に1以上の修飾を有する定常ドメインまたはそのフラグメントの

、FcRnへの結合速度および解離速度を測定するために、BIAcore速度論的分析を用いることができる。BIAcore速度論的分析は、アミノ酸残基251-256、285-290、308-314、385-389および／または428-436中に1以上の修飾を有する定常ドメインまたはそのフラグメントの、チップからの結合および解離を、その表面上の固定化FcRnで分析することを含む（第5.1節および後記実施例を参照されたい）。

【００９３】

5.2.3. 配列決定

アミノ酸残基251-256、285-290、308-314、385-389および／または428-436中に1以上の修飾を有する定常ドメインまたはそのフラグメントをコードするヌクレオチド配列を直接的に配列決定するためには、当技術分野で公知の種々の配列決定反応のいずれかを用いることができる。配列決定反応の具体例は、MaximおよびGilbert（Proc. Natl. Acad. Sci. USA, 74: 560,1977）またはSanger（Proc. Natl. Acad. Sci. USA, 74: 5463,1977）により開発された技術に基づくものを包含する。また、マススペクトロメトリー（例えば、PCT公開WO 94/16101, Cohenら, Adv. Chromatogr., 36:127-162,1996およびGriffinら, Appl. Biochem. Biotechnol., 38:147-159,1993を参照されたい）を含む種々の自動配列決定法のいずれかを用いることも意図される（Bio/Techniques, 19: 448,1995）。

【００９４】

5.3 抗体の組換え製造方法

本発明の抗体またはそのフラグメントは、抗体の合成のための当技術分野で公知の任意の方法、特に、化学合成または好ましくは組換え発現技術により製造することができる。

【００９５】

抗体をコードするヌクレオチド配列は、当業者に入手可能な任意の情報（すなわち、Genbank、文献または常套的なクローニング）から得ることができる。特定の抗体またはそのエピトープ結合性フラグメントをコードする核酸を含有するクローンが入手可能でないが、該抗体分子またはそのエピトープ結合性フラグメントの配列が公知である場合には、免疫グロブリンをコードする核酸を化学的に合成したり、あるいは該配列の3'および5'末端にハイブリダイズしうる合成プライマーを使用するPCR増幅により、または例えば該抗体をコードするcDNAライブラリーからのcDNAクローンを同定するために特定の遺伝子配列に特異的なオリゴヌクレオチドプローブを使用するクローニングにより、適当な入手源（例えば、抗体を発現するよう選択されたハイブリドーマ細胞のような該抗体を発現する任意の組織または細胞から作製されたcDNAライブラリーまたは抗体cDNAライブラリー、あるいは該組織または細胞から単離された核酸、好ましくはポリA^+ RNA）から得ることが可能である。ついで、PCRにより得られた増幅された核酸を、当技術分野で良く知られた任意の方法を用いて、複製可能なクローニングベクター中にクローニングすることができる。

【００９６】

該抗体のヌクレオチド配列を決定したら、例えば、該抗体のエピトープ結合性ドメイン領域（好ましくは、FcRnとの相互作用に関与する該抗体のヒンジ-Fc領域）中にアミノ酸の置換、欠失および／または挿入を導入することにより、異なるアミノ酸配列を有する抗体を作製するために、ヌクレオチド配列の操作のための当技術分野で良く知られた方法、例えば、組換えDNA技術、部位特異的突然変異誘発、PCRなど（例えば、Sambrookら, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; およびAusubel ら編, 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY（それらの両方の全体を参照により本明細書に組み入れることとする）に記載の技術を参照されたい）を用いて、該抗体のヌクレオチド配列を操作することができる。好ましい実施形態においては、アミノ酸残基251-256、285-290、308-314、385-389および428-436中に1以上の修飾を有する抗体を作製する。

【００９７】

抗体の組換え発現は、該抗体をコードするヌクレオチド配列を含有する発現ベクターの構築を要する。抗体分子または抗体の重もしくは軽鎖またはそれらの一部（好ましくは、重または軽鎖可変領域を含有するものであるが必ずしもそれが必要なわけではない）をコー

ドするヌクレオチド配列を得たら、当技術分野で良く知られた技術を用いる組換えDNA技術により、該抗体分子の製造のためのベクターを製造することができる。したがって、抗体コードヌクレオチド配列を含有するポリヌクレオチドを発現させることによるタンパク質の製造方法が本明細書に記載されている。抗体コード配列と適当な転写および翻訳制御シグナルとを含有する発現ベクターを構築するためには、当業者に良く知られた方法を用いることができる。これらの方法は、例えば、in vitro組換えDNA技術、合成技術、およびin vivo遺伝子組換えを包含する。したがって、本発明は、FcRnとの相互作用に関与するアミノ酸残基中に1以上の修飾を有する抗体分子の定常領域をコードするヌクレオチド配列を含んでなる複製可能なベクターを提供する（例えば、PCT公開WO 86/05807; PCT公開WO 89/01036; および米国特許第5,122,464号を参照されたい）。重鎖可変領域、軽鎖可変領域、該重鎖および軽鎖の両方の可変領域、該重鎖および／または軽鎖可変領域のエピトープ結合性フラグメント、あるいは抗体の相補性決定領域（CDR）の1以上をコードするヌクレオチド配列を、そのような発現用ベクター中にクローニングすることができる。

【００９８】

該発現ベクターを通常の技術により宿主細胞中に導入し、ついで該トランスフェクト化細胞を通常の技術により培養して、FcRnに対する増加した親和性および増加したin vivo半減期を有する抗体を得る。したがって、本発明は、アミノ酸残基251-256、285-290、308-314、385-389および／または428-436中に1以上の修飾を有する抗体、定常ドメインまたはそのFcRn結合性フラグメントをコードするポリヌクレオチド（好ましくは、これは、機能しうる形で異種プロモーターに連結されている）を含有する宿主細胞を含む。

【００９９】

本発明の抗体分子を発現させるためには、種々の宿主-発現ベクター系を使用することができる。そのような宿主-発現系は、関心のあるコード配列が産生され次いで精製されうるビヒクルを意味するだけでなく、適当なヌクレオチドコード配列で形質転換またはトランスフェクトされた場合に本発明の抗体分子をin situで発現しうる細胞をも意味する。これらは、抗体コード配列を含有する組換えバクテリオファージDNA、プラスミドDNAまたはコスミドDNA発現ベクターで形質転換された細菌（例えば、E. coliおよびB. subtilis）、抗体コード配列を含有する組換え酵母発現ベクターで形質転換された酵母（例えば、SaccharomycesおよびPichia）のような微生物、抗体コード配列を含有する組換えウイルス発現ベクター（例えば、バキュロウイルス）に感染した昆虫細胞系、抗体コード配列を含有する組換えプラスミド発現ベクター（例えば、Tiプラスミド）で形質転換された又は組換えウイルス発現ベクター（例えば、カリフラワーモザイクウイルス（CaMV）およびタバコモザイクウイルス（TMV））に感染した植物細胞系、および哺乳類細胞のゲノムに由来するプロモーター（例えばメタロチオネインプロモーター）又は哺乳類ウイルスに由来するプロモーター（例えば、アデノウイルス後期プロモーター、ワクシニアウイルス7.5Kプロモーター）を含有する組換え発現構築物を保持する哺乳類細胞系（例えば、COS、CHO、BHK、293、3T3およびNSO細胞）を包含するが、それらに限定されるものではない。好ましくは、大腸菌のような細菌細胞、およびより好ましくは、特に全組換え抗体分子の発現には真核細胞を、組換え抗体分子の発現に使用する。例えば、ヒトサイトメガロウイルス由来の主要中間（major intermediate）初期遺伝子プロモーターエレメントのようなベクターと組合せたチャイニーズハムスター卵巣細胞（CHO）のような哺乳類細胞は、抗体のための有効な発現系である（Foeckingら, Gene, 45: 101,1986, およびCockettら, Bio/Technology, 8: 2, 1990）。

【０１００】

細菌系においては、発現される抗体分子に意図される用途に応じて多数の発現ベクターが有利に選択されうる。例えば、抗体分子の医薬組成物の製造のために大量のそのようなタンパク質を製造したい場合には、容易に精製される高レベルの融合タンパク質産物の発現を導くベクターが望ましいかもしれない。そのようなベクターは、大腸菌（E. coli）発現ベクターpUR278（Rutherら, EMBO, 12: 1791,1983）（この場合、融合タンパク質が産生されるよう、該抗体コード配列は個々に、lacZコード領域とインフレームで該ベクター

中に連結されうる）、およびpINベクター（Inouye および Inouye, Nucleic Acids Res., 13: 3101-3109,1985ならびにVan Heeke およびSchuster, J. Biol. Chem., 24: 5503-5509,1989）を包含するが、これらに限定されるものではない。

【０１０１】

昆虫細胞系においては、外来遺伝子を発現させるためのベクターとして、Autographa californica核多角体病ウイルス（AcNPV）を使用する。該ウイルスはSpodoptera frugiperda中で増殖する。該抗体コード配列を該ウイルスの非必須領域（例えば、多角体遺伝子）中に個々にクローニングし、AcNPVプロモーター（例えば、多角体プロモーター）の制御下に配置することができる。

【０１０２】

哺乳類宿主細胞においては、本発明の抗体分子を発現させるために、ウイルスに基づく多数の発現系を使用することができる。発現ベクターとしてアデノウイルスを使用する場合には、関心のある抗体コード配列をアデノウイルス転写／翻訳制御複合体（例えば、後期プロモーターおよびトリパータイトリーダー配列）に連結しすることができる。ついでこのキメラ遺伝子をin vitroまたはin vivo組換えによりアデノウイルスゲノム中に挿入することができる。該ウイルスゲノムの非必須領域（例えば、領域E1またはE3）中での挿入は、感染宿主中で該抗体分子を発現しうる生存可能な組換えウイルスを与える（例えば、LoganおよびShenk, Proc. Natl. Acad. Sci. USA, 81: 355-359,1984を参照されたい）。挿入された抗体コード配列の効率的な翻訳のためには、特異的開始シグナルも必要なことがある。これらのシグナルはATG開始コドンおよび隣接配列を含む。さらに、該全インサートの翻訳が保証されるためには、該開始コドンは所望のコード配列のリーディングフレームと同調していなければならない。これらの外因性翻訳制御シグナルおよび開始コドンは、天然物および合成物の両方を含む種々の起源に由来しうる。発現の効率は、適当な転写エンハンサーエレメント、転写ターミネーターなどを含有させることにより向上させることが可能である（例えば、Bitterら, Methods in Enzymol., 153: 516-544,1987を参照されたい）。

【０１０３】

また、該抗体配列の発現をモジュレーションし又は所望の特定の様態で該抗体を修飾しプロセシングする宿主細胞株を選択することができる。タンパク質産物のそのような修飾（例えば、グリコシル化）およびプロセシング（例えば、切断）は該抗体の機能に重要でありうる。種々の宿主細胞は、タンパク質および遺伝子産物の翻訳後プロセシングおよび修飾のための特徴的かつ特異的なメカニズムを有する。発現された抗体の適切な修飾およびプロセシングが保証されるよう、適当な細胞系または宿主系を選択することができる。この目的には、該遺伝子産物の一次転写産物の適当なプロセシング、グリコシル化およびリン酸化のための細胞機構を有する真核宿主細胞を使用することができる。そのような哺乳類宿主細胞は、CHO、VERY、BHK、HeLa、COS、MDCK、293、3T3、W138および特に骨髄腫細胞、例えばNS0細胞および関連細胞系を包含するが、これらに限定されるものではない。例えば、Morrisonら, 米国特許第5,807,715号（その全体を参照により本明細書に組み入れることとする）を参照されたい。

【０１０４】

組換え抗体の長期的な高収率の製造のためには、安定な発現が好ましい。例えば、該抗体分子を安定に発現する細胞系を操作することができる。ウイルス複製起点を含有する発現ベクターを使用する代わりに、適当な発現制御エレメント（例えば、プロモーター、エンハンサー、配列、転写ターミネーター、ポリアデニル化部位など）により制御されるDNAおよび選択マーカーで宿主細胞を形質転換することができる。該外来DNAの導入の後、操作された細胞を強化培地中で1～2日間増殖させ、ついで選択培地に交換することができる。該組換えプラスミド中の選択マーカーは該選択に対する耐性を付与し、細胞がその染色体中に該プラスミドを安定に組込みフォーカスを形成するまで増殖するのを可能にし、そしてそれをクローニングし、細胞系にまで増殖させることができる。この方法は、該抗体分子を発現する細胞系を操作するために有利に用いられうる。そのような操作された細胞

系は、該抗体分子と直接的または間接的に相互作用する組成物のスクリーニングおよび評価において特に有用であり得る。

【０１０５】

以下のものを含む（それらに限定されるものではない）多数の選択系を用いることが可能であり、単純ヘルペスウイルスチミジンキナーゼ（Wiglerら，Cell，11:223，1977）、ヒポキサンチン-グアニンホスホリボシルトランスフェラーゼ（SzybalskaおよびSzybalski，Proc. Natl. Acad. Sci. USA，48:202，1992）およびアデニンホスホリボシルトランスフェラーゼ（Lowyら，Cell，22:8-17，1980）遺伝子は、それぞれtk⁻、hgprt⁻またはaprt⁻細胞において使用することができる。また、以下の遺伝子に関する選択の基礎として、代謝拮抗物質耐性を用いることができる：dhfr，これはメトトレキセートに対する耐性を付与する（Wiglerら，Natl. Acad. Sci. USA，77:357，1980およびO'Hareら，Proc. Natl. Acad. Sci. USA，78: 1527,1981）；gpt，これはミコフェノール酸に対する耐性を付与する（MulliganおよびBerg，Proc. Natl. Acad. Sci. USA，78: 2072,1981）；neo，これはアミノグリコシドG418に対する耐性を付与する（WuおよびWu，Biotherapy，3: 87-95，1991; Tolstoshev，Ann. Rev. Pharmacol. Toxicol.，32: 573-596,1993; Mulligan，Science，260: 926-932,1993; ならびにMorganおよびAnderson，Ann. Rev. Biochem.，62: 191-217,1993; ならびにMay，TIB TECH，11 (5): 155-2 15,1993）、およびhygro，これはハイグロマイシンに対する耐性を付与する（Santerreら，Gene，30: 147,1984）。所望の組換えクローンを選択するためには、組換えDNA技術の当技術分野で一般に公知の方法を常套的に適用することが可能であり、そのような方法は、例えば、Ausubelら(編)，1993，Current Protocols in Molecular Biology，John Wiley & Sons，NY; Kriegler，1990，Gene Transfer and Expression，A Laboratory Manual，Stockton Press，NY; 第12章および第13章，Dracopoliら(編)，1994，Current Protocols in Human Genetics，John Wiley & Sons，NY; ならびにColberre-Garapinら，J. Mol. Biol.，150: 1,1981（それらの全体を参照により本明細書に組み入れることとする）に記載されている。

【０１０６】

抗体分子の発現レベルはベクターの増幅により増加させることができる（総説としては、BebbingtonおよびHentschel，1987，The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning，Vol. 3. Academic Press，New Yorkを参照されたい）。抗体を発現するベクター系中のマーカーが増幅可能な場合には、宿主細胞の培養内に存在するインヒビターのレベルの増加が該マーカー遺伝子のコピー数を増加させる。該増幅領域は該抗体遺伝子と結合しているため、該抗体の産生も増加する（Crouseら，Mol. Cell. Biol.，3: 257,1983）。

【０１０７】

該宿主細胞は、本発明の2つの発現ベクター（第1ベクターは重鎖由来ポリペプチドをコードし、第2ベクターは軽鎖由来ポリペプチドをコードする）でコトランスフェクトすることができる。それらの2つのベクターは、重鎖ポリペプチドおよび軽鎖ポリペプチドの等しい発現を可能にする同じ選択マーカー、または両方のプラスミドの維持を保証する異なる選択マーカーを含有しうる。あるいは、重鎖および軽鎖の両方のポリペプチドをコードしておりそれらを発現しうる単一のベクターを使用することができる。そのような場合には、過剰な毒性遊離重鎖を避けるために、該軽鎖は該重鎖の前に配置されるべきである（Proudfoot，Nature，322: 52,1986; およびKohler，Proc. Natl. Acad. Sci. USA，77: 2197,1980）。該重鎖および軽鎖のコード配列はcDNAまたはゲノムDNAを含みうる。

【０１０８】

本発明の抗体分子が組換え発現により産生されたら、免疫グロブリン分子の精製のための当技術分野で公知の任意の方法、例えば、クロマトグラフィー（例えば、イオン交換クロマトグラフィー、アフィニティークロマトグラフィー、特に、プロテインA精製後の特異的抗原に関するアフィニティークロマトグラフィー、およびサイズカラムクロマトグラフィー）、遠心分離、溶解度差、またはタンパク質の精製のための任意の他の標準的な技術により、それを精製することができる。さらに、本発明の抗体またはそのフラグメントは

、精製を促進する本明細書に記載の又は当技術分野で公知の異種ポリペプチド配列に融合させることができる。

【０１０９】

5.3.1. 抗体コンジュゲート

本発明は、融合タンパク質を生成するよう異種ポリペプチド（すなわち、無関係なポリペプチドまたはその一部、好ましくは、該ポリペプチドの少なくとも10、少なくとも20、少なくとも30、少なくとも40、少なくとも50、少なくとも60、少なくとも70、少なくとも80、少なくとも90または少なくとも100アミノ酸）に組換え的に融合した又は化学的に結合（コンジュゲーション）（共有結合および非共有結合の両方を含む）した抗体を含む。該融合は必ずしも直接的である必要はなく、リンカー配列を介して生じうる。また、異種ポリペプチドに融合または結合した抗体は、当技術分野で公知の方法を用いるin vitroイムノアッセイおよび精製方法において使用することができる。例えば、PCT公開番号WO 93/21232; EP 439,095; Naramuraら, Immunol. Lett., 39: 91-99, 1994; 米国特許第5,474,981号; Gilliesら, PNAS, 89: 1428-1432,1992; およびFellら,J. Immunol., 146: 2446-2452,1991（それらの全体を参照により本明細書に組み入れることとする）を参照されたい。

【０１１０】

抗体はマーカー配列、例えば精製を促進するペプチドに融合させることが可能である。好ましい実施形態においては、該マーカーアミノ酸配列は、とりわけ、pQEベクター（QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311）において提供されるタグのようなヘキサヒスチジンペプチド（それらの多くは商業的に入手可能である）である。例えばGentzら, Proc. Natl. Acad. Sci. USA, 86: 821-824,1989に記載されているとおり、ヘキサヒスチジンは、該融合タンパク質の簡便な精製をもたらす。精製に有用な他のペプチドタグは、インフルエンザ赤血球凝集素タンパク質に由来するエピトープに相当する赤血球凝集素「HA」タグ（Wilsonら, Cell, 37: 767 1984）、および「flag」タグ（Knappikら, Biotechniques, 17 (4): 754-761,1994）を包含するが、これらに限定されるものではない。

【０１１１】

本発明はまた、in vivo半減期を増加させたい診断もしくは治療剤または任意の他の分子に結合した抗体を含む。該抗体は、例えば、与えられた治療計画の有効性を判定するために、例えば、臨床試験法の一部として疾患、障害または感染の発生または進行をモニターするために診断的に使用することができる。検出は、検出可能な物質に該抗体を結合させることにより容易にすることができる。検出可能な物質の具体例は、種々の酵素、補欠分子族、蛍光物質、発光物質、生物発光物質、放射性物質、陽電子放射性金属、および非放射性常磁性金属イオンを包含する。検出可能な物質は、当技術分野で公知の技術を用いて該抗体に直接的に又は中間物（例えば、当技術分野で公知のリンカー）を介して間接的に結合または連結させることができる。本発明に従い診断用に使用する抗体に結合されうる金属イオンに関しては、例えば、米国特許第4,741,900号を参照されたい。適当な酵素の具体例は、ホースラディッシュペルオキシダーゼ、アルカリホスファターゼ、β-カラクトシダーゼまたはアセチルコリンエステラーゼを包含し、適当な補欠分子族複合体の具体例は、ストレプトアビジン／ビオチンおよびアビジン／ビオチンを包含し、適当な蛍光物質の具体例は、ウンベリフェロン、フルオレセイン、フルオレセインイソチオシアナート、ローダミン、ジクロロトリアジニルアミンフルオレセイン、ダンシルクロリドまたはフィコエリトリンを包含し、発光物質の具体例はルミノールを包含し、生物発光物質の具体例はルシフェラーゼ、ルシフェリンおよびエクオリンを包含し、適当な放射性物質の具体例は^{125}I、^{131}I、^{111}Inまたは^{99m}Tcを包含する。

【０１１２】

抗体は、細胞毒素（例えば、静細胞剤または殺細胞剤）、治療剤または放射性元素（例えば、α放射体、γ放射体など）のような治療用部分に結合させることができる。細胞毒素または細胞傷害剤は、細胞に有害な任意の物質を包含する。具体例は、パクリタクソール

、サイトカラシンB、グラミシジンD、臭化エチジウム、エメチン、マイトマイシン、エトポシド、テノポシド、ビンクリスチン、ビンブラスチン、コルヒチン、ドキソルビシン、ダウノルビシン、ジヒドロキシアントラシンジオン、ミトザントロン、ミトラマイシン、アクチノマイシンD、1-デヒドロテストステロン、グルココルチコイド、プロカイン、テトラカイン、リドカイン、プロパノロールおよびピューロマイシンならびにそれらの類似体またはホモログを包含する。治療剤は、代謝拮抗剤（例えば、メトトレキセート、6-メルカプトプリン、6-チオグアニン、シタラビン、5-フルオロウラシルデカルバジン）、アルキル化剤（例えば、メクロルエタミン、チオエパクロラムブシル、メルファラン、カルムスチン（BSNU）およびロムスチン（CCNU）、シクロトスファミド、ブスルファン、ジブロモマンニトール、ストレプトゾトシン、マイトマイシンCおよびシスジクロロジアミン白金（II）（DDP）シスプラチン）、アントラサイクリン（例えば、ダウノルビシン（旧称ダウノマイシン）およびドキソルビシン）、抗生物質（例えば、ダクチノマイシン（旧称アクチノマイシン））、ブレオマイシン、ミトラマイシンおよびアントラマイシン（AMC）および抗有糸分裂剤（例えば、ビンクリスチンおよびビンブラスチン）を包含するが、これらに限定されるものではない。

【０１１３】

さらに、抗体は、与えられた生物学的応答を修飾する治療剤または薬物部分に結合させることができる。治療剤または薬物部分は古典的な化学療法剤に限定されると解釈されるべきではない。例えば、該薬物部分は、所望の生物活性を有するタンパク質またはポリペプチドでありうる。そのようなタンパク質は、例えば、アブリン、リシンA、シュードモナス外毒素またはジフテリア毒素のような毒素；腫瘍壊死因子、α-インターフェロン（IFN-α）、β-インターフェロン（IFN-β）、神経成長因子（NGF）、血小板由来増殖因子（PDGF）、組織プラスミノーゲンアクチベーター（TPA）、アポトーシス剤（例えば、TNF-α、TNF-β、AIM I（PCT公開番号WO 97/33899に開示されているとおり））、AIM II（PCT公開番号WO 97/34911を参照されたい）、Fasリガンド（Takahashiら, J. Iminunol., 6: 1567-1574, 1994）およびVEGI（PCT公開番号WO 99/23105）、血栓剤または抗血管新生剤（例えば、アンジオスタチンまたはエンドスタチン）のようなタンパク質；あるいは例えばリンホカイン（例えば、インターロイキン-1（「IL-1」）、インターロイキン-2（「IL-2」）、インターロイキン-6（「IL-6」）、顆粒球マクロファージコロニー刺激因子（「GM-CSF」）および顆粒球コロニー刺激因子（「G-CSF」））または増殖因子（例えば、成長ホルモン（「GH」））のような生物応答調節剤を包含しうる。

【０１１４】

そのような治療用部分を抗体に結合させるための技術は良く知られている。例えば、Arnonら, “Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy” (Monoclonal Antibodies And Cancer Therapy, Reisfeldら(編), 1985, pp. 243-56, Alan R. Liss, Inc.) ; Hellstromら, “Antibodies For Drug Delivery” (Controlled Drug Delivery (2nd Ed.), Robinsonら(編), 1987, pp. 623-53, Marcel Dekker, Inc.); Thorpe, “Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review” (Monoclonal Antibodies'84 : Biological And Clinical Applications, Pincheraら(編), 1985, pp. 475-506); “Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy” (Monoclonal Antibodies For Cancer Detection And Therapy, Baldwinら(編), 1985, pp. 303-16, Academic Press); およびThorpeら, Immunol. Recombinant expression vector., 62: 119-58,1982を参照されたい。

【０１１５】

単独で又は細胞傷害性（細胞毒性）因子および／またはサイトカインと共に投与される、治療用部分に結合した又は結合していない抗体またはそのフラグメントは、治療剤として使用することができる。

【０１１６】

あるいは、Segal, 米国特許第4,676,980号（その全体を参照により本明細書に組み入れる

こととする）に記載のとおり抗体ヘテロコンジュゲートを形成させるために、抗体を二次抗体に結合させることが可能である。

【０１１７】

また、抗体を固体支持体に結合させることが可能であり、それは標的抗原の精製またはイムノアッセイに特に有用である。そのような固体支持体は、ガラス、セルロース、ポリアクリルアミド、ナイロン、ポリスチレン、ポリビニルクロリドまたはポリプロピレンを包含するが、これらに限定されるものではない。

【０１１８】

5.4　融合タンパク質を生産する方法

融合タンパク質は標準的な組換えDNA技法または、例えばペプチド合成機の使用によるタンパク質合成技法によって生産することができる。例えば、ある融合タンパク質をコードする核酸分子は、自動DNA合成機を含む従来の技法で合成することができる。あるいはまた、遺伝子断片のPCR増幅を、2つの連続的な遺伝子断片、それらは後にアニーリングされ再増幅されてキメラ遺伝子配列を生成することができるが、それらの遺伝子断片の間に相補的なオーバーハングを生ずるアンカープライマーを用いて行うことができる(例えば、Current Protocols in Molecular Biology, Ausubelら編, John Wiley & Sons, 1992を参照せよ)。さらに、生物活性分子をコードする核酸を、Fcドメインまたはそのフラグメントを含んでいる発現ベクター中にクローン化してその生物活性分子が定常ドメインまたはそのフラグメントとインフレームで連結するようにすることができる。

【０１１９】

抗体の定常領域にポリペプチドを融合させるまたは結合させる方法は当業界では公知である。例えば、米国特許第5,336,603号、第5,622,929号、第5,359,046号、第5,349,053号、第5,447,851号、第5,723,125号、第5,783,181号、第5,908,626号、第5,844,095号、および第5,112,946号；EP307,434；EP367,166；EP394,827；PCT公開WO 91/06570、WO 96/04388、WO 96/22024、WO 97/34631、およびWO 99/04813；Ashkenaziら, Proc. Natl. Acad. Sci. USA, 88:10535-10539, 1991；Traunecker ら, Nature, 331:84-86, 1988；Zhengら, J. Immunol., 154:5590-5600, 1995；およびVilら, Proc. Natl. Acad. Sci. USA, 89:11337-11341, 1992(これらはその全体を本明細書中に参照により組み入れる)を参照されたい。

【０１２０】

生物活性分子をコードするヌクレオチド配列は当業者であれば入手できる情報のいかなるものからでも得ることができ(例えば、Genbank、文献から、または日常行っているようなクローニングによって)、FcRnに対しての親和性を増大させた定常ドメインまたはそのフラグメントをコードするヌクレオチド配列は、本明細書に記載の技法を用いて産生させた突然変異体の配列分析によって決定することができ、またはGenbankもしくは文献から得ることができる。融合タンパク質をコードするヌクレオチド配列は適切な発現ベクター、すなわち、挿入された、タンパク質をコードする配列の転写と翻訳のために必要なエレメントを含んでいるベクター中に挿入することができる。タンパク質をコードする該配列を発現させるために、種々の宿主-ベクター系を本発明で用いることができる。そのような系としては限定はされないが、ウイルス(例えばワクシニアウイルス、アデノウイルス、他)を感染させた哺乳類細胞系；ウイルス(例えばバキュロウイルス)を感染させた昆虫細胞系；酵母ベクターを含んでいる酵母などの微生物；またはバクテリオファージ、DNA、プラスミドDNA、もしくはコスミドDNAで形質転換させた細菌が挙げられる。ベクターの発現エレメントはそれらの強度および特異性が様々である。用いる宿主-ベクター系に応じて、多数の好適な転写および翻訳エレメントのうちのいずれか1つを用いることができる。

【０１２１】

融合タンパク質の発現は当業界で既知のプロモーターまたはエンハンサーエレメントのいずれによっても制御することができる。融合タンパク質をコードする遺伝子の発現を制御するために用いることのできるプロモーターとしては、限定はされないが、SV40初期プロ

モーター領域(BernoistとChambon, Nature, 290:304-310, 1981)、ラウス肉腫ウイルスの3'の長い末端反復配列(long terminal repeat)中に含まれるプロモーター(Yamamotoら, Cell, 22:787-797, 1980)、ヘルペスチミジンキナーゼプロモーター(Wagnerら, Proc. Natl. Acad. Sci. U.S.A., 78:1441-1445, 1981)、メタロチオネイン遺伝子の調節配列(Brinsterら, Nature, 296:39-42, 1982)、テトラサイクリン(Tet)プロモーター(Gossenら, Proc. Nat. Acad. Sci. USA, 89:5547-5551, 1995)；β-ラクタマーゼプロモーター(Villa-Kamaroffら, Proc. Natl. Acad. Sci. U.S.A., 75:3727-3731, 1978)またはtacプロモーター(DeBoerら, Proc. Natl. Acad. Sci. U.S.A., 80:21-25, 1983；また、「組換え細菌由来の有用なタンパク質(Useful proteins from recombinant bacteria)」, Scientific American, 242:74-94, 1980をも参照せよ)などの原核生物発現ベクター；ノパリンシンセターゼプロモーター領域(Herrera-Estrellaら, Nature, 303:209-213, 1983)またはカリフラワーモザイクウイルス35S RNA プロモーター(Gardnerら, Nucl. Acids Res., 9:2871, 1981)、および光合成酵素であるリブロース二リン酸カルボキシラーゼのプロモーター(Herrera-Estrellaら, Nature, 310:115-120, 1984)を含んでいる植物の発現ベクター；GAl 4 プロモーター、ADC(アルコール脱水素酵素)プロモーター、PGK(ホスホグリセロールキナーゼ)プロモーター、アルカリホスファターゼプロモーターなどの酵母またはその他の真菌由来のプロモーターエレメント、ならびに下記の動物の転写制御領域(これらは組織特異性を示し、トランスジェニック動物で用いられてきたものである)：膵臓腺房細胞(pancreatic acinar cell)中で活性のあるエラスターゼI遺伝子制御領域(Swiftら, Cell, 38:639-646, 1984; Ornitzら, 50:399-409, Cold Spring Harbor Symp. Quant. Biol., 1986；MacDonald, Hepatology 7:425-515, 1987)；膵臓β細胞中で活性のあるインスリン遺伝子制御領域(Hanahan, Nature 315:115-122, 1985)、リンパ系細胞で活性のある免疫グロブリン遺伝子制御領域(Grosschedlら, Cell, 38:647-658, 1984；Adamesら, Nature, 318:533-538, 1985；Alexanderら, Mol. Cell. Biol., 7:1436-1444, 1987)、精巣、乳房、リンパ系および肥満細胞で活性のあるマウス乳腺腫瘍ウイルス制御領域(Lederら, Cell, 45:485-495, 1986)、肝臓で活性のあるアルブミン遺伝子制御領域(Pinkertら, Genes and Devel., 1: 268-276, 1987)、肝臓で活性のあるα-フェトプロテイン遺伝子制御領域(Krumlaufら, Mol. Cell. Biol., 5:1639-1648, 1985; Hammerら, Science, 235:53-58, 1987)、肝臓で活性のあるα1-アンチトリプシン遺伝子制御領域(Kelseyら, Genes and Devel., 1:161-171, 1987)、骨髄性細胞で活性のあるβ-グロビン遺伝子制御領域(Mogramら, Nature, 315:338-340, 1985; Kolliasら, Cell, 46:89-94, 1986; 脳の希突起膠細胞で活性のあるミエリン塩基性タンパク質遺伝子制御領域(Readheadら, Cell, 48:703-712, 1987)；骨格筋で活性のあるミオシン軽鎖-2遺伝子制御領域(Sani, Nature, 314:283-286, 1985)；神経細胞で活性のあるニューロン特異的エノラーゼ(NSE)(Morelliら, Gen. Virol., 80:571-83, 1999)；神経細胞で活性のある脳由来神経栄養因子(BDNF)遺伝子制御領域(Tabuchiら, Biochem. Biophysic. Res. Comprising., 253:818-823, 1998)；星状細胞で活性のあるグリア線維酸性タンパク(glial fibrillary acidic protein：GFAP)プロモーター(Gomesら, Braz. J. Med. Biol. Res., 32(5):619-631, 1999; Morelliら, Gen. Virol., 80:571-83, 1999)、ならびに視床下部で活性のあるゴナドトロピン放出ホルモン遺伝子制御領域(Masonら, Science, 234;1372-1378, 1986)が挙げられる。

【０１２２】

特定の1実施形態においては、融合タンパク質の発現は構成的プロモーターによって調節される。別の1実施形態においては、融合タンパク質の発現は誘導性プロモーターによって調節される。これらの実施形態に従えば、プロモーターは組織特異的プロモーターとすることができる。

【０１２３】

特定の1実施形態においては、融合タンパク質をコードする核酸と機能的に連結されたプロモーター、1つ以上の複製起点、および任意で1つ以上の選択マーカー(例えば、抗生物質耐性遺伝子)を含んでなるベクターが用いられる。

【０１２４】

哺乳類の宿主細胞では、多数のウイルスに基づく発現系を用いることができる。アデノウイルスが発現ベクターとして用いられる場合には、融合タンパク質をコードする配列はアデノウイルス転写/翻訳制御複合体、例えば後期プロモーターおよび三部分からなる(tripartite)リーダー配列と連結することができる。次いでこのキメラ遺伝子を、in vitroまたはin vivo組換えでアデノウイルスゲノム中に挿入することができる。ウイルスゲノムの非必須領域(例えば、E1またはE3領域)中へ挿入すると、感染した宿主中で生存可能で、かつ抗体分子を発現することができる組換えウイルスとなろう(LoganとShenk, Proc. Natl. Acad. Sci. USA, 81:355-359, 1984を参照せよ)。挿入された融合タンパク質コード配列の効率的な翻訳には、特異的な開始シグナルも必要でありうる。そのようなシグナルには、ATG開始コドンおよび隣接配列が含まれる。さらに、インサート全体の翻訳を確実に行うためには、該開始コドンは所望のコード配列のリーディングフレームと一致していなければならない。これらの外因性翻訳制御シグナルおよび開始コドンは、種々の起源のものとすることができ、天然および合成のいずれのものとすることもできる。発現の効率は、適切な転写エンハンサーエレメント、転写ターミネーター、その他を含ませることによって増強することができる(Bitterら, Methods in Enzymol., 153:516-544, 1987を参照されたい)。

【０１２５】

融合タンパク質をコードする遺伝子のインサート含んでいる発現ベクターは、次の3つの一般的なアプローチで同定することができる：(a)核酸ハイブリダイゼーション、(b)「マーカー」遺伝子機能の存在または不在、および(c)挿入された配列の発現。第1のアプローチでは、発現ベクター中の、融合タンパク質をコードする遺伝子の存在は、その融合タンパク質をコードする挿入された遺伝子と相同な配列を含んでなるプローブを用いて核酸ハイブリダイゼーションで検出することができる。第2のアプローチでは、ベクター中の、融合タンパク質をコードするヌクレオチド配列の挿入によって起こる、特定の「マーカー」遺伝子機能(例えば、チミジンキナーゼ活性、抗生物質耐性、形質転換表現型、バキュロウイルス封入体形成、その他)の存在または不在に基づいて、組換えベクター/宿主系を同定し、選択することができる。例えば、該融合タンパク質をコードしているヌクレオチド配列がそのベクターのマーカー遺伝子配列内に挿入された場合には、該融合タンパク質をコードする遺伝子インサートを含んでいる組換え体はマーカー遺伝子機能の不在によって同定することができる。第3のアプローチでは、組換え発現ベクターはその組換え体によって発現された遺伝子産物(すなわち、融合タンパク質)をアッセイすることによって同定することができる。そのようなアッセイは例えば、in vitroアッセイ系でその融合タンパク質の物理的または機能的性質、例えば抗生物活性分子抗体との結合に基づいて行うことができる。

【０１２６】

さらに、挿入された配列の発現をモジュレートするか、または所望の特異的様式で遺伝子産物を修飾およびプロセシングする宿主細胞株を選択することができる。特定のプロモーターからの発現を特定のインデューサーを存在させて増大させることができる；このように遺伝子工学的に産生させる融合タンパク質の発現を制御することができる。さらに、種々の宿主細胞は翻訳時および翻訳後のプロセシングと修飾のための特徴的で特異的な機構を有している(例えば、糖鎖付加、タンパク質のリン酸化)。発現される外来タンパク質の所望の修飾とプロセシングを確実に行うために、適切な細胞系または宿主系を選択することができる。例えば、細菌系で発現させると糖鎖が付加されていない産物が産生されることとなり、酵母で発現させると糖鎖が付加された産物が産生されることとなろう。一次転写産物の適切なプロセシング、遺伝子産物の糖鎖付加およびリン酸化のための細胞性機構を備えている真核生物の宿主細胞を用いることができる。そのような哺乳類の宿主細胞としては、限定はされないが、CHO、VERY、BHK、HeLa、COS、MDCK、293、3T3、WI38、および特に例えば、SK-N-AS、SK-N-FI、SK-N-DZヒト神経芽細胞腫(Sugimotoら、J. Natl. Cancer Inst., 73:51-57, 1984)、SK-N-SHヒト神経芽細胞腫(Biochim. Biophys. Acta, 704:450-460, 1982)、Daoyヒト小脳髄芽腫(Heら, Cancer Res., 52:1144-1148, 1992)、DBTRG

-05MG膠芽腫細胞(Kruseら, 1992, In Vitro Cell. Dev. Biol., 28A:609-614, 1992)、IMR-32ヒト神経芽細胞腫(Cancer Res., 30:2110-2118, 1970)、1321N1ヒト星状細胞腫(Proc. Natl. Acad. Sci. USA, 74:4816, 1997)、MOG-G-CCMヒト星状細胞腫(Br. J. Cancer, 49:269, 1984)、U87MGヒト膠芽腫-星状細胞腫(Acta Pathol. Microbiol. Scand., 74:465-486, 1968)、A172ヒト膠芽腫(Olopadeら, Cancer Res., 52:2523-2529, 1992)、C6ラット神経膠腫細胞(Bendaら, Science, 161:370-371, 1968)、Neuro-2aマウス神経芽細胞腫(Proc. Natl. Acad. Sci. USA, 65: 129-136, 1970)、NB41A3マウス神経芽細胞腫(Proc. Natl. Acad. Sci. USA, 48:1184-1190, 1962)、SCPヒツジ脈絡叢(Bolinら, J. Virol. Methods, 48:211-221, 1994)、G355-5, PG-4ネコ正常星状細胞(Haapalaら, J. Virol., 53:827-833, 1985)、Mpfフェレット脳(Trowbridgeら, In Vitro, 18:952-960, 1982)、ならびに例えば,CRL7030およびHs578BstなどのCTX TNA2ラット正常大脳皮質(Radanyら, Proc. Natl. Acad. Sci. USA, 89:6467-6471, 1992)などの正常細胞系などの神経細胞系が挙げられる。さらに、種々のベクター/宿主発現系が異なる程度でプロセシング反応に影響を及ぼすことができる。

【０１２７】

長期にわたり高収率で組換えタンパク質を産生させるためには、安定な発現が好ましい。例えば、融合タンパク質を安定に発現する細胞系を操作することができる。ウイルス性の複製起点を含んでいる発現ベクターを用いるよりはむしろ、宿主細胞を適切な発現制御エレメント(例えば、プロモーター、エンハンサー、配列、転写ターミネーター、ポリアデニル化部位、その他)によって制御されているＤＮＡと選択マーカーで形質転換することができる。外来DNAの導入後に、操作された細胞を1～2日間、富化培地で増殖させ、次いで選択培地に交換することができる。組換えプラスミド中の選択マーカーは選択に対する耐性を付与し、細胞がそのプラスミドを染色体中に安定に組み込み、増殖して細胞増殖巣を形成し、次いでそれがクローン化されて細胞系へと拡大させることができる。この方法は、区別して発現されるかまたは経路遺伝子タンパク質を発現する細胞系を操作するために有利に用いることができる。そのような操作された細胞系は、区別して発現された、または経路遺伝子タンパク質の内在性の活性に影響を及ぼす化合物のスクリーニングおよび評価に特に有用でありうる。

【０１２８】

多数の選択系を用いることができ、そのようなものとしては、限定はされないが、単純ヘルペスウイルスチミジンキナーゼ(Wiglerら, Cell, 11:223, 1997)、ヒポキサンチン-グアニンホスホリボシルトランスフェラーゼ(SzybalskaとSzybalski, Proc. Natl. Acad. Sci. USA, 48:2026, 1962)、およびアデニンホスホリボシルトランスフェラーゼ(Lowyら, 1980, Cell, 22:817)遺伝子が挙げられ、それらの遺伝子はそれぞれtk-、hgprt-、またはaprt-細胞で用いることができる。また、代謝拮抗物質耐性を次の遺伝子についての選択に基づいて用いることもできる：dhfr、これはメトトレキセートへの耐性を付与する(Wiglerら, Natl. Acad. Sci. USA, 77:3567, 1980; O'Hareら, Proc. Natl. Acad. Sci. USA, 78:1527, 1981)；gpt、これはミコフェノール酸に対する耐性を付与する(MulliganとBerg, Proc. Natl. Acad. Sci. USA, 78:2072, 1981)；neo、これはアミノグリコシドG-418に対する耐性を付与する(Colberre-Garapinら, J. Mol. Biol., 150:1, 1981)；およびhygro、これはハイグロマイシンに対する耐性を付与する(Santerreら, Gene, 30:147, 1984)。

【０１２９】

本発明の融合タンパク質は組換え発現によって産生された場合には、タンパク質を精製する方法として当業界で知られている、例えばクロマトグラフィー(例えば、イオン交換、アフィニティー、とりわけプロテインAなどの特異的抗原に対するアフィニティー、およびサイズで分けるカラムクロマトグラフィー)、遠心分離、示差溶解度、またはタンパク質を精製するためのその他の標準的な技法のいずれによっても精製することができる。

【０１３０】

5.5. 抗体の予防的および治療的使用

本発明は、疾患、障害、または感染に伴う症状を予防、治療、または改善するために、抗体を動物、好ましくは哺乳類、最も好ましくはヒトに投与することを含む、抗体に基づく治療を包含する。本発明の予防用および治療用化合物は、限定はされないが、抗体、および抗体をコードする核酸を含む。抗体は当業界では公知の、または本明細書に記載の製薬上許容される組成物で提供することができる。

【０１３１】

疾患、障害、または感染のアンタゴニストとして機能する本発明の抗体は、疾患、障害、または感染に伴う1種以上の症状を治療、予防、または改善するために、動物、好ましくは哺乳類、最も好ましくはヒトに投与することができる。例えば、ウイルス抗原とそれの宿主細胞受容体との間の相互作用を破壊する、または防止する抗体をウイルス感染に伴う1種以上の症状を治療、予防、または改善するために、動物、好ましくは哺乳類、および最も好ましくはヒトに投与することができる。

【０１３２】

特定の1実施形態においては、抗体またはそのフラグメントは、ウイルス抗原または細菌抗原がそれの宿主細胞受容体と結合することを、該抗体の不在下での抗原のそれの宿主細胞受容体との結合と比較して、少なくとも99%、少なくとも95%、少なくとも90%、少なくとも85%、少なくとも80%、少なくとも75%、少なくとも70%、少なくとも60%、少なくとも50%、少なくとも45%、少なくとも40%、少なくとも45%、少なくとも35%、少なくとも30%、少なくとも25%、少なくとも20%、または少なくとも10%、防止する。別の1実施形態においては、抗体の組み合わせが、ウイルス抗原または細菌抗原がそれの宿主細胞受容体と結合することを、該抗体の不在下での抗原のそれの宿主細胞受容体との結合と比較して、少なくとも99%、少なくとも95%、少なくとも90%、少なくとも85%、少なくとも80%、少なくとも75%、少なくとも70%、少なくとも60%、少なくとも50%、少なくとも45%、少なくとも40%、少なくとも45%、少なくとも35%、少なくとも30%、少なくとも25%、少なくとも20%、または少なくとも10%、防止する。好ましい1実施形態においては、該抗体はRSV感染を治療または予防するために用いられる。

【０１３３】

ウイルス抗原または細菌抗原がそれの宿主受容体と結合することを防止はしないが、ウイルスまたは細菌の複製を阻害またはダウンレギュレートする抗体はまた、ウイルス感染または細菌感染に伴う1種以上の症状を治療、予防、または改善するために投与することもできる。ウイルスまたは細菌の複製を阻害またはダウンレギュレートする抗体の能力は、本明細書に記載の技法またはその他の当業界では公知の技法によって測定することができる。例えば、ウイルスの複製の阻害またはダウンレギュレーションはその動物中のウイルス力価を検出することによって測定することができる。

【０１３４】

特定の1実施形態においては、抗体がウイルスの複製または細菌の複製を、該抗体の不在下でのウイルスの複製または細菌の複製と比較して、少なくとも99%、少なくとも95%、少なくとも90%、少なくとも85%、少なくとも80%、少なくとも75%、少なくとも70%、少なくとも60%、少なくとも50%、少なくとも45%、少なくとも40%、少なくとも45%、少なくとも35%、少なくとも30%、少なくとも25%、少なくとも20%、または少なくとも10%、阻害またはダウンレギュレートする。別の1実施形態においては、抗体の組み合わせがウイルスの複製または細菌の複製を、該抗体の不在下でのウイルスの複製または細菌の複製と比較して、少なくとも99%、少なくとも95%、少なくとも90%、少なくとも85%、少なくとも80%、少なくとも75%、少なくとも70%、少なくとも60%、少なくとも50%、少なくとも45%、少なくとも40%、少なくとも45%、少なくとも35%、少なくとも30%、少なくとも25%、少なくとも20%、または少なくとも10%、阻害またはダウンレギュレートする。

【０１３５】

抗体はまた、癌細胞の増殖または転移を防止、阻害、または低下させるためにも用いることができる。特定の1実施形態においては、抗体が癌細胞の増殖または転移を、該抗体の不在下での増殖または転移と比較して、少なくとも99%、少なくとも95%、少なくとも90%

、少なくとも85%、少なくとも80%、少なくとも75%、少なくとも70%、少なくとも60%、少なくとも50%、少なくとも45%、少なくとも40%、少なくとも45%、少なくとも35%、少なくとも30%、少なくとも25%、少なくとも20%、または少なくとも10%、阻害または低下させる。別の1実施形態においては、抗体の組み合わせが癌の増殖または転移を、該抗体の不在下での増殖または転移と比較して、少なくとも99%、少なくとも95%、少なくとも90%、少なくとも85%、少なくとも80%、少なくとも75%、少なくとも70%、少なくとも60%、少なくとも50%、少なくとも45%、少なくとも40%、少なくとも45%、少なくとも35%、少なくとも30%、少なくとも25%、少なくとも20%、または少なくとも10%、阻害または低下させる。癌の例としては、限定はされないが、白血病(例えば、急性リンパ性白血病および急性骨髄性白血病などの急性白血病)、新生物、腫瘍(例えば、線維肉腫、粘液肉腫、脂肪肉腫、軟骨肉腫、骨原性肉腫、脊索腫、血管肉腫、内皮肉腫、リンパ管肉腫、リンパ管内皮肉腫、滑膜腫、中皮腫、ユーイング腫瘍、平滑筋肉腫、横紋筋肉腫、結腸癌、膵臓癌、乳癌、卵巣癌、前立腺癌、扁平上皮癌、基底細胞癌、腺癌、汗腺癌、脂腺癌、乳頭状癌、乳頭腺癌、嚢胞腺癌、髄様癌、気管支原性癌、腎細胞癌、肝癌、胆管癌、絨毛癌、精上皮腫、胎児性癌、ウィルムス腫瘍、子宮頚癌、精巣腫瘍、肺癌、小細胞肺癌、膀胱癌、上皮癌、神経膠腫、星状腫、髄芽腫、頭蓋咽頭腫、上衣細胞腫、松果体腫、血管芽細胞腫、聴神経腫、乏突起膠腫、髄膜腫、黒色腫、神経芽細胞腫、および網膜芽細胞腫)、重鎖病、転移、または制御されない細胞増殖を特徴とする任意の疾患または障害が挙げられる。

【０１３６】

抗体はまた、炎症性障害を有する動物、特に哺乳類が経験する炎症を低下させるために用いることができる。特定の1実施形態においては、抗体が動物の炎症を、該抗体が投与されないときの動物の炎症と比較して、少なくとも99%、少なくとも95%、少なくとも90%、少なくとも85%、少なくとも80%、少なくとも75%、少なくとも70%、少なくとも60%、少なくとも50%、少なくとも45%、少なくとも40%、少なくとも45%、少なくとも35%、少なくとも30%、少なくとも25%、少なくとも20%、または少なくとも10%、低下させる。別の1実施形態においては、抗体の組み合わせが、動物の炎症を、該抗体が投与されないときの動物の炎症と比較して、少なくとも99%、少なくとも95%、少なくとも90%、少なくとも85%、少なくとも80%、少なくとも75%、少なくとも70%、少なくとも60%、少なくとも50%、少なくとも45%、少なくとも40%、少なくとも45%、少なくとも35%、少なくとも30%、少なくとも25%、少なくとも20%、または少なくとも10%、低下させる。炎症性障害の例には、限定はされないが、関節リウマチ、脊椎関節症、炎症性腸疾患、および喘息が含まれる。

【０１３７】

特定の実施形態においては、炎症(または癌)の治療に用いられる抗体は改変した抗$\alpha_v\beta_3$抗体、好ましくはVitaxin抗体である(PCT公開WO 98/33919およびWO 00/78815(双方ともHuseらによる)を参照すればよく、これらの双方はその全体を本明細書中に参照により組み入れる)。

【０１３８】

抗体はまた、移植片の拒絶反応を防止するためにも用いることができる。抗体はまた、凝血塊形成を防止するために用いることもできる。さらに、免疫応答のアゴニストとして機能する抗体も、疾患、障害、または感染に伴う1種以上の症状を治療、予防、または改善するために、動物、好ましくは哺乳類、最も好ましくはヒトに投与することができる。

【０１３９】

1種以上の抗原と免疫特異的に結合する1種以上の抗体を体内で局所的にまたは全身的に、治療剤として用いることができる。本発明の抗体はまた、他のモノクローナルもしくはキメラ抗体、またはリンホカインもしくは造血性成長因子(例えばIL-2、IL-3、およびIL-7など)と組み合わせて有利に用いることができ、その組み合わせたものは、例えば、その抗体と相互作用するエフェクター細胞の数または活性を増大させるのに役立つ。本発明の抗体はまた、他のモノクローナルもしくはキメラ抗体、またはリンホカインもしくは造血性成長因子(例えばIL-2、IL-3、およびIL-7など)と組み合わせて有利に用いることができ、その組み合わせたものは、例えば、免疫応答を増大させるのに役立つ。本発明の抗体は

また、疾患、障害、または感染の治療のために使用される1種以上の薬剤、例えば抗癌剤、抗炎症剤、または抗ウイルス剤などと組み合わせて有利に用いることができる。抗癌剤の例としては、限定はされないが、シスプラチン、イホスファミド、パクリタキセル、タキサン、トポイソメラーゼI阻害剤(例えば、CPT-11、トポテカン、9-AC、およびGG-211)、ゲムシタビン、ビノレルビン、オキサリプラチン、5-フルオロウラシル(5-FU)、ロイコボリン、ビノレルビン、テモダール(temodal)、およびタキソールが挙げられる。抗ウイルス剤の例としては、限定はされないが、サイトカイン(例えば、IFN-α、IFN-β、IFN-γ)、逆転写酵素の阻害剤(例えば、AZT、3TC、D4T、ddC、ddI、d4T、3TC、アデフォビル、エファビレンツ、デラビルジン、ネビラピン、アバカビル、およびその他のジデオキシヌクレオシドまたはジデオキシフルオロヌクレオシド)、リバビリンなどのウイルスmRNAキャッピングの阻害剤、HIVプロテアーゼ阻害剤などのプロテアーゼの阻害剤(例えば、アンプレナビル、インジナビル、ネルフィナビル、リトナビル、およびサキナビル)、アムホテリシンB、糖タンパクプロセシングの阻害剤としてのカスタノスペルミン、インフルエンザウイルスノイラミニダーゼ阻害剤などのノイラミニダーゼの阻害剤(例えば、ザナミビルおよびオセルタミビル)、トポイソメラーゼI阻害剤(例えば、カンプトテシンおよびその類似体)、アマンタジン、およびリマンタジンが挙げられる。抗炎症剤の例としては、限定はされないが、COX-2阻害剤(例えば、メロキシカム、セレコキシブ、ロフェコキシブ、フロスリド(flosulide)、およびSC-58635、ならびにMK-966)、イブプロフェンおよびインドメタシンなどの非ステロイド抗炎症薬、ならびにステロイド(例えば、デフラザコート、デキサメタゾン、およびメチルプレドニゾロン)が挙げられる。

【０１４０】

特定の1実施形態においては、動物に投与される抗体はその動物と同一種の種起源のものまたは種反応性のものである。従って、好ましい1実施形態においては、ヒト抗体もしくはヒト化抗体、またはヒトもしくはヒト化抗体をコードする核酸は治療または予防のためにヒトの患者に投与される。

【０１４１】

好ましい1実施形態においては、in vivoでの半減期が延長された免疫グロブリンを受動免疫療法(治療または予防のいずれかの目的で)で用いることができる。半減期が延長されているので、受動免疫療法または予防は治療剤の投与をより低い投与量で、および/またはより少ない頻度で行うことができ、その結果副作用がより少なく、患者のコンプライアンスがより良好となり、治療/予防にかかる費用がより低くなるなどがもたらされる。好ましい1実施形態においては、該治療剤/予防剤はRSVと結合する抗体、例えばSYNAGIS(登録商標)またはその他の抗RSV抗体である。そのような抗RSV抗体および投与方法については米国特許出願第09/724,396号および第09/724,531号に開示されており、双方とも「抗RSV抗体を予防および治療のために投与/投薬する方法(Methods of Administering/Dosing Anti-RSV Antibodies For Prophylaxis and Treatment)」と題されたもので、双方ともYoungらにより、2000年11月28日に出願され、これらの出願の一部継続出願はそれぞれSerial No.　　および　　(弁理士名簿番号はそれぞれ10271-048および10271-047)であり、これらも「抗RSV抗体を予防および治療のために投与/投薬する方法(Methods of Administering/Dosing Anti-RSV Antibodies For Prophylaxis and Treatment)」と題され、Youngらによるものであり、これらの全てはその全体を本明細書中に参照により組み入れる。また、上記の第5.1節に記載の抗RSV抗体も含まれる。

【０１４２】

特定の1実施形態においては、動物に投与される融合タンパク質はその動物と同一種の種起源のものまたは種反応性のものである。従って、好ましい1実施形態においては、ヒト融合タンパク質またはヒト融合タンパク質をコードする核酸は治療または予防のためにヒトの被験者に投与される。

【０１４３】

5.6.　融合タンパク質およびコンジュゲート分子の予防的および治療的使用

本発明は融合タンパク質に基づくおよびコンジュゲート分子に基づく治療法を包含し、そ

の治療法は融合タンパク質またはコンジュゲート分子を疾患、障害、または感染に伴う症状を予防、治療、または改善するために、動物、好ましくは哺乳類、最も好ましくはヒトに投与することを含むものである。本発明の予防用および治療用化合物には、限定はされないが、融合タンパク質および融合タンパク質をコードする核酸、ならびにコンジュゲート分子が含まれる。融合タンパク質およびコンジュゲート分子は、当業界では公知の、または本明細書に記載の製薬上許容される組成物で提供することができる。

【０１４４】

疾患、障害、または感染のアンタゴニストとして機能する本発明の融合タンパク質およびコンジュゲート分子は、疾患、障害、または感染に伴う1種以上の症状を治療、予防、または改善するために、動物、好ましくは哺乳類、最も好ましくはヒトに投与することができる。さらに、免疫応答のアゴニストとして機能する本発明の融合タンパク質およびコンジュゲート分子は、疾患、障害、または感染に伴う1種以上の症状を治療、予防、または改善するために、動物、好ましくは哺乳類、最も好ましくはヒトに投与することができる。

【０１４５】

1種以上の融合タンパク質とコンジュゲート分子を体内で局所的にまたは全身的に、治療剤として用いることができる。本発明の融合タンパク質とコンジュゲート分子はまた、モノクローナルもしくはキメラ抗体、またはリンホカインもしくは造血性成長因子(例えばIL-2、IL-3、およびIL-7など)と組み合わせて有利に用いることができ、その組み合わせたものは、例えば、その抗体と相互作用するエフェクター細胞の数または活性を増大させるのに役立つ。本発明の融合タンパク質とコンジュゲート分子はまた、モノクローナルもしくはキメラ抗体、またはリンホカインもしくは造血性成長因子(例えばIL-2、IL-3、およびIL-7など)と組み合わせて有利に用いることができ、その組み合わせたものは、例えば、免疫応答を増大させるのに役立つ。本発明の融合タンパク質とコンジュゲート分子はまた、疾患、障害、または感染の治療のために用いられる1種以上の薬剤、例えば抗癌剤、抗炎症剤、または抗ウイルス剤と組み合わせて有利に用いることができる。抗癌剤の例としては、限定はされないが、シスプラチン、イホスファミド、パクリタキセル、タキサン、トポイソメラーゼI阻害剤(例えば、CPT-11、トポテカン、9-AC、およびGG-211)、ゲムシタビン、ビノレルビン、オキサリプラチン、5-フルオロウラシル(5-FU)、ロイコボリン、ビノレルビン、テモダール、およびタキソールが挙げられる。抗ウイルス剤の例としては、限定はされないが、サイトカイン(例えば、IFN-α、IFN-β、IFN-γ)、逆転写酵素の阻害剤(例えば、AZT、3TC、D4T、ddC、ddI、d4T、3TC、アデフォビル、エファビレンツ、デラビルジン、ネビラピン、アバカビル、およびその他のジデオキシヌクレオシドまたはジデオキシフルオロヌクレオシド)、リバビリンなどのウイルスmRNAキャッピングの阻害剤、HIVプロテアーゼ阻害剤などのプロテアーゼの阻害剤(例えば、アンプレナビル、インジナビル、ネルフィナビル、リトナビル、およびサキナビル)、アムホテリシンB、糖タンパクプロセシングの阻害剤としてのカスタノスペルミン、インフルエンザウイルスノイラミニダーゼ阻害剤などのノイラミニダーゼの阻害剤(例えば、ザナミビルおよびオセルタミビル)、トポイソメラーゼI阻害剤(例えば、カンプトテシンおよびその類似体)、アマンタジン、およびリマンタジンが挙げられる。抗炎症剤の例としては、限定はされないが、COX-2阻害剤(例えば、メロキシカム、セレコキシブ、ロフェコキシブ、フロスリド、およびSC-58635、ならびにMK-966)、イブプロフェンおよびインドメタシンなどの非ステロイド抗炎症薬、ならびにステロイド(例えば、デフラザコート、デキサメタゾン、およびメチルプレドニゾロン)が挙げられる。

【０１４６】

5.7. 抗体または融合タンパク質の投与

本発明は、本発明の抗体、または本発明の抗体を含む医薬組成物の有効量を被験者に投与することによる、疾患、障害、または感染に伴う1種以上の症状を治療、予防、および改善する方法を提供する。本発明はまた、本発明の融合タンパク質もしくはコンジュゲート分子、または本発明の融合タンパク質もしくはコンジュゲート分子を含む医薬組成物の有

効量を被験者に投与することによる、疾患、障害、または感染に伴う1種以上の症状を治療、予防、および改善する方法をも提供する。好ましい1態様においては、抗体または融合タンパク質もしくはコンジュゲート分子は実質的に精製されたものである(すなわち、その効果を制限する、または望ましくない副作用を生じる物質を実質的に含まない)。特定の1実施形態においては、該被験者は動物、好ましくは、非霊長類(例えば、ウシ、ブタ、馬、猫、イヌ、ラット、その他)および霊長類(例えばカニクイザルなどのサル、およびヒト)などの哺乳類である。好ましい1実施形態においては、該被験者はヒトである。

【０１４７】

種々の送達系が公知であり、本発明の抗体または融合タンパク質もしくはコンジュゲート分子を投与するために用いることができ、そのようなものとしては、例えば、リポソーム中への被包、微粒子、マイクロカプセル、該抗体または融合タンパク質を発現することのできる組換え細胞、受容体介在性エンドサイトーシス(例えば、WuとWu, J. Biol. Chem., 262:4429-4432, 1987を参照せよ)、レトロウイルスベクターまたはその他のベクターの一部分としての核酸の構築、その他が挙げられる。抗体、融合タンパク質、もしくはコンジュゲート分子、または医薬組成物を投与する方法としては、限定はされないが、非経口投与(例えば、皮内、筋肉内、腹腔内、静脈内、および皮下)、硬膜外、および粘膜(例えば、鼻腔内、および経口経路)が挙げられる。特定の1実施形態においては、抗体、融合タンパク質、コンジュゲート分子、または医薬組成物は筋肉内、静脈内、または皮下に投与される。該組成物はいずれの都合のよい経路によっても、例えば、注入またはボーラス注射により、上皮または粘膜皮膚の内腔(lining)(例えば、口腔粘膜、直腸および腸粘膜、その他)を経由する吸収により投与することができ、および他の生物活性剤とともに投与することができる。投与は全身的または局所的に行うことができる。さらに、肺への投与も、例えば吸入器またはネブライザーを用いて、エアロゾル化剤とともに製剤化することによって使用することができる。例えば、米国特許第6,019,968号、第5,985,320号、第5,985,309号、第5,934,272号、第5,874,064号、第5,855,913号、第5,290,540号、および第4,880,078号；ならびにPCT公開No. WO 92/19244；WO 97/32572；WO 97/44013；WO 98/31346；およびWO 99/66903を参照されたい(これらの各々はその全体を本明細書中に参照により組み入れる)。好ましい1実施形態においては、抗体、融合タンパク質、コンジュゲート分子、または医薬組成物は、Alkermes AIR™肺薬剤送達技法(Alkermes, Inc., Cambridge, MA)を用いて投与される。

【０１４８】

本発明はまた、抗体、融合タンパク質、またはコンジュゲート分子が、抗体、融合タンパク質、またはコンジュゲート分子の量を表示したアンプルまたはサシェット(sachette)などの密封容器にパッケージングされることをも提供する。1実施形態においては、該抗体、融合タンパク質、またはコンジュゲート分子は、乾燥した、滅菌済の凍結乾燥粉末または水非含有濃縮物を含む密封容器として供給され、例えば水または生理食塩水を用いて被験者への投与に適した濃度となるように再構成することができる。好ましくは、該抗体、融合タンパク質、またはコンジュゲート分子は乾燥した滅菌済の凍結乾燥粉末として、密封容器中に入れた形で、少なくとも5mg、より好ましくは少なくとも10mg、少なくとも15mg、少なくとも25mg、少なくとも35mg、少なくとも45mg、少なくとも50mg、または少なくとも75mgの単位投与量で供給される。その凍結乾燥した抗体、融合タンパク質、またはコンジュゲート分子はその元の容器において2～8℃の間で保存されるべきで、その抗体、融合タンパク質、またはコンジュゲート分子は再構成後12時間以内に、好ましくは6時間以内、5時間以内、3時間以内、または1時間以内に投与すべきである。また別の1実施形態においては、抗体、融合タンパク質、またはコンジュゲート分子は、液状の形態で抗体、融合タンパク質、またはコンジュゲート分子の量および濃度を表示した密封容器で供給される。好ましくは、その抗体、融合タンパク質、またはコンジュゲート分子の液状の形態は、密封容器において少なくとも1mg/mL、より好ましくは少なくとも2.5mg/mL、少なくとも5mg/mL、少なくとも8mg/mL、少なくとも10mg/mL、少なくとも15mg/kg、または少なくとも25mg/mLで供給される。

【０１４９】
特定の1実施形態においては、本発明の医薬組成物を治療を必要とする領域に局所的に投与することが望ましい場合がある：このことは、例えば、限定するものではないが、局所注入により、注射により、またはインプラントにより達成することができ、そのインプラントはシアラスティック(sialastic)膜などの膜を含む多孔性、非多孔性、もしくはゼラチン状材料、または繊維である。好ましくは、抗体または融合タンパク質を投与する場合には、その抗体または融合タンパク質が吸収しない材料を用いることに気を配らなければならない。
【０１５０】
別の1実施形態においては、該組成物は小胞中、とりわけリポソーム中に入れて送達することができる(Langer, Science, 249:1527-1533, 1990; Treatら, 「感染性疾患と癌の治療におけるリポソーム(Liposomes in the Therapy of Infectious Disease adn Cancer)」中, Lopez-BersteinとFidler(編), Liss, New York, pp.353-365(1989); Lopez-Berestein, 同書, pp.317-327を参照せよ；一般的には同書を参照せよ)。
【０１５１】
また別の1実施形態においては、該組成物は制御放出または徐放性系で送達することができる。1種以上の抗体、または1種以上の融合タンパク質を含んでいる徐放性製剤を生産するために、当業者には公知のいずれの技法も用いることができる。例えば、米国特許第4,526,938号；PCT公開 WO 91/05548；PCT公開 WO 96/20698；Ningら,「徐放性ゲルを用いるヒト結腸癌異種移植片の腫瘍内放射免疫療法(Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel)」, Radiotherapy & Oncology, 39:179-189, 1996; Songら,「長期間循環するエマルジョンの抗体が介在する肺へのターゲティング(Antibody Mediated Lung Targeting of Long-Circulating Emulsions)」, PDA Journal of Pharmaceutical Science & Technology, 50:372-397, 1995; Cleekら,「心臓血管系への適用のためのbFGF抗体用の生物分解性ポリマー担体(Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application)」, Pro. Intl. Symp. Control. Rel. Bioact. Mater., 24:853-854, 1997; およびLamら, 「局所への送達のための組換えヒト化モノクローナル抗体のマイクロカプセル化(Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery)」, Proc. Int'l. Symp. Control Rel. Bioact. Mater., 24:759-760, 1997を参照されたい(これらの各々はその全体を本明細書中に参照により組み入れる)。1実施形態においては、制御放出系にポンプを用いることができる(Langer, 上述の文献; Sefton, CRC Crit. Ref. Biomed. Eng., 14:20, 1987; Buchwaldら, Surgery, 88:507, 1980; およびSaudekら, N. Engl. J. Med., 321:574, 1989を参照せよ)。別の1実施形態においては、抗体または融合タンパク質の制御放出を達成するためにポリマー材料を用いることができる(例えば、「制御放出の医学的応用(Medical Applications of Controlled Release)」, LangerとWise(編), CRC Pres., Boca Raton, Florida(1974); 「制御された薬剤バイオアベイラビリティー、製剤のデザインと性能(Controlled Drug Bioavailability, Drug Product Design and Performance)」, SmolenとBall(編), Wiley, New York(1984); RangerとPeppas, J. Macromol. Sci. Rev. Macromol. Chem., 23:61, 1983を参照されたい; また、Levyら, Science, 228:190, 1985; Duringら, Ann. Neurol., 25:351, 1989; Howardら, J. Neurosurg., 71:105, 1989); 米国特許第5,679,377号; 米国特許第5,916,597号; 米国特許第5,912,015号; 米国特許第5,989,463号; 米国特許第5,128,326号;PCT公開No. WO 99/15154; およびPCT公開No. WO 99/20253をも参照せよ)。また別の1実施形態においては、制御放出系を治療の標的(例えば肺)の近傍に置くことができ、そうすることによって全身投与量のごく一部しか必要としないこととなる(例えば、Goodson, 「制御放出の医学的応用(Medical Applications of Controlled Release)」中, 上述の文献, 第2巻, pp.115-138(1984)を参照せよ)。
【０１５２】
その他の制御放出系についてはLangerによる総説(Science, 249:1527-1533, 1990)に述べ

られている。

【０１５３】

本発明の組成物が抗体または融合タンパク質をコードする核酸である特定の1実施形態においては、その核酸を適切な核酸発現ベクターの一部として構築し、それを細胞内に入るように、例えば、レトロウイルスベクター(米国特許第4,980,286号を参照せよ)によって、または直接注入によって、または微粒子ボンバードメント(microparticle bombardment)(例えば、遺伝子銃；Biolistic, Dupont)、または脂質もしくは細胞表面受容体もしくはトランスフェクト剤を用いたコーティングを用いて、または核内に入ることが知られているホメオボックス様ペプチドと連結させて投与することによって(例えば、Joliotら, Proc. Natl. Acad. Sci. USA, 88:1864-1868, 1991)、その他で投与することによって、それがコードする抗体または融合タンパク質の発現を促進するためにin vivoで投与することができる。あるいはまた、核酸を細胞内に導入し、相同組換えによって発現のために宿主細胞のDNA中に組み込むことができる。

【０１５４】

本発明はまた、医薬組成物をも提供する。そのような組成物は抗体、融合タンパク質、またはコンジュゲート分子の予防上または治療上有効な量および製薬上許容される担体を含んでなる。特定の1実施形態においては、「製薬上許容される」という用語は、連邦政府または州政府の薬事当局によって承認されていること、または米国薬局方、もしくは動物、より特にはヒトに用いるための一般的に認められた他の薬局方に挙げられていることを意味する。「担体」という用語は希釈剤、アジュバント(例えばフロイント完全および不完全アジュバント)、水酸化アルミニウムなどの鉱物ゲル、リゾレシチンなどの界面活性物質、プルロニックポリオール、多価陰イオン、ペプチド、油性エマルジョン、キーホールリンペットヘモシアニン、ジニトロフェノール、ならびに、BCG(カルメット-ゲラン杆菌 (Bacille Calmette-Guerin))およびコリネバクテリウム・パルバム(Corynebacterium parvum)などのヒトに有用なものとなりうるアジュバント、賦形剤、または該治療剤とともに投与されるビヒクル(vehicle)を意味する。そのような製薬上の担体は水および油などの無菌の液体とすることができる。その油には、落花生油、大豆油、鉱油、ごま油、および類似のものなどの、石油、動物、植物、または合成物起源のものが含まれる。該医薬組成物が静脈内に投与される場合には、水が好ましい担体である。生理食塩水溶液ならびに水性デキストロース、およびグリセロール溶液も、特に注射用溶液のための液状担体として用いることができる。好適な製薬用賦形剤には、デンプン、ブドウ糖、乳糖、ショ糖、ゼラチン、麦芽、イネ、小麦粉、チョーク、シリカゲル、ステアリン酸ナトリウム、モノステアリン酸グリセロール、タルク、塩化ナトリウム、脱脂粉乳、グリセロール、プロピレン、グリコール、水、エタノール、および類似のものが含まれる。所望により、また該組成物は少量の湿潤剤もしくは乳化剤、またはpH緩衝剤を含むことができる。これらの組成物は、溶液、懸濁液、エマルジョン、錠剤、丸剤、カプセル剤、粉末、徐放性製剤、および類似のものの形態を取ることができる。経口製剤には、医薬品等級のマンニトール、乳糖、デンプン、ステアリン酸マグネシウム、ナトリウムサッカリン、セルロース、炭酸マグネシウムなどの標準的な担体を含ませることができる。好適な製薬用担体の例については「レミントンの製薬科学(Remington's Pharmaceutical Sciences)」(E. W. Martinによる)中に記載されている。このような組成物は、抗体もしくはそのフラグメント、または融合タンパク質、またはコンジュゲート分子の予防上または治療上有効な量を、好ましくは精製された形態で、好適な量の担体と共に含有させて、患者への適切な投与のための形態が提供されることとなろう。その製剤は投与様式に適したものでなければならない。

【０１５５】

好ましい1実施形態においては、該組成物はヒトへの静脈内投与に適合した医薬組成物として慣例の方法に従って製剤化される。典型的には、静脈内投与用の組成物は無菌で等張の水性バッファー中の溶液である。必要に応じて、該組成物はまた、可溶化剤およびリグノカインなどの局所麻酔剤を注射部位の疼痛を軽減するために含むことができる。

【０１５６】
通常は、本発明の組成物の成分は別々にまたは一緒に混合して単位剤形で、例えば、乾燥した凍結乾燥粉末、または無水の濃縮物として活性物質の量を表示したアンプルまたはサシェットなどの密封容器で供給される。該組成物が注入で投与される場合には、組成物を無菌の医薬品等級の水または生理食塩水を含有している注入ボトルに調剤することができる。該組成物が注射によって投与される場合には、無菌の注射用水または生理食塩水のアンプルを提供して、その成分が投与前に混合されるようにすることができる。
【０１５７】
本発明の組成物は中性のまたは塩の形態として製剤化することができる。製薬上許容される塩は、塩酸、リン酸、酢酸、シュウ酸、酒石酸、その他に由来する陰イオンとともに形成されるもの、およびナトリウム、カリウム、アンモニウム、カルシウム、水酸化鉄、イソプロピルアミン、トリエチルアミン、2-エチルアミノエタノール、ヒスチジン、プロカイン、その他に由来する陽イオンとともに形成されるものを含む。
【０１５８】
疾患、障害、または感染に伴う1種以上の症状を治療、予防、または改善するために有効なものとなろう本発明の組成物の量は標準的な臨床技法によって決定することができる。その製剤で用いられる正確な投与量は、投与経路、被験者の年齢、および疾患、障害、または感染の重篤度に依存し、開業医の判断および各患者の状況に従って定められるべきである。有効量はin vitroまたは動物モデル(例えば、コットンラットまたはカニクイザル)での試験系から導かれた用量-応答曲線を外挿して得ることができる。
【０１５９】
融合タンパク質については、被験者に投与される、治療上または予防上有効な投与量は、体重1kgあたり約0.001～50mg、好ましくは約0.01～25mg/kg体重、より好ましくは約0.1～20mg/kg体重、およびさらにより好ましくは約1～10mg/kg体重、2～9mg/kg体重、3～8mg/kg体重、4～7mg/kg体重、または5～6mg/kg体重の範囲である。抗体については、被験者に投与される、治療上または予防上有効な投与量は、典型的には被験者の体重1kgあたり0.1～200mgである。好ましくは、被験者に投与される投与量は、被験者の体重1kgあたり0.1mg～20mgであり、より好ましくは、被験者に投与される投与量は、被験者の体重1kgあたり1mg～10mgである。しかし、その投与量は、その分子のin vivoでの半減期がどの程度増大されているかに依存するだろう。通常は、ヒト抗体およびヒト融合タンパク質は、他種から得た融合タンパク質の抗体よりも外来ポリペプチドへの免疫応答のためにヒトの体内での半減期がより長い。従って、ヒト抗体またはヒト融合タンパク質ではより少ない投与量で投与頻度をより少なくすることが可能であることがしばしばある。さらに、抗体、融合タンパク質、またはコンジュゲート分子の投与の投与量と頻度はまた、例えば脂質化などの改変によって、抗体または融合タンパク質の取り込みと組織への浸透(例えば肺への)を増大させることによって、低減することができる。
【０１６０】
抗体、融合タンパク質、またはコンジュゲート分子の治療上または予防上有効な量を用いる被験者の治療には、単一治療、または好ましくは、一連の治療を含むことができる。好ましい1例においては、被験者は、約0.1～30mg/kg体重の範囲の抗体、融合タンパク質、またはコンジュゲート分子を用いて、1週間に1回で約1～10週間、好ましくは2～8週間、より好ましくは約3～7週間、さらにより好ましくは約4、5、または6週間治療される。他の実施形態では、本発明の医薬組成物は、1日1回、1日2回、または1日3回投与される。他の実施形態においては、本発明の医薬組成物は、週1回、週2回、2週間に1回、月に1回、6週間毎に1回、2ヶ月毎に1回、年に2回、または年1回投与される。また治療に用いる抗体、融合タンパク質、またはコンジュゲート分子の有効投与量は特定の治療経過にわたって増加または減少させることができることは理解されよう。
【０１６１】
5.7.1. 遺伝子治療
特定の1実施形態においては、抗体または融合タンパク質をコードする配列を含んでいる

核酸は、疾患、障害、または感染に伴う1種以上の症状を治療、予防、または改善するために遺伝子治療によって投与することができる。遺伝子治療とは、被験者に発現された、または発現しうる核酸を投与することによって行われる治療法を意味する。本発明のこの実施形態においては、該核酸はそれがコードする、治療または予防効果を介在する抗体または融合タンパク質を産生する。

【０１６２】

当業界で利用可能な遺伝子治療の方法のいずれも本発明に従って用いることができる。代表的な方法を下記で説明する。

【０１６３】

遺伝子治療の方法に関しての一般的な概説は、Goldspielら，Clinical Pharmacy，12:488-505，1993；WuとWu，Biotherapy，3:87-95，1991；Tolstoshev，Ann. Rev. Pharmacol. Toxicol.，32:573-596，1993；Mulligan，Science，260:926-932，1993；およびMorganとAnderson，Ann. Rev. biochem.，62:191-217，1993；TIBTECH 11(5):155-215，1993を参照せよ。用いることのできる、組換えDNA技術の当業界で一般的に知られている方法については、Ausubelら(編)，「分子生物学の今日のプロトコール(Current Protocols in Molecular Biology)」，John Wiley & Sons，NY(1993)；およびKriegler，「遺伝子導入と発現、実験室マニュアル(Gene Transfer and Expression，A Laboratory Manual)」，Stockton Press，NY(1990)に述べられている。

【０１６４】

好ましい1態様においては、本発明の組成物は、抗体をコードする核酸を含んでなり、その核酸は好適な宿主中で該抗体を発現する発現ベクターの一部である。特に、そのような核酸はプロモーターを有しており、そのプロモーターは好ましくは異種プロモーターで、機能的に抗体をコードする領域と連結され、そのプロモーターは誘導性または構成的なものであり、また、任意で、組織特異的なものである。別の特定の1実施形態においては、抗体をコードする配列およびその他の所望の配列をゲノム中の所望の部位での相同組換えを促進する領域と隣接させた核酸分子が用いられて、従って、その抗体をコードする核酸の染色体内での発現が提供される(KollerとSmithies，Proc. Natl. Acad. Sci. USA，86:8932-8935，1989；およびZijlstraら，Nature，342:435-438，1989)。

【０１６５】

別の好ましい1態様においては、本発明の組成物は,融合タンパク質をコードする核酸を含んでなり、その核酸は好適な宿主中でその融合タンパク質を発現する発現ベクターの一部である。特に、そのような核酸はプロモーターを有しており、そのプロモーターは好ましくは異種プロモーターで、機能的に融合タンパク質をコードする領域と連結され、そのプロモーターは誘導性または構成的なものであり、また、任意で、組織特異的なものである。別の特定の1実施形態においては、融合タンパク質をコードする配列およびその他の所望の配列をゲノム中の所望の部位での相同組換えを促進する領域と隣接させた核酸分子が用いられて、従って、その融合タンパク質をコードする核酸の染色体内での発現が提供される。

【０１６６】

被験者へのその核酸の送達は、直接的に、その場合には被験者はその核酸または核酸担持ベクターに直接的に暴露されることとなるが、または間接的に、その場合には細胞はまずその核酸でin vitroで形質転換され、次いで被験者内に移植されることとなる。これらの2種類のアプローチはそれぞれ、in vivo、またはex vivo遺伝子治療法として知られている。

【０１６７】

特定の1実施形態においては、該核酸配列は直接的に、in vivoで、それがコードする産物を産生するために発現するところへ投与される。このことは当業界で公知の多数の方法のいずれによっても達成することができ、例えば、その核酸配列を適切な核酸発現ベクターの一部として構築し、それらが細胞内となるように投与することにより、その投与は例えば、欠陥のある、または弱毒化したレトロウイルスまたはその他のウイルスベクターを用

いた感染(米国特許第4,980,286号を参照せよ)、または裸のＤＮＡの直接注射、または微粒子ボンバードメント(例えば、遺伝子銃；Biolistic, Dupont)、または脂質もしくは細胞表面受容体もしくはトランスフェクト剤を用いたコーティング、リポソーム、微粒子、もしくはマイクロカプセル中への被包、または核内に入ることが知られているペプチドと連結させて投与することによって、受容体介在性エンドサイトーシス(例えば、WuとWu, J. Biol. Chem., 262:4429-4432, 1987を参照せよ) (この方法は受容体を特異的に発現している細胞のタイプを標的とするために用いることができる)に対するリガンド対象物と連結して投与することによるなどである。別の1実施形態においては、核酸-リガンド複合体を形成させることができ、その複合体中ではリガンドはエンドソームを破壊するための膜融合性ウイルスペプチドを含み、それによって該核酸がリソソームによる分解を避けることができる。別の1実施形態においては、in vivoでの細胞特異的取り込み及び発現のために、特異的受容体を標的とすることによって、その核酸をターゲティングすることができる(例えば、PCT公開WO 92/06180; WO 92/22635; WO 92/20316; WO 93/14188; WO 93/20221を参照せよ)。あるいはまた、該核酸を細胞内に導入し、宿主細胞のDNA内に発現のために相同組換えによって組み込ませることができる(KollerとSmithies, Proc. Natl. Acad. Sci. USA, 86:8932-8935, 1989; およびZijlstraら, Nature, 342:435-438, 1989)。

【０１６８】

特定の1実施形態においては、抗体または融合タンパク質をコードしている核酸配列を含んでいるウイルスベクターが用いられる。例えば、レトロウイルスベクターを用いることができる(Millerら, Meth. Enzymol., 217:581-599, 1993を参照せよ)。これらのレトロウイルスベクターはウイルスゲノムの正しいパッケージングと宿主細胞DNA中への組み込みのために必要なコンポーネントを含んでいる。遺伝子治療に用いられる抗体または融合タンパク質をコードする核酸配列は1種以上のベクター中にクローン化することができ、それによってそのヌクレオチド配列の被験者内への送達を容易にする。レトロウイルスベクターに関するより詳細な説明はBoesenら, Biotherapy, 6:291-302, 1994に見つけることができ、この文献は、造血幹細胞を化学療法に対してより耐性なものとするためにmdr1遺伝子を造血幹細胞に送達するためのレトロウイルスベクターの使用を記載する。遺伝子治療でのレトロウイルスベクターの使用について説明している他の参照文献としては、次のものがある：Clowesら, J. Clin. Invest., 93:644-651, 1994; Kleinら, Blood 83:1467-1473, 1994; SalmonsとGunzberg, Human Gene Therapy, 4:129-141, 1993; およびGrossmanとWilson, Curr. Opin. in Genetics and Devel., 3:110-114, 1993。

【０１６９】

アデノウイルスは遺伝子治療に用いることのできる他のウイルスベクターである。アデノウイルスは呼吸上皮への遺伝子の送達のためには特に魅力的なビヒクルである。アデノウイルスは自然状態で呼吸上皮に感染し、そこで軽度の疾患を引き起こす。アデノウイルスに基づく送達系の他の標的としては、肝臓、中枢神経系、内皮細胞、および筋肉がある。アデノウイルスは、非分裂細胞に感染することができるという利点を有する。KozarskyとWilson, Current Opinion in Genetics and Development, 3:499-503, 1993は、アデノウイルスに基づく遺伝子治療についての概説を示す。Boutら, Human Gene Therapy, 5:3-10, 1994はアカゲザルの呼吸上皮への遺伝子の導入のためのアデノウイルスベクターの使用を実証した。遺伝子治療におけるアデノウイルスの使用の他の例については、次の文献中に見ることができる：Rosenfeldら、Science, 252:431-434, 1991; Rosenfeldら, Cell, 68:143-155, 1992; Mastrangeliら, J. Clin. Invest., 91:225-234, 1993; PCT公開 WO 94/12649; およびWangら, Gene Therapy, 2:775-783, 1995。好ましい1実施形態においては、アデノウイルスベクターが用いられる。

【０１７０】

またアデノ関連ウイルス(AAV)は、遺伝子治療での使用が提案されている(例えば、Walshら, Proc. Soc. Exp. Biol. Med., 204:289-300, 1993、および米国特許第5,436,146号を参照せよ)。

【０１７１】

遺伝子治療の別のアプローチには、電気穿孔法、リポフェクション、リン酸カルシウム介在トランスフェクション、またはウイルス感染などの方法による組織培養中の細胞への遺伝子の導入が含まれる。通常は、その導入方法には、その細胞への選択マーカーの導入が含まれる。次いで、その細胞を選択下に置き、導入された遺伝子を取り込み、かつ発現している細胞を単離する。それらの細胞が次いで被験者へ送達される。

【０１７２】

この実施形態においては、該核酸は得られた組換え細胞のin vivo投与前に細胞に導入される。そのような導入は当業界では公知のいずれの方法によっても行うことができ、そのような方法としては限定はされないが、トランスフェクション、電気穿孔法、マイクロインジェクション、該核酸配列を含んでいるウイルスベクターまたはバクテリオファージベクターを用いた感染、細胞融合、染色体介在遺伝子導入、ミクロセル介在(microcellmediated)遺伝子導入、スフェロプラスト融合、その他が挙げられる。外来遺伝子の細胞内への導入には当業界で公知の多数の技法があり(例えば、LoefflerとBehr, Meth. Enzymol., 217:599-618, 1993; Cohenら, Meth. Enzymol., 217:618-644,1993; およびClin. Pharma. Ther., 29:69-92, 1985を参照せよ)、レシピエントの細胞の発達上および生理学的に必要な機能が混乱されない限りは、本発明に従って用いることができる。そのような技法は該核酸の細胞への安定な導入を提供するものであるべきであり、それによってその核酸が細胞で発現可能となり、好ましくはその子孫の細胞に遺伝可能であり、かつ発現可能である。

【０１７３】

上記の結果得られた組換え細胞を被験者に、当業界では公知の種々の方法で送達することができる。組換え血液細胞(例えば、造血幹細胞、または前駆細胞)は静脈内に投与することが好ましい。用いようとする細胞の量は所望の効果、患者の状態、その他に依存し、当業者であれば決定することができる。

【０１７４】

遺伝子治療の目的で核酸を導入できる細胞は、所望の、利用可能な細胞タイプであればいずれも包含されるが、そのような細胞としては限定はされないが、上皮細胞、内皮細胞、ケラチノサイト、線維芽細胞、筋肉細胞、肝細胞；Tリンパ球、Bリンパ球、単球、マクロファージ、好中球、好酸球、巨核球、顆粒球などの血液細胞；種々の幹細胞または前駆細胞、特に造血幹細胞または前駆細胞、例えば、骨髄、臍帯血、末梢血、胎児肝臓、その他から得られるような細胞が挙げられる。

【０１７５】

好ましい1実施形態においては、遺伝子治療に用いられる細胞は被験者にとって自己のものである。

【０１７６】

遺伝子治療に組換え細胞が用いられる1実施形態においては、抗体または融合タンパク質をコードする核酸配列は、それらが組換え細胞またはその子孫によって発現可能なものとなるように細胞中に導入され、次いでその組換え細胞は治療効果を求めてin vivoに投与される。特定の1実施形態においては、幹細胞または前駆細胞が用いられる。単離が可能でin vitroで維持できるいずれの幹細胞および/または前駆細胞も本発明のこの実施形態に従って用いうる可能性がある(例えば、PCT公開 WO 94/08598; StempleとAnderson, Cell, 71;973-985, 1992; Rheinwald, Meth. Cell Bio., 21A:229, 1980; およびPittelkowとScott, Mayo Clinic Proc., 61:771, 1986を参照せよ)。

【０１７７】

特定の1実施形態においては、遺伝子治療の目的で導入されることとなる核酸はそのコード領域と機能的に連結された誘導性プロモーターを含んでなり、適切な転写インデューサーの存在または不在を制御することによってその核酸の発現が制御可能となる。

【０１７８】

5.8. 治療上または予防上の有用性の特徴とその実証

本発明の抗体、融合タンパク質、またはコンジュゲート分子は種々の方法で特徴を調べる

ことができる。特に、本発明の抗体はある抗原に対しての免疫特異的な結合能をアッセイすることができる。そのようアッセイは、溶液中で(例えば、Houghten, Bio/Techniques, 13:412-421, 1992)、ビーズ上で(Lam, Nature, 354:82-84,1991)、チップ上で(Fodor, Nature, 364:555-556, 1993)、細菌で(米国特許第5,223,409号)、胞子で(米国特許第5,571,698号、第5,403,484号、および第5,223,409号)、プラスミドで(Cullら, Proc. Natl. Acad. Sci. USA, 89:1865-1869, 1992)、またはファージで(ScottとSmith, Science, 249:386-390, 1990; Devlin, Science, 249:404-406, 1990; Cwirlaら, Proc. Natl. Acad. Sci. USA, 87:6378-6382, 1990; およびFelici, J. Mol. Biol., 222:301-310, 1991)(これらの参照文献の各々はその全体を本明細書中に参照により組み入れる)行うことができる。ある抗原またはその断片と免疫特異的に結合することが判明している抗体については、その抗原に対する特異的親和性の程度をアッセイすることができる。

【０１７９】

本発明の抗体またはそのフラグメントはある抗原に対する免疫特異的結合およびその他の抗原との交差反応性について当業界で既知のどの方法を用いてもアッセイすることができる。免疫特異的結合および交差反応性を分析するために用いることのできるイムノアッセイとしては、限定はされないが、ウエスタンブロット、ラジオイムノアッセイ、ELISA(酵素免疫測定法)、「サンドイッチ」イムノアッセイ、免疫沈降アッセイ、沈降素反応、ゲル拡散沈降反応、免疫拡散アッセイ、凝集アッセイ、補体結合アッセイ、免疫放射線測定法、蛍光イムノアッセイ、プロテインAイムノアッセイ、などの技法を使用する競合的または非競合的アッセイを多数あるうちの少数であるが挙げることができる。そのようなアッセイは当業界ではよく知られており、日常的に行われている(例えば、Ausbelら(編), 1994, 「分子生物学の今日のプロトコール」"Current Protocols in Molecular Biology", 第1巻, John Wiley & Sons, Inc., New Yorkを参照すればよく、これはその全体を本明細書中に参照により組み入れる)。代表的なイムノアッセイについて下記に簡潔に記載する(しかし限定することは意図していない)。

【０１８０】

免疫沈降反応のプロトコールは通常は、細胞の集団をタンパク質ホスファターゼおよび/またはプロテアーゼインヒビター(例えば、EDTA、PMSF、アプロチニン、バナジン酸ナトリウム)を添加したRIPAバッファー(1% NP-40またはTriton X-100, 1% デオキシコール酸ナトリウム、0.1% SDS、0.15M NaCl、0.01M リン酸ナトリウムpH7.2、1%トラジロール)などの溶解バッファー中で溶解させ、対象の抗体を細胞溶解物に添加し、一定時間(例えば1から4時間)40℃でインキュベートし、プロテインAおよび/またはプロテインGセファロースビーズを細胞溶解物に添加し、40℃で約1時間以上インキュベートし、溶解バッファー中でそのビーズを洗って、それをSDS/サンプルバッファー中に再懸濁する、ことを含んでなる。対象の抗体が特定の抗原を免疫沈降させる能力は、例えばウエスタンブロット分析などによって評価することができる。当業者であれば、抗体の抗原との結合を増大させ、バックグラウンドを低減させるために改変しうるパラメーターに関して(例えば、セファロースビーズで細胞溶解物をプレクリーニングすることなど)知っているであろう。免疫沈降法のプロトコールについてさらに知るためには、例えば、Ausbelら(編), 1994, 「分子生物学の今日のプロトコール」"Current Protocols in Molecular Biology", 第1巻, John Wiley & Sons, Inc., New Yorkの10.16.1項を参照せよ。

【０１８１】

ウエスタンブロット分析は通常は、タンパク質サンプルを調製し、そのタンパク質サンプルをポリアクリルアミドゲル電気泳動にかけ(例えば、抗原の分子量の如何によって8%-20% SDS-PAGE)、そのタンパク質サンプルをポリアクリルアミドゲルから、ニトロセルロース、PVDF、またはナイロンの膜へ移し、その膜をブロッキング液(例えば、3% BSAまたは脱脂乳を添加したPBS)中でブロックし、洗浄バッファー(例えばPBS-Tween 20)中でその膜を洗い、ブロッキングバッファーで希釈した一次抗体(対象の抗体)で膜をブロックし、その膜を洗浄バッファーで洗い、ブロッキングバッファーで希釈した酵素(例えば西洋ワサビペルオキシダーゼまたはアルカリホスファターゼ)または放射性分子(例えば、^{32}Pまた

は^{125}I)を結合させた二次抗体(この二次抗体は一次抗体を認識するもので、例えば抗ヒト抗体である)でその膜をブロックし、その膜を洗浄バッファーで洗い、該抗原の存在を検出する、ことを含んでなる。当業者であれば、検出されるシグナルを増強させ、バックグラウンドノイズを低減させるために改変しうるパラメーターに関して知っているであろう。ウエスタンブロットのプロトコールについてさらに知るためには、例えば、Ausbelら(編), 1994, 「分子生物学の今日のプロトコール」"Current Protocols in Molecular Biology", 第1巻, John Wiley & Sons, Inc., New Yorkの10.8.1項を参照せよ。

【０１８２】

ELISAは、抗原を調製し、96ウエルのマイクロタイタープレートのウエルをその抗原でコーティングし、酵素(例えば西洋ワサビペルオキシダーゼまたはアルカリホスファターゼ)などの検出可能な化合物を結合させた対象の抗体をウエルに添加し、一定時間インキュベートし、抗原の存在を検出する、ことを含んでなる。ELISAでは対象の抗体は検出可能な化合物と結合させる必要はない；その替わりに、検出可能な化合物と結合させた二次抗体(これは対象の抗体を認識する)をウエルに添加することができる。さらに、ウエルを抗原でコーティングする替わりに、該抗体をウエルにコーティングすることができる。その場合には、検出可能な化合物と結合させた二次抗体を、そのコーティングされたウエルへの抗原の添加後に、添加することができる。当業者であれば、検出されるシグナルを増強させ、当業界で既知であるELISAの他の変動要素を改変しうるパラメーターに関して知っているであろう。ELISAについてさらに知るためには、例えば、Ausbelら(編), 1994, 「分子生物学の今日のプロトコール」"Current Protocols in Molecular Biology", 第1巻, John Wiley & Sons, Inc., New Yorkの11.2.1項を参照せよ。

【０１８３】

該抗体の抗原への結合の親和性、および抗体-抗原相互作用の脱離速度は競合的結合アッセイによって測定することができる。競合的結合アッセイの1例はラジオイムノアッセイで、標識抗原(例えば、^{3}Hまたは^{125}I)を対象の抗体と、標識していない抗原の量を増加させつつ共存させてインキュベートし、標識抗原に結合した抗体を検出する、ことを含んでなる。本発明の抗体、またはそのフラグメントのその抗原に対する親和性および結合の脱離速度はスキャッチャード分析による飽和度のデータから測定することができる。二次抗体との競合は、また、ラジオイムノアッセイを用いても測定することができる。その場合には、該抗原を、標識化合物(例えば、^{3}Hまたは^{125}I)を結合させた本発明の抗体またはそのフラグメントと、標識していない二次抗体の量を増加させつつ共存させてインキュベートする。

【０１８４】

好ましい1実施形態においては、BIAcore反応速度分析は抗体の抗原に対する結合の結合速度および脱離速度を測定するために用いられる。BIAcore反応速度分析は、固定化抗体をその表面に有するチップへの抗原の結合とそれからの抗原の解離を分析することを含んでなる(後述の実施例の節を参照せよ)。

【０１８５】

本発明の抗体、および融合タンパク質とコンジュゲート分子はまた、抗原のそれの宿主細胞レセプターへの結合を阻害する能力を当業者には既知の技法を用いてアッセイすることができる。例えば、あるウイルス抗原と結合するレセプターを発現している細胞は、抗体の存在下または不在下でウイルスと接触させて、ウイルス抗原の結合を阻害するその抗体の能力を、例えば、フローサイトメトリーまたはシンチレーションカウンターによって測定することができる。抗原とそれの宿主細胞レセプターとの間の相互作用の検出を行うために、抗原または抗体を、放射性標識(例えば、^{32}P、^{35}S、および^{125}I)または蛍光標識(例えば、イソチオシアン酸フルオレセイン、ローダミン、フィコエリスリン、フィコシアニン、アロフィコシアニン、o-フタルアルデヒド、およびフルオレサミン)などの検出可能な化合物を用いて標識することができる。あるいはまた、抗原がそのレセプターと結合することを抗体が阻害する能力はセルフリーアッセイで測定することができる。例えば、ウイルスまたはウイルス抗原(例えば、RSV F糖タンパク質)はセルフリーアッセイで抗体

と接触させ、ウイルスまたはウイルス抗原がそれの宿主細胞レセプターと結合することをその抗体が阻害する能力を測定することができる。好ましくは、その抗体は固相担体上に固定化され、その抗原は検出可能な化合物で標識されたものである。あるいはまた、該抗原を固相担体上に固定化し、該抗体を検出可能な化合物で標識されたものとすることもできる。該抗原は部分的にまたは完全に精製されたもの(例えば、他のポリペプチドが部分的にまたは完全に存在しないもの)、または細胞溶解物の一部とすることができる。さらに、該抗原は、ウイルス抗原およびグルタチオン-S-トランスフェラーゼなどのドメインを含んでなる融合タンパク質とすることができる。あるいはまた、抗原を当業者に周知の技法を用いてビオチン化することができる(例えば、ビオチン化キット, Pierce Chemicals; Rockford, IL)。

【０１８６】

本発明の抗体、融合タンパク質、およびコンジュゲート分子はまた、当業者には既知の技法を用いて、ウイルスまたは細菌の複製を阻害またはダウンレギュレートする能力をアッセイすることができる。例えば、ウイルスの複製は、例えば、Johnsonら, Journal of Infectious Disease, 176:1215-1224, 1997などに述べられているプラークアッセイを用いてアッセイすることができる。本発明の抗体、融合タンパク質、およびコンジュゲート分子はまた、ウイルスまたは細菌のポリペプチドの発現を阻害またはダウンレギュレートする能力をアッセイすることができる。当業者に既知の技法としては、限定はされないが、ウエスタンブロット分析、ノーザンブロット分析、およびRT-PCRが含まれ、それらはウイルスまたは細菌のポリペプチドの発現を測定するために用いることができる。さらに、本発明の抗体、融合タンパク質、およびコンジュゲート分子は合胞体形成を阻害する能力についてアッセイすることができる。

【０１８７】

本発明の抗体、融合タンパク質、コンジュゲート分子、および組成物はヒトに用いる前に、好ましくはin vitroでまず試験し、次いでin vivoで所望の治療または予防活性を試験する。例えば、本発明の特定の抗体、特定の融合タンパク質、特定のコンジュゲート分子、または特定の組成物の投与を行うかどうかを決めるために用いることのできるin vitroアッセイには、in vitro細胞培養アッセイが含まれ、そのアッセイ法は、被験者の組織サンプルを培養液中で増殖させ、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物に暴露するか、またはそれらを投与し、その組織サンプルに及ぼす本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物の影響を観察するものである。種々の特別な実施形態においては、in vitroアッセイは、ある疾患または障害に関与する細胞型の代表的な細胞を用いて行うことができ、その細胞型に本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物が所望の効果を及ぼすか否かを調べることができる。好ましくは、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物は、ヒトへ投与する前に、in vitroアッセイおよび動物モデル系でも試験される。

【０１８８】

治療に用いるための本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物は、それらの毒性について適切な動物モデル系で試験することができ、そのような動物としては、限定はされないが、ラット、マウス、ウシ、サル、およびウサギが含まれる。抗体、融合タンパク質、コンジュゲート分子、または組成物の毒性をin vivoで試験するためには、当業界で既知の動物モデル系のどれでも用いることができる。

【０１８９】

ウイルス感染の治療または予防における有効性は、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物のウイルスの複製を阻害する能力、ウイルスの伝播を阻害する能力、またはウイルスがその宿主中にそのウイルス自体を確立することを妨げる能力、またはウイルス感染に伴う1種以上の症状を防止する、改善する、または緩和する能力を検出することによって示すことができる。その処置は、例えば、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物の投与後にウイルスロードの低減、1種以上の症状の改善、または死亡率および/もしくは疾病率の減少があれば、治療的なものと

考えられる。本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物は、ウイルスの複製を阻害する能力、またはウイルスロードを低減させる能力についてin vitroおよびin vivoアッセイで試験することもできる。

【０１９０】

細菌感染の治療または予防における有効性は、本発明の抗体、融合タンパク質、または組成物の細菌の複製を阻害する能力、または細菌感染に伴う1種以上の症状を防止する、改善する、または緩和する能力を検出することによって示すことができる。その処置は、例えば、本発明の抗体、融合タンパク質、または組成物の投与後に、細菌数の低減、1種以上の症状の改善、または死亡率および/もしくは疾病率の減少があれば、治療的なものと考えられる。

【０１９１】

癌の治療における有効性は、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物の癌細胞の増殖もしくは転移を阻害もしくは低減する能力、または癌に伴う1種以上の症状を、改善する、または緩和する能力を検出することによって示すことができる。その処置は、例えば、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物の投与後に、癌細胞の増殖もしくは転移の低減、癌に伴う1種以上の症状の改善、または死亡率および/もしくは疾病率の減少があれば、治療的なものと考えられる。本発明の抗体、融合タンパク質、または組成物は、腫瘍形成を低減させる能力について、in vitro、ex vivo、およびin vivoのアッセイで試験することができる。

【０１９２】

炎症性障害の治療における有効性は、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物の、動物における炎症を低減もしくは阻害する能力、または炎症性障害に伴う1種以上の症状を改善する、もしくは緩和する能力を検出することによって示すことができる。その処置は、例えば、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物の投与後に、1種以上の症状の改善があれば、治療的なものと考えられる。

【０１９３】

本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物は、in vitroまたはin vivoでサイトカイン(例えば、IFN-α、IFN-β、IFN-γ、IL-2、IL-3、IL-4、IL-5、IL-6、IL-10、IL-12、およびIL-15)ならびに活性化マーカー(例えば、CD28、ICOS、およびSLAM)の発現を誘導する能力を試験することができる。サイトカインおよび活性化マーカーの発現レベルを測定するために、当業者には既知の技法を用いることができる。例えば、サイトカインの発現レベルは、サイトカインのRNAのレベルを、例えば、RT-PCRおよびノーザンブロット分析を用いて分析することによって、および、例えば免疫沈降反応を行った後にウエスタンブロット分析もしくはELISAを用いてサイトカインのレベルを分析することによって、測定することができる。

【０１９４】

本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物は、免疫細胞、好ましくはヒトの免疫細胞(例えば、T細胞、B細胞、およびナチュラルキラー細胞)の生物活性を変調させる能力について、in vitroおよびin vivoで試験することができる。本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物の、免疫細胞の生物活性を変調させる能力は、抗原の発現の検出、免疫細胞の増殖の検出、シグナル分子の活性化の検出、免疫細胞のエフェクター機能の検出、または免疫細胞の分化の検出によって評価することができる。これらの活性を測定するために当業者には既知の技法を用いることができる。例えば、細胞増殖は^{3}H-チミジン取り込みアッセイおよびトリパンブルーを用いた細胞数の計数によってアッセイすることができる。例えば、抗原の発現はイムノアッセイで調べることができ、そのアッセイには、限定はされないが、ウエスタンブロット、免疫組織化学、ラジオイムノアッセイ、ELISA(酵素免疫測定法)、「サンドイッチ」イムノアッセイ、免疫沈降アッセイ、沈降反応、ゲル拡散沈降反応、免疫拡散アッセイ、凝集アッセイ、補体結合アッセイ、イムノラジオメトリックアッセイ、蛍光イムノアッセイ、プロテ

インAイムノアッセイ、およびFACS分析などの技法を用いた競合的および非競合的アッセイ系が含まれる。シグナル分子の活性化は、例えば、キナーゼアッセイおよび電気泳動シフトアッセイ(EMSA)によってアッセイすることができる。

【０１９５】

本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物はまた、疾患、障害、または感染に罹患している動物、好ましくは哺乳類、最も好ましくはヒトの生存期間を、少なくとも25%、好ましくは少なくとも50%、少なくとも60%、少なくとも75%、少なくとも85%、少なくとも95%、もしくは少なくとも99%、延長させる能力について試験することもできる。さらに、本発明の抗体、融合タンパク質、コンジュゲート分子、または組成物はまた、疾患、障害、または感染に罹患している動物、好ましくは哺乳類、最も好ましくはヒトの入院期間を、少なくとも60%、好ましくは少なくとも75%、少なくとも85%、少なくとも95%、もしくは少なくとも99%、短縮させる能力について試験することもできる。本発明の抗体または組成物のin vivoにおける機能を分析するために当業者には既知の技法を用いることができる。

【０１９６】

5.9. 抗体および融合タンパク質の診断的用途

本発明の標識した抗体、融合タンパク質、およびコンジュゲート分子は、疾患、障害、または感染の検出、診断、またはモニターのための診断用途で用いることができる。本発明は、疾患、障害、または感染の検出または診断法であって、(a)被験者の細胞または組織サンプル中での抗原の発現を、その抗原と免疫特異的に結合する1種以上の抗体を用いてアッセイし、(b)その抗原のレベルを対照のレベル、例えば正常な組織サンプル中のレベルと比較し、アッセイした抗原のレベルが対照のレベルと比較して増加していれば疾患、障害、または感染を示すこととなることを含んでなる方法、を提供する。本発明はまた、疾患、障害、または感染の検出または診断法であって、(a)被験者の細胞または組織サンプル中での抗原の発現を、その抗原と結合する本発明の1種以上の融合タンパク質またはコンジュゲート分子を用いてアッセイし、(b)その抗原のレベルを対照のレベル、例えば正常な組織サンプル中のレベルと比較し、アッセイした抗原のレベルが対照のレベルと比較して増加していれば疾患、障害、または感染を示すこととなることを含んでなる方法、も提供する。従って、該融合タンパク質またはコンジュゲート分子は、リガンド、サイトカイン、もしくは増殖因子などの生物活性分子、およびヒンジ-Fc領域もしくはそれのフラグメントを含んでなり、そのような融合タンパク質またはコンジュゲート分子は検出される抗原と結合することができる。

【０１９７】

本発明の抗体は、生物学的サンプル中の抗原のレベルを古典的な免疫組織学的方法を用いてアッセイするために用いることができ、そのような方法は本明細書中に記載されているか、または当業者には既知のものである(例えば、Jalkanenら, J. Cell. Biol., 101:976-985, 1985; Jalkanenら, J. Cell. Biol., 105:3087-3096, 1987)。タンパク質の遺伝子からの発現を検出するのに有用な他の抗体をベースとする方法としては、酵素免疫測定法(ELISA)およびラジオイムノアッセイ(RIA)などのイムノアッセイが含まれる。抗体を用いるアッセイでの適切な標識については当業界では既知であり、そのようなものとしては、アルカリホスファターゼ、グルコースオキシダーゼなどの酵素標識；ヨウ素(^{125}I、^{131}I)、炭素(^{14}C)、イオウ(^{35}S)、トリチウム(^{3}H)、インジウム(^{121}In)、およびテクネチウム(^{99m}Tc)などのラジオアイソトープ；ルミノールなどの発光物質による標識；フルオレセインおよびローダミンなどの蛍光標識が含まれる。

【０１９８】

融合タンパク質は生物学的サンプル中の抗原レベルを、例えば、当業者には既知のSDS-PAGEおよびイムノアッセイを用いてアッセイすることができる。

【０１９９】

本発明の1態様は、ヒトの疾患、障害、または感染の検出および診断である。1実施形態においては、診断法は、a)抗原と免疫特異的に結合する標識抗体の有効量を被験者に投与し

(例えば、非経口、皮下、または腹腔内に)、b)投与後、抗原が発現されている被験者体内の部位へ標識抗体が優先的に濃縮されるまでの時間(および結合しなかった標識分子がバックグラウンドレベルまで除去されるまでの時間)を待ち、c)バックグラウンドレベルを測定し、d)被験者体内の標識抗体を検出して、バックグラウンドレベルを超える標識抗体が検出されれば、その被験者が疾患、障害、または感染を有していることが示される、ことを含んでなる。この実施形態では、該抗体は、当業者には既知のイメージングシステムを用いて検出しうるイメージング成分を用いて標識される。バックグラウンドレベルは種々の方法で測定することができ、そのような方法では、特定のシステムであらかじめ測定して定められた標準値と、検出された標識分子の量を比較することを含む。

【０２００】

別の1実施形態においては、診断法は、a)抗原またはその他の分子と結合する標識融合タンパク質またはコンジュゲート分子の有効量を被験者に投与し(例えば、非経口、皮下、または腹腔内に)、b)投与後、該抗原またはその他の該分子が発現されている被験者体内の部位へ標識融合タンパク質またはコンジュゲート分子が優先的に濃縮されるまでの時間(および結合しなかった標識分子がバックグラウンドレベルまで除去されるまでの時間)を待ち、c)バックグラウンドレベルを測定し、d)被験者体内の標識融合タンパク質またはコンジュゲート分子を検出して、バックグラウンドレベルを超える標識融合タンパク質が検出されれば、その被験者が疾患、障害、または感染を有していることが示される、ことを含んでなる。この実施形態では、該融合タンパク質またはコンジュゲート分子は、リガンド、サイトカイン、または増殖因子などの生物活性物質、およびヒンジ-Fc領域もしくはそれのフラグメントを含んでなり、そのような癒合タンパク質またはコンジュゲート分子はイメージング成分を用いて標識して、検出しようとする抗原と結合することができる。

【０２０１】

被験者の身体の大きさおよび用いるイメージングシステムによって診断用イメージを作り出すために必要なイメージング成分の量が定められることは当業界では理解されよう。放射性同位元素成分の場合、ヒト被験者に対して、注射される放射能の量は通常は約5から20ミリキュリー(mCi)の^{99m}Tcである。次いで、該標識抗体は、特定のタンパク質を含有している細胞の位置へ優先的に蓄積される。in vivoでの腫瘍イメージングについては、「腫瘍イメージング：癌の放射線化学的検出」"Tumor Imaging:The Radiochemical Detection of Cancer", S. W. BurchielとB. A. Rhodes編, Masson Publishing Inc.(1982)中のS.W.Burchielら, 「放射性標識抗体およびそのフラグメントの免疫薬物動態学」"Immunopharmacokinetics of Radiolabeled Antibodies and Their fragments", 第13章に述べられている。

【０２０２】

いくつかの変動要因（そのような要因としては、用いる標識のタイプおよび投与方法が含まれる）に応じて、被験者体内の部位へ標識分子が優先的に濃縮され、結合しなかった標識分子がバックグラウンドレベルまで除去されるために投与後必要な時間は、6から48時間、または6から24時間、または6から12時間である。別の1実施形態においては、その時間は投与後5から20日間または5から10日間である。

【０２０３】

1実施形態においては、疾患、障害、または感染のモニタリングは、疾患、障害、または感染を診断する方法を繰り返すこと、例えば、最初の診断の1ヶ月後、最初の診断の6ヶ月後、最初の診断の1年後などに繰り返すことによって行われる。

【０２０４】

被験者体内の標識分子の存在は、in vivoスキャンニングのための方法として当業界で既知の方法を用いて検出することができる。それらの方法は、用いる標識のタイプの如何によって変わる。当業者であれば、特定の標識を検出するために適切な方法を決定することができよう。本発明の診断方法に用いることのできる方法と装置としては、限定はされないが、コンピューター断層撮影法(CT)、陽電子放射断層撮影法(PET)、核磁気共鳴映像法(MRI)、および超音波検査法などの全身スキャンが含まれる。

【０２０５】
特定の1実施形態においては、該分子は放射性同位元素で標識され、患者体内での検出は放射能に応答性の手術器具を用いて行われる(Thurstonら，米国特許第5,441,050号)。別の1実施形態においては、該分子は蛍光化合物で標識され、患者体内での検出は蛍光に応答性のスキャンニング装置を用いて行われる。別の1実施形態においては、該分子は陽電子放射性金属で標識され、患者体内での検出は陽電子放射断層撮影法を用いて行われる。また別の1実施形態においては、該分子は常磁性標識され、患者体内での検出は核磁気共鳴映像法(MRI)で行われる。
【０２０６】
5.10. キット
本発明はまた、本発明の医薬組成物の1種以上の成分で満たされた1つ以上の容器を含んでなる医薬パックまたはキットをも提供する。任意でそのような容器には、一般医薬品または生物学的製剤の製造、使用、または販売を規制する薬事当局によって指定された形式の通知を添付することができ、その通知はヒトへの投与のための製造、使用、または販売のその薬事当局による承認内容を反映したものである。
【０２０７】
本発明は上述の方法を用いることのできるキットを提供する。1実施形態においては、キットは、本発明の抗体、融合タンパク質、またはコンジュゲート分子を、好ましくは精製された形で、1つ以上の容器に入れたものを含んでなる。特定の1実施形態においては、本発明のキットは対照として実質的に単離された抗原を含んでなる。好ましくは、本発明のキットはさらに、キット中に含まれる抗原と反応しない対照の抗体、融合タンパク質、またはコンジュゲート分子を含んでなる。別の特定の1実施形態においては、本発明のキットは、抗体、融合タンパク質、またはコンジュゲート分子の抗原との結合を検出するための手段を含んでいる(例えば、該抗体、融合タンパク質、またはコンジュゲート分子は、蛍光化合物、酵素基質、放射性化合物、もしくは発光化合物などの検出可能な物質と結合させることができ、または一次抗体を認識する二次抗体を検出可能な物質と結合させることができる)。特定の実施形態においては、該キットには組換えで産生させた、または化学的に合成した抗原を含むことができる。該キット中に提供される抗原はまた、固相担体に付着させることもできる。より特定の1実施形態においては、上述のキットの検出手段としては、抗原を付着させる固相担体が含まれる。そのようなキットはまた、付着させていない、レポーターで標識した抗ヒト抗体を含むこともできる。その実施形態においては、該抗体の該抗原への結合はそのレポーターを標識した抗体の結合によって検出することができる。
【０２０８】
5.11. 修飾したIgGヒンジ-Fcフラグメントの半減期が延長されているか調べるためのin vitroおよびin vivoアッセイ
修飾IgGおよびそれのFcRnフラグメントの IgG定常ドメインのFcRnとの結合能は、種々のin vitroアッセイで特徴を調べることができる。WardによるPCT公開WO 97/34631は、種々の方法を詳細に開示しており、その全体を本明細書中に参照により組み入れる。
【０２０９】
例えば、修飾IgGまたはそれのフラグメントのの FcRnとの結合の能力を、野生型IgG、修飾IgG、またはそのフラグメントの結合の能力と比較するために、野生型IgGを放射性標識して、in vitroでFcRnを発現している細胞と反応させることができる。次いでその細胞に結合した画分の放射能を測定し、比較することができる。このアッセイに用いられるFcRnを発現している細胞は、好ましくは、B10.DBA/2マウスの肺由来のマウス肺末梢血管内皮細胞(B10, D2.PCE)およびC3H/HeJマウス由来のSV40で形質転換させた内皮細胞(SVEC)(Kimら，J. Immunol., 40:457-465, 1994)などの内皮細胞系である。しかし、その他のタイプの細胞（例えば生後10日～14日目の乳児マウスから単離した腸刷子縁であり、これは十分な数のFcRnを発現している）を用いることもできる。あるいはまた、対象としている種の組換えFcRnを発現している哺乳類細胞を用いることもできる。修飾IgGの結合した画分、また

は野生型の結合した画分の放射能を計数した後、その結合した分子を界面活性剤で抽出し、細胞のユニット数あたりの放出されたものの比率を計算し、比較することができる。

【０２１０】

修飾IgGのFcRnに対する親和性は表面プラスモン共鳴(SPR)測定を、例えば前述のBIAcore 2000(BIAcore Inc.)を用いて測定することができる(Popovら, Mol. Immunol., 33:493-502, 1996; Karlssonら, J. Immunol. Methods, 145:229-240, 1991、これらは双方ともその全体を本明細書中に参照により組み入れる)。この方法では、FcRn分子はBIAcoreセンサーチップ(例えば、PharmaciaのCM5チップ)と結合され、修飾IgGの固定化FcRnへの結合は、特定の流速でBIA evaluation 2.1ソフトウエアを用いてセンサーグラムを得て、それに基づいてその修飾IgG、定常ドメイン、またはそれらのフラグメントのFcRnとの結合速度および脱離速度を計算することができる。

【０２１１】

修飾IgGまたはそれのフラグメント、および野生型IgGのFcRnに対する相対親和性は単純競合結合アッセイで測定することもできる。FcRnが固定化されている96ウエルのプレートのウエルに種々の量の非標識修飾IgGまたは野生型IgGを添加する。次いで、一定量の放射標識野生型IgGを各ウエルに添加する。結合した画分の放射能百分率を非標識修飾IgGまたは野生型IgGの量に対してプロットし、その曲線の勾配から修飾ヒンジ-Fcの相対的親和性を計算することができる。

【０２１２】

さらに、修飾IgGまたはそれのフラグメント、および野生型IgGのFcRnに対する親和性は、飽和試験およびスキャッチャード分析によっても測定することができる。

【０２１３】

FcRnによる修飾IgGまたはそれのフラグメントの細胞を横切る移送は、放射性標識IgGまたはそれのフラグメントおよびFcRnを発現している細胞を用いたin vitro移送アッセイにより、細胞単層の一方の側の放射能をもう一方の側の放射能と比較することによって測定することができる。あるいはまた、そのような移送はin vivoで生後10日から14日後の乳児マウスに放射性標識した修飾IgGを与え、定期的に血液中の放射能を計測することによって測定することができ、それは腸を通過するIgGの循環系(またはその他の標的組織のいずれかのもの、例えば肺)への移送を示している。腸を通過する用量依存性のIgG移送の阻害を試験するために、放射性標識および非標識のIgGの一定比率の混合物をマウスに与え、血漿中の放射能を定期的に測定することができる(Kimら, Eur. J. Immunol., 24:2429-2434, 1994)。

【０２１４】

修飾IgGまたはそれのフラグメントの半減期はKimら(Eur. J. Immunol., 24:542, 1994)によって報告されている方法に従って薬物動態学的実験によって測定することができ、この文献はその全体を本明細書中に参照により組み入れる。この方法では、放射性標識した修飾IgGまたはそれのフラグメントは、マウスに静脈内注射され、その血漿中濃度は、例えば注射後3分から72時間の時点などにおける時間の関数として定期的に測定される。そのようにして得られたクリアランス曲線は2相性、すなわちα-相およびβ-相、となるはずである。該修飾IgGまたはそれのフラグメントのin vivoでの半減期の決定のために、β-相のクリアランス速度が計算され、野生型IgGのクリアランス速度と比較する。

【０２１５】

6. 実施例

下記の実施例はin vivoでの半減期が延長されている修飾ヒンジーFcフラグメントの作成、単離、および特性決定を説明するものである。

【０２１６】

6.1. ライブラリーの構築

6.1.1. 試薬

化学物質は全て分析用グレードのものを用いた。制限酵素およびDNA修飾酵素はNew England Biolabs, Inc.(Beverly, MA)から購入した。オリゴヌクレオチドはNWG Biotech, Inc.

(High Point, NC)で合成されたものである。pCANTAB5Eファージミドベクター，抗E-tag-西洋ワサビペルオキシダーゼコンジュゲート、TG1大腸菌(E.coli)株、IgG Sepharose 6 Fast FlowおよびHiTrap プロテインAカラムはAPBiotech, Inc.(Piscataway, NJ)から購入した。VCSM 13ヘルパーファージおよびQuick change mutagenesis kitはStratagene(La Jolla, CA)から入手した。CJ236大腸菌株はBio-Rad(Richmond, CA)から購入した。BCA Protein Assay Reagent KitはPierce(Rockford, IL)から入手した。Lipofectamine 2000はInvitrogen, Inc.(Carlsbad, CA)から購入した。

【０２１７】

6.1.2. マウスとヒトのFcRnの発現と精製

ヒトおよびマウスのFcRnのアミノ酸配列はそれぞれ配列番号84および85である（Firanら，Intern. Immunol., 13:993-1002, 2001およびPopovら，Mol. Immunol., 33:521-530, 1996も参照されたい、これら双方の文献はその全体を本明細書中に参照により組み入れる）。ヒトFcRnはまた、標準的なPCRプロトコールを用いてヒト胎盤cDNA(Clonetech, Palo Alto, CA)からのヒトβ2-ミクログロブリン(Kabatら，1991，「免疫学的興味深いタンパク質の配列」"Sequences of Proteins of Immunological Interest", U.S.Public Health Service, National Institute of Health, Washington, DC)とヒトα鎖のコドン23から267(Soryら，J. Exp. Med.,180:2377-2381, 1994)の単離後、得ることができる。軽鎖と重鎖はそれらの天然のシグナル配列(Kabatら，1991，上述の文献；Storyら，上述の文献)とともにpFastBac DUALおよびpFastBac1 バクミド中にそれぞれクローン化し、ウイルスストックをSpodoptera frugiperda細胞(Sf9)中に製造者(Invitrogen, Carlbad, CA)の使用説明書に従って作成した。High-Five細胞は感染多重度を3として、市販のプロトコールを用いて(Invitrogen)、α鎖およびβ2鎖をコードするバキュロウイルスで感染させた。組換えヒトFcRnは下記のとおり精製した：感染させた昆虫細胞の上清を50mM MES(2-N-[モルホリノ]エタンスルホン酸)pH 6.0に対して透析し、10mLのヒトIgG Sepharose 6 Fast Flowカラム(APBiotech, Piscataway, NJ)にアプライした。樹脂を200mLの50mM MES pH6.0で洗い、FcRnを0.1M Tris-Cl pH8.0で溶出した。精製FcRnを50mM MES pH6.0に対して透析し、急速凍結し、－70℃で保存した。タンパク質の純度はSDS-PAGEおよびHPLCによって調べた。

【０２１８】

6.1.3. TAAを含有するssDNAウラシル鋳型の調製

ライブラリーの構築はKunkel法(Kunkelら，Methods Enzymol., 154:367-382, 1987)に由来する部位特異的突然変異誘発法に基づいて行った。MEDI-493 ヒトIgG1(Johnsonら，J. Infect. Disease, 176:1215-1224, 1997)由来のヒトヒンジ-Fc遺伝子のアミノ酸残基226-478(Kabatの番号付けによる、Kabatら，1991，上述の文献)をpCANTAB5Eファージミドベクター中にSfiI/NotI断片としてクローン化した。4種類のライブラリーは、251, 252, 254, 255, 256(ライブラリー1)、308, 309, 311, 312, 314(ライブラリー2)、385, 386, 387, 389(ライブラリー3)、および428, 433, 434, 436(ライブラリー4)の位置にランダムな変異を導入することによって作成した。簡潔に述べれば、4つの別々のヒンジ-Fc鋳型をオーバーラップ伸長法(Hoら，Gene, 15:51-59, 1989)によってPCRを用いて作成し、その各々には252(ライブラリー1)、310(ライブラリー2)、384(ライブラリー3)、および429(ライブラリー4)の位置に1個の停止コドンTAAを含んでおり、そのため、突然変異したファージミドのみがFcをディスプレイするファージを生ずる。

【０２１９】

次いで、TAA含有の一本鎖DNA(TAAssDNA)の各々を次のとおり調製した：4種類のTAA含有ファージミドのうちの1つを含んでいる1個のCJ236大腸菌コロニーを、10μg/mLのクロラムフェニコールおよび100μg/mLのアンピシリンを添加した10mLの2 x YT培地中で増殖させた。OD600＝1となったところでVCSM 13ヘルパーファージを終濃度が10^{10}pfu/mLとなるように添加した。2時間後、その培養液を0.25μg/mLのウリジン、10μg/mLのクロラムフェニコール、30μg/mLのカナマイシン、および100μg/mLのアンピシリンを添加した500mLの2 x YT培地に移し、37℃で一晩増殖させた。標準的なプロトコール(Sambrookら，1989，「

分子クローニング、実験室マニュアル」"Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor, New York, 第1-3巻)に従ってファージをPEG6000で沈殿させ、Qiaprep Spin M13 Kit(Qiagen, Valencia, CA)を用いて製造者の使用説明書に従って精製した。ウラシルを含有するTAAssDNA鋳型の各々を10から30μg、0.6μgの下記のリン酸化オリゴヌクレオチド(ランダマイズした部分は下線を付した)と、50mM Tris-HCl, 10mM $MgCl_2$,pH7.5中で最終容量を250μLとして結合させた：

ライブラリー1：

5'-CATGTGACCTCAGGSNNSNNSNNGATSNNSNNGGTGTCCTTGGGTTTTGGGGGG-3'(配列番号120)

ライブラリー2：

5'-GCACTTGTACTCCTTGCCATTSNNCCASNNSNNGTGSNNSNNGGTGAGGACGC-3'(配列番号121)

ライブラリー3：

5'-GGTCTTGTAGTTSNNCTCSNNSNNSNNATTGCTCTCCC-3'(配列番号122)

ライブラリー4：

5'-GGCTCTTCTGCGTSNNGTGSNNSNNCAGAGCCTCATGSNNCACGGAGCATGAG-3'(配列番号122)

上記でN＝A、C、T、またはG、　S＝GまたはCである。

【０２２０】

6.1.4　ヘテロ二本鎖DNAの合成

適切な縮重オリゴヌクレオチドを、標準的なプロトコールを用いてT4ポリヌクレオチドキナーゼの存在下でリン酸化した。10から30μgのssDNA U鋳型および0.6μgのリン酸化オリゴヌクレオチドを、10mM $MgCl_2$, pH7.5を含有する50mM Tris-HClで最終容量を250μLとしてその中で結合させ、90℃で2分間、50℃で3分間、および20℃で5分間インキュベートした。ヘテロ二本鎖DNAの合成は、0.4mM ATP、1mM dNTP、および6mM DTTの存在下でT4 DNAリガーゼおよびT7 DNAポリメラーゼの双方を30ユニット添加して行い、その混合物を20℃で4時間インキュベートした。次いで、このようにして作成したヘテロ二本鎖DNAをQiagen Qiaquick DNA purification Kit(Qiagen, CA)を用いて精製および脱塩した。

【０２２１】

6.1.5　電気穿孔法

電気穿孔法で取り込み可能な300μLのTG1大腸菌細胞を、1から5μgのヘテロ二本鎖DNAとともに、2.5kVの電場で200Ωと25μFキャパシタンスを用いて、ライブラリーのサイズが1 x 10^8(ライブラリー1および2)、または1 x 10^7(ライブラリー3および4)となるまで、電気的に穿孔させた。その細胞を2mLのSOC培地中に再懸濁し、この方法を6から10回反復した。多様性については組換え大腸菌の滴定によって評価した。電気パルスをあてた細胞を50mLのSOC培地中で37℃で30分間振盪しつつインキュベートし、遠心し、100μg/mLのアンピシリンおよび10^{10}pfu/mLのVCSM 13ヘルパーファージを含んでいる500mLの2xYT中に再懸濁した。その培養物を37℃で一晩インキュベートし、細胞を遠心してペレットとした。変異したヒンジ-Fc部分をGIIIコートタンパク質上に発現している、上清中のファージを既に報告されているとおり(Sambrookら, 1989, 上述の文献)PEG6000で沈澱させ、5mLの20mM MES, pH6.0中に再懸濁した。

【０２２２】

6.2　ライブラリーのパニング

ファージをELISAをベースとするアプローチを用いてパニングした。96ウエルのELISAプレートを、炭酸ナトリウムバッファーpH9.0中に0.01mg/mLのマウスFcRnを含む液100μL/ウエルで、4℃で一晩コーティングし、次いで、4%脱脂粉乳で37℃で2時間ブロックした。そのコーティングしたプレートの各ウエルに、5%の粉乳および0.05%のTween 20含有の20mM MES, pH6.0中にファージを懸濁させた液(総計約10^{13}個のファージ)の100-150μLを入れ、37℃で2～3時間、振盪しつつインキュベートした。

【０２２３】

インキュベーション後、ウエルを0.2%のTween 20および0.3M NaCl含有の20mM MES, pH6.0で、室温で約30回洗った。結合したファージを、100μL/ウエルのPBS, pH7.4で37℃にて30分間溶出させた。

【０２２４】
次いで、溶出したファージを、対数増殖期の大腸菌細胞培養液に添加し、100μg/mLのアンピシリンおよび10^{10}pfu/mLのVCSM 13ヘルパーファージを添加した250mLの2xYT中で37℃で一晩増殖させた。増殖したファージを遠心して集めた後、PEGで沈殿させ、パニングプロセスは総計6回まで反復した。
【０２２５】
308-314の位置の残基(H310およびW313を固定)に変異を含んでいるファージライブラリーで、FcRnに対する親和性がより高いヒンジ-Fc領域を発現しているファージを表IVに示す各パニングプロセスによって濃厚化した。251-256の位置の残基(I253を固定)に変異のあるライブラリー、および428-436の位置の残基(H429, E430, A431, L432, およびH435を固定)に変異のあるライブラリーのパニング結果は表VおよびVIにそれぞれ示している。さらに、385-389の位置の残基(E388を固定)に変異のあるライブラリーのパニング結果は表VIIに示している。
【表４】

表 IV

ライブラリー(残基 308-314; H310 および W313 固定)のパニング
pCANTAB5E-KUNKEL-muFcRn(マウス FcRn)

パニング	アウトプット		濃厚化比
	+ FcRn	- FcRn	
第１ラウンド	1.1×10^5	0.5×10^5	2
第２ラウンド	1×10^4	0.2×10^4	5
第３ラウンド	9×10^4	0.3×10^4	30
第４ラウンド	3×10^5	2×10^4	15

【０２２６】
【表５】

表 V

ライブラリー(残基 251-256; I253 固定)のパニング
pCANTAB5E-KUNKEL-muFcRn

パニング	アウトプット		濃厚化比
	+ FcRn	- FcRn	
第１ラウンド	2.5×10^5	1×10^5	2.5
第２ラウンド	6×10^4	2×10^4	3.0
第３ラウンド	8×10^5	4×10^4	20
第４ラウンド	1.2×10^6	5×10^4	24
第５ラウンド	3.0×10^6	6×10^4	50

【０２２７】

【表６】

表VI

ライブラリー(残基428-436; H429、E430、A431、L432、およびH435固定)のパニング
pCANTAB5E-KUNKEL-muFcRn

パニング	アウトプット		濃厚化比
	+ FcRn	- FcRn	
第１ラウンド	2.3×10^5	0.9×10^5	2.5
第２ラウンド	3×10^4	1×10^4	3
第３ラウンド	2×10^5	2×10^4	10
第４ラウンド	8×10^5	5×10^4	16

【０２２８】
【表７】

表VII

ライブラリー(残基385-389; E388固定)のパニング
pCANTAB5E-KUNKEL-muFcRn

パニング	アウトプット		濃厚化比
	+ FcRn	- FcRn	
第１ラウンド	4.2×10^5	3.8×10^5	1.1
第２ラウンド	5×10^4	0.3×10^4	17
第３ラウンド	3.5×10^5	1×10^4	35
第４ラウンド	5.5×10^5	4×10^4	14
第５ラウンド	7.5×10^5	5×10^4	15
第６ラウンド	2×10^6	1×10^5	20

【０２２９】
6.3 パニングで単離されたクローンの同定
各パニングプロセスの後、ファージを単離し、FcRnと結合した発現されたペプチドをコードする核酸を、ABI3000ゲノム分析機(Applied Biosystems, Foster City, CA)を用いるジデオキシヌクレオチド配列決定法(Sangerら, Proc. Natl. Acad. Sci. USA, 74:5463-5467, 1977)などの標準的な配列決定法で配列を調べた。
【０２３０】
パニングの結果、308-314の位置の残基(H310およびW313を固定)に変異を含んでいるファージライブラリーから2個の変異体が単離され、251-256の位置の残基(I253を固定)に変異のあるライブラリーからは13個の変異体、428-436の位置の残基(H429, E430, A431, L432, およびH435を固定)に変異のあるライブラリーからは6個の変異体、385-389の位置の残基(E388を固定)に変異のあるライブラリーからは9個の変異体が単離された。ライブラリ

ーから単離された変異体は表VIIIに列挙している。

【表８】

表VIII

パニングによって単離された変異体

ライブラリー	変異体*
251-256	Leu **Tyr** Ile **Thr** Arg **Glu** (配列番号90)
	*Leu **Tyr** Ile Ser Arg Thr* (配列番号91)
	*Leu **Tyr** Ile Ser Arg **Ser*** (配列番号92)
	*Leu **Tyr** Ile Ser Arg **Arg*** (配列番号93)

ライブラリー	変異体*
	Leu Tyr Ile Ser Arg Gln (配列番号 94)
	Leu Trp Ile Ser Arg Thr (配列番号 95)
	Leu **Tyr** Ile Ser **Leu Gln** (配列番号 96)
	Leu **Phe** Ile Ser Arg **Asp** (配列番号 97)
	Leu **Phe** Ile Ser Arg **Thr** (配列番号 98)
	Leu **Phe** Ile Ser Arg **Arg** (配列番号 99)
	Leu **Phe** Ile **Thr Gly Ala** (配列番号 100)
	Leu **Ser** Ile Ser Arg **Glu** (配列番号 101)
	Arg Thr Ile Ser **Ile Ser** (配列番号 102)
308-314	**Thr Pro** His **Ser** Asp Trp Leu (配列番号 103)
	Ile Pro His **Glu** Asp Trp Leu (配列番号 104)
385-389	**Arg Thr Arg** Glu **Pro** (配列番号 105)
	Asp Pro Pro Glu Ser (配列番号 106)
	Ser Asp Pro Glu **Pro** (配列番号 107)
	Thr Ser His Glu Asn (配列番号 108)
	Ser Lys Ser Glu Asn (配列番号 109)
	His Arg Ser Glu Asn (配列番号 110)
	Lys Ile Arg Glu Asn (配列番号 111)
	Gly **Ile Thr** Glu **Ser** (配列番号 112)
	Ser Met Ala Glu **Pro** (配列番号 113)
428-436	*Met His Glu Ala Leu Arg Tyr His His* (配列番号 114)
	Met His Glu Ala Leu His **Phe** His **His** (配列番号 115)
	Met His Glu Ala Leu **Lys Phe** His **His** (配列番号 116)
	Met His Glu Ala Leu **Ser Tyr** His **Arg** (配列番号 117)
	Thr His Glu Ala Leu His **Tyr** His **Thr** (配列番号 118)
	Met His Glu Ala Leu His **Tyr** His **Tyr** (配列番号 119)

*置換されている残基は太字で示している。

【０２３１】
表VIIIで下線を付した配列はパニングの最終ラウンドで10から20回生じた配列に対応しており、イタリックで示した配列はパニングの最終ラウンドで2から5回生じた配列に対応している。下線付きでもイタリックでもない配列はパニングの最終ラウンドで1回生じたも

のである。

【０２３２】

6.4 可溶性変異型ヒンジ-Fc領域の発現と精製

変異型のヒンジ-Fcフラグメントをコードする遺伝子を適切な制限酵素で切断し、発現ベクター、例えばVβpelBhis(Ward, J. Mol. Biol., 224:885-890, 1992)中に再クローン化した。その他のいずれかのタイプのタグ配列、例えばc-mycタグ、デカペプチドタグ（Huseら, Science, 246:1275-1281, 1989)、Flag™(Immunex)タグなどを含むベクターを用いることができる。大腸菌などの組換えクローンを増殖させて、可溶性ヒンジ-Fcフラグメントの発現を誘導し、それは培地からまたは細胞溶解物に浸透圧ショックを与えた後該細胞溶解物から、用いたタグに基づいて単離することができ、または当業者にはよく知られた他の精製方法のいずれかで単離することができ、下記に列挙する方法によって特徴を調べることができる。

【０２３３】

6.5 IgG1変異体の構築、産生、および精製

代表的なFc変異、例えばI253A、M252Y/S254T/T256E、M252W、M252Y、M252Y/T256Q、M252F/T256D、V308T/L309P/Q311S、G385D/Q386P/N389S、G385R/Q386T/P387R/N389P、H433K/N434F/Y436H、およびN434F/Y436などをヒトIgG1 MEDI-493(SYNAGIS(登録商標))(Johnsonら, 1997, 上述の文献)中に組み入れた。Quick Change Mutagenesis kit(Stratagene, La Jolla, CA)を製造者の使用説明書に従って用いて重鎖を部位特異的突然変異誘発にかけ、配列をABI3000(Applied Biosystems, Foster City, CA)シーケンサーを用いてジデオキシヌクレオチド配列決定法によって確認した。IgG1/V_HがIgG1/V_Lと同時分泌されるヒト胎児腎細胞293でCMV前初期プロモーターおよびジシストロニックオペロンを用いて種々の構築物が一過性に発現された(Johnsonら, 1997, 上述の文献)。トランスフェクションはLipofectamine 2000(Invitrogen, Carlsbad, CA)と標準的なプロトコールを用いて行った。IgGはならし培地から直接的に1mLのHiTrapプロテインAカラムで製造者(APBiotech)の説明書に従って精製した。

【０２３４】

6.6 変異させたヒンジ-Fc領域の特徴の検討

6.6.1 in vitroでのHPLCおよびSDS-PAGEでの特徴の検討

精製後、修飾ヒンジ-Fcフラグメントの分子量および結合特性などの一般的な特徴については、SDS-PAGEおよびHPLCを含む当業者にはよく知られた方法で検討することができる。

【０２３５】

FcRn結合アッセイ

修飾ヒンジ-Fcフラグメントの結合活性は、放射性標識した野生型ヒンジ-Fcまたは修飾ヒンジ-FcをマウスまたはヒトのFcRnを発現している細胞とインキュベートすることによって測定することができる。典型的には、SV40で形質転換させた内皮細胞(SVEC)(Kimら, J. Immunol., 40:457-465, 1994) などの内皮細胞系が用いられる。ヒンジ-Fcフラグメントと37℃で16-18時間インキュベートした後、細胞を培地で洗い、次いで50mM リン酸バッファー, pH7.5中に5mM Na_2EDTAを含む液と5分間インキュベートして引き離す。10^7個の細胞あたりの放射能を測定する。

【０２３６】

次いで、細胞を、0.3mg/mL PMSF、25mg/mLペプスタチン、および0.1mg/mLアプロチニンを含有する2mLの2.5mg/mL CHAPS、0.1M Tris-HCl, pH8.0中に再懸濁し、室温で30分間インキュベートする。次いで、その細胞懸濁液を遠心し、上清を分離する。その上清の放射能を測定し、10^7個の細胞あたりの抽出されたヒンジ-Fcフラグメントの量を計算するために用いる。

【０２３７】

野生型ヒトIgG1とマウスおよびヒトFcRnとの相互作用のK_d(それぞれ269および2527nM)は他の研究者らによって測定された値とよく一致する(それぞれ265および2350nM、Firanら, 2001, 上述の文献)。I253Aの変異は他の研究者らが報告しているとおり(Kimら, Eur. J.

Immunol., 29:2819-2825, 1991; Shieldsら, J. Biol. Chem., 276:6591-6604, 2001)事実上ヒトおよびマウスFcRnと結合しない。この変異体はマイクロ中和アッセイにおいて野生型分子(SYNAGIS(登録商標))と同じ比活性を保持しているので(Johnsonら, 1997, 上述の文献；データはここには示していない)、このことは抗体の折り畳みの誤りの結果ではない。

【０２３８】

マウスおよびヒトのFcRnに対しての結合親和性が増大しているヒトIgG1変異体を作成した(表VIII)。複合体の安定性の改善は、全体としてヒトIgG1-ヒトFcRnペアは、ヒトIgG1-マウスFcRnよりも低く、野生型IgG1と比較すると、それぞれ30-(N434F/Y436HでΔΔG＝2.0kcal/モル)倍、および11-(M252Y/S254Y/S254T/T256EでΔΔG＝1.4kcal/モル)倍であった。しかし、ヒトおよびマウスFcRnと比較すると最も重要な位置が変化しておらず、IgG1-マウスFcRn複合体の安定性が最も増加していたのは(ΔΔG＞1.3kcal/モル)、252、254、256の位置での変異(M252Y/S254T/T256EおよびM252W)、ならびに433、434、436の位置での変異(H433K/N434F/Y436HおよびN434F/Y436H)であった。同様にして、これらと同じ変異がIgG1-ヒトFcRn相互作用に最も強い影響を与えることが見出され、複合体の安定性も最も大きな増大をもたらした(ΔΔG＞1.0kcal/モル)。308、309、311、385、386、387、および389の位置での置換は、ヒトまたはマウスのFcRnの関与する複合体の安定性にほとんどまたは全く影響を及ぼさなかった(ΔΔG＜0.5kcal/モル)。Fc-FcRn結合部位の中央に位置する残基は周辺部分に位置する残基に比べ複合体の安定性の改善に有意に大きな寄与をする(図9)。

【０２３９】

ヒトFcのマウスFcRnへの効率的な結合にはいくつかの野生型Fcの残基を必要とすることは明らかである。例えば、ロイシンは251の位置で、アルギニンは255で、アスパラギン酸は310で、ロイシンは314で、メチオニンは428で非常によく保存されている(図6)。別の特異性の傾向は、308、309、および311の位置でありそれぞれトレオニン、プロリン、およびセリンは対応する野生型の残基よりも非常に好都合である(図6)。しかし、この強いコンセンサスを有する配列が生ずることは親和性の増大の程度とは相関しないが、それはV308T/L309P/Q311Sは、ヒトおよびマウスのFcRnの双方との結合に対して野生型IgG1とよりは良好だがその程度が2倍未満だからである(表IX)。

【０２４０】

親和性の増大は1箇所の「ホットスポット」の位置の残基の置換に強く依存している。例えば、M252Yでの単一の変異はマウスFcRnとの結合を9倍増大させるが、さらに変異箇所を追加してもほとんど(M252Y/S254T/T256Eの場合)または全く(M252Y/T256Qの場合)追加効果はない。同様の傾向がヒトのレセプターでも程度は低いながら観察される。M252Y/S254T/T256EはM252Yと比較すると2.5倍という顕著な親和性の改善を示す。このことはおそらく、ヒトとマウスのFcRnの結合部位の相異を反映しているのであろう(WestとBjorkman, Biochemistry, 39:9698-9708, 2000)。

【０２４１】

マウスFcRnに対しての親和性の著しい増加(ΔΔG＞1.3kcal/モル)を示しているファージ由来IgG1変異体は、野生型IgG1と比較するとpH7.2でレセプターへの著しく強い結合活性を示した(図8A-H)。親和性が中等度に増大している(ΔΔG＜0.3kcal/モル)IgG1変異体は、pH7.2では結合は非常に弱い(データはここに示していない)。これに対して、ヒトFcRnに対する親和性が大きく増大している(ΔΔG＞1.0kcal/モル)IgG1変異体では、野生型IgG1と比較するとpH7.4での結合はごくわずかであった(図8A-H)。

【表９】

表IX

IgG1/Fc変異体のマウスおよびヒトFcRnへの結合の解離定数と相対的自由エネルギー変化*

変異体	解離定数 Fc/マウスFcRn (nM)	ΔΔG(nM) (kcal/モル)	解離定数 Fc/ヒトFcRn (mM)	ΔΔG (kcal/モル)
野生型	269±1		2527±117	
I253A	NB	NA	NB	NA
M252Y/S254T/T256E	27±6	1.4	225±10	1.4
M252W	30±1	1.3	408±24	1.1
M252Y	41±7	1.1	532±37	0.9
M252Y/T256Q	39±8	1.1	560±102	0.9
M252F/T256D	52±9	1.0	933±170	0.6
V308T/L309P/Q311S	153±23	0.3	1964±84	0.1
G385D/Q386P/N389S	187±10	0.2	2164±331	0.1
G385R/Q386T/P387R/N389P	147±24	0.4	1620±61	0.3
H433K/N434F/Y436H	14±2	1.8	399±47	1.1
N434F/Y436H	9±1	2.0	493±7	1.0

*アフィニティー測定は上述のとおりBIAcoreで行った。残基の番号はEUにしたがった(Kabatら, 1991, 上述の文献)。自由エネルギー変化の差は野生型のΔGと変異型の反応のΔGとの差として計算した(ΔΔG=ΔG野生型−ΔG変異体)。NB：結合なし、NA：適用なし。

【０２４２】

FcRn介在型移送アッセイ

このアッセイはPCT公開 WO 97/34631で開示されているプロトコールに従ったものである。放射性標識した、修飾ヒンジ-Fcフラグメントの種々の濃度のもの(1μg/mL～1mg/mL)をトランスウエルの片側に添加し、FcRnを発現している細胞単層が介在するそのフラグメントの移送を、そのトランスウエルのもう一方の側の放射能を測定することによって定量することができる。

【０２４３】

6.6.2 in vivo薬物動態研究

修飾IgGヒンジ-Fcの半減期を測定するために、修飾ヒンジ-Fcフラグメントを^{125}Iで放射性標識し(比活性は約10^7cpm/μg)、食塩水(pH7.2)中に溶解する。その溶液をBALB/Cマウス(Harlan, Indianapolis, IN)、このマウスはあらかじめ胸腺をブロックするためにNaI含有の水を与えておいたものであるが、そのマウスに液量を150μL以下で放射能は10×10^6～50×10^6cpmのものを静脈内注射する。種々の時点、例えば注射後3分から72時間までマウスの後眼窩洞から採血して血液をヘパリンを添加した毛細管に入れ、各サンプルから採取した血漿の放射能を計測する。

【０２４４】
図10に示すデータを得るために、アッセイする各分子について10匹のマウスを用い、1匹あたり2.5μgの抗体を注射した。血清中の抗体のレベルは抗ヒトIgGELISAを用いて測定した(図10)。マウスFcRnに対する親和性と血清中の持続性には逆相関があるものと思われる。このことはおそらく、pH7.2で観察された変異体の著しい量の結合によって、その分子の隔離(すなわち、血清中への放出の欠如)がもたらされたことによるものと思われる。予備的データでは(ここには示していない)それらの変異体の肺への輸送が増大していることが示唆されている。さらに、これらの変異体のヒトFcRnに対する結合はマウスFcRnに対するものよりも低いので(図8A-H参照)、血清中の抗体レベルは霊長類およびヒトではより高いものと予測される。

【０２４５】
6.6.3 表面プラスモン共鳴分析
可溶性のマウスおよびヒトFcRnと固定化ヒトIgG1変異体との相互作用を、BIAcore 3000装置(Pharmacia Biosensor, Uppsala, Sweden)を用いて表面プラスモン共鳴の検出によってモニターした。ゲルろ過では親和性測定を妨害するような凝集物（van der Merweら, EMBO J., 12:4945-4954, 1993; van der Merweら, Biochemistry, 33:10149-10160, 1994)は検出されなかった。タンパク質濃度はヒトおよびマウスFcRnの双方についてはビシンコニン酸(BCA)法で計算し、IgG1の野生型および変異型については280nmでの1%減衰係数1.5を用いて計算した。IgG1は、Amine Coupling Kitを報告されているとおり用いて(Johnsonら, 上述の文献)、CM5センサーチップのデキストランマトリクス(Pharmacia Biosensor)に結合させた。10mM酢酸ナトリウム,pH5.0中でタンパク質濃度の範囲は3～5μg/mLであった。活性化時間は流速を10μL/分として7分間とし、固定化時間は流速を10μL/分として10分間から20分間の間とした。過剰の反応性エステルは70μLの1.0 塩酸メタノールアミン, pH8.5を注射してクエンチした。このことによって、典型的には、500から4000共鳴ユニット(RU)の固定化がもたらされた。ヒトおよびマウスFcRnはバッファーを0.05% Tween20含有の50mM PBSバッファー, pH6.0に交換した。希釈はその同じバッファー中で行った。結合実験は全て25℃で濃度範囲を120から1μg/mLとし流速を5から10μL/分として行った；データは25から50分間収集し、表面を再生させるためにPBSバッファーpH7.2の1分間のパルスを3回行った。FcRnもコーティングされていない細胞上を流し、これらのブランクのランでのセンサーグラムをIgG1を結合させたチップで得られたものから差し引いた。各ランはソフトウエアBIAevaluation 3.1(Pharmacia)を用いて分析した。会合定数(K_A)は、非特異的結合を補正した後、有利の反応体と複合体の平衡状態での濃度を測定することによるスキャッチャード分析から求めた。結合の平衡状態を見るBIAcore実験で(Karlssonら, 1991, 上述の文献; van der Merweら, 1993, 上述の文献; van der Merweら, 1994, 上述の文献; Raghavanら, Immunity, 1:303-315, 1994; Malchiodiら, J. Exp. Med., 182:1833-1845, 1995)、複合体の濃度は定常状態応答として直接測定することができる。遊離のアナライト(ヒトまたはマウスFcRn)の濃度はアナライト全体の濃度に等しいが、それはアナライトがサンプルの注入の際に常に補給されるからである。センサーチップ上の遊離のリガンドの濃度は複合体の濃度から、および表面の総結合能から$K_A = R_{eq}/C(R_{max} - R_{eq})$として得ることができるが、この式でCは遊離アナライトの濃度、R_{eq}は定常状態応答、R_{max}は表面の総結合能である。式を整理し直すと$R_{eq}/C = K_A R_{max} - K_A R_{eq}$となる。

【０２４６】
従って種々のアナライト濃度でのR_{eq}/C対R_{eq}のプロットは直線となり、その直線から、K_Aを計算することができる(表IX参照)。誤差は2または3回の測定の標準偏差として推定し、それは＜20%であった。

【０２４７】
ライブラリー1から4のパニング後(図6、表VIII)に同定された代表的な変異をヒトIgG1のFc部分に導入した。種々の濃度のヒトまたはマウスFcRnを固定化IgG1変異体上に注入すると、濃度依存性の結合が認められた。変異体M252Y/S254T/T256Eのマウスおよびヒト FcRnへの平衡結合の典型的な共鳴プロフィールを図7AおよびBに示す。見かけのK_Aを推定する

ために、FcRnの濃度範囲として120から1μg/mLを用いた。全例で平衡(または近似的平衡)結合レベルに50分間以内に到達した。タンパク質の総屈折率に及ぼす非特異的影響の結果生ずるRUの増加を推定するために、FcRnのコーティングされていない細胞への結合を測定し、これらのブランクのランからのセンサーグラムをIgG1結合チップで得られたものから差し引いた。変異体M252Y/S254T/T256Eのマウスおよびヒト FcRnへの結合のスキャッチャードプロットは図7CおよびDに示す。それらのプロットは全て直線であり、見かけのK_Aは傾きから計算した。測定は2回または3回重複して行い、固定化IgGがそのもともとの結合活性を保持していることを確認した。

【０２４８】

マウスIgG1上にはマウスFcRnのための2箇所の等価でない結合部位、それらの親和性は＜130nMおよび6μMであるが(Sanchezら, Biochemistry, 38:9471-9476, 1999; Schuckら, Mol. Immunol., 36:1117-1125, 1999; GhetieとWard, Ann. Rev. Immunol., 18:739-766, 2000)、それらの結合部位があるので、IgG1が固定化FcRnと結合する際に生ずる親和性効果を避けるためにレセプターを溶液中で用いた。このことと一致して、IgGではなくFcRnをバイオセンサーチップ上に固定化した場合には、常に高い親和性が観察されている(Popovら, 1996, 上述の文献; VaughnとBjorkman, Biochemistry, 36:9374-9380, 1997; MartinとBjorkman, Biochemistry, 38:12639-12647, 1999; WestとBjorkman、Biochemistry, 39:9698-9708, 2000)。本発明者らの実験でのBIAcoreの条件下では、より高い親和性の会合(すなわち、単一のリガンド-レセプター)に対応する相互作用が主として測定され、それはスキャッチャードプロットの直線性と一致している(図7CおよびD)。

【０２４９】

BIAcore分析はまた、野生型IgG1とIgG1変異体の親和性を比較するためにも用いた。マウスFcRnに対してpH6.0で親和性の著しい増加(ΔΔG≧1.0kcal/モル) を示すファージ由来のIgG1変異体はマウスレセプターとpH7.2で著しい結合をも示し、そのSPRシグナル$_{pH7.4}$/SPRシグナル$_{pH6.0}$は飽和状態で＞0.6であった。マウスFcRnに対してのpH6.0での親和性の増大が中程度である(ΔΔG＜0.4kcal/モル)IgG1変異体は、pH7.2でマウスのレセプターへの結合は非常に弱かった。これに対して、ヒトFcRnに対してのpH6.0での親和性の増大が大きい(ΔΔG≧1.0kcal/モル)IgG1変異体ではヒトレセプターとpH7.4ではごくわずかな結合しか示さず、そのSPRシグナル$_{pH7.4}$/SPRシグナル$_{pH6.0}$は飽和状態で＜0.15であった。

【０２５０】

当業者であれば、本明細書に記載の本発明の特定の実施形態に対して等価な多数のものを理解し、日常的な実験をさらに行うことなく確認することができよう。そのような等価物は添付の特許請求の範囲によって包含されることを意図している。

【０２５１】

本明細書中で言及した刊行物、特許、および特許出願はその全てを、個々の刊行物、特許、および特許出願を特定してかつ個別に本明細書中に参照により組み入れることを指示した場合と同程度に、本明細書中に参照により組み入れる。

【図面の簡単な説明】

【０２５２】

【図１】FcRn受容体との相互作用に関与することが確認された残基の位置を示すIgGヒンジ-Fc領域の構造を示す。

【図２】ヒンジ領域（配列番号82）、CH2ドメイン（配列番号80）およびCH3ドメイン（配列番号81）を含有するヒトIgG1ヒンジ-Fc領域のアミノ酸配列（配列番号83）を示す。

【図３Ａ】ヒトFcRn（配列番号84）のアミノ酸配列を示す。

【図３Ｂ】マウスFcRn（配列番号85）のアミノ酸配列を示す。

【図４】ヒトIgG1ヒンジ-Fc領域（配列番号83）のアミノ酸配列を示す。ここで、アミノ酸置換により突然変異した野生型残基は下線付き太字で示されている。

【図５】ファージ提示修飾ヒンジ-Fcライブラリーに関するパニング法の概要図である。

【図６】スクリーニングしたライブラリー中の変異体位置における選択された突然変異残基の出現の概要を示す。

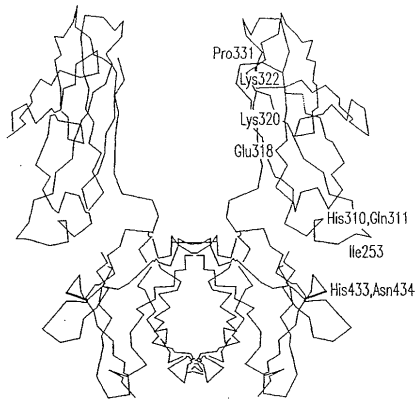
【図７】（A）は、M252Y/S254T/T256E置換を有する固定化IgG1へのマウスFcRnの結合を示す。（B）は、固定化IgG1/M252Y/S254T/T256Eへのヒト FcRnの結合を示す。（C）および（D）は、非特異的結合に関する補正後のそれぞれ（A）および（B）におけるデータのスキャッチャード解析を示す。

【図８】（A）～（D）は、非特異的結合に関する補正後のそれぞれ（A）野生型ヒトIgG1、（B）M252Y/S254T/T256E、（C）H433K/N434F/Y436H、および（D）G385D/G386P/N389SへのpH 6.0およびpH 7.4におけるマウスFcRnの結合のBIAcore分析からの結果を示す。（E）～（H）は、非特異的結合に関する補正後のそれぞれ（E）野生型ヒトIgG1、（F）M252Y/S254T/T256E、（G）H433K/N434F/Y436H、および（H）G385D/Q386P/N389SへのpH 6.0およびpH 7.4におけるヒトFcRnの結合のBIAcore分析からの結果を示す。

【図９】図9は、Deisenhofer, 1981, Biochemistry 20: 2361-2370のヒトIgG1構造に基づくヒトIgG1のFcフラグメントの表面の空間充填型模型を示す。

【図１０】野生型定常ドメインを有する抗体（SYNAGIS（登録商標））（白正方形）、または突然変異M252Y/S254T/T256E（白丸）、G385D/Q386P/N389S（黒正方形）およびH433K/N434F/Y436H（黒丸）を含有する定常ドメインを有する抗体の、時間（日単位）経過に対する血清濃度（[Mab]ng/ml）における変化を示す。

【図１】



【図２】

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                  10                  15
|----------------Hinge-----------------------------------|----
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30
-----------------------------------------------------------------
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45
-----------------------------------------------------------------
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60
-----------------------------------------------------CH2-------
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80
-----------------------------------------------------------------
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95
-----------------------------------------------------------------
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110
-----------------------------------------------------------------
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125
-------------------------------------------|---------------------
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140
-----------------------------------------------------------------
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160
-----------------------------------------------------------CH3---
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175
-----------------------------------------------------------------
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190
-----------------------------------------------------------------
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205
-----------------------------------------------------------------
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220
-----------------------------------------------------------------
Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
------------------------------|
```

【図３Ａ】

```
Met Gly Val Pro Arg Pro Gln Pro Trp Ala Leu Gly Leu Leu Leu Phe
1               5                  10                  15
Leu Leu Pro Gly Ser Leu Gly Ala Glu Ser His Leu Ser Leu Leu Tyr
            20                  25                  30
His Leu Thr Ala Val Ser Ser Pro Ala Pro Gly Thr Pro Ala Phe Trp
        35                  40                  45
Val Ser Gly Trp Leu Gly Pro Gln Gln Tyr Leu Ser Tyr Asn Ser Leu
    50                  55                  60
Arg Gly Glu Ala Glu Pro Cys Gly Ala Trp Val Trp Glu Asn Gln Val
65                  70                  75                  80
Ser Trp Tyr Trp Glu Lys Glu Thr Thr Asp Leu Arg Ile Lys Glu Lys
                85                  90                  95
Leu Phe Leu Glu Ala Phe Lys Ala Leu Gly Gly Lys Gly Pro Tyr Thr
            100                 105                 110
Leu Gln Gly Leu Leu Gly Cys Glu Leu Gly Pro Asp Asn Thr Ser Val
        115                 120                 125
Pro Thr Ala Lys Phe Ala Leu Asn Gly Glu Glu Phe Met Asn Phe Asp
    130                 135                 140
Leu Lys Gln Gly Thr Trp Gly Gly Asp Trp Pro Glu Ala Leu Ala Ile
145                 150                 155                 160
Ser Gln Arg Trp Gln Gln Gln Asp Lys Ala Ala Asn Lys Glu Leu Thr
                165                 170                 175
Phe Leu Leu Phe Ser Cys Pro His Arg Leu Arg Glu His Leu Glu Arg
            180                 185                 190
Gly Arg Gly Asn Leu Glu Trp Lys Glu Pro Pro Ser Met Atg Leu Lys
        195                 200                 205
Ala Arg Pro Ser Ser Pro Gly Phe Ser Val Leu Thr Cys Ser Ala Phe
    210                 215                 220
Ser Phe Tyr Pro Pro Glu Leu Gln Leu Arg Phe Leu Arg Asn Gly Leu
225                 230                 235                 240
Ala Ala Gly Thr Gly Gln Gly Asp Phe Gly Pro Asn Ser Asp Gly Ser
                245                 250                 255
Phe His Ala Ser Ser Ser Leu Thr Val Lys Ser Gly Asp Glu His His
            260                 265                 270
Tyr Cys Cys Ile Val Gln His Ala Gly Leu Ala Gln Pro Leu Arg Val
        275                 280                 285
Glu Leu Glu Ser Pro Ala Lys Ser Ser Val Leu Val Val Gly Ile Val
    290                 295                 300
Ile Gly Val Leu Leu Leu Thr Ala Ala Ala Val Gly Gly Ala Leu Leu
305                 310                 315                 320
Trp Arg Arg Met Arg Ser Gly Leu Pro Ala Pro Trp Ile Ser Leu Arg
                325                 330                 335
Gly Asp Asp Thr Gly Val Leu Leu Pro Thr Pro Gly Glu Ala Gln Asp
            340                 345                 350
Ala Asp Leu Lys Asp Val Asn Val Ile Pro Ala Thr Ala
        355                 360                 365
```

A

【図３Ｂ】

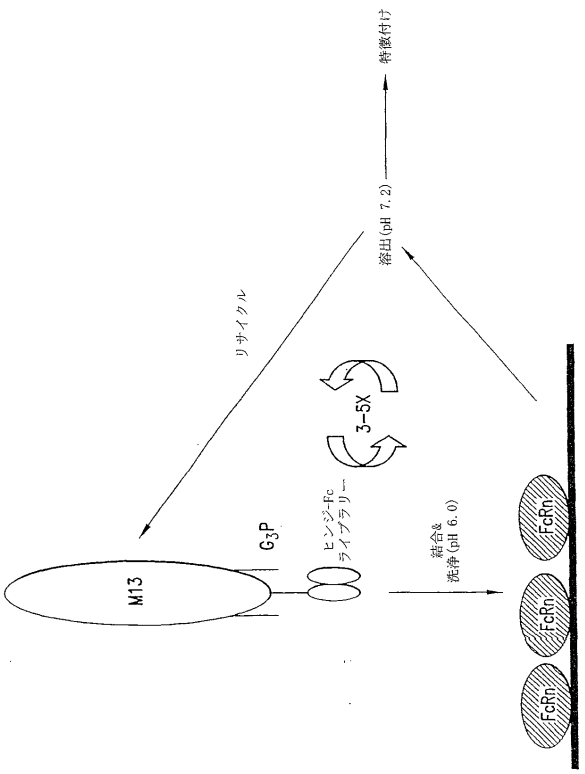
```
Met Gly Met Pro Leu Pro Trp Ala Leu Ser Leu Leu Leu Val Leu Leu
1               5                  10                  15
Pro Gln Thr Trp Gly Ser Glu Thr Arg Pro Pro Leu Met Tyr His Leu
            20                  25                  30
Thr Ala Val Ser Asn Pro Ser Thr Gly Leu Pro Ser Phe Trp Ala Thr
        35                  40                  45
Gly Trp Leu Gly Pro Gln Gln Tyr Leu Thr Tyr Asn Ser Leu Arg Gln
    50                  55                  60
Glu Ala Asp Pro Cys Gly Ala Trp Val Trp Glu Asn Gln Val Ser Trp
65                  70                  75                  80
Tyr Trp Glu Lys Glu Thr Thr Asp Leu Lys Ser Lys Glu Gln Leu Phe
                85                  90                  95
Leu Glu Ala Leu Lys Thr Leu Glu Lys Ile Leu Asn Gly Thr Tyr Thr
            100                 105                 110
Leu Gln Gly Leu Leu Gly Cys Glu Leu Ala Ser Asp Asn Ser Ser Val
        115                 120                 125
Pro Thr Ala Val Phe Ala Leu Asn Gly Glu Glu Phe Met Lys Phe Asn
    130                 135                 140
Pro Arg Ile Gly Asn Trp Thr Gly Glu Trp Pro Glu Thr Glu Ile Val
145                 150                 155                 160
Ala Asn Leu Trp Met Lys Gln Pro Asp Ala Ala Arg Lys Glu Ser Glu
                165                 170                 175
Phe Leu Leu Asn Ser Cys Pro Glu Arg Leu Leu Gly His Leu Glu Arg
            180                 185                 190
Gly Arg Arg Asn Leu Glu Trp Lys Glu Pro Pro Ser Met Arg Leu Lys
        195                 200                 205
Ala Arg Pro Gly Asn Ser Gly Ser Ser Val Leu Thr Cys Ala Ala Phe
    210                 215                 220
Ser Phe Tyr Pro Pro Glu Leu Lys Phe Arg Phe Leu Arg Asn Gly Leu
225                 230                 235                 240
Ala Ser Gly Ser Gly Asn Cys Ser Thr Gly Pro Asn Gly Asp Gly Ser
                245                 250                 255
Phe His Ala Trp Ser Leu Leu Glu Val Lys Arg Gly Asp Glu His His
            260                 265                 270
Tyr Gln Cys Gln Val Glu His Glu Gly Leu Ala Gln Pro Leu Thr Val
        275                 280                 285
Asp Leu Asp Ser Ser Ala Arg Ser Ser Val Pro Val Val Gly Ile Val
    290                 295                 300
Leu Gly Leu Leu Leu Val Val Val Ala Ile Ala Gly Gly Val Leu Leu
305                 310                 315                 320
Trp Gly Arg Met Arg Ser Gly Leu Pro Ala Pro Trp Leu Ser Leu Ser
                325                 330                 335
Gly Asp Asp Ser Gly Asp Leu Leu Pro Gly Gly Asn Leu Pro Pro Glu
            340                 345                 350
Ala Glu Pro Gln Gly Ala Asn Ala Phe Pro Ala Thr Ser
        355                 360                 365
```

B

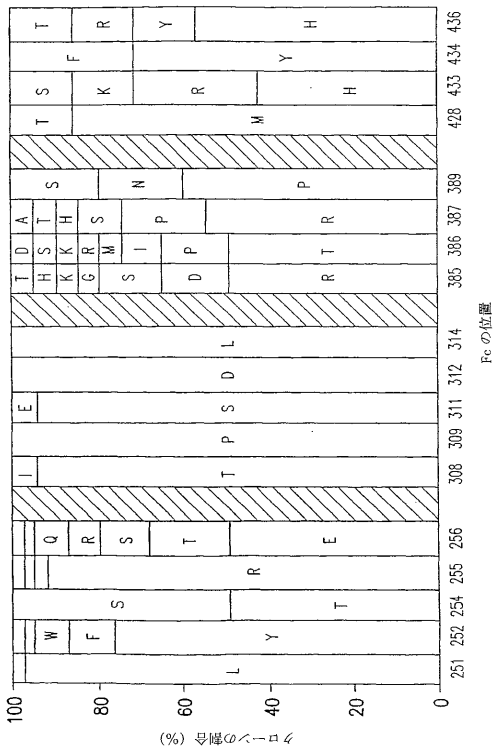
【図４】

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                  10                  15
------------------Hinge-------------------------------|----
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30
-----------------------------------------------------------------
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        40                  45                  50
-----------------------------------------------------------------
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60
-----------------------------------------------------CH2------
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80
-----------------------------------------------------------------
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95
-----------------------------------------------------------------
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110
-----------------------------------------------------------------
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125
-----------------------------------------------|----------------
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140
-----------------------------------------------------------------
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160
-----------------------------------------------------CH3-
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175
-- --------------------------------------------------------------
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190
-----------------------------------------------------------------
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205
-----------------------------------------------------------------
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220
-----------------------------------------------------------------
Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
------------------------------|
```

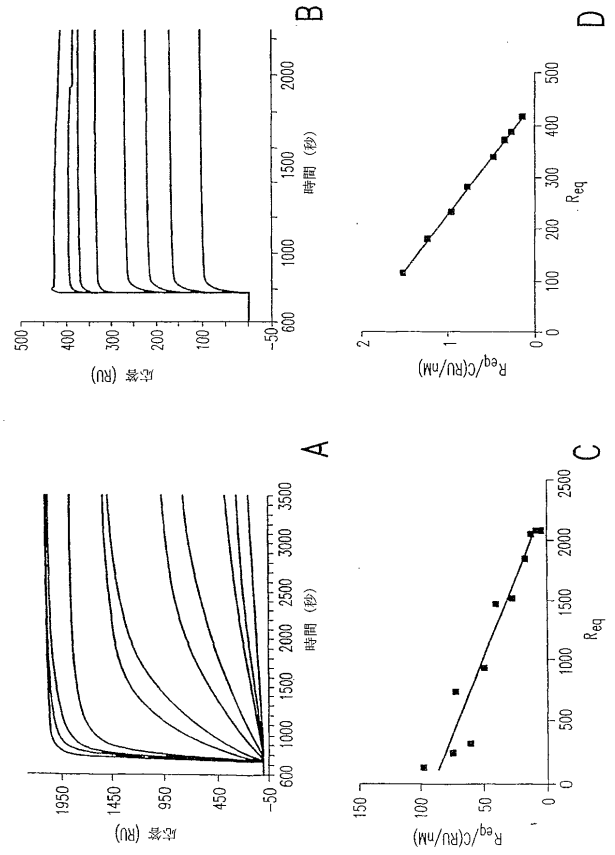
【図５】



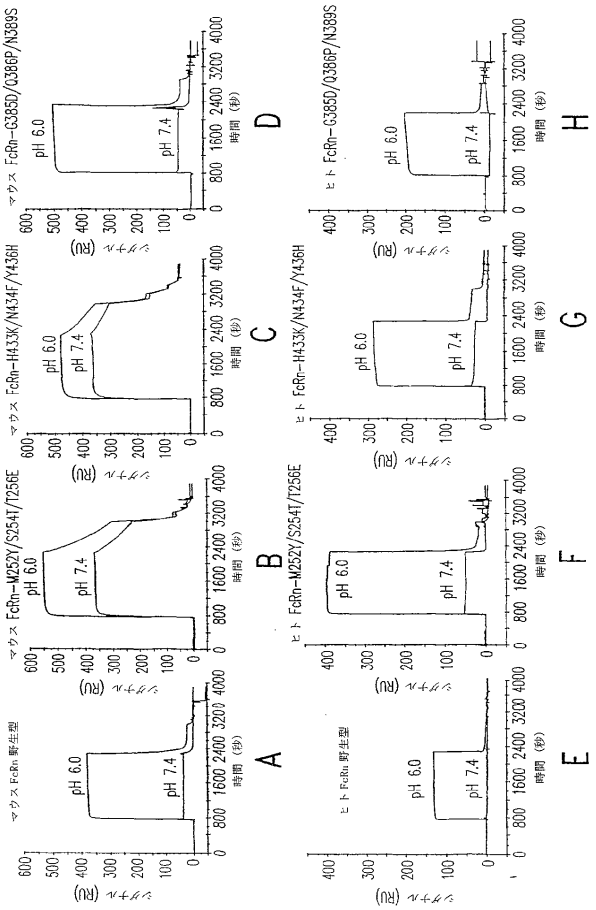
【図６】


プロトンの割合 (%)
Fc の位置

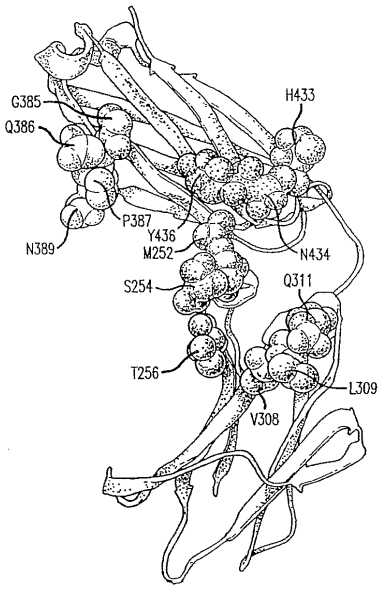
【図７】


A
B
C
D
結合 (RU)
時間 (秒)
Req/C(RU/nM)
Req

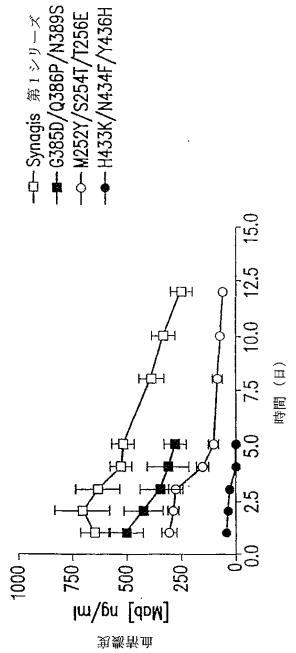
【図８】


A
B
C
D
E
F
G
H
マウス FcRn 野生型
マウス FcRn-M252Y/S254T/T256E
マウス FcRn-H433K/N434F/Y436H
マウス FcRn-G385D/Q386P/N389S
ヒト FcRn 野生型
ヒト FcRn-M252Y/S254T/T256E
ヒト FcRn-H433K/N434F/Y436H
ヒト FcRn-G385D/Q386P/N389S
pH 6.0
pH 7.4
シグナル (RU)
時間 (秒)

【図９】


G385
Q386
P387
N389
M252
Y436
H433
N434
S254
Q311
T256
L309
V308

【図１０】


Synagis 第１シリーズ
G385D/Q386P/N389S
M252Y/S254T/T256E
H433K/N434F/Y436H
血清濃度
[Mab] ng/ml
1000
750
500
250
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
時間（日）

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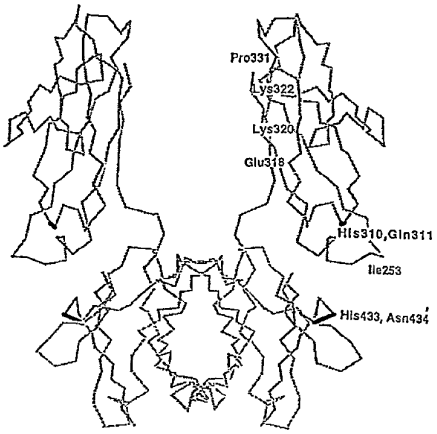
[Continued on next page]

(54) Title: MOLECULES WITH EXTENDED HALF-LIVES, COMPOSITIONS AND USES THEREOF



(57) Abstract: The present invention provides molecules, including IgGs, non-IgG immunoglobulins, proteins and non-protein agents, that have increased *in vivo* half-lives due to the presence of an IgG constant domain, or a portion thereof that binds the FcRn, having one or more amino acid modifications that increase the affinity of the constant domain or fragment for FcRn. Such proteins and molecules with increased half-lives have the advantage that smaller amounts and or less frequent dosing is required in the therapeutic, prophylactic or diagnostic use of such molecules.

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Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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MOLECULES WITH EXTENDED HALF-LIVES, COMPOSITIONS AND USES THEREOF

This application claims the benefit of United States provisional application Serial Nos. 60/254,884, filed December 12, 2000, and 60/289,760, filed May 9, 2001, both of which are incorporated by reference herein in their entireties. This invention was made, in part, with United States Government support under award number A139167 from the National Institute of Health. The United States Government may have certain rights in the invention.

1. INTRODUCTION

The present invention relates to molecules whose *in vivo* half-lives are increased by modification of an IgG constant domain, or FcRn (Fc Receptor-neonate) binding domain thereof. Specifically, these molecules have amino acid modifications that increase the affinity of the constant domain or fragment thereof for the FcRn. Increasing the half-life of therapeutic and diagnostic IgGs and other bioactive molecules using methods of the invention has many benefits including reducing the amount and/or frequency of dosing of these molecules, for example, in vaccines, passive immunotherapy and other therapeutic and prophylactic methods. The invention further relates to fusion proteins containing all or a portion (a FcRn binding portion) of an IgG constant domain having one or more of these amino acid modifications and a non-IgG protein or non-protein molecule conjugated to such a modified IgG constant domain, where the presence of the modified IgG constant domain increases the *in vivo* half-life of the non-IgG protein or molecule.

2. BACKGROUND OF THE INVENTION

The use of immunoglobulins as therapeutic agents has increased dramatically in recent years and have expanded to different areas of medical treatments. Such uses include treatment of agammaglobulinemia and hypogammaglobulinemia, as immunosuppressive agents for treating autoimmune diseases and graft-vs.-host (GVH) diseases, the treatment of lymphoid malignancies, and passive immunotherapies for the treatment of various systemic and infectious diseases. Also, immunoglobulins are useful as *in vivo* diagnostic tools, for example, in diagnostic imaging procedures.

One critical issue in these therapies is the persistence of immunoglobulins in the circulation. The rate of immunoglobulin clearance directly affects the amount and frequency of dosage of the immunoglobulin. Increased dosage and frequency of dosage may cause adverse effects in the patient and also increase medical costs.

IgG is the most prevalent immunoglobulin class in humans and other mammals and is utilized in various types of immunotherapies and diagnostic procedures. The mechanism of IgG catabolism in the circulation has been elucidated through studies related to the transfer of passive immunity from mother to fetus/neonate through the placenta or yolk sac or through colostrum (maternofetal transfer of IgG via transcytosis) in rodents (Brambell, *Lancet*, ii:1087-1093, 1966; Rodewald, *J. Cell Biol.*, 71:666-670, 1976; Morris *et al.*, In: *Antigen Absorption by the Gut*, pp. 3-22, 1978, University Park Press, Baltimore; Jones *et al.*, *J. Clin. Invest.*, 51:2916-2927, 1972).

The involvement of certain receptors in the maternofetal transmission of maternal IgGs was first suggested by Brambell's group in their study on the intestinal absorption of maternal antibodies from ingested milk in newborn rats (Halliday, *Proc. R. Soc. B.*, 143:408-413, 1955; Halliday, *Proc. R. Soc. B.*, 144:427-430, 1955; Halliday, *Proc. R. Soc. B.*, 148:92-103, 1957; Morris, *Proc. R. Soc. B.*, 148:84-91, 1957; Brambell *et al.*, *Proc. R. Soc. B.*, 149:1-11, 1958; Morris, *Proc. R. Soc. B.*, 160:276-292, 1964). Brambell *et al.* suggested, based on the observation that heterologous IgGs interfered with the transmission of a specific antibody, that IgG molecules from various species might have sufficiently similar structures or sequences that bind to common receptors (Brambell *et al.*, *Proc. R. Soc. B.*, 149:1-11, 1958).

A high-affinity Fc receptor, FcRn, has been implicated in this transfer mechanism. The FcRn receptor has been isolated from duodenal epithelial brush borders of suckling rats (Rodewald *et al.*, *J. Cell Biol.*, 99:154s-164s, 1984; Simister *et al.*, *Eur. J. Immunol.*, 15:733-738, 1985) and the corresponding gene has been cloned (Simister *et al.*, *Nature*, 337:184, 1989 and *Cold Spring Harbor Symp. Quant. Biol., LIV*, 571-580, 1989). The later clonings of FcRn-encoding genes from mice (Ahouse *et al.*, *J. Immunol.*, 151:6076-6088, 1993) and humans (Story *et al.*, *J. Exp. Med.*, 180:2377-2381, 1994) demonstrate high homology of these sequences to the rat FcRn, suggesting a similar mechanism of maternofetal transmission of IgGs involving FcRn in these species.

Meanwhile, a mechanism for IgG catabolism was also proposed by Brambell's group (Brambell *et al.*, *Nature*, 203:1352-1355, 1964; Brambell, *Lancet*, ii:1087-1093, 1966). They proposed that a proportion of IgG molecules in the circulation are bound by certain cellular receptors (*i.e.*, FcRn), which are saturable, whereby the IgGs

are protected from degradation and eventually recycled into the circulation; on the other hand, IgGs which are not bound by the receptors are degraded. The proposed mechanism was consistent with the IgG catabolism observed in hypergammaglobulinemic or hypogammaglobulinemic patients. Furthermore, based on his studies as well as others (see, *e.g.,* Spiegelberg *et al.*, *J. Exp. Med.*, 121:323-338, 1965; Edelman *et al.*, *Proc. Natl. Acad. Sci. USA*, 63:78-85, 1969), Brambell also suggested that the mechanisms involved in maternofetal transfer of IgG and catabolism of IgG may be either the same or, at least, very closely related (Brambell, *Lancet*, ii:1087-1093, 1966). Indeed, it was later reported that a mutation in the Fc-hinge fragment caused concomitant changes in catabolism, maternofetal transfer, neonatal transcytosis, and, particularly, binding to FcRn (Ghetie *et al.*, *Immunology Today*, 18(12):592-598, 1997).

These observations suggested that portions of the IgG constant domain control IgG metabolism, including the rate of IgG degradation in the serum through interactions with FcRn. Indeed, increased binding affinity for FcRn increased the serum half-life of the molecule (Kim *et al.*, *Eur. J. Immunol.*, 24:2429-2434, 1994; Popov *et al.*, *Mol. Immunol.*, 33:493-502, 1996; Ghetie *et al.*, *Eur. J. Immunol.*, 26:690-696, 1996; Junghans *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:5512-5516, 1996; Israel *et al.*, *Immunol.*, 89:573-578, 1996).

Various site-specific mutagenesis experiments in the Fc region of mouse IgGs have led to identification of certain critical amino acid residues involved in the interaction between IgG and FcRn (Kim *et al.*, *Eur. J. Immunol.*, 24:2429-2434, 1994; Medesan *et al.*, *Eur. J. Immunol.*, 26:2533, 1996; Medesan *et al.*, *J. Immunol.*, 158:2211-2217, 1997). These studies and sequence comparison studies found that isoleucine at position 253, histidine at position 310, and histidine at position 435 (according to Kabat numbering, Kabat *et al.*, In: *Sequences of Proteins of Immunological Interest*, US Department of Health and Human Services, 1991, which is hereby incorporated by reference in its entirety), are highly conserved in human and rodent IgGs, suggesting their importance in IgG-FcRn binding.

Additionally, various publications describe methods for obtaining physiologically active molecules whose half-lives are modified either by introducing an FcRn-binding polypeptide into the molecules (WO 97/43316; U.S. Patent No. 5,869,046; U.S. Patent No. 5,747,035; WO 96/32478; WO 91/14438) or by fusing the molecules with antibodies whose FcRn-binding affinities are preserved but affinities for other Fc receptors have been greatly reduced (WO 99/43713) or fusing with FcRn binding domains of

antibodies (WO 00/09560; U.S. Patent No. 4,703,039). However, none of these publications disclose specific mutants in the IgG constant domain that affect half-life.

Prior studies have demonstrated that certain constant domain mutations actually reduce binding to FcRn and, thereby, reduce the IgG *in vivo* half-life. PCT publication WO 93/22332 (by Ward *et al.*) discloses various recombinant mouse IgGs whose *in vivo* half-lives are reduced by mutations between about residue 253 and about residue 434. Particularly, substitutions of isoleucine at position 253; histidine at position 310; glutamine at position 311; His at position 433; and asparagine at position 434 were found to reduce IgG half-life.

Modulation of IgG molecules by amino acid substitution, addition, or deletion to increase or reduce affinity for FcRn is also disclosed in WO 98/23289; however, the publication does not list any specific mutants that exhibit either longer or shorter *in vivo* half-lives.

In fact, only one mutant of mouse IgG1 that actually exhibited increased half-life, the triple mutation Thr252 to Ala, Thr254 to Ser, and Thr256 to Phe, has been identified (WO 97/34631).

In view of the pharmaceutical importance of increasing the *in vivo* half-lives of immunoglobulins and other bioactive molecules, there is a need to develop modified IgGs and FcRn-binding fragments thereof, (particularly modified human IgGs) that confer increased *in vivo* half-life on immunoglobulins and other bioactive molecules.

3. SUMMARY OF THE INVENTION

The present invention is based upon the inventors' identification of several mutations in the constant domain of a human IgG molecule that increase the affinity of the IgG molecule for the FcRn. In particular, the present inventors have screened libraries of human IgG1 constant domains with random amino acid mutations introduced into particular regions of the constant domain for increased affinity for FcRn. Such random mutations were made in the regions of residues 251-256, 285-290, and 308-314, all of which are in CH2 domain, and 385-389 and 428-436, which are in CH3 domain, of human IgG1 hinge-Fc regions (residues as depicted in Figure 2 (SEQ ID NO:83 or analogous residues in hinge-Fc regions of other IgG molecules as determined by sequence alignment). As used herein, all residues of the IgG constant domain are numbered according to Kabat *et al.* (Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1991, which is incorporated by reference herein in its entirety) and as presented in Figure 2 (SEQ ID NO:83), and include corresponding residues in other IgG constant domains as

determined by sequence alignment. The *in vivo* half-life, or persistence in serum or other tissues of a subject, of antibodies, and other therapeutic agents and other bioactive molecules is an important clinical parameter which determines the amount and frequency of antibody (or any other pharmaceutical molecule) administration. Accordingly, such molecules, including antibodies, with increased half-life are of significant pharmaceutical importance.

Thus, the present invention relates to a modified molecule (preferably a protein, but may be a non-protein agent) that has an increased *in vivo* half-life by virtue of the presence of a modified IgG constant domain, or FcRn-binding portion thereof (preferably the Fc or hinge-Fc domain) (preferably from a human IgG) wherein the IgG constant domain, or fragment thereof, is modified (*e.g.*, by amino acid substitution, deletion or insertion) to increase the affinity for the FcRn. In a particular embodiment, the present invention relates to modified IgGs, whose *in vivo* half-lives are extended by the modification of amino acid residues identified to be involved in the interaction of the hinge-Fc domain with the FcRn receptor. Preferably, the constant domain or fragment thereof has higher affinity for FcRn at pH 6.0 than at pH 7.4. Such modifications may also alter (*i.e.*, increase or decrease) the bioavailability (*e.g.*, transport to mucosal surfaces, or other target tissues) of the molecules. The invention also relates to other types of immunoglobulins or fragments thereof (*i.e.*, non-IgG immunoglobulins), non-immunoglobulin proteins and non-protein agents that are fused or conjugated to, or engineered to contain, an IgG constant domain, or FcRn-binding fragment thereof, having one or more such amino acid modifications.

In preferred embodiments, the present invention provides molecules, particularly, immunoglobulins whose *in vivo* half-lives are extended by the presence of an IgG constant domain, or FcRn binding fragment thereof (preferably, Fc or hinge-Fc domain), that has modifications of one or more of amino acid residues 251-256, 285-290, 308-314, 385-389, and 428-436 that increase the affinity of the constant domains or fragments thereof for FcRn. In certain embodiments, these modifications preferably exclude residues 252, 254, and 256, in particular when the IgG constant domain or fragment thereof, is murine. In particular embodiments, the modification is at one or more surface-exposed residues, and the modification is a substitution with a residue of similar charge, polarity or hydrophobicity to the residue being substituted. In preferred embodiments, the modified IgG constant domain, or fragment thereof, binds with higher affinity to FcRn at pH 6.0 than at pH 7.4. In a preferred embodiment, the constant domain, or fragment thereof, is modified by substitution of one or more of amino acid residues 251-256, 285-

290, 308-314, 385-389, and 428-436 that increase the affinity of the constant domain or FcRn-binding fragments thereof for FcRn. In certain embodiments, substitutions of residue 252 with leucine, residue 254 with serine, and/or residue 256 with phenylalanine are excluded, particularly when the constant domain or fragment thereof is derived from a mouse IgG.

In specific embodiments, the invention provides immunoglobulins or other bioactive molecules that contain an IgG1 constant domain, or FcRn-binding fragment thereof (preferably Fc or hinge-Fc domain) (preferably human), having amino acid modifications at one or more of position 308, 309, 311, 312, and 314, more specifically, having substitutions at one or more of positions 308, 309, 311, 312 and 314 with threonine, proline, serine, aspartic acid and leucine respectively. In another embodiment, residues at one or more of positions 308, 309, and 311 are substituted with isoleucine, proline, and glutamic acid, respectively. In yet another embodiment, residues at one or more of positions 308, 309, 311, 312, and 314, are substituted with threonine, proline, serine, aspartic acid, and leucine, respectively. The invention further relates to combinations of these amino acid substitutions.

Furthermore, the invention provides immunoglobulins or other bioactive molecules that contain an IgG1 constant domain, or FcRn-binding fragment thereof (preferably, Fc or hinge-Fc domain) (preferably human), having amino acid modifications at one or more of positions 251, 252, 254, 255, and 256, more specifically, having substitutions at one or more of these positions. In specific embodiments, residue 251 is substituted with leucine or arginine, residue 252 is substituted with tyrosine, phenylalanine, serine, tryptophan or threonine, residue 254 is substituted with threonine or serine, residue 255 is substituted with leucine, glycine, isoleucine or arginine, and/or residue 256 is substituted with serine, arginine, glutamine, glutamic acid, aspartic acid, alanine, asparagine or threonine. In a more specific embodiment, residue 251 is substituted with leucine, residue 252 is substituted with tyrosine, residue 254 is substituted with threonine or serine, and/or residue 255 is substituted with arginine. In yet another specific embodiment, residue 252 is substituted with phenylalanine and/or residue 256 is substituted with aspartic acid. In a preferred embodiment, residue 251 is substituted with leucine, residue 252 is substituted with tyrosine, residue 254 is substituted with threonine or serine, and/or residue 255 is substituted with arginine. The invention further relates to any combination of these substitutions.

Furthermore, the invention provides immunoglobulins or other bioactive molecules that contain an IgG1 constant domain, or FcRn-binding fragment thereof

(preferably, Fc or hinge-Fc domain) (preferably human), having amino acid modifications at one or more of positions 428, 433, 434, and 436, more specifically, having substitutions at one or more of these positions. In specific embodiments, residue 428 is substituted with methionine, threonine, leucine, phenylalanine, or serine, residue 433 is substituted with lysine, arginine, serine, isoleucine, proline, glutamine, or histidine, residue 434 is substituted with phenylalanine, tyrosine, or histidine, and/or residue 436 is substituted with histidine, asparagine, arginine, threonine, lysine, methionine, or threonine. In a more specific embodiment, residues at one or more positions 433, 434, and 436 are substituted with lysine, phenylalanine, and histidine, respectively. In a preferred embodiment, residue 428 is substituted with methionine and/or residue 434 is substituted with tyrosine.

Furthermore, the invention provides immunoglobulins or other bioactive molecules that contain an IgG1 constant domain, or FcRn-binding fragment thereof (preferably, Fc or hinge-Fc domain) (preferably human), having amino acid modifications at one or more positions 385, 386, 387, and 389, more specifically, having substitutions at one or more of these positions. In specific embodiments, residue 385 is substituted with arginine, aspartic acid, serine, threonine, histidine, lysine, or alanine, residue 386 is substituted with threonine, proline, aspartic acid, serine, lysine, arginine, isoleucine, or methionine, residue 387 is substituted with arginine, histidine, serine, threonine, alanine, or proline and/or residue 389 is substituted with proline or serine. In more specific embodiments, residues at one or more positions 385, 386, 387, and 389 are substituted with arginine, threonine, arginine, and proline, respectively. In yet another specific embodiment, residues at one or more positions 385, 386, and 389 are substituted with aspartic acid, proline, and serine, respectively.

Molecules of the invention include any combination of the above-described substitutions at one or more of residues 251, 252, 254, 255, 256, 308, 309, 311, 312, 385, 386, 387, 389, 428, 433, 434, and/or 436. In a preferred embodiment, the molecule of the invention contains a Fc region, or FcRn-binding domain thereof, having one or more of the following substitutions: leucine at residue 251, tyrosine at residue 252, threonine or serine at residue 254, arginine at residue 255, threonine at residue 308, proline at residue 309, serine at residue 311, aspartic acid at residue 312, leucine at residue 314, arginine at residue 385, threonine at residue 386, arginine at residue 387, proline at residue 389, methionine at residue 428, and/or tyrosine at residue 434.

Included within the invention are pharmaceutical compositions and methods of prophylaxis and therapy using modified immunoglobulins, proteins and other bioactive molecules of the invention having extended half-lives. Also included are methods of

diagnosis using modified immunoglobulins, proteins and other bioactive molecules of the invention having extended half-lives. In a specific embodiment, the invention provides an anti-respiratory syncytial virus (RSV) antibody useful to treat or prevent RSV infection, such as SYNAGIS® (see U.S. Patent No. 5,824,307 and Johnson *et al.*, *J. Infectious Disease* 176:1215-1224, 1997, both of which are incorporated by reference in their entireties), and other anti-RSV antibodies, including variants of SYNAGIS® (see United States patent Application Serial No., 09/724,396, filed November 28, 2000, United States patent Application Serial No. 09/724,531, filed November 28, 2000, United States patent Application Serial No. ___, filed November 28, 2001 (attorney docket no. 10271-047), and United States patent Application Serial No. ___, filed November 28, 2001 (attorney docket no. 10271-048), all entitled "Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment," all by Young *et al.*, all of which are incorporated by reference herein in their entireties, particularly the sequences of heavy and light chain variable domains and CDRs of anti-RSV antibodies disclosed therein), which has one or more amino acid modifications in the constant domain that increase the affinity of the antibody for FcRn and that has an increased *in vivo* half-life (see also, Section 5.1 *infra*).

3.1 DEFINITIONS

The term "IgG Fc region" as used herein refers the portion of an IgG molecule that correlates to a crystallizable fragment obtained by papain digestion of an IgG molecule. The Fc region consists of the C-terminal half of the two heavy chains of an IgG molecule that are linked by disulfide bonds. It has no antigen binding activity but contains the carbohydrate moiety and the binding sites for complement and Fc receptors, including the FcRn receptor (see below). The Fc fragment contains the entire second constant domain CH2 (residues 231-340 of human IgG1, according to the Kabat numbering system) (*e.g.*, SEQ ID NO:80) and the third constant domain CH3 (residues 341-447) (*e.g.*, SEQ ID NO:81).

The term "IgG hinge-Fc region" or "hinge-Fc fragment" as used herein refers to a region of an IgG molecule consisting of the Fc region (residues 231-447) and a hinge region (residues 216-230; *e.g.*, SEQ ID NO:82) extending from the N-terminus of the Fc region. An example of the amino acid sequence of the human IgG1 hinge-Fc region is SEQ ID NO:83.

The term "constant domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The

constant domain contains the CH1, CH2 and CH3 domains of the heavy chain and the CHL domain of the light chain.

The term "FcRn receptor" or "FcRn" as used herein refers to an Fc receptor ("n" indicates neonatal) which is known to be involved in transfer of maternal IgGs to a fetus through the human or primate placenta, or yolk sac (rabbits) and to a neonate from the colostrum through the small intestine. It is also known that FcRn is involved in the maintenance of constant serum IgG levels by binding the IgG molecules and recycling them into the serum. The binding of FcRn to IgG molecules is strictly pH-dependent with optimum binding at pH 6.0. FcRn comprises a heterodimer of two polypeptides, whose molecular weights are approximately 50 kD and 15 kD, respectively. The extracellular domains of the 50 kD polypeptide are related to major histocompatibility complex (MHC) class I α-chains and the 15 kD polypeptide was shown to be the non-polymorphic β_2-microglobulin (β_2-m). In addition to placenta and neonatal intestine, FcRn is also expressed in various tissues across species as well as various types of endothelial cell lines. It is also expressed in human adult vascular endothelium, muscle vasculature and hepatic sinusoids and it is suggested that the endothelial cells may be most responsible for the maintenance of serum IgG levels in humans and mice. The amino acid sequences of human FcRn and murine FcRn are indicated by SEQ ID NO:84 and SEQ ID NO:85, respectively. Homologs of these sequences having FcRn activity are also included.

The term "*in vivo* half-life" as used herein refers to a biological half-life of a particular type of IgG molecule or its fragments containing FcRn-binding sites in the circulation of a given animal and is represented by a time required for half the quantity administered in the animal to be cleared from the circulation and/or other tissues in the animal. When a clearance curve of a given IgG is constructed as a function of time, the curve is usually biphasic with a rapid α-phase which represents an equilibration of the injected IgG molecules between the intra- and extra-vascular space and which is, in part, determined by the size of molecules, and a longer β-phase which represents the catabolism of the IgG molecules in the intravascular space. The term "*in vivo* half-life" practically corresponds to the half life of the IgG molecules in the β-phase.

An "isolated" or "purified" antibody or fusion protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of an antibody or a fusion protein in which the antibody or the fusion protein is separated from cellular components of the cells from which it is isolated or recombinantly

produced. Thus, an antibody or a fusion protein that is substantially free of cellular material includes preparations of antibody or fusion protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of contaminating protein. When the antibody or the fusion protein is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the antibody or the fusion protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly such preparations of the antibody or the fusion protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the antibody or antibody fragment of interest. In a preferred embodiment of the present invention, antibodies are isolated or purified. In another preferred embodiment of the invention, fusion proteins are isolated or purified.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. An "isolated" nucleic acid molecule does not include cDNA molecules within a cDNA library. In a preferred embodiment of the invention, nucleic acid molecules encoding antibodies are isolated or purified. In another preferred embodiment of the invention, nucleic acid molecules encoding fusion proteins are isolated or purified.

The term "host cell" as used herein refers to the particular subject cell transfected with a nucleic acid molecule or infected with phagemid or bacteriophage and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

The names of amino acids referred to herein are abbreviated either with three-letter or one-letter symbols.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are

then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:2264-2268, modified as in Karlin and Altschul, 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score-50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, http://www.ncbi.nlm.nih.gov). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *CABIOS* 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

4. DESCRIPTION OF THE FIGURES

FIG. 1 shows the structure of the IgG hinge-Fc region indicating the locations of the residues identified to be involved in the interaction with the FcRn receptor (Ghetie *et al.*, *Immunology Today*, 18(12):592-598, 1997).

FIG. 2 shows the amino acid sequence of the human IgG1 hinge-Fc region (SEQ ID NO:83) containing a hinge region (SEQ ID NO:82), CH2 domain (SEQ ID NO:80), and CH3 domain (SEQ ID NO:81).

FIGS. 3 (A and B) show the amino acid sequences of (A) human FcRn (SEQ ID NO:84) and (B) mouse FcRn (SEQ ID NO:85), respectively.

FIG. 4 shows the amino acid sequence of the human IgG1 hinge-Fc region (SEQ ID NO:83), in which wild-type residues which are mutated by amino acid substitutions are indicated in underlined bold-face.

FIG. 5 shows a schematic diagram of panning process for the phage-displayed modified hinge-Fc library.

FIG. 6 shows a summary of the occurrence of selected mutant residues at the variant positions in the libraries screened.

FIGS. 7 (A-D). (A) shows the binding of murine FcRn to immmobilized IgG1 having M252Y/S254T/T256E substitutions. Murine FcRn was injected at 10 different concentrations ranging from 1nM to 556 nM over a surface on which 4000 resonance units (RU) of IgG1 had been coupled. After equilibrium was reached, residual bound protein was eluted with a pulse of PBS, pH 7.4. (B) shows the binding of human FcRn to immmobilized IgG1/M252Y/S254T/T256E. Murine FcRn was injected at 8 different concentrations ranging from 71 nM to 2.86 μM over a surface on which 1000 RU of IgG1 had been coupled. After equilibrium was reached, residual bound protein was eluted with a pulse of PBS, pH 7.4. (C) and (D) show scatchard analyses of the data in (A) and (B), respectively, after correction for nonspecific binding. R_{eq} is the corrected equilibrium response at a given concentration C. The plots are linear with correlation coefficients of 0.97 and 0.998, respectively. The apparent K_d are 24 nM and 225 nM, respectively.

FIGS. 8 (A-H). (A)-(D) show the results from BIAcore analysis of the binding of murine FcRn at pH 6.0 and pH 7.4 to (A) wild type human IgG1, (B) M252Y/S254T/T256E, (C) H433K/N434F/Y436H, and (D) G385D/G386P/N389S, respectively, after correction for nonspecific binding. Murine FcRn was injected at a concentration of 1.1 μm over a surface on which 1000 RU of wild type IgG1, 1000 RU of M252Y/S254T/T256E, 955 RU of H433K/N434F/Y436H, and 939 RU of G385D/Q386P/N389S had been coupled. (E)-(H) show the results from BIAcore analysis

of the binding of human FcRn at pH 6.0 and pH 7.4 to (E) wild type human IgG1, (F) M252Y/S254T/T256E, (G) H433K/N434F/Y436H, and (H) G385D/Q386P/N389S, respectively, after correction for nonspecific binding. Human FcRn was injected at a concentration of 1.4 μm over a surface on which 1000 RU of wild type IgG1, 1000 RU of M252Y/S254T/T256E, 955 RU of H433K/N434F/Y436H, and 939 RU of G385D/Q386P/N389S had been coupled.

FIG. 9 shows the space-filling model of the surface of the Fc fragment of a human IgG1 based upon the human IgG1 structure of Deisenhofer, 1981, *Biochemistry* 20:2361-2370. Residues are color-coded according to the gain of free energy of stabilization of the Fc-FcRn complex: red, substitutions at these positions were found to increase affinity b a factor of at least 2.5 times in the Fc/human FcRn interaction and of at least 5 time in the Fc/mouse FcRn interaction; blue, substitutions at those positions were found to increase affinity by a factor of less than 2 times in both the Fc-human FcRn and Fc-mouse FcRn interaction. The figure was drawn using Swiss pdb viewer (Guex and Peitsch, 1997, *Electrophoresis* 18:2714-2723).

FIG. 10 shows the changes in serum concentration ([Mab] ng/ml) over time (in days) of antibody having a wild type constant domain (SYNAGIS®) (open squares), or constant domains with the following mutations: M252Y/S254T/T256E (open circles), G385D/Q386P/N389S (solid squares), and H433K/N434F/Y436H (solid circles). Antibody concentration was determined using anti-human IgG ELISA.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to molecules, particularly proteins, more particularly immunoglobulins, that have an increased *in vivo* half-life and comprise an IgG constant domain, or fragment thereof that binds to an FcRn (preferably a Fc or hinge-Fc domain), that contains one or more amino acid modifications relative to a wild type IgG constant domain which modifications increase the affinity of the IgG constant domain, or fragment thereof, for the FcRn. In a preferred embodiment, the invention particularly relates to the modification of human or humanized IgGs and other bioactive molecules containing FcRn-binding portions of human IgGs, which have particular use in human therapy, prophylaxis and diagnosis.

5.1 MOLECULES WITH INCREASED *IN VIVO* HALF-LIVES

The present invention is based upon identification of amino acid modifications in particular portions of the IgG constant domain that interact with the FcRn, which modifications increase the affinity of the IgG, or fragment thereof, for the FcRn. Accordingly, the invention relates to molecules, preferably proteins, more preferably immunoglobulins, that comprise an IgG constant domain, or FcRn binding fragment thereof (preferably a Fc or hinge-Fc domain fragment), having one or more amino acid modifications (*i.e.*, substitutions, insertions or deletions) in one or more regions that interact with the FcRn, which modifications increase the affinity of the IgG or fragment thereof, for the FcRn, and also increase the *in vivo* half-life of the molecule. In preferred embodiments, the one or more amino acid modifications are made in one or more of residues 251-256, 285-290, 308-314, 385-389, and 428-436 of the IgG hinge-Fc region (for example, as in the human IgG1 hinge-Fc region depicted in Figure 4, SEQ ID NO:83), or analogous residues thereof, as determined by amino acid sequence alignment, in other IgG hinge-Fc regions. In a preferred embodiment, the amino acid modifications are made in a human IgG constant domain, or FcRn-binding domain thereof. In a certain embodiment, the modifications are not made at residues 252, 254, or 256 (*i.e.*, all are made at one or more of residues 251, 253, 255, 285-290, 308-314, 385-389, or 428-436) of the IgG constant domain. In a more preferred embodiment, the amino acid modifications are not the substitution with leucine at residue 252, with serine at 254, and/or with phenylalanine at position 256. In particular, in preferred embodiments, such modifications are not made when the IgG constant domain, hinge-Fc domain, hinge-Fc domain or other FcRn-binding fragment thereof is derived from a mouse.

The amino acid modifications may be any modification, preferably at one or more of residues 251-256, 285-290, 308-314, 385-389, and 428-436, that increases the *in vivo* half-life of the IgG constant domain, or FcRn-binding fragment thereof (*e.g.*, Fc or hinge-Fc domain), and any molecule attached thereto, and increases the affinity of the IgG, or fragment thereof, for FcRn. Preferably, the one or more modifications also result in a higher binding affinity of the constant domain, or FcRn-binding fragment thereof, for FcRn at pH 6.0 than at pH 7.4. In other embodiments, the modifications alter (*i.e.*, increase or decrease) bioavailability of the molecule, in particular, alters (*i.e.*, increases or decreases) transport (or concentration or half-life) of the molecule to mucosal surfaces (*e.g.*, of the lungs) or other portions of a target tissue. In a preferred embodiment, the amino acid modifications alter (preferably, increase) transport or concentration or half-life of the

molecule to the lungs. In other embodiments, the amino acid modifications alter (preferably, increase) transport (or concentration or half-life) of the molecule to the heart, pancreas, liver, kidney, bladder, stomach, large or small intestine, respiratory tract, lymph nodes, nervous tissue (central and/or peripheral nervous tissue), muscle, epidermis, bone, cartilage, joints, blood vessels, bone marrow, prostate, ovary, uterine, tumor or cancer tissue, etc. In a preferred embodiment, the amino acid modifications do not abolish, or, more preferably, do not alter, other immune effector or receptor binding functions of the constant domain, for example, but not limited to complement fixation, ADCC and binding to FcγRI, FcγRII, and FcγRIII, as can be determined by methods well-known and routine in the art. In another preferred embodiment, the modified FcRn binding fragment of the constant domain does not contain sequences that mediate immune effector functions or other receptor binding. Such fragments may be particularly useful for conjugation to a non-IgG or non-immunoglobulin molecule to increase the *in vivo* half-life thereof. In yet another embodiment, the effector functions are selectively altered (*e.g.*, to reduce or increase effector functions).

In preferred embodiments, the amino acid modifications are substitutions at one or more of residues 308, 309, 311, 312 and 314, particularly a substitution with threonine at position 308, proline at position 309, serine at position 311, aspartic acid at position 312, and/or leucine at position 314. Alternatively, the modification is the substitution with an isoleucine at position 308, proline at position 309, and/or a glutamic acid at position 311. In yet another embodiment, residues at one or more of positions 308, 309, 311, 312, and 314, are substituted with threonine, proline, leucine, alanine, and alanine, respectively. Accordingly, in certain embodiments the residue at position 308 is substituted with threonine or isoleucine, the residue at position 309 is substituted with proline, the residue at position 311 is substituted with serine, glutamic acid or leucine, the residue at position 312 is substituted with alanine, and/or the residue at position 314 is substituted with leucine or alanine. In a preferred embodiment, the substitution is a threonine at position 308, a proline at position 309, a serine at position 311, an aspartic acid at position 312, and/or a leucine at position 314.

In preferred embodiments, the amino acid modifications are substitutions at one or more of residues 251, 252, 254, 255, and 256. In specific embodiments, residue 251 is substituted with leucine or arginine, residue 252 is substituted with tyrosine, phenylalanine, serine, tryptophan or threonine, residue 254 is substituted with threonine or serine, residue 255 is substituted with arginine, leucine, glycine, or isoleucine, and/or residue 256 is substituted with serine, arginine, glutamine, glutamic acid, aspartic acid,

alanine, asparagine or threonine. In a more specific embodiment, residue 251 is substituted with leucine, residue 252 is substituted with tyrosine, residue 254 is substituted with threonine or serine, residue 255 is substituted with arginine, and/or residue 256 is substituted with glutamic acid.

In preferred embodiments, the amino acid modifications are substitutions at one or more of residues 428, 433, 434, and 436. In specific embodiments, residue 428 is substituted with threonine, methionine, leucine, phenylalanine, or serine, residue 433 is substituted with lysine, arginine, serine, isoleucine, proline, glutamine or histidine, residue 434 is substituted with phenylalanine, tyrosine, or histidine, and/or residue 436 is substituted with histidine, asparagine, arginine, threonine, lysine, or methionine. In a more specific embodiment, residues at position 428 and/or 434 are substituted with methionine, and/or histidine respectively.

In preferred embodiments, the amino acid modifications are substitutions at one or more of residues 385, 386, 387, and 389, more specifically, having substitutions at one or more of these positions. In specific embodiments, residue 385 is substituted with arginine, aspartic acid, serine, threonine, histidine, lysine, alanine or glycine, residue 386 is substituted with threonine, proline, aspartic acid, serine, lysine, arginine, isoleucine, or methionine, residue 387 is substituted with arginine, proline, histidine, serine, threonine, or alanine, and/or residue 389 is substituted with proline, serine or asparagine. In more specific embodiments, residues at one or more positions 385, 386, 387, and 389 are substituted with arginine, threonine, arginine, and proline, respectively. In yet another specific embodiment, residues at one or more positions 385, 386, and 389 are substituted with aspartic acid, proline, and serine, respectively.

In particular embodiments, amino acid modifications are made at one or a combination of residues 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, and/or 436, particularly where the modifications are one or more of the amino acid substitutions described immediately above for these residues.

In a preferred embodiment, the molecule of the invention contains a Fc region, or FcRn-binding domain thereof, having one or more of the following substitutions: leucine at residue 251, tyrosine at residue 252, threonine or serine at residue 254, arginine at residue 255, threonine at residue 308, proline at residue 309, serine at residue 311, aspartic acid at residue 312, leucine at residue 314, arginine at residue 385, threonine at residue 386, arginine at residue 387, proline at residue 389, methionine at residue 428, and/or tyrosine at residue 434.

In a preferred embodiment, the FcRn binding domain has a substitution at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 or all 18 of residues 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, and/or 436.

Amino acid modifications can be made by any method known in the art and many such methods are well known and routine for the skilled artisan. For example, but not by way of limitation, amino acid substitutions, deletions and insertions may be accomplished using any well-known PCR-based technique. Amino acid substitutions may be made by site-directed mutagenesis (see, for example, Zoller and Smith, *Nucl. Acids Res.* 10:6487-6500, 1982; Kunkel, *Proc. Natl. Acad. Sci USA* 82:488, 1985, which are hereby incorporated by reference in their entireties). Mutants that result in increased affinity for FcRn and increased *in vivo* half-life may readily be screened using well-known and routine assays, such as those described in Section 5.11, *infra*. In a preferred method, amino acid substitutions are introduced at one or more residues in the IgG constant domain or FcRn-binding fragment thereof and the mutated constant domains or fragments are expressed on the surface of bacteriophage which are then screened for increased FcRn binding affinity (see, in particular, Section 5.2 and 5.11, *infra)*.

Preferably, the amino acid residues to be modified are surface exposed residues. Additionally, in making amino acid substitutions, preferably the amino acid residue to be substituted is a conservative amino acid substitution, for example, a polar residue is substituted with a polar residue, a hydrophilic residue with a hydrophilic residue, hydrophobic residue with a hydrophobic residue, a positively charged residue with a positively charged residue, or a negatively charged residue with a negatively charged residue. Moreover, preferably, the amino acid residue to be modified is not highly or completely conserved across species and/or is critical to maintain the constant domain tertiary structure or to FcRn binding. For example, but not by way of limitation, modification of the histidine at residue 310 is not preferred.

Specific mutants of the Fc domain that have increased affinity for FcRn were isolated after the third-round panning (as described in Section 6) from a library of mutant human IgG1 molecules having mutations at residues 308-314 (histidine at position 310 and tryptophan at position 313 are fixed), those isolated after the fifth-round panning of the library for residues 251-256 (isoleucine at position 253 is fixed), those isolated after fourth-round panning of the library for residues 428-436 (histidine at position 429, glutamic acid at position 430, alanine at position 431, leucine at position 432, and histidine at position 435 are fixed), and those isolated after sixth-round panning of the library for residues 385-389 (glutamic acid at position 388 is fixed) are listed in Table I. The wild type human IgG1 has

a sequence Val-Leu-His-Gln-Asp-Trp-Leu (SEQ ID NO:86) at positions 308-314, Leu-Met-Ile-Ser-Arg-Thr (SEQ ID NO:87) at positions 251-256, Met-His-Glu-Ala-Leu-His-Asn-His-Tyr (SEQ ID NO:88) at positions 428-436, and Gly-Gln-Pro-Glu-Asn (SEQ ID NO:89) at positions 385-389.

Table I

MUTANTS ISOLATED BY PANNING

LIBRARY	MUTANTS*
251-256	Leu **Tyr** Ile **Thr** Arg **Glu** (SEQ ID NO:90)
	*Leu **Tyr** Ile Ser Arg Thr* (SEQ ID NO:91)
	*Leu **Tyr** Ile Ser Arg **Ser*** (SEQ ID NO:92)
	*Leu **Tyr** Ile Ser Arg **Arg*** (SEQ ID NO:93)
	*Leu **Tyr** Ile Ser Arg **Gln*** (SEQ ID NO:94)
	*Leu **Trp** Ile Ser Arg Thr* (SEQ ID NO:95)
	Leu **Tyr** Ile Ser **Leu Gln** (SEQ ID NO:96)
	Leu **Phe** Ile Ser Arg **Asp** (SEQ ID NO:97)
	Leu **Phe** Ile Ser Arg **Thr** (SEQ ID NO:98)
	Leu **Phe** Ile Ser Arg **Arg** (SEQ ID NO:99)
	Leu **Phe** Ile **Thr Gly Ala** (SEQ ID NO:100)
	Leu **Ser** Ile Ser Arg **Glu** (SEQ ID NO:101)
	Arg Thr Ile Ser **Ile Ser** (SEQ ID NO:102)
308-314	**Thr Pro** His **Ser** Asp Trp Leu (SEQ ID NO:103)
	Ile Pro His **Glu** Asp Trp Leu (SEQ ID NO:104)
385-389	**Arg Thr Arg** Glu **Pro** (SEQ ID NO:105)
	Asp Pro** Pro Glu **Ser (SEQ ID NO:106)
	Ser Asp Pro Glu **Pro** (SEQ ID NO:107)
	Thr Ser His Glu Asn (SEQ ID NO:108)
	Ser Lys Ser Glu Asn (SEQ ID NO:109)
	His Arg Ser Glu Asn (SEQ ID NO:110)
	Lys Ile Arg Glu Asn (SEQ ID NO:111)

LIBRARY	MUTANTS*
	Gly **Ile Thr** Glu **Ser** (SEQ ID NO:112)
	Ser Met Ala Glu **Pro** (SEQ ID NO:113)
428-436	*Met His Glu Ala Leu **Arg Tyr** His **His*** (SEQ ID NO:114)
	Met His Glu Ala Leu His **Phe** His **His** (SEQ ID NO:115)
	Met His Glu Ala Leu **Lys Phe** His **His** (SEQ ID NO:116)
	Met His Glu Ala Leu **Ser Tyr** His **Arg** (SEQ ID NO:117)
	Thr His Glu Ala Leu His **Tyr** His **Thr** (SEQ ID NO:118)
	Met His Glu Ala Leu **His Tyr** His **Tyr** (SEQ ID NO:119)

* Substituting residues are indicated in bold face

The underlined sequences in Table I correspond to sequences that occurred 10 to 20 times in the final round of panning and the sequences in italics correspond to sequences that occurred 2 to 5 times in the final round of panning. Those sequences that are neither underlined nor italicized occurred once in the final round of panning.

In one preferred embodiment, the invention provides modified immunoglobulin molecules (*e.g.*, various antibodies) that have increased *in vivo* half-life and affinity for FcRn relative to unmodified molecules (and, in preferred embodiments, altered bioavailabilty such as increased or decreased transport to mucosal surfaces or other target tissues). Such immunoglobulin molecules include IgG molecules that naturally contain an FcRn binding domain and other non-IgG immunoglobulins (*e.g.*, IgE, IgM, IgD, IgA and IgY) or fragments of immunoglobulins that have been engineered to contain an FcRn-binding fragment (*i.e.*, fusion proteins comprising non-IgG immunoglobulin or a portion thereof and an FcRn binding domain). In both cases the FcRn-binding domain has one or more amino acid modifications that increase the affinity of the constant domain fragment for FcRn.

The modified immunoglobulins include any immunoglobulin molecule that binds (preferably, immunospecifically, *i.e.*, competes off non-specific binding), as determined by immunoassays well known in the art for assaying specific antigen-antibody binding) an antigen and contains an FcRn-binding fragment. Such antibodies include, but are not limited to, polyclonal, monoclonal, bi-specific, multi-specific, human, humanized, chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, disulfide-linked Fvs, and fragments containing either a VL or VH domain or even a complementary

determining region (CDR) that specifically binds an antigen, in certain cases, engineered to contain or fused to an FcRn binding domain.

The IgG molecules of the invention, and FcRn-binding fragments thereof, are preferably IgG1 subclass of IgGs, but may also be any other IgG subclasses of given animals. For example, in humans, the IgG class includes IgG1, IgG2, IgG3, and IgG4; and mouse IgG includes IgG1, IgG2a, IgG2b, IgG2c and IgG3. It is known that certain IgG subclasses, for example, mouse IgG2b and IgG2c, have higher clearance rates than, for example, IgG1 (Medesan *et al.*, *Eur. J. Immunol.*, 28:2092-2100, 1998). Thus, when using IgG subclasses other than IgG1, it may be advantageous to substitute one or more of the residues, particularly in the CH2 and CH3 domains, that differ from the IgG1 sequence with those of IgG1, thereby increasing the *in vivo* half-life of the other types of IgG.

The immunoglobulins (and other proteins used herein) may be from any animal origin including birds and mammals. Preferably, the antibodies are human, rodent (*e.g.*, mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described *infra* and, for example, in U.S. Patent No. 5,939,598 by Kucherlapati *et al.*

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide or may be specific for heterologous epitopes, such as a heterologous polypeptide or solid support material. See, *e.g.*, PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, *et al.*, *J. Immunol.*, 147:60-69, 1991; U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny *et al.*, *J. Immunol.*, 148:1547-1553, 1992.

The antibodies of the invention include derivatives that are otherwise modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding antigen and/or generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of

tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas, pp. 563-681 (Elsevier, N.Y., 1981) (both of which are incorporated herein by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with an antigen of interest or a cell expressing such an antigen. Once an immune response is detected, *e.g.*, antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells. Hybridomas are selected and cloned by limiting dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding the antigen. Ascites fluid, which generally contains high levels of antibodies, can be generated by inoculating mice intraperitoneally with positive hybridoma clones.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and $F(ab')_2$ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). $F(ab')_2$ fragments contain the complete light chain, and the variable region, the CH1 region and the hinge region of the heavy chain.

For example, antibodies can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains, such as Fab and Fv or disulfide-bond stabilized Fv, expressed from a repertoire or combinatorial antibody library (*e.g.*, human or murine). Phage expressing an

antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage, including fd and M13. The antigen binding domains are expressed as a recombinantly fused protein to either the phage gene III or gene VIII protein. Alternatively, the modified FcRn binding portion of immunoglobulins of the present invention can be also expressed in a phage display system. Examples of phage display methods that can be used to make the immunoglobulins, or fragments thereof, of the present invention include those disclosed in Brinkman *et al.*, *J. Immunol. Methods*, 182:41-50, 1995; Ames *et al.*, *J. Immunol. Methods*, 184:177-186, 1995; Kettleborough *et al.*, *Eur. J. Immunol.*, 24:952-958, 1994; Persic *et al.*, *Gene*, 187:9-18, 1997; Burton *et al.*, *Advances in Immunology*, 57:191-280, 1994; PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/1 1236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired fragments, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax *et al.*, *BioTechniques*, 12(6):864-869, 1992; and Sawai *et al.*, *AJRI*, 34:26-34, 1995; and Better *et al.*, *Science*, 240:1041-1043, 1988 (each of which is incorporated by reference in its entirety). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston *et al.*, *Methods in Enzymology*, 203:46-88, 1991; Shu *et al.*, *PNAS*, 90:7995-7999, 1993; and Skerra *et al.*, *Science*, 240:1038-1040, 1988.

For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a constant region derived from a human immunoglobulin. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison,

Science, 229:1202, 1985; Oi *et al.*, *BioTechniques*, 4:214 1986; Gillies *et al.*, *J. Immunol. Methods*, 125:191-202, 1989; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. See, *e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; Riechmann *et al.*, *Nature*, 332:323, 1988, which are incorporated herein by reference in their entireties. Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101 and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology*, 28(4/5):489-498, 1991; Studnicka *et al.*, *Protein Engineering*, 7(6):805-814, 1994; Roguska *et al.*, *Proc Natl. Acad. Sci. USA*, 91:969-973, 1994), and chain shuffling (U.S. Patent No. 5,565,332), all of which are hereby incorporated by reference in their entireties.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741, each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.*, 13:65-93, 1995. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.*, PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806;

5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entireties. In addition, companies such as Abgenix, Inc. (Freemont, CA), Medarex (NJ) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers *et al.*, *Bio/technology*, 12:899-903, 1988).

In particular embodiments, the modified antibodies have *in vivo* therapeutic and/or prophylactic uses. Examples of therapeutic and prophylactic antibodies which may be so modified include, but are not limited to, SYNAGIS® (MedImmune, MD) which is a humanized anti-respiratory syncytial virus (RSV) monoclonal antibody for the treatment of patients with RSV infection; HERCEPTIN® (Trastuzumab) (Genentech, CA) which is a humanized anti-HER2 monoclonal antibody for the treatment of patients with metastatic breast cancer; REMICADE® (infliximab) (Centocor, PA) which is a chimeric anti-TNFα monoclonal antibody for the treatment of patients with Crone's disease; REOPRO® (abciximab) (Centocor) which is an anti-glycoprotein IIb/IIIa receptor on the platelets for the prevention of clot formation; ZENAPAX® (daclizumab) (Roche Pharmaceuticals, Switzerland) which is an immunosuppressive, humanized anti-CD25 monoclonal antibody for the prevention of acute renal allograft rejection. Other examples are a humanized anti-CD18 $F(ab')_2$ (Genentech); CDP860 which is a humanized anti-CD18 $F(ab')_2$ (Celltech, UK); PRO542 which is an anti-HIV gp120 antibody fused with CD4 (Progenics/Genzyme Transgenics); Ostavir which is a human anti Hepatitis B virus antibody (Protein Design Lab/Novartis); PROTOVIR™ which is a humanized anti-CMV IgG1 antibody (Protein Design Lab/Novartis); MAK-195 (SEGARD) which is a murine anti-TNF-α $F(ab')_2$ (Knoll Pharma/BASF); IC14 which is an anti-CD14 antibody (ICOS Pharm); a humanized anti-VEGF IgG1 antibody (Genentech); OVAREX™ which is a murine anti-CA 125 antibody (Altarex); PANOREX™ which is a murine anti-17-IA cell surface antigen IgG2a antibody (Glaxo Wellcome/Centocor); BEC2 which is a murine anti-idiotype (GD3 epitope) IgG antibody (ImClone System); IMC-C225 which is a chimeric anti-EGFR IgG antibody (ImClone System); VITAXIN™ which is a humanized anti-αVβ3 integrin antibody (Applied Molecular Evolution/MedImmune); Campath 1H/LDP-03 which is a humanized anti CD52 IgG1 antibody (Leukosite); Smart M195 which is a humanized anti-CD33 IgG antibody (Protein Design Lab/Kanebo); RITUXAN™ which is a chimeric anti-CD20 IgG1

antibody (IDEC Pharm/Genentech, Roche/Zettyaku); LYMPHOCIDE™ which is a humanized anti-CD22 IgG antibody (Immunomedics); Smart ID10 which is a humanized anti-HLA antibody (Protein Design Lab); ONCOLYM™ (Lym-1) is a radiolabelled murine anti-HLA DIAGNOSTIC REAGENT antibody (Techniclone); ABX-IL8 is a human anti-IL8 antibody (Abgenix); anti-CD11a is a humanized IgG1 antibody (Genetech/Xoma); ICM3 is a humanized anti-ICAM3 antibody (ICOS Pharm); IDEC-114 is a primatied anti-CD80 antibody (IDEC Pharm/Mitsubishi); ZEVALIN™ is a radiolabelled murine anti-CD20 antibody (IDEC/Schering AG); IDEC-131 is a humanized anti-CD40L antibody (IDEC/Eisai); IDEC-151 is a primatized anti-CD4 antibody (IDEC); IDEC-152 is a primatized anti-CD23 antibody (IDEC/Seikagaku); SMART anti-CD3 is a humanized anti-CD3 IgG (Protein Design Lab); 5G1.1 is a humanized anti-complement factor 5 (C5) antibody (Alexion Pharm); D2E7 is a humanized anti-TNF-α antibody (CAT/BASF); CDP870 is a humanized anti-TNF-α Fab fragment (Celltech); IDEC-151 is a primatized anti-CD4 IgG1 antibody (IDEC Pharm/SmithKline Beecham); MDX-CD4 is a human anti-CD4 IgG antibody (Medarex/Eisai/Genmab); CDP571 is a humanized anti-TNF-α IgG4 antibody (Celltech); LDP-02 is a humanized anti-α4β7 antibody (LeukoSite/Genentech); OrthoClone OKT4A is a humanized anti-CD4 IgG antibody (Ortho Biotech); ANTOVA™ is a humanized anti-CD40L IgG antibody (Biogen); ANTEGREN™ is a humanized anti-VLA-4 IgG antibody (Elan); MDX-33 is a human anti-CD64 (FcγR) antibody (Medarex/Centeon); SCH55700 is a humanized anti-IL-5 IgG4 antibody (Celltech/Schering); SB-240563 and SB-240683 are humanized anti-IL-5 and IL-4 antibodies, respectively, (SmithKline Beecham); rhuMab-E25 is a humanized anti-IgE IgG1 antibody (Genentech/Norvartis/Tanox Biosystems); IDEC-152 is a primatized anti-CD23 antibody (IDEC Pharm); ABX-CBL is a murine anti CD-147 IgM antibody (Abgenix); BTI-322 is a rat anti-CD2 IgG antibody (Medimmune/Bio Transplant); Orthoclone/OKT3 is a murine anti-CD3 IgG2a antibody (ortho Biotech); SIMULECT™ is a chimeric anti-CD25 IgG1 antibody (Novartis Pharm); LDP-01 is a humanized anti-β_2-integrin IgG antibody (LeukoSite); Anti-LFA-1 is a murine anti CD18 F(ab')$_2$ (Pasteur-Merieux/Immunotech); CAT-152 is a human anti-TGF-β_2 antibody (Cambridge Ab Tech); and Corsevin M is a chimeric anti-Factor VII antibody (Centocor).

In specific embodiments, the invention provides modified antibodies having one or more of the mutations described herein and that immunospecifically bind RSV, *e.g.*, SYNAGIS®. The present invention also provides modified antibodies having one or more of the mutations described herein and that comprise a variable heavy (VH) and/or variable light (VL) domain having the amino acid sequence of any VH and/or VL domain listed in

Table III. The present invention further encompasses anti-RSV antibodies comprising one or more VH complementarity determining regions (CDRs) and/or one or more VL CDRs having the amino acid sequence of one or more VH CDRs and/or VL CDRS listed in Table III or one or more of the CDRs listed in Table II wherein one or more of the bolded and underlined residues has an amino acid substitution, preferably that increases the affinity of the antibody for RSV. In specific embodiments, the antibody to be modified is AFFF, p12f2, p12f4, p11d4, Ale109, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L215B10, A13A11, A1H5, A4B4(1), A4B4L1FR-S28R, A4B4-F52S.

Table II.

CDR Sequences of SYNAGIS®

CDR	Sequence	SEQ ID NO:
VH1	T**S**GMSVG	1
VH2	DIWWD**D**K**KD**YNPSLK**S**	2
VH3	**S**MI**T**N**W**YFDV	3
VL1	**KCQLS**VGYMH	4
VL2	DT**SKLA**S	5
VL3	FQGS**G**YP**F**T	6

Table III
ANTI-RSV ANTIBODIES

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
SYNAGIS	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNWYFDV (SEQ ID NO:3)	SEQ ID NO:8	KCQLSVGYMH (SEQ ID NO:4)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
AFFF	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNEYFDV (SEQ ID NO:12)	SEQ ID NO:13	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQESGYPFT (SEQ ID NO:16)
p12f2	SEQ ID NO:17	TPGMSVG (SEQ ID NO:18)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:21	SLSSRVGYMH (SEQ ID NO:22)	DTFYLSS (SEQ ID NO:23)	FQGSGYPFT (SEQ ID NO:6)
p12f4	SEQ ID NO:24	TPGMSVG (SEQ ID NO:18)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:26	SLSSRVGYMH (SEQ ID NO:22)	DTRGLPS (SEQ ID NO:27)	FQGSGYPFT (SEQ ID NO:6)
p11d4	SEQ ID NO:28	TPGMSVG (SEQ ID NO:18)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:30	SPSSRVGYMH (SEQ ID NO:31)	DTMRLAS (SEQ ID NO:32)	FQGSGYPFT (SEQ ID NO:6)
Ale109	SEQ ID NO:33	TAGMSVG (SEQ ID NO:10)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:34	SLSSRVGYMH (SEQ ID NO:22)	DTFKLSS (SEQ ID NO:35)	FQGSGYPFT (SEQ ID NO:6)
A12a6	SEQ ID NO:36	TAGMSVG (SEQ ID NO:10)	DIWWDGKKDYNPSLKD (SEQ ID NO:37)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:38	SASSRVGYMH (SEQ ID NO:39)	DTFKLSS (SEQ ID NO:35)	FQGSGYPFT (SEQ ID NO:6)
A13c4	SEQ ID NO:40	TAGMSVG (SEQ ID NO:10)	DIWWDGKKSYNPSLKD (SEQ ID NO:41)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:42	SLSSRVGYMH (SEQ ID NO:22)	DTMYQSS (SEQ ID NO:43)	FQGSGYPFT (SEQ ID NO:6)
A17d4	SEQ ID NO:44	TAGMSVG (SEQ ID NO:10)	DIWWDDKKSYNPSLKD (SEQ ID NO:45)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:46	LPSSRVGYMH (SEQ ID NO:47)	DTMYQSS (SEQ ID NO:43)	FQGSGYPFT (SEQ ID NO:6)

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
A4B4	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:49	SASSRVGYMH (SEQ ID NO:39)	DTFFLDS (SEQ ID NO:50)	FQGSGYPFT (SEQ ID NO:6)
A8C7	SEQ ID NO:51	TAGMSVG (SEQ ID NO:10)	DIWWDDKKSYNPSLKD (SEQ ID NO:45)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:52	SPSSRVGYMH (SEQ ID NO:31)	DTRYQSS (SEQ ID NO:53)	FQGSGYPFT (SEQ ID NO:6)
IX-493L1FR	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNWYFDV (SEQ ID NO:3)	SEQ ID NO:54	SASSSVGYMH (SEQ ID NO:14)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
H3-3F4	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
M3H9	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTYKQTS (SEQ ID NO:57)	FQGSGYPFT (SEQ ID NO:6)
Y10H6	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:58	SASSSVGYMH (SEQ ID NO:14)	DTRYLSS (SEQ ID NO:59)	FQGSGYPFT (SEQ ID NO:6)
DG	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
AFFF(1)	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNFYFDV (SEQ ID NO:12)	SEQ ID NO:60	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSFYPFT (SEQ ID NO:61)
6H8	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:62	SASSSVGYMH (SEQ ID NO:14)	DTFKLLS (SEQ ID NO:63)	FQGSGYPFT (SEQ ID NO:6)
L1-7E5	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:64	SASSRVGYMH (SEQ ID NO:39)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
L215B10	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:65	SASSSVGYMH (SEQ ID NO:14)	DTFRLAS (SEQ ID NO:66)	FQGSGYPFT (SEQ ID NO:6)
A13A11	SEQ ID NO:67	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:68	SPSSRVGYMH (SEQ ID NO:31)	DTYRHSS (SEQ ID NO:69)	FQQSGYPFT (SEQ ID NO:5)

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
A1H5	SEQ ID NO:70	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKD (SEQ ID NO:25)	DMEPNFYFDV (SEQ ID NO:29)	SEQ ID NO:71	SASSSVGYMH (SEQ ID NO:72)	DTFFHRS (SEQ ID NO:23)	FQGSGYPFT (SEQ ID NO:6)
A4B4(1)	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKD (SEQ ID NO:19)	DMEPNFYFDV (SEQ ID NO:20)	SEQ ID NO:74	SASSRVGYMH (SEQ ID NO:39)	DTLLLDS (SEQ ID NO:75)	FQGSGYPFT (SEQ ID NO:6)
A4B4LIF R-S28R	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKD (SEQ ID NO:19)	DMEPNFYFDV (SEQ ID NO:20)	SEQ ID NO:11	SASSRVGYMH (SEQ ID NO:39)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
A4B4 F52S	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKD (SEQ ID NO:19)	DMEPNFYFDV (SEQ ID NO:20)	SEQ ID NO:76	SASSRVGYMH (SEQ ID NO:39)	DTSELDS (SEQ ID NO:77)	FQGSGYPFT (SEQ ID NO:6)

In other embodiments, the antibody is a modified anti-$\alpha_v\beta_3$ antibody, preferably a Vitaxin antibody (see, PCT publications WO 98/33919 and WO 00/78815, both by Huse *et al.*, and both of which are incorporated by reference herein in their entireties).

Modified IgGs of the present invention having longer half-lives than wild type may also include IgGs whose bioactive sites, such as antigen-binding sites, Fc-receptor binding sites, or complement-binding sites, are modified by genetic engineering to increase or reduce such activities compared to the wild type.

Modification of these and other therapeutic antibodies to increase the *in vivo* half-life permits administration of lower effective dosages and/or less frequent dosing of the therapeutic antibody. Such modification to increase *in vivo* half-life can also be useful to improve diagnostic immunoglobulins as well, for example, permitting administration of lower doses to achieve sufficient diagnostic sensitivity.

The present invention also provides fusion proteins comprising a bioactive molecule and an hinge-Fc region or a fragment thereof (preferably human) having one or more modifications (*i.e.*, substitutions, deletions, or insertions) in amino acid residues identified to be involved in the interaction between the hinge-Fc region and the FcRn receptor. In particular, the present invention provides fusion proteins comprising a bioactive molecule recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a CH2 domain having one or more modifications in amino acid residues 251-256, 285-290, and/or amino acid residues 308-314, and/or to a CH3 domain having one or more modifications in amino acid residues 385-389 and/or 428-436, in particular, one or more of the amino acid substitutions discussed above. The fusion of a bioactive molecule to a constant domain or a fragment thereof with one or more of such modifications increases the *in vivo* half-life of the bioactive molecule.

In a preferred embodiment, fusion proteins of the invention comprise a bioactive molecule recombinantly fused or chemically conjugated to a CH2 domain having one or more amino acid residue substitutions in amino acid residues 251-256, 285-290, and/or amino acid residues 308-314, and/or to a CH3 domain having one or more modifications in amino acid residues 385-389 and/or 428-436. In certain embodiments, a fusion protein comprises a CH2 domain of IgG molecule in which amino acid residues 253, 310, and 313 are not modified. In another embodiments, a fusion protein comprises a CH3 domain of IgG molecule in which amino acid residues 388, 429, 430, 431, 432, and 435 are not modified.

A bioactive molecule can be any polypeptide or synthetic drug known to one of skill in the art. Preferably, a bioactive molecule is a polypeptide consisting of at least 5,

preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acid residues. Examples of bioactive polypeptides include, but are not limited to, various types of antibodies, cytokines (*e.g.*, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-15, IFN-γ, IFN-α, and IFN-β), cell adhesion molecules (*e.g.*, CTLA4, CD2, and CD28), ligands (*e.g.*, TNF-α,, TNF-β, and an anti-angiogenic factor such as endostatin), receptors, antibodies and growth factors (*e.g.*, PDGF, EGF, NGF, and KGF).

A bioactive molecule can also be a therapeutic moiety such as a cytotoxin (*e.g.*, a cytostatic or cytocidal agent), a therapeutic agent or a radioactive element (*e.g.*, alpha-emitters, gamma-emitters, etc.). Examples of cytostatic or cytocidal agents include, but are not limited to, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The present invention also provides polynucleotides comprising a nucleotide sequence encoding a modified IgG of the invention and fragments thereof which contain the modified FcRn binding sites with increased affinity and vectors comprising said polynucleotides. Furthermore, the invention includes polynucleotides that hybridize under stringent or lower stringent hybridization conditions to polynucleotides encoding modified IgGs of the present invention.

The nucleotide sequence of modified IgGs and the polynucleotides encoding the same may be obtained by any methods known in the art, including general DNA sequencing method, such as dideoxy chain termination method (Sanger sequencing), and oligonucleotide priming in combination with PCR, respectively.

5.2. IDENTIFICATION OF MUTATIONS WITHIN THE HINGE-FC REGION OF IMMUNOGLOBULIN MOLECULES

One or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and 428-436 of the constant domain may be introduced utilizing any technique known to those of skill in the art. The constant domain or fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and 428-436 may be screened by, for example, a binding assay to identify the constant domain or fragment thereof with increased affinity for the FcRn receptor (*e.g.*, as described in section 5.11, *infra*). Those modifications in the hinge-Fc domain or the fragments thereof which increase the affinity of the constant domain or fragment thereof for the FcRn receptor can be introduced into antibodies to increase the *in vivo* half-lives of said antibodies. Further, those modifications in the constant domain or the fragment thereof which increase the affinity of the constant domain or fragment thereof for the FcRn can be fused to bioactive molecules to increase the *in vivo* half-lives of said bioactive molecules (and, preferably alter (increase or decrease) the bioavailability of the molecule, for example, to increase or decrease transport to mucosal surfaces (or other target tissue) (*e.g.*, the lungs).

5.2.1. MUTAGENESIS

Mutagenesis may be performed in accordance with any of the techniques known in the art including, but not limited to, synthesizing an oligonucleotide having one or more modifications within the sequence of the constant domain of an antibody or a fragment thereof (*e.g.*, the CH2 or CH3 domain) to be modified. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to about 75 nucleotides or more in length is preferred, with about 10 to about 25 or more residues on both sides of the junction of the sequence being altered. A number of such primers introducing a variety of different mutations at one or more positions may be used to generated a library of mutants.

The technique of site-specific mutagenesis is well known in the art, as exemplified by various publications (see, *e.g.*,. Kunkel *et al.*, *Methods Enzymol.*, 154:367-82, 1987, which is hereby incorporated by reference in its entirety). In general, site-directed mutagenesis is performed by first obtaining a single-stranded vector or melting

apart of two strands of a double stranded vector which includes within its sequence a DNA sequence which encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as T7 DNA polymerase, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform or transfect appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement. As will be appreciated, the technique typically employs a phage vector which exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially available and their use is generally well known to those skilled in the art. Double stranded plasmids are also routinely employed in site directed mutagenesis which eliminates the step of transferring the gene of interest from a plasmid to a phage.

Alternatively, the use of PCR™ with commercially available thermostable enzymes such as *Taq* DNA polymerase may be used to incorporate a mutagenic oligonucleotide primer into an amplified DNA fragment that can then be cloned into an appropriate cloning or expression vector. See, *e.g.*, Tomic *et al.*, *Nucleic Acids Res.*, 18(6):1656, 1987, and Upender *et al.*, *Biotechniques*, 18(1):29-30, 32, 1995, for PCR™ - mediated mutagenesis procedures, which are hereby incorporated in their entireties. PCR™ employing a thermostable ligase in addition to a thermostable polymerase may also be used to incorporate a phosphorylated mutagenic oligonucleotide into an amplified DNA fragment that may then be cloned into an appropriate cloning or expression vector (see *e.g.*, Michael, *Biotechniques*, 16(3):410-2, 1994, which is hereby incorporated by reference in its entirety).

Other methods known to those of skill in art of producing sequence variants of the Fc domain of an antibody or a fragment thereof can be used. For example, recombinant vectors encoding the amino acid sequence of the constant domain of an antibody or a fragment thereof may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants.

5.2.2. PANNING

Vectors, in particular, phage, expressing constant domains or fragments thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436 can be screened to identify constant domains or fragments thereof

having increased affinity for FcRn to select out the highest affinity binders from a population of phage. Immunoassays which can be used to analyze binding of the constant domain or fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436 to the FcRn include, but are not limited to, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, and fluorescent immunoassays. Such assays are routine and well known in the art (see, *e.g.*, Ausubel *et al.*, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly herein below (but are not intended by way of limitation). BIAcore kinetic analysis can also be used to determine the binding on and off rates of a constant domain or a fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436 to the FcRn. BIAcore kinetic analysis comprises analyzing the binding and dissociation of a constant domain or a fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436 from chips with immobilized FcRn on their surface (see section 5.1 and the Example section *infra*).

5.2.3. SEQUENCING

Any of a variety of sequencing reactions known in the art can be used to directly sequence the nucleotide sequence encoding constant domains or fragments thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert (*Proc. Natl. Acad. Sci. USA*, 74:560, 1977) or Sanger (*Proc. Natl. Acad. Sci. USA*, 74:5463, 1977). It is also contemplated that any of a variety of automated sequencing procedures can be utilized (*Bio/Techniques*, 19:448, 1995), including sequencing by mass spectrometry (see, *e.g.*, PCT Publication No. WO 94/16101, Cohen *et al.*, *Adv. Chromatogr.*, 36:127-162, 1996, and Griffin *et al.*, *Appl. Biochem. Biotechnol.*, 38:147-159, 1993).

5.3. RECOMBINANT METHODS OF PRODUCING ANTIBODIES

The antibodies of the invention or fragments thereof can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

The nucleotide sequence encoding an antibody may be obtained from any information available to those of skill in the art (*i.e.*, from Genbank, the literature, or by routine cloning). If a clone containing a nucleic acid encoding a particular antibody or an epitope-binding fragment thereof is not available, but the sequence of the antibody molecule or epitope-binding fragment thereof is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A^+ RNA, isolated from any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5 'ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et al.*, 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and Ausubel *et al.*, eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence by, for example, introducing amino acid substitutions, deletions, and/or insertions into the epitope-binding domain regions of the antibodies and preferably, into the hinge-Fc regions of the antibodies which are involved in the interaction with the FcRn. In a preferred embodiment, antibodies having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and 428-436 are generated.

Recombinant expression of an antibody requires construction of an expression vector containing a nucleotide sequence that encodes the antibody. Once a nucleotide sequence encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably, but not necessarily, containing the heavy or light chain variable region) has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and

appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding the constant region of the antibody molecule with one or more modifications in the amino acid residues involved in the interaction with the FcRn (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464). The nucleotide sequence encoding the heavy-chain variable region, light-chain variable region, both the heavy-chain and light-chain variable regions, an epitope-binding fragment of the heavy- and/or light-chain variable region, or one or more complementarity determining regions (CDRs) of an antibody may be cloned into such a vector for expression.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody having an increased affinity for the FcRn and an increased *in vivo* half-life. Thus, the invention includes host cells containing a polynucleotide encoding an antibody, a constant domain or a FcRn binding fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436, preferably, operably linked to a heterologous promoter.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; and tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; and mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3 and NSO cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for

the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, *Gene*, 45:101, 1986, and Cockett *et al.*, *Bio/Technology*, 8:2, 1990).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, *EMBO*, 12:1791, 1983), in which the antibody coding sequence may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; and pIN vectors (Inouye & Inouye, *Nucleic Acids Res.*, 13:3101-3109, 1985 and Van Heeke & Schuster, *J. Biol. Chem.*, 24:5503-5509, 1989).

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized to express an antibody molecule of the invention. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region El or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, *Proc. Natl. Acad. Sci. USA*, 81:355-359, 1984). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of

appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bitter *et al.*, *Methods in Enzymol.*, 153:516-544, 1987).

In addition, a host cell strain may be chosen which modulates the expression of the antibody sequences, or modifies and processes the antibody in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the antibody. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the antibody expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, W138, and in particular, myeloma cells such as NS0 cells, and related cell lines, see, for example, Morrison *et al.*, U.S. Patent No. 5,807,715, which is hereby incorporated by reference in its entirety.

For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and

al., *Proc. Natl. Acad. Sci. USA*, 78:1527, 1981); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, *Biotherapy*, 3:87-95, 1991; Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.*, 32:573-596, 1993; Mulligan, *Science*, 260:926-932, 1993; and Morgan and Anderson, *Ann. Rev. Biochem.*, 62: 191-217, 1993; and May, *TIB TECH*, 11(5):155-2 15, 1993); and *hygro*, which confers resistance to hygromycin (Santerre *et al.*, *Gene*, 30:147, 1984). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.* (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; in Chapters 12 and 13, Dracopoli *et al.* (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY; and Colberre-Garapin *et al.*, *J. Mol. Biol.*, 150:1, 1981, which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, 1987, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. Academic Press, New York). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse *et al.*, *Mol., Cell. Biol.*, 3:257, 1983).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides or different selectable markers to ensure maintenance of both plasmids. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature*, 322:52, 1986; and Kohler, *Proc. Natl. Acad. Sci. USA*, 77:2 197, 1980). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by recombinant expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange,

affinity, particularly by affinity for the specific antigen after Protein A purification, and sizing column chromatography), centrifugation, differential solubility, or by any other standard techniques for the purification of proteins. Further, the antibodies of the present invention or fragments thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

5.3.1. ANTIBODY CONJUGATES

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to heterologous polypeptides (*i.e.*, an unrelated polypeptide; or portion thereof, preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids of the polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. Antibodies fused or conjugated to heterologous polypeptides may also be used in *in vitro* immunoassays and purification methods using methods known in the art. See *e.g.*, PCT Publication No. WO 93/21232; EP 439,095; Naramura *et al.*, *Immunol. Lett.*, 39:91-99, 1994; U.S. Patent 5,474,981; Gillies *et al.*, *PNAS*, 89:1428-1432, 1992; and Fell *et al.*, *J. Immunol.*, 146:2446-2452, 1991, which are incorporated herein by reference in their entireties.

Antibodies can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:821-824, 1989, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, *Cell*, 37:767 1984) and the "flag" tag (Knappik *et al.*, *Biotechniques*, 17(4):754-761, 1994).

The present invention also encompasses antibodies conjugated to a diagnostic or therapeutic agent or any other molecule for which *in vivo* half-life is desired to be increased. The antibodies can be used diagnostically to, for example, monitor the development or progression of a disease, disorder or infection as part of a clinical testing procedure to, *e.g.*, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent

materials, bioluminescent materials, radioactive materials, positron emitting metals, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{111}In or ^{99m}Tc.

An antibody may be conjugated to a therapeutic moiety such as a cytotoxin (*e.g.*, a cytostatic or cytocidal agent), a therapeutic agent or a radioactive element (*e.g.*, alpha-emitters, gamma-emitters, etc.). Cytotoxins or cytotoxic agents include any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Further, an antibody may be conjugated to a therapeutic agent or drug moiety that modifies a given biological response. Therapeutic agents or drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α-interferon (IFN-α), β-interferon (IFN-β),

nerve growth factor (NGF), platelet derived growth factor (PDGF), tissue plasminogen activator (TPA), an apoptotic agent (*e.g.*, TNF-α, TNF-β, AIM I as disclosed in PCT Publication No. WO 97/33899), AIM II (see, PCT Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *J. Iminunol.*, 6:1567-1574, 1994), and VEGI (PCT Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent (*e.g.*, angiostatin or endostatin); or a biological response modifier such as, for example, a lymphokine (*e.g.*, interleukin-1 ("IL- 1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), and granulocyte colony stimulating factor ("G-CSF")), or a growth factor (*e.g.*, growth hormone ("GH")).

Techniques for conjugating such therapeutic moieties to antibodies are well known; see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), 1985, pp. 243-56, Alan R. Liss, Inc.); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery (2nd Ed.)*, Robinson *et al.* (eds.), 1987, pp. 623-53, Marcel Dekker, Inc.); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), 1985, pp. 475-506); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.),1985, pp. 303-16, Academic Press; and Thorpe *et al.*, *Immunol. Recombinant expression vector.*, 62:119-58, 1982.

An antibody or fragment thereof, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

5.4 METHODS OF PRODUCING FUSION PROTEINS

Fusion proteins can be produced by standard recombinant DNA techniques or by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. For example, a nucleic acid molecule encoding a fusion protein can be synthesized by conventional techniques

including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g., Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, 1992). Moreover, a nucleic acid encoding a bioactive molecule can be cloned into an expression vector containing the Fc domain or a fragment thereof such that the bioactive molecule is linked in-frame to the constant domain or fragment thereof.

Methods for fusing or conjugating polypeptides to the constant regions of antibodies are known in the art. See, *e.g.*, U.S. Patent Nos. 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, 5,723,125, 5,783,181, 5,908,626, 5,844,095, and 5,112,946; EP 307,434; EP 367,166; EP 394,827; PCT publications WO 91/06570, WO 96/04388, WO 96/22024, WO 97/34631, and WO 99/04813; Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA*, 88: 10535-10539, 1991; Traunecker *et al.*, *Nature*, 331:84-86, 1988; Zheng *et al.*, *J. Immunol.*, 154:5590-5600, 1995; and Vil *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:11337-11341, 1992, which are incorporated herein by reference in their entireties.

The nucleotide sequence encoding a bioactive molecule may be obtained from any information available to those of skill in the art (*e.g.*, from Genbank, the literature, or by routine cloning), and the nucleotide sequence encoding a constant domain or a fragment thereof with increased affinity for the FcRn may be determined by sequence analysis of mutants produced using techniques described herein, or may be obtained from Genbank or the literature. The nucleotide sequence coding for a fusion protein can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. A variety of host-vector systems may be utilized in the present invention to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

The expression of a fusion protein may be controlled by any promoter or enhancer element known in the art. Promoters which may be used to control the expression of the gene encoding fusion protein include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 290:304-310, 1981), the promoter contained in the 3'

long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, *Cell*, 22:787-797, 1980), the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445, 1981), the regulatory sequences of the metallothionein gene (Brinster *et al.*, *Nature*, 296:39-42, 1982), the tetracycline (Tet) promoter (Gossen *et al.*, *Proc. Nat. Acad. Sci. USA*, 89:5547-5551, 1995); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 75:3727-3731, 1978), or the *tac* promoter (DeBoer, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 80:21-25, 1983; see also "Useful proteins from recombinant bacteria" in *Scientific American*, 242:74-94, 1980); plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella *et al.*, *Nature*, 303:209-213, 1983) or the cauliflower mosaic virus 35S RNA promoter (Gardner, *et al.*, *Nucl. Acids Res.*, 9:2871, 1981), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella *et al.*, *Nature*, 310:115-120, 1984); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, *Cell* 38:639-646, 1984; Ornitz *et al.*, 50:399-409, *Cold Spring Harbor Symp. Quant. Biol.*, 1986; MacDonald, *Hepatology* 7:425-515, 1987); insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature* 315:115-122, 1985), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, *Cell*, 38:647-658, 1984; Adames *et al.*, *Nature* 318:533-538, 1985; Alexander *et al.*, *Mol. Cell. Biol.*, 7:1436-1444, 1987), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, *Cell*, 45:485-495, 1986), albumin gene control region which is active in liver (Pinkert *et al.*, *Genes and Devel.*, 1:268-276, 1987), α-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, *Mol. Cell. Biol.*, 5:1639-1648, 1985; Hammer *et al.*, *Science*, 235:53-58, 1987; α 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, *Genes and Devel.*, 1:161-171, 1987), beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, *Nature*, 315:338-340, 1985; Kollias *et al.*, *Cell*, 46:89-94, 1986; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, *Cell*, 48:703-712, 1987); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature*, 314:283-286, 1985); neuronal-specific enolase (NSE) which is active in neuronal cells (Morelli *et al.*, *Gen. Virol.*, 80:571-83, 1999); brain-derived neurotrophic factor (BDNF) gene control region which is active in neuronal cells (Tabuchi *et al.*, *Biochem. Biophysic. Res. Comprising.*, 253:818-823,

1998); glial fibrillary acidic protein (GFAP) promoter which is active in astrocytes (Gomes *et al.*, Braz. J. Med. Biol. Res., 32(5):619-631, 1999; Morelli *et al.*, *Gen. Virol.*, 80:571-83, 1999) and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, *Science*, 234:1372-1378, 1986).

In a specific embodiment, the expression of a fusion protein is regulated by a constitutive promoter. In another embodiment, the expression of a fusion protein is regulated by an inducible promoter. In accordance with these embodiments, the promoter may be a tissue-specific promoter.

In a specific embodiment, a vector is used that comprises a promoter operably linked to a fusion protein-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the fusion protein coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, *Proc. Natl. Acad. Sci. USA*, 81:355-359, 1984). Specific initiation signals may also be required for efficient translation of inserted fusion protein coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bitter *et al.*, *Methods in Enzymol.*, 153:516-544, 1987).

Expression vectors containing inserts of a gene encoding a fusion protein can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a gene encoding a fusion protein in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted gene encoding the fusion protein. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to

antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a nucleotide sequence encoding a fusion protein in the vector. For example, if the nucleotide sequence encoding the fusion protein is inserted within the marker gene sequence of the vector, recombinants containing the gene encoding the fusion protein insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the gene product (*i.e.*, fusion protein) expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the fusion protein in *in vitro* assay systems, *e.g.*, binding with anti-bioactive molecule antibody.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered fusion protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system will produce an unglycosylated product and expression in yeast will produce a glycosylated product. Eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, neuronal cell lines such as, for example, SK-N-AS, SK-N-FI, SK-N-DZ human neuroblastomas (Sugimoto *et al.*, *J. Natl. Cancer Inst.*, 73: 51-57, 1984), SK-N-SH human neuroblastoma (*Biochim. Biophys. Acta*, 704: 450-460, 1982), Daoy human cerebellar medulloblastoma (He *et al.*, *Cancer Res.*, 52: 1144-1148, 1992) DBTRG-05MG glioblastoma cells (Kruse *et al.*, 1992, *In Vitro Cell. Dev. Biol.*, 28A:609-614, 1992), IMR-32 human neuroblastoma (*Cancer Res.*, 30: 2110-2118, 1970), 1321N1 human astrocytoma (*Proc. Natl Acad. Sci. USA*, 74: 4816, 1997), MOG-G-CCM human astrocytoma (Br. J. Cancer, 49: 269, 1984), U87MG human glioblastoma-astrocytoma (*Acta Pathol. Microbiol. Scand.*, 74: 465-486, 1968), A172 human glioblastoma (Olopade *et al.*, *Cancer Res.*, 52: 2523-2529, 1992), C6 rat glioma cells (Benda *et al.*, Science, 161: 370-371, 1968), Neuro-2a mouse neuroblastoma (*Proc. Natl. Acad. Sci. USA*, 65: 129-136, 1970), NB41A3 mouse neuroblastoma (*Proc. Natl. Acad. Sci. USA*, 48: 1184-1190, 1962), SCP sheep choroid plexus (Bolin *et al.*, *J. Virol. Methods*, 48: 211-221, 1994), G355-5, PG-4 Cat normal astrocyte (Haapala *et al.*, *J. Virol.*, 53: 827-833, 1985), Mpf

ferret brain (Trowbridge *et al.*, *In Vitro,* 18: 952-960, 1982), and normal cell lines such as, for example, CTX TNA2 rat normal cortex brain (Radany *et al.*, *Proc. Natl. Acad. Sci. USA,* 89: 6467-6471, 1992) such as, for example, CRL7030 and Hs578Bst. Furthermore, different vector/host expression systems may effect processing reactions to different degrees.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the fusion protein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines that express the differentially expressed or pathway gene protein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the differentially expressed or pathway gene protein.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, *Cell,* 11:223, 1997), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA,* 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, *Cell,* 22:817, 1980) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, *Natl. Acad. Sci.USA*, 77:3567, 1980; O'Hare, *et al.*, *Proc. Natl. Acad. Sci. USA,* 78:1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA,* 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, *J. Mol. Biol.,* 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, *Gene*, 30:147, 1984) genes.

Once a fusion protein of the invention has been produced by recombinant expression, it may be purified by any method known in the art for purification of a protein, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

5.5. PROPHYLACTIC AND THERAPEUTIC USES OF ANTIBODIES

The present invention encompasses antibody-based therapies which involve administering antibodies to an animal, preferably a mammal, and most preferably a human, for preventing, treating, or ameliorating symptoms associated with a disease, disorder, or infection. Prophylactic and therapeutic compounds of the invention include, but are not limited to, antibodies and nucleic acids encoding antibodies. Antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

Antibodies of the present invention that function as antagonists of a disease, disorder, or infection can be administered to an animal, preferably a mammal and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with the disease, disorder, or infection. For example, antibodies which disrupt or prevent the interaction between a viral antigen and its host cell receptor may be administered to an animal, preferably a mammal and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with a viral infection.

In a specific embodiment, an antibody or fragment thereof prevents a viral or bacterial antigen from binding to its host cell receptor by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to antigen binding to its host cell receptor in the absence of said antibodies. In another embodiment, a combination of antibodies prevent a viral or bacterial antigen from binding to its host cell receptor by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to antigen binding to its host cell receptor in the absence of said antibodies. In a preferred embodiment, the antibody is used to treat or prevent RSV infection

Antibodies which do not prevent a viral or bacterial antigen from binding its host cell receptor but inhibit or downregulate viral or bacterial replication can also be administered to an animal to treat, prevent or ameliorate one or more symptoms associated with a viral or bacterial infection. The ability of an antibody to inhibit or downregulate viral or bacterial replication may be determined by techniques described herein or otherwise known in the art. For example, the inhibition or downregulation of viral replication can be determined by detecting the viral titer in the animal.

In a specific embodiment, an antibody inhibits or downregulates viral or bacterial replication by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at

least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to viral or bacterial replication in absence of said antibody. In another embodiment, a combination of antibodies inhibit or downregulate viral or bacterial replication by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to viral or bacterial replication in absence of said antibodies.

Antibodies can also be used to prevent, inhibit or reduce the growth or metastasis of cancerous cells. In a specific embodiment, an antibody inhibits or reduces the growth or metastasis of cancerous cells by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the growth or metastasis in absence of said antibody. In another embodiment, a combination of antibodies inhibits or reduces the growth or metastasis of cancer by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the growth or metastasis in absence of said antibodies. Examples of cancers include, but are not limited to, leukemia (*e.g.*, acute leukemia such as acute lymphocytic leukemia and acute myelocytic leukemia), neoplasms, tumors (*e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma), heavy chain disease, metastases, or any disease or disorder characterized by uncontrolled cell growth.

Antibodies can also be used to reduce the inflammation experienced by animals, particularly mammals, with inflammatory disorders. In a specific embodiment, an antibody reduces the inflammation in an animal by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the inflammation in an animal in the not administered said antibody. In another embodiment, a combination of antibodies reduce the inflammation in an animal by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the inflammation in an animal in not administered said antibodies. Examples of inflammatory disorders include, but are not limited to, rheumatoid arthritis, spondyloarthropathies, inflammatory bowel disease and asthma.

In certain embodiments, the antibody used for treatment of inflammation (or cancer) is a modified anti-$\alpha_v\beta_3$ antibody, preferably a Vitaxin antibody (see, PCT publications WO 98/33919 and WO 00/78815, both by Huse *et al.*, and both of which are incorporated by reference herein in their entireties).

Antibodies can also be used to prevent the rejection of transplants. Antibodies can also be used to prevent clot formation. Further, antibodies that function as agonists of the immune response can also be administered to an animal, preferably a mammal, and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with the disease, disorder, or infection.

One or more antibodies that immunospecifically bind to one or more antigens may be used locally or systemically in the body as a therapeutic. The antibodies of this invention may also be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the number or activity of effector cells which interact with the antibodies. The antibodies of this invention may also be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the immune response. The antibodies of this invention may also be advantageously utilized in combination with one or more drugs used to treat a disease, disorder, or infection such as, for example anti-cancer agents, anti-inflammatory agents or anti-viral agents. Examples of anti-cancer agents include, but are not limited to, isplatin, ifosfamide, paclitaxel, taxanes, topoisomerase I inhibitors (*e.g.*, CPT-11, topotecan,

9-AC, and GG-211), gemcitabine, vinorelbine, oxaliplatin, 5-fluorouracil (5-FU), leucovorin, vinorelbine, temodal, and taxol. Examples of anti-viral agents include, but are not limited to, cytokines (*e.g.*, IFN-α, IFN-β, IFN-γ), inhibitors of reverse transcriptase (*e.g.*, AZT, 3TC, D4T, ddC, ddI, d4T, 3TC, adefovir, efavirenz, delavirdine, nevirapine, abacavir, and other dideoxynucleosides or dideoxyfluoronucleosides), inhibitors of viral mRNA capping, such as ribavirin, inhibitors of proteases such HIV protease inhibitors (*e.g.*, amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir,), amphotericin B, castanospermine as an inhibitor of glycoprotein processing, inhibitors of neuraminidase such as influenza virus neuraminidase inhibitors (*e.g.*, zanamivir and oseltamivir), topoisomerase I inhibitors (*e.g.*, camptothecins and analogs thereof), amantadine, and rimantadine. Examples of anti-inflammatory agents include, but are not limited to, nonsteroidal anti-inflammatory drugs such as COX-2 inhibitors (*e.g.*, meloxicam, celecoxib, rofecoxib, flosulide, and SC-58635, and MK-966), ibuprofen and indomethacin, and steroids (*e.g.*, deflazacort, dexamethasone and methylprednisolone).

In a specific embodiment, antibodies administered to an animal are of a species origin or species reactivity that is the same species as that of the animal. Thus, in a preferred embodiment, human or humanized antibodies, or nucleic acids encoding human or human, are administered to a human patient for therapy or prophylaxis.

In preferred embodiments, immunoglobulins having extended *in vivo* half-lives are used in passive immunotherapy (for either therapy or prophylaxis). Because of the extended half-life, passive immunotherapy or prophylaxis can be accomplished using lower doses and/or less frequent administration of the therapeutic resulting in fewer side effects, better patient compliance, less costly therapy/prophylaxis, etc. In a preferred embodiment, the therapeutic/prophylactic is an antibody that binds RSV, for example, SYNAGIS® or other anti-RSV antibody. Such anti-RSV antibodies, and methods of administration are disclosed in U.S. patent application Serial Nos. 09/724,396 and 09/724,531, both entitled "Methods of Administering/Dosing Anti-RSV Antibodies For Prophylaxis and Treatment," both by Young *et al.*, both filed November 28, 2000, and continuation-in-part applications of these applications, Serial Nos. _____ and _____, respectively (attorney docket Nos. 10271-048 and 10271-047, respectively), also entitled "Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment," by Young *et al.*, all which are incorporated by reference herein in their entireties. Also included are the anti-RSV antibodies described in Section 5.1, *supra.*

In a specific embodiment, fusion proteins administered to an animal are of a species origin or species reactivity that is the same species as that of the animal. Thus, in a

preferred embodiment, human fusion proteins or nucleic acids encoding human fusion proteins, are administered to a human subject for therapy or prophylaxis.

5.6. PROPHYLACTIC AND THERAPEUTIC USES OF FUSION PROTEINS AND CONJUGATED MOLECULES

The present invention encompasses fusion protein-based and conjugated molecule-based therapies which involve administering fusion proteins or conjugated molecules to an animal, preferably a mammal and most preferably a human, for preventing, treating, or ameliorating symptoms associated with a disease, disorder, or infection. Prophylactic and therapeutic compounds of the invention include, but are not limited to, fusion proteins and nucleic acids encoding fusion proteins and conjugated molecules. Fusion proteins and conjugated molecules may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

Fusion proteins and conjugated molecules of the present invention that function as antagonists of a disease, disorder, or infection can be administered to an animal, preferably a mammal, and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with the disease, disorder, or infection. Further, fusion proteins and conjugated molecules of the present invention that function as agonists of the immune response may be administered to an animal, preferably a mammal, and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with the disease, disorder, or infection.

One or more fusion proteins and conjugated molecules may be used locally or systemically in the body as a therapeutic. The fusion proteins and conjugated molecules of this invention may also be advantageously utilized in combination with monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the number or activity of effector cells which interact with the antibodies. The fusion proteins and conjugated molecules of this invention may also be advantageously utilized in combination with monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the immune response. The fusion proteins and conjugated molecules of this invention may also be advantageously utilized in combination with one or more drugs used to treat a disease, disorder, or infection such as, for example anti-cancer agents, anti-inflammatory agents or anti-viral agents. Examples of anti-cancer agents include, but are not limited to, isplatin, ifosfamide, paclitaxel, taxanes,

topoisomerase I inhibitors (*e.g.*, CPT-11, topotecan, 9-AC, and GG-211), gemcitabine, vinorelbine, oxaliplatin, 5-fluorouracil (5-FU), leucovorin, vinorelbine, temodal, and taxol. Examples of anti-viral agents include, but are not limited to, cytokines (*e.g.*, IFN-α, IFN-β, IFN-γ), inhibitors of reverse transcriptase (*e.g.*, AZT, 3TC, D4T, ddC, ddI, d4T, 3TC, adefovir, efavirenz, delavirdine, nevirapine, abacavir, and other dideoxynucleosides or dideoxyfluoronucleosides), inhibitors of viral mRNA capping, such as ribavirin, inhibitors of proteases such HIV protease inhibitors (*e.g.*, amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir,), amphotericin B, castanospermine as an inhibitor of glycoprotein processing, inhibitors of neuraminidase such as influenza virus neuraminidase inhibitors (*e.g.*, zanamivir and oseltamivir), topoisomerase I inhibitors (*e.g.*, camptothecins and analogs thereof), amantadine, and rimantadine. Examples of anti-inflammatory agents include, but are not limted to, nonsteroidal anti-inflammatory drugs such as COX-2 inhibitors (*e.g.*, meloxicam, celecoxib, rofecoxib, flosulide, and SC-58635, and MK-966), ibuprofen and indomethacin, and steroids (*e.g.*, deflazacort, dexamethasone and methylprednisolone).

5.7. ADMINISTRATION OF ANTIBODIES OR FUSION PROTEINS

The invention provides methods of treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administrating to a subject of an effective amount of an antibody of the invention, or pharmaceutical composition comprising an antibody of the invention. The invention also provides methods of treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of a fusion protein or conjugated molecule of the invention, or a pharmaceutical composition comprising a fusion protein or conjugated molecules of the invention. In a preferred aspect, an antibody or fusion protein or conjugated molecule, is substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side-effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats etc.) and a primate (*e.g.*, monkey such as a cynomolgous monkey and a human). In a preferred embodiment, the subject is a human.

Various delivery systems are known and can be used to administer an antibody or fusion protein or conjugated molecule of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the

antibody or fusion protein, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, *J. Biol. Chem.*, 262:4429-4432, 1987), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering an antibody, a fusion protein or conjugated molecule, or pharmaceutical composition include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, antibodies, fusion proteins, conjugated molecules, or pharmaceutical compositions are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety. In a preferred embodiment, an antibody, a fusion protein, conjugated molecules, or a pharmaceutical composition is administered using Alkermes AIR™ pulmonary drug delivery technology (Alkermes, Inc., Cambridge, MA).

The invention also provides that an antibody, a fusion protein, or conjugated molecule is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of antibody, fusion protein, or conjugated molecule. In one embodiment, the antibody, fusion protein, or conjugated molecule is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the antibody, fusion protein, or conjugated molecule is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, or at least 75 mg. The lyophilized antibody, fusion protein, or conjugated molecule should be stored at between 2 and 8°C in its original container and the antibody, fusion protein, or conjugated molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, an antibody, fusion protein, or conjugated molecule is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the antibody, fusion protein, or conjugated molecule.

Preferably, the liquid form of the antibody, fusion protein, or conjugated molecule is supplied in a hermetically sealed container at least 1 mg/ml, more preferably at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, or at least 25 mg/ml.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering an antibody or a fusion protein, care must be taken to use materials to which the antibody or the fusion protein does not absorb.

In another embodiment, the composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science,* 249:1527-1533, 1990; Treat *et al.*, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327; see generally ibid.).

In yet another embodiment, the composition can be delivered in a controlled release or sustained release system. Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more antibodies, or one or more fusion proteins. See, *e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.*, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," *Radiotherapy & Oncology,* 39:179-189, 1996; Song *et al.*, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," *PDA Journal of Pharmaceutical Science & Technology,* 50:372-397, 1995; Cleek *et al.*, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," *Pro. Intl. Symp. Control. Rel. Bioact. Mater.*, 24:853-854, 1997; and Lam *et al.*, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," *Proc. Int'l. Symp. Control Rel. Bioact. Mater.*, 24:759-760, 1997, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled release system (see Langer, supra; Sefton, *CRC Crit. Ref. Biomed. Eng.*, 14:20, 1987; Buchwald *et al.*, *Surgery*, 88:507, 1980; and Saudek *et al.*, *N. Engl. J. Med.*, 321:574, 1989). In another embodiment, polymeric materials can be used to achieve controlled release of antibodies or fusion proteins (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J., Macromol. Sci. Rev. Macromol. Chem.*, 23:61, 1983; see also Levy *et al.*, *Science,* 228:190, 1985; During *et al.*, *Ann. Neurol.*, 25:351, 1989;

Howard *et al.*, *J. Neurosurg.*, 7 1:105, 1989); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer, *Science*, 249:1527-1533, 1990).

In a specific embodiment where the composition of the invention is a nucleic acid encoding an antibody or fusion protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded antibody or fusion protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:1864-1868, 1991), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a prophylactically or therapeutically effective amount of an antibody, fusion protein or conjugated molecule, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's complete and incomplete, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful adjuvants for humans such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and

aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a prophylactically or therapeutically effective amount of the antibody or fragment thereof, or fusion protein or conjugated molecule, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the composition of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disease, disorder, or infection can be determined by standard clinical techniques. The precise dose to be employed in the formulation will depend on the route of administration, the age of the subject, and the seriousness of the disease, disorder, or infection, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model (*e.g.*, the cotton rat or Cynomolgous monkey) test systems.

For fusion proteins, the therapeutically or prophylactically effective dosage administered to a subject ranges from about 0.001 to 50 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. For antibodies, the therapeutically or prophylactically effective dosage administered to a subject is typically 0.1 mg/kg to 200 mg/kg of the subject's body weight. Preferably, the dosage administered to a subject is between 0.1 mg/kg and 20 mg/kg of the subject's body weight and more preferably the dosage administered to a subject is between 1 mg/kg to 10 mg/kg of the subject's body weight. The dosage will, however, depend upon the extent to which the *in vivo* half-life of the molecule has been increased Generally, human antibodies and human fusion proteins have longer half-lives within the human body than antibodies of fusion proteins from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies or human fusion proteins and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies, fusion proteins, or conjugated molecules may be reduced also by enhancing uptake and tissue penetration (*e.g.*, into the lung) of the antibodies or fusion proteins by modifications such as, for example, lipidation.

Treatment of a subject with a therapeutically or prophylactically effective amount of an antibody, fusion protein, or conjugated molecule can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with an antibody, fusion protein, or conjugated molecule in the range of between about 0.1 to 30 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. In other embodiments, the pharmaceutical composition of the invention is administered once a day, twice a day, or three times a day. In other embodiments, the pharmaceutical composition is administered once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a

year or once per year. It will also be appreciated that the effective dosage of the antibody, fusion protein, or conjugated molecule used for treatment may increase or decrease over the course of a particular treatment.

5

5.7.1. GENE THERAPY

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or fusion proteins, are administered to treat, prevent or ameliorate one or more symptoms associated with a disease, disorder, or infection, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded antibody or fusion protein that mediates a therapeutic or prophylactic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel *et al.*, *Clinical Pharmacy,* 12:488-505, 1993; Wu and Wu, *Biotherapy,* 3:87-95, 1991; Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.*, 32:573-596, 1993; Mulligan, *Science,* 260:926-932, 1993; and Morgan and Anderson, *Ann. Rev. biochem.* 62:191-217, 1993; *TIBTECH* 11(5):155-215, 1993. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, a composition of the invention comprises nucleic acids encoding an antibody, said nucleic acids being part of an expression vector that expresses the antibody in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA,* 86:8932-8935, 1989; and Zijlstra *et al.*, *Nature,* 342:435-438, 1989).

In another preferred aspect, a composition of the invention comprises nucleic acids encoding a fusion protein, said nucleic acids being a part of an expression vector that expression the fusion protein in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the coding region of a

fusion protein, said promoter being inducible or constitutive, and optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the coding sequence of the fusion protein and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the fusion protein encoding nucleic acids.

Delivery of the nucleic acids into a subject may be either direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the subject. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, *e.g.*, by infection using defective or attenuated retroviral or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, *J. Biol. Chem.*, 262:4429-4432, 1987) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180; WO 92/22635; WO 92/20316; WO 93/14188; WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, *Proc. Natl. Acad. Sci. USA,* 86:8932-8935, 1989; and Zijlstra *et al.*, *Nature,* 342:435-438, 1989).

In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an antibody or a fusion protein are used. For example, a retroviral vector can be used (see Miller *et al.*, *Meth. Enzymol.,* 217:581-599, 1993). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody or a fusion protein to

be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the nucleotide sequence into a subject. More detail about retroviral vectors can be found in Boesen *et al.*, *Biotherapy,* 6:291-302, 1994, which describes the use of a retroviral vector to deliver the mdr 1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, *J. Clin. Invest.*, 93:644-651, 1994; Klein *et al.*, Blood 83:1467-1473, 1994; Salmons and Gunzberg, *Human Gene Therapy,* 4:129-141, 1993; and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.,* 3:110-114, 1993.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development,* 3:499-503, 1993, present a review of adenovirus-based gene therapy. Bout *et al.*, *Human Gene Therapy*, 5:3-10, 1994, demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, *Science,* 252:431-434, 1991; Rosenfeld *et al.*, *Cell,* 68:143-155, 1992; Mastrangeli *et al.*, *J. Clin. Invest.,* 91:225-234, 1993; PCT Publication WO 94/12649; and Wang *et al.*, *Gene Therapy,* 2:775-783, 1995. In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (see, *e.g.*,Walsh *et al.*, *Proc. Soc. Exp. Biol. Med.,* 204:289-300, 1993, and U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcellmediated gene transfer,

spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, *Meth. Enzymol.*, 217:599-618, 1993; Cohen *et al.*, *Meth. Enzymol.*, 217:618-644, 1993; and *Clin. Pharma. Ther.*, 29:69-92, 1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the subject.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody or a fusion protein are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see *e.g.*, PCT Publication WO 94/08598; Stemple and Anderson, *Cell*, 7 1:973-985, 1992; Rheinwald, *Meth. Cell Bio.*, 21A:229, 1980; and Pittelkow and Scott, *Mayo Clinic Proc.*, 61:771, 1986).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

5.8. CHARACTERIZATION AND DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

Antibodies, fusion proteins, and conjugated molecules of the present invention may be characterized in a variety of ways. In particular, antibodies of the invention may be assayed for the ability to immunospecifically bind to an antigen. Such an assay may be performed in solution (*e.g.*, Houghten, *Bio/Techniques,* 13:412-421, 1992), on beads (Lam, *Nature,* 354:82-84, 1991, on chips (Fodor, *Nature,* 364:555-556, 1993), on bacteria (U.S. Patent No. 5,223,409), on spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. USA,* 89:1865-1869, 1992) or on phage (Scott and Smith, *Science,* 249:386-390, 1990; Devlin, *Science,* 249:404-406, 1990; Cwirla *et al.*, *Proc. Natl. Acad. Sci. USA,* 87:6378-6382, 1990; and Felici, *J. Mol. Biol.,* 222:301-310, 1991) (each of these references is incorporated herein in its entirety by reference). Antibodies that have been identified to immunospecifically bind to an antigen or a fragment thereof can then be assayed for their specificity affinity for the antigen.

The antibodies of the invention or fragments thereof may be assayed for immunospecific binding to an antigen and cross-reactivity with other antigens by any method known in the art. Immunoassays which can be used to analyze immunospecific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40°C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40°C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to

immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, *e.g.*, an anti-human antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*, ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*, ^{3}H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of the present invention or a fragment thereof for the antigen and the binding off-rates can be determined from the saturation data by scatchard analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with an antibody of the present invention or a fragment thereof conjugated to a labeled compound (*e.g.*, ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibodies to an antigen. BIAcore kinetic analysis comprises analyzing the binding and dissociation of an antigen from chips with immobilized antibodies on their surface (see the Example section *infra*).

The antibodies of the invention as well as fusion proteins and conjugated molecules can also be assayed for their ability to inhibit the binding of an antigen to its host cell receptor using techniques known to those of skill in the art. For example, cells expressing the receptor for a viral antigen can be contacted with virus in the presence or absence of an antibody and the ability of the antibody to inhibit viral antigen's binding can measured by, for example, flow cytometry or a scintillation counter. The antigen or the antibody can be labeled with a detectable compound such as a radioactive label (*e.g.*, ^{32}P, ^{35}S, and ^{125}I) or a fluorescent label (*e.g.*, fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine) to enable detection of an interaction between the antigen and its host cell receptor. Alternatively, the ability of antibodies to inhibit an antigen from binding to its receptor can be determined in cell-free assays. For example, virus or a viral antigen (*e.g.*, RSV F glycoprotein) can be contacted in a cell-free assay with an antibody and the ability of the antibody to inhibit the virus or the viral antigen from binding to its host cell receptor can be determined. Preferably, the antibody is immobilized on a solid support and the antigen is labeled with a detectable compound. Alternatively, the antigen is immobilized on a solid support and the antibody is labeled with a detectable compound. The antigen may be partially or completely purified (*e.g.*, partially or completely free of other polypeptides) or part of a cell lysate. Further, the antigen may be a fusion protein comprising the viral antigen and a domain such as glutathionine-S-transferase. Alternatively, an antigen can be biotinylated using techniques

well known to those of skill in the art (*e.g.*, biotinylation kit, Pierce Chemicals; Rockford, IL).

The antibodies, fusion proteins, and conjugated molecules of the invention can also be assayed for their ability to inhibit or downregulate viral or bacterial replication using techniques known to those of skill in the art. For example, viral replication can be assayed by a plaque assay such as described, *e.g.*, by Johnson *et al.*, *Journal of Infectious Diseases*, 176:1215-1224, 1997. The antibodies, fusion proteins, and conjugated molecules of the invention of the invention can also be assayed for their ability to inhibit or downregulate the expression of viral or bacterial polypeptides. Techniques known to those of skill in the art, including, but not limited to, Western blot analysis, Northern blot analysis, and RT-PCR, can be used to measure the expression of viral or bacterial polypeptides. Further, the antibodies, fusion proteins, and conjugated molecules of the invention of the invention can be assayed for their ability to prevent the formation of syncytia.

The antibodies, fusion proteins, conjugated molecules, and compositions of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays which can be used to determine whether administration of a specific antibody, a specific fusion protein, a specific conjugated molecule, or a composition of the present invention is indicated, include *in vitro* cell culture assays in which a subject tissue sample is grown in culture, and exposed to or otherwise administered an antibody, a fusion protein, conjugated molecule, or composition of the present invention, and the effect of such an antibody, a fusion protein, conjugated molecule, or a composition of the present invention upon the tissue sample is observed. In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a disease or disorder, to determine if an antibody, a fusion protein, conjugated molecule, or composition of the present invention has a desired effect upon such cell types. Preferably, the antibodies, the fusion proteins, the conjugated molecules, or compositions of the invention are also tested in *in vitro* assays and animal model systems prior to administration to humans.

Antibodies, fusion proteins, conjugated molecules, or compositions of the present invention for use in therapy can be tested for their toxicity in suitable animal model systems, including but not limited to rats, mice, cows, monkeys, and rabbits. For *in vivo* testing for the toxicity of an antibody, a fusion protein, a conjugated molecule, or a composition, any animal model system known in the art may be used.

Efficacy in treating or preventing viral infection may be demonstrated by detecting the ability of an antibody, a fusion protein, a conjugated molecule, or a composition

of the invention to inhibit the replication of the virus, to inhibit transmission or prevent the virus from establishing itself in its host, or to prevent, ameliorate or alleviate one or more symptoms associated with viral infection. The treatment is considered therapeutic if there is, for example, a reduction is viral load, amelioration of one or more symptoms or a decrease in mortality and/or morbidity following administration of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention. Antibodies, fusion proteins, conjugated molecules, or compositions of the invention can also be tested for their ability to inhibit viral replication or reduce viral load in *in vitro* and *in vivo* assays.

Efficacy in treating or preventing bacterial infection may be demonstrated by detecting the ability of an antibody, a fusion protein or a composition of the invention to inhibit the bacterial replication, or to prevent, ameliorate or alleviate one or more symptoms associated with bacterial infection. The treatment is considered therapeutic if there is, for example, a reduction is bacterial numbers, amelioration of one or more symptoms or a decrease in mortality and/or morbidity following administration of an antibody, a fusion protein or a composition of the invention.

Efficacy in treating cancer may be demonstrated by detecting the ability of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention to inhibit or reduce the growth or metastasis of cancerous cells or to ameliorate or alleviate one or more symptoms associated with cancer. The treatment is considered therapeutic if there is, for example, a reduction in the growth or metastasis of cancerous cells, amelioration of one or more symptoms associated with cancer, or a decrease in mortality and/or morbidity following administration of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention. Antibodies, fusion proteins or compositions of the invention can be tested for their ability to reduce tumor formation in *in vitro*, *ex vivo*, and *in vivo* assays.

Efficacy in treating inflammatory disorders may be demonstrated by detecting the ability of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention to reduce or inhibit the inflammation in an animal or to ameliorate or alleviate one or more symptoms associated with an inflammatory disorder. The treatment is considered therapeutic if there is, for example, a reduction is in inflammation or amelioration of one or more symptoms following administration of an antibody, a fusion proteins, a conjugated molecule, or a composition of the invention.

Antibodies, fusion proteins, conjugated molecules, or compositions of the invention can be tested *in vitro* and *in vivo* for the ability to induce the expression of cytokines (*e.g.*, IFN-α, IFN-β, IFN-γ, IL-2, IL-3, IL-4, IL-5, IL-6, IL10, IL-12, and IL-15) and

activation markers (*e.g.*, CD28, ICOS, and SLAM). Techniques known to those of skill in the art can be used to measure the level of expression of cytokines and activation markers. For example, the level of expression of cytokines can be measured by analyzing the level of RNA of cytokines by, for example, RT-PCR and Northern blot analysis, and by analyzing the level of cytokines by, for example, immunoprecipitation followed by Western blot analysis or ELISA.

Antibodies, fusion proteins, conjugated molecules, or compositions of the invention can be tested *in vitro* and *in vivo* for their ability to modulate the biological activity of immune cells, preferably human immune cells (*e.g.*, T-cells, B-cells, and Natural Killer cells). The ability of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention to modulate the biological activity of immune cells can be assessed by detecting the expression of antigens, detecting the proliferation of immune cells, detecting the activation of signaling molecules, detecting the effector function of immune cells, or detecting the differentiation of immune cells. Techniques known to those of skill in the art can be used for measuring these activities. For example, cellular proliferation can be assayed by ^{3}H-thymidine incorporation assays and trypan blue cell counts. Antigen expression can be assayed, for example, by immunoassays including, but are not limited to, competitive and non-competitive assay systems using techniques such as Western blots, immunohistochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and FACS analysis. The activation of signaling molecules can be assayed, for example, by kinase assays and electrophoretic shift assays (EMSAs).

Antibodies, fusion proteins, conjugated molecules, or compositions of the invention can also be tested for their ability to increase the survival period of animals, preferably mammals and most preferably humans, suffering from a disease, disorder, or infection by at least 25%, preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99%. Further, antibodies, fusion proteins, conjugated molecules, or compositions of the invention can be tested for their ability reduce the hospitalization period of animals, preferably mammals and most preferably humans, suffering from a disease, disorder, or infection by at least 60%, preferably at least 75%, at least 85%, at least 95%, or at least 99%. Techniques known to those of skill in the art can be used to analyze the function of the antibodies or compositions of the invention *in vivo*.

5.9. DIAGNOSTIC USES OF ANTIBODIES AND FUSION PROTEINS

Labeled antibodies, fusion proteins, and conjugated molecules of the invention can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders or infections. The invention provides for the detection or diagnosis of a disease, disorder or infection, comprising: (a) assaying the expression of an antigen in cells or a tissue sample of a subject using one or more antibodies that immunospecifically bind to the antigen; and (b) comparing the level of the antigen with a control level, *e.g.*, levels in normal tissue samples, whereby an increase in the assayed level of antigen compared to the control level of the antigen is indicative of the disease, disorder or infection. The invention also provides for the detection or diagnosis of a disease, disorder or infection, comprising (a) assaying the expression of an antigen in cells or a tissue sample of a subject using one or fusion proteins or conjugated molecules of the invention that bind to the antigen; and (b) comparing the level of the antigen with a control level, e.g., levels in normal tissue samples, whereby an increase of antigen compared to the control level of the antigen is indicative of the disease, disorder or infection. Accordingly, the fusion protein or conjugated molecule comprises a bioactive molecule such as a ligand, cytokine or growth factor and the hinge-Fc region or fragments thereof, wherein the fusion protein or conjugated molecule is capable of binding to an antigen being detected.

Antibodies of the invention can be used to assay antigen levels in a biological sample using classical immunohistological methods as described herein or as known to those of skill in the art (*e.g.*, see Jalkanen *et al.*, *J. Cell. Biol.*, 101:976-985, 1985; Jalkanen *et al.*, *J. Cell . Biol.*, 105:3087-3096, 1987). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, alkaline phosphatase, glucose oxidase; radioisotopes, such as iodine (^{125}I, ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{121}In), and technetium (^{99m}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine.

Fusion proteins can be used to assay antigen levels in a biological sample using, for example, SDS-PAGE and immunoassays known to those of skill in the art.

One aspect of the invention is the detection and diagnosis of a disease, disorder, or infection in a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled antibody that immunospecifically binds to an antigen; b) waiting for a

time interval following the administration for permitting the labeled antibody to preferentially concentrate at sites in the subject where the antigen is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled antibody in the subject, such that detection of labeled antibody above the background level indicates that the subject has the disease, disorder, or infection. In accordance with this embodiment, the antibody is labeled with an imaging moiety which is detectable using an imaging system known to one of skill in the art. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

In another embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled fusion protein or conjugated molecule that binds to an antigen or some other molecule; b) waiting for a time interval following the administration for permitting the labeled fusion protein or conjugated molecule to preferentially concentrate at sites in the subject where the antigen or other molecule is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled fusion protein or conjugated molecule in the subject, such that detection of labeled fusion protein above the background level indicates that the subject has the disease, disorder, or infection. In accordance with this embodiment, the fusion protein or conjugated molecule comprises a bioactive molecule such as a ligand, cytokine or growth factor and a hinge-Fc region or a fragment thereof, wherein said fusion protein or conjugated molecule is labeled with an imaging moiety and is capable of binding to the antigen being detected.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments," Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours.

In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In one embodiment, monitoring of a disease, disorder or infection is carried out by repeating the method for diagnosing the disease, disorder or infection, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the subject using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston *et al.*, U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

5.10. KITS

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody, fusion protein, or conjugated molecule, of the invention, preferably in a purified form, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated antigen as a control. Preferably, the kits of the present invention further comprise a control antibody, fusion protein, or conjugated molecule which does not react with the antigen included in the kit. In another specific embodiment, the kits of the present invention contain a means for

detecting the binding of an antibody, fusion protein, or conjugated molecule, to an antigen (*e.g.*, the antibody, fusion protein, or conjugated molecule, may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized antigen. The antigen provided in the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above-described kit includes a solid support to which antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the antigen can be detected by binding of the said reporter-labeled antibody.

5.11 *IN VITRO* AND *IN VIVO* ASSAYS FOR EXTENDED HALF-LIFE OF MODIFIED IGG HINGE-FC FRAGMENTS

The binding ability of modified IgGs and molecules comprising an IgG constant domain of FcRn fragment thereof to FcRn can be characterized by various *in vitro* assays. PCT publication WO 97/34631 by Ward discloses various methods in detail and is incorporated herein in its entirety by reference.

For example, in order to compare the ability of the modified IgG or fragments thereof to bind to FcRn with that of the wild type IgG, the modified IgG or fragments thereof and the wild type IgG can be radio-labeled and reacted with FcRn-expressing cells *in vitro*. The radioactivity of the cell-bound fractions can be then counted and compared. The cells expressing FcRn to be used for this assay are preferably endothelial cell lines including mouse pulmonary capillary endothelial cells (B10, D2.PCE) derived from lungs of B10.DBA/2 mice and SV40 transformed endothelial cells (SVEC) (Kim *et al.*, *J. Immunol.*, 40:457-465, 1994) derived from C3H/HeJ mice. However, other types of cells, such as intestinal brush borders isolated from 10- to 14-day old suckling mice, which express sufficient number of FcRn can be also used. Alternatively, mammalian cells which express recombinant FcRn of a species of choice can be also utilized. After counting the radioactivity of the bound fraction of modified IgG or that of wild type, the bound molecules can be then extracted with the detergent, and the percent release per unit number of cells can be calculated and compared.

Affinity of modified IgGs for FcRn can be measured by surface plasmon resonance (SPR) measurement using, for example, a BIAcore 2000 (BIAcore Inc.) as

described previously (Popov *et al.*, *Mol. Immunol.*, 33:493-502, 1996; Karlsson *et al.*, *J. Immunol. Methods*, 145:229-240, 1991, both of which are incorporated by reference in their entireties). In this method, FcRn molecules are coupled to a BlAcore sensor chip (*e.g.*, CM5 chip by Pharmacia) and the binding of modified IgG to the immobilized FcRn is measured at a certain flow rate to obtain sensorgrams using BIA evaluation 2.1 software, based on which on- and off-rates of the modified IgG, constant domains, or fragments thereof, to FcRn can be calculated.

Relative affinities of modified IgGs or fragments thereof, and the wild type IgG for FcRn can be also measured by a simple competition binding assay. Unlabeled modified IgG or wild type IgG is added in different amounts to the wells of a 96-well plate in which FcRn is immobilize. A constant amount of radio-labeled wild type IgG is then added to each well. Percent radioactivity of the bound fraction is plotted against the amount of unlabeled modified IgG or wild type IgG and the relative affinity of the modified hinge-Fc can be calculated from the slope of the curve.

Furthermore, affinities of modified IgGs or fragments thereof, and the wild type IgG for FcRn can be also measured by a saturation study and the Scatchard analysis.

Transfer of modified IgG or fragments thereof across the cell by FcRn can be measured by *in vitro* transfer assay using radiolabeled IgG or fragments thereof and FcRn-expressing cells and comparing the radioactivity of the one side of the cell monolayer with that of the other side. Alternatively, such transfer can be measured *in vivo* by feeding 10- to 14-day old suckling mice with radiolabeled, modified IgG and periodically counting the radioactivity in blood samples which indicates the transfer of the IgG through the intestine to the circulation (or any other target tissue, *e.g.*, the lungs). To test the dose-dependent inhibition of the IgG transfer through the gut, a mixture of radiolabeled and unlabeled IgG at certain ratio is given to the mice and the radioactivity of the plasma can be periodically measured (Kim *et al.*, *Eur. J. Immunol.*, 24:2429-2434, 1994).

The half-life of modified IgG or fragments thereof can be measure by pharmacokinetic studies according to the method described by Kim *et al.* (*Eur. J. of Immuno.* 24:542, 1994), which is incorporated by reference herein in its entirety. According to this method, radiolabeled modified IgG or fragments thereof is injected intravenously into mice and its plasma concentration is periodically measured as a function of time, for example, at 3 minutes to 72 hours after the injection. The clearance curve thus obtained should be biphasic, that is, α-phase and β-phase. For the determination of the *in vivo* half-life of the modified IgGs or fragments thereof, the clearance rate in β-phase is calculated and compared with that of the wild type IgG.

6. EXAMPLES

The following examples illustrate the production, isolation, and characterization of modified hinge-Fc fragments that have longer *in vivo* half-lives.

6.1 LIBRARY CONSTRUCTION

6.1.1 REAGENTS

All chemicals were of analytical grade. Restriction enzymes and DNA-modifying enzymes were purchased from New England Biolabs, Inc. (Beverly, MA). Oligonucleotides were synthesized by MWG Biotech, Inc. (High Point, NC). pCANTAB5E phagemid vector, anti-E-tag-horseradish peroxydase conjugate, TG1 *E. Coli* strain, IgG Sepharose 6 Fast Flow and HiTrap protein A columns were purchased from APBiotech, Inc. (Piscataway, NJ). VCSM13 helper phage and the Quick change mutagenesis kit were obtained from Stratagene (La Jolla, CA). CJ236 *E. coli* strain was purchased from Bio-Rad (Richmond, CA). BCA Protein Assay Reagent Kit was obtained from Pierce (Rockford, IL). Lipofectamine 2000 was purchased from Invitrogen, Inc. (Carlsbad, CA).

6.1.2 EXPRESSION AND PURIFICATION OF MURINE AND HUMAN FCRN

The amino acid sequences of human and mouse FcRn are SEQ ID NOs. 84 and 85, respectively (see also Firan *et al.*, *Intern. Immunol.*, 13:993-1002, 2001 and Popov *et al.*, *Mol. Immunol.*, 33:521-530, 1996, both of which are incorporated herein by reference in their entireties). Human FcRn was also obtained following isolation from human placenta cDNA (Clontech, Palo Alto, CA) of the genes for human β2-microglobulin (Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, U.S. Public Health Service, National Institutes of Health, Washington, DC) and codons -23 to 267 of the human α chain (Story *et al.*, *J. Exp. Med.*, 180:2377-2381, 1994) using standard PCR protocols. Light and heavy chains along with their native signal sequence (Kabat *et al.*, 1991, supra; Story *et al.*, *supra*) were cloned in pFastBac DUAL and pFastBac1 bacmids, respectively, and viral stocks produced in *Spodoptera frugiperda* cells (Sf9) according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). High-Five cells were infected at a multiplicity of infection of 3 with the baculoviruses encoding α and β2 chains using commercially available protocols (Invitrogen). Recombinant human FcRn was purified as follows: supernatant of infected insect cells was dialyzed into 50 mM MES (2-N-[Morpholino]ethansulfonic acid) pH 6.0 and

applied to a 10 ml human IgG Sepharose 6 Fast Flow column (APBiotech, Piscataway, NJ). Resin was washed with 200 ml 50 mM MES pH 6.0 and FcRn eluted with 0.1 M Tris-Cl pH 8.0. Purified FcRn was dialyzed against 50 mM MES pH 6.0, flash frozen and stored at -70°C. The purity of proteins was checked by SDS-PAGE and HPLC.

6.1.3 PREPARATION OF TAA-CONTAINING ssDNA URACIL TEMPLATE

Construction of the libraries was based on a site directed mutagenesis strategy derived from the Kunkel method (Kunkel *et al.*, *Methods Enzymol.* 154:367-382, 1987). A human hinge-Fc gene spanning amino acid residues 226-478 (Kabat numbering, Kabat *et al.*, 1991, *supra*) derived from MEDI-493 human IgG1 (Johnson *et al.*, *J. Infect. Disease*, 176:1215-1224, 1997), was cloned into the pCANTAB5E phagemid vector as an SfiI/NotI fragment. Four libraries were generated by introducing random mutations at positions 251, 252, 254, 255, 256 (library 1), 308, 309, 311, 312, 314 (library 2), 385, 386, 387, 389 (library 3) and 428, 433, 434, 436 (library 4). Briefly, four distinct hinge-Fc templates were generated using PCR by overlap extension (Ho *et al.*, *Gene*, 15:51-59, 1989), each containing one TAA stop codon at position 252 (library 1), 310 (library 2), 384 (library 3) or 429 (library 4), so that only mutagenized phagemids will give rise to Fc-displaying phage.

Each TAA-containing single-stranded DNA (TAAssDNA) was then prepared as follows: a single CJ236 *E. coli* colony harboring one of the four relevant TAA-containing phagemids was grown in 10 ml 2 x YT medium supplemented with 10 μg/ml chloramphenicol and 100 μg/ml ampicillin. At OD_{600} = 1, VCSM13 helper phage was added to a final concentration of 10^{10} pfu/ml. After 2 hours, the culture was transferred to 500 ml of 2 x YT medium supplemented with 0.25 μg/ml uridine, 10 μg/ml chloramphenicol, 30 μg/ml kanamycin, 100 μg/ml ampicillin and grown overnight at 37°C. Phage were precipitated with PEG6000 using standard protocols (Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York, Vols. 1-3) and purified using the Qiaprep Spin M13 Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. 10 to 30 μg of each uracil-containing TAAssDNA template was then combined with 0.6 μg of the following phosphorylated oligonucleotides (randomized regions underlined) in 50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.5 in a final volume of 250 μl:

Library 1:

5'-CATGTGACCTCAGGSNNSNNSNNGATSNNSNNGGTGTCCTTGGGTTTT

GGGGGG-3' (SEQ ID NO:120)
Library 2:
5'-GCACTTGTACTCCTTGCCATTSNNCCASNNSNNGTGSNNSNNGGTGA
GGACGC-3' (SEQ ID NO:121)
Library 3:
5'-GGTCTTGTAGTTSNNCTCSNNSNNSNNATTGCTCTCCC-3' (SEQ ID NO:122)
Library 4:
5'-GGCTCTTCTGCGTSNNGTGSNNSNNCAGAGCCTCATGSNNCACGGAGC
ATGAG-3' (SEQ ID NO:123)
where N = A, C, T or G and S = G or C.

6.1.4 SYNTHESIS OF HETERODUPLEX DNA

Appropriate, degenerate oligonucleotides were phosphorylated in the presence of T4 polynucleotide kinase using the standard protocol. Ten to 30 μg of ssDNA U template and 0.6 μg of phosphorylated oligonucleotide were combined in 50 mM Tris-HCl containing 10 mM $MgCl_2$, pH 7.5, to a final volume of 250 μl and incubated at 90°C for 2 minutes, 50°C for 3 minutes, and 20°C for 5 minutes. Synthesis of the heteroduplex DNA was carried out by adding 30 units of both T4 DNA ligase and T7 DNA polymerase in the presence of 0.4 mM ATP, 1 mM dNTPs and 6 mM DTT and the mixture was incubated for 4 hours at 20°C. The heteroduplex DNA thus produced was then purified and desalted using Qiagen Qiaquick DNA purification Kit (Qiagen, CA).

6.1.5 ELECTROPORATION

300 μl electrocompetent TG1 *E. coli* cells were electroporated with 1 to 5 μg of the heteroduplex DNA in a 2.5 kV field using 200 Ω and 25 μF capacitance until a library size of 1 x 10^8 (library 1 and 2) or 1 x 10^7 (library 3 and 4) was reached. The cells were resuspended in 2 ml SOC medium and the procedure was repeated 6 to 10 times. The diversity was assessed by titration of recombinant *E. coli*. The pulsed cells were incubated in 50 ml SOC medium for 30 minutes at 37°C under agitation, centrifuged, and resuspended in 500 ml 2xYT containing 100 μg/ml ampicillin and 10^{10} pfu/ml of VCSM13 helper phage. The culture was incubated overnight at 37°C and the cells were pelleted by centrifugation. The phage in the supernatant which express mutated hinge-Fc portion on its GIII-coat protein were precipitated with PEG6000 as previously described (Sambrook *et al.*, 1989, *supra*) and resuspended in 5 ml of 20 mM MES, pH 6.0.

6.2 PANNING OF THE LIBRARY

Phage were panned using an ELISA-based approach. A 96-well ELISA plate was coated with 100 µl/well of 0.01 mg/ml murine FcRn in sodium carbonate buffer, pH 9.0, at 4°C overnight and then blocked with 4% skimmed milk at 37°C for 2 hours. In each well of the coated plate, 100-150 µl of the phage suspension (about 10^{13} phage in total) in 20 mM MES, pH 6.0, containing 5% milk and 0.05% Tween 20, were placed and incubated at 37°C for two to three hours with agitation.

After the incubation, the wells were washed with 20 mM MES, pH 6.0, containing 0.2% Tween 20 and 0.3 M NaCl about thirty times at room temperature. The bound phage were eluted with 100 µl/well of PBS, pH 7.4, at 37°C for 30 minutes.

The eluted phage were then added to the culture of exponentially growing *E. coli* cells and propagation was carried out overnight at 37°C in 250 ml 2xYT supplemented with 100 µg/ml ampicillin and 10^{10} pfu/ml of VCSM13 helper phage. Propagated phage were collected by centrifugation followed by precipitation with PEG and the panning process was repeated up to a total of six times.

For the phage library containing mutations in residues 308-314 (H310 and W313 fixed), the phage expressing hinge-Fc region with higher affinities for FcRn were enriched by each panning process as shown in Table IV. The panning results of the library for the mutations in the residues 251-256 (I253 fixed) and that of the library for the mutations in the residues 428-436 (H429, E430, A431, L432, and H435 fixed), are shown in Tables V and VI, respectively. Furthermore, the panning results of the library for the mutations in the residues 385-389 (E388 fixed) is shown in Table VII.

Table IV

PANNING OF LIBRARY (RESIDUES 308-314; H310 AND W313 FIXED) pCANTAB5E-KUNKEL-muFcRn (MURINE FcRn)

PANNING	OUTPUT		ENRICHMENT RATIO
	+ FcRn	- FcRn	
1st Round	1.1×10^5	0.5×10^5	2
2nd Round	1×10^4	0.2×10^4	5
3rd Round	9×10^4	0.3×10^4	30
4th Round	3×10^5	2×10^4	15

Table V

PANNING OF LIBRARY (RESIDUES 251-256; I253 FIXED)
pCANTAB5E-KUNKEL-muFcRn

PANNING	OUTPUT		ENRICHMENT RATIO
	+ FcRn	- FcRn	
1st Round	2.5×10^5	1×10^5	2.5
2nd Round	6×10^4	2×10^4	3.0
3rd Round	8×10^5	4×10^4	20
4th Round	1.2×10^6	5×10^4	24
5th Round	3.0×10^6	6×10^4	50

Table VI

PANNING OF LIBRARY (RESIDUES 428-436; H429, E430, A431, L432, AND H435 FIXED) pCANTAB5E-KUNKEL-muFcRn

PANNING	OUTPUT		ENRICHMENT RATIO
	+ FcRn	- FcRn	
1st Round	2.3×10^5	0.9×10^5	2.5
2nd Round	3×10^4	1×10^4	3
3rd Round	2×10^5	2×10^4	10
4th Round	8×10^5	5×10^4	16

Table VII

PANNING OF LIBRARY (RESIDUES 385-389; E388 FIXED)
pCANTAB5E-KUNKEL-muFcRn

PANNING	OUTPUT		ENRICHMENT RATIO
	+ FcRn	- FcRn	
1st Round	4.2×10^5	3.8×10^5	1.1
2nd Round	5×10^4	0.3×10^4	17
3rd Round	3.5×10^5	1×10^4	35
4th Round	5.5×10^5	4×10^4	14
5th Round	7.5×10^5	5×10^4	15
6th Round	2×10^6	1×10^5	20

6.3 IDENTIFICATION OF ISOLATED CLONES FROM PANNING

After each panning process, phage were isolated and the nucleic acids encoding the expressed peptides which bound to FcRn were sequenced by a standard sequencing method such as by dideoxynucleotide sequencing (Sanger *et al.*, *Proc. Natl. Acad. Sci USA,* 74:5463-5467, 1977) using a ABI3000 genomic analyzer (Applied Biosystems, Foster City, CA).

As a result of panning, two mutants were isolated from the phage library containing mutations in residues 308-314 (H310 and W313 fixed), thirteen mutants from the library for residues 251-256 (I253 fixed), six mutants from the library for residues 428-436 (H429, E430, A431, L432, and H435 fixed), and nine mutants from the library for residues 385-389 (E388 fixed). The mutants isolated from the libraries are listed in Table VIII.

Table VIII

MUTANTS ISOLATED BY PANNING

LIBRARY	MUTANTS*
251-256	Leu **Tyr** Ile **Thr** Arg **Glu** (SEQ ID NO:90)
	*Leu **Tyr** Ile Ser Arg Thr* (SEQ ID NO:91)
	*Leu **Tyr** Ile Ser Arg **Ser*** (SEQ ID NO:92)
	*Leu **Tyr** Ile Ser Arg **Arg*** (SEQ ID NO:93)

LIBRARY	MUTANTS*
	*Leu **Tyr** Ile Ser Arg **Gln*** (SEQ ID NO:94)
	*Leu **Trp** Ile Ser Arg Thr* (SEQ ID NO:95)
	Leu **Tyr** Ile Ser **Leu Gln** (SEQ ID NO:96)
	Leu **Phe** Ile Ser Arg **Asp** (SEQ ID NO:97)
	Leu **Phe** Ile Ser Arg **Thr** (SEQ ID NO:98)
	Leu **Phe** Ile Ser Arg **Arg** (SEQ ID NO:99)
	Leu **Phe** Ile **Thr Gly Ala** (SEQ ID NO:100)
	Leu **Ser** Ile Ser Arg **Glu** (SEQ ID NO:101)
	Arg Thr Ile Ser **Ile Ser** (SEQ ID NO:102)
308-314	**Thr Pro** His **Ser** Asp Trp Leu (SEQ ID NO:103)
	Ile Pro His **Glu** Asp Trp Leu (SEQ ID NO:104)
385-389	**Arg Thr Arg** Glu **Pro** (SEQ ID NO:105)
	Asp Pro** Pro Glu **Ser (SEQ ID NO:106)
	Ser Asp Pro Glu **Pro** (SEQ ID NO:107)
	Thr Ser His Glu Asn (SEQ ID NO:108)
	Ser Lys Ser Glu Asn (SEQ ID NO:109)
	His Arg Ser Glu Asn (SEQ ID NO:110)
	Lys Ile Arg Glu Asn (SEQ ID NO:111)
	Gly **Ile Thr** Glu **Ser** (SEQ ID NO:112)
	Ser Met Ala Glu **Pro** (SEQ ID NO:113)
428-436	*Met His Glu Ala Leu **Arg Tyr** His **His*** (SEQ ID NO:114)
	Met His Glu Ala Leu His **Phe** His **His** (SEQ ID NO:115)
	Met His Glu Ala Leu **Lys Phe** His **His** (SEQ ID NO:116)
	Met His Glu Ala Leu **Ser Tyr** His **Arg** (SEQ ID NO:117)
	Thr His Glu Ala Leu His **Tyr** His **Thr** (SEQ ID NO:118)
	Met His Glu Ala Leu His **Tyr** His **Tyr** (SEQ ID NO:119)

* Substituting residues are indicated in bold face

The underlined sequences in Table VIII correspond to sequences that occurred 10 to 20 times in the final round of panning and the sequences in italics correspond to sequences that occurred 2 to 5 times in the final round of panning. Those sequences that are neither underlined nor italicized occurred once in the final round of panning.

6.4 EXPRESSION AND PURIFICATION OF SOLUBLE MUTANT HINGE-FC REGION

The genes encoding mutated hinge-Fc fragments are excised with appropriate restriction enzymes and recloned into an expression vector, for example, VβpelBhis (Ward, *J. Mol. Biol.*, 224:885-890, 1992). Vectors containing any other type of tag sequence, such as *c-myc* tag, decapeptide tag (Huse *et al.*, *Science*, 246:1275-1281, 1989), Flag™ (Immunex) tags, can be used. Recombinant clones, such as *E. coli*, are grown and induced to express soluble hinge-Fc fragments, which can be isolated from the culture media or cell lysate after osmotic shock, based on the tag used, or by any other purification methods well known to those skilled in the art and characterized by the methods as listed below.

6.5 CONSTRUCTION, PRODUCTION AND PURIFICATION OF IgG1 VARIANTS

Representative Fc mutations such as I253A, M252Y/S254T/T256E, M252W, M252Y, M252Y/T256Q, M252F/T256D, V308T/L309P/Q311S, G385D/Q386P/N389S, G385R/Q386T/P387R/N389P, H433K/N434F/Y436H, and N434F/Y436 were incorporated into the human IgG1 MEDI-493 (SYNAGIS®) (Johnson *et al.*, 1997, *supra*). The heavy chain was subjected to site-directed mutagenesis using a Quick Change Mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions and sequences were verified by didoxynucleotide sequencing using a ABI3000 (Applied Biosystems, Foster City, CA) sequencer. The different constructions were expressed transiently in human embryonic kidney 293 cells using a CMV immediate-early promoter and dicistronic operon in which IgG1/V_H is cosecreted with IgG1/V_L (Johnson *et al.*, 1997, *supra*). Transfection was carried out using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and standard protocols. IgGs were purified from the conditioned media directly on 1 ml HiTrap protein A columns according to the manufacterer's instructions (APBiotech).

6.6 CHARACTERIZATION OF MUTATED HINGE-FC REGION

6.6.1 *IN VITRO* CHARACTERIZATION HPLC AND SDS-PAGE

Following the purification, general characteristics such as molecular weight and bonding characteristics of the modified hinge-Fc fragments may be studied by various methods well known to those skilled in the art, including SDS-PAGE and HPLC.

FcRn binding assay

Binding activity of modified hinge-Fc fragments can be measured by incubating radio-labeled wild-type hinge-Fc or modified hinge-Fc with the cells expressing either mouse or human FcRn. Typically, endothelial cell lines such as SV40 transformed endothelial cells (SVEC) (Kim *et al.*, *J. Immunol.*, 40:457-465, 1994) are used. After incubation with the hinge-Fc fragments at 37°C for 16-18 hours, the cells are washed with medium and then detached by incubation with 5 mM Na_2EDTA in 50 mM phosphate buffer, pH 7.5, for 5 minutes. The radioactivity per 10^7 cells is measured.

Then, the cells are resuspended in 2 ml of 2.5 mg/ml CHAPS, 0.1 M Tris-HCl pH 8.0 containing 0.3 mg/ml PMSF, 25 mg/ml pepstatin and 0.1 mg/ml aprotinin and incubated for 30 minutes at room temperature. The cell suspension is then centrifuged and the supernatant separated. The radioactivity of the supernatant is measured and used to calculate the amount of the hinge-Fc fragments extracted per 10^7 cells.

The K_d for the interaction of wild type human IgG1 with murine and human FcRn (269 and 2527 nM, respectively) agree well with the values determined by others (265 and 2350 nM, respectively, Firan *et al.*, 2001, *supra*). The I253A mutation virtually abolishes binding to human and murine FcRn, as reported by others (Kim *et al.*, *Eur. J. Immunol.*, 29:2819-2825, 1991; Shields *et al.*, *J. Biol. Chem.*, 276:6591-6604, 2001). This is not the result of misfolding of the antibody as this mutant retains the same specific activity than the wild type molecule (SYNAGIS®) in a microneutralization assay (Johnson *et al.*, 1997, *supra*; data not shown).

Human IgG1 mutants with increased binding affinity towards both murine and human FcRn were generated (Table VIII). Improvements in complex stability were overall less marked for the human IgG1-human FcRn pair than for the human IgG1-murine FcRn compared to wild type IgG1 were 30-($\Delta\Delta G$ = 2.0 kcal/mol for N434F/Y436H) and 11-($\Delta\Delta G$ = 1.4 kcal/mol for M252Y/S254Y/S254T/T256E) fold, respectively. However, ranking of the most critical positions remain unchanged when comparing human and murine FcRn: the largest increases in IgG1-murine FcRn complex stability ($\Delta\Delta G$ > 1.3 kcal/mol) occurred on

mutations at positions 252, 254, 256 (M252Y/S254T/T256E and M252W) and 433, 434, 436 (H433K/N434F/Y436H and N434F/Y436H). Likewise, the same mutations were found to have the most profound impact on the IgG1-human FcRn interaction and also resulted in the largest increases in complex stability ($\Delta\Delta G > 1.0$ kcal/mol). Substitutions at positions 308, 309, 311, 385, 386, 387 and 389 had little or no effect on the stability of the complexes involving human or murine FcRn ($\Delta\Delta G < 0.5$ kcal/mol). Residues at the center of the Fc-FcRn combining site contribute significantly more to improvement in complex stability than residues at the periphery (FIG. 9).

Efficient binding of human Fc to murine FcRn apparently requires the presence of several wild type Fc residues. For example, leucine is very conserved at 251, arginine at 255, aspartic acid at 310, leucine at 314 and methionine at 428 (FIG. 6). Another specificity trend is observed when one considers positions 308, 309, and 311 where threonine, proline, and serine, respectively, are very strongly favored over the corresponding wild type residues (FIG. 6). However, generation of this strong consensus sequences does not correlate with the magnitude of increase in affinity as V308T/L309P/Q311S binds less than 2-fold better than the wild type IgG1 to both human and murine FcRn (Table IX).

Increases in affinity can be strongly dependent upon residue substitution at one 'hot spot' position. For example, the single mutation M252Y causes an increase in binding to murine FcRn by 9-fold, whereas additional mutations bring little (M252Y/S254T/T256E) or no (M252Y/T256Q) added benefit. The same trend is observed for the human receptor, although to a lesser extent. Indeed, M252Y/S254T/T256E shows a marked improvement of 2.5-fold in affinity compared to M252Y. This probably reflects the differences between the binding site of human and murine FcRn (West and Bjorkman, *Biochemistry*, 39:9698-9708, 2000).

Phage-derived IgG1 mutants exhibiting a significant increase in affinity towards murine FcRn ($\Delta\Delta G > 1.3$ kcal/mol) also showed significant binding activity to the receptor at pH 7.2 when compared to wild type IgG1 (FIGs. 8A-H). IgG1 mutants with moderate increase in affinity ($\Delta\Delta G < 0.3$ kcal/mol) bound very poorly at pH 7.2 (data not shown). In contrast, IgG1 mutants with large ($\Delta\Delta G > 1.0$ kcal/mol) increase in affinity towards human FcRn exhibited only minimal binding at pH 7.4 when compared to wild type IgG1 (FIGs. 8A-H).

Table IX

DISSOCIATION CONSTANTS AND RELATIVE FREE ENERGY CHANGES FOR THE BINDING OF IgG1/FC MUTANTS TO MURINE AND HUMAN FcRn*

MUTANT	Dissociation Constant Fc/Murine FcRn (nM)	ΔΔG (kcal/mol)	Dissociation Constant Fc/Human FcRn (mM)	ΔΔG (kcal/mol)
wild type	269 ± 1		2527 ± 117	
I253A	NB	NA	NB	NA
M252Y/S254T/T256E	27 ± 6	1.4	225 ± 10	1.4
M252W	30 ± 1	1.3	408 ± 24	1.1
M252Y	41 ± 7	1.1	532 ± 37	0.9
M252Y/T256Q	39 ± 8	1.1	560 ± 102	0.9
M252F/T256D	52 ± 9	1.0	933 ± 170	0.6
V308T/L309P/Q311S	153 ± 23	0.3	1964 ± 84	0.1
G385D/Q386P/N389S	187 ± 10	0.2	2164 ± 331	0.1
G385R/Q386T/P387R/N389P	147 ± 24	0.4	1620 ± 61	0.3
H433K/N434F/Y436H	14 ± 2	1.8	399 ± 47	1.1
N434F/Y436H	9 ± 1	2.0	493 ± 7	1.0

*Affinity measurements were carried out by BIAcore as described above. Residue numbering is according to EU (Kabat *et al.*, 1991, *supra*). Differences in free energy changes are calculated as the differences between the Δgs of wild type and mutant reactions ($\Delta\Delta G = \Delta G_{wild\ type} - \Delta G_{mutant}$). NB, no binding. NA, not-applicable.

FcRn-mediated transfer assay

This assay follows the protocol disclosed in PCT publication WO 97/34631. Radiolabeled modified hinge-Fc fragments at various concentration (1 μg/ml-1 mg/ml) are added to the one side of the transwell and the transfer of the fragments mediated by FcRn-expressing monolayer of the cells can be quantitated by measuring the radioactivity on the other side of the transwell.

6.6.2 *IN VIVO* PHARMACOKINETIC STUDY

In order to determine the half-life of the modified IgG hinge-Fc, modified hinge-Fc fragments are radiolabelled with ^{125}I (approximate specific activity of 10^7 cpm/μg) and dissolved in saline (pH 7.2). The solution is injected intravenously into BALB/c mice (Harlan, Indianapolis, IN), which have been given NaI-containing water previously to block the thyroid, in a volume not more than 150 μl and with a radioactivity of 10×10^6-50×10^6 cpm. The mice are bled from the retro-orbital sinus at various time points, for example, at 3 minutes to 72 hours after the injection, into heparinized capillary tubes and the plasma collected from each sample is counted for radioactivity.

To generate the data provided in FIG. 10, 10 animals were used for each molecule assayed with 2.5 μg of antibody injected per animal. Antibody serum levels were determined using an anti-human IgG ELISA (FIG. 10). There seems to be an inverse correlation between affinity to mouse FcRn and persistence in serum. This might be due to the significant amount of binding of the mutants observed at pH 7.2, which leads to the sequestration (i.e., lack of release in the serum) of the molecules. Preliminary data (not shown) suggests increased transport of the mutants to the lung. Additionally, since the mutants exhibit lower levels of binding to human FcRn than murine FcRn (see FIGS. 8A-H), antibody serum levels are expected to be higher in primates and humans.

6.6.3 SURFACE PLASMON RESONANCE ANALYSES

The interaction of soluble murine and human FcRn with immobilized human IgG1 variants was monitored by surface plasmon resonance detection using a BIAcore 3000 instrument (Pharmacia Biosensor, Uppsala, Sweden). No aggregated material which could interfere with affinity measurements (van der Merwe *et al.*, *EMBO J.*, 12:4945-4954, 1993; van der Merwe *et al., Biochemistry*, 33:10149-10160, 1994) was detected by gel filtration. Protein concentrations were calculated by the bicinchoninic acid (BCA) method for both human and murine FcRn or using the 1% extinction coefficient at 280 nm of 1.5 for IgG1 wild type and variants. The latter were coupled to the dextran matrix of a CM5 sensor chip (Pharmacia Biosensor) using an Amine Coupling Kit as described (Johnson *et al.*, *supra*). The protein concentrations ranged from 3-5 μg/ml in 10 mM sodium acetate, pH 5.0. The activation period was set for 7 minutes at a flow rate of 10 μl/min and the immobilization period was set to between 10 and 20 minutes at a flow rate of 10 μl/min. Excess reactive esters were quenched by injection of 70 μl of 1.0 methanolamine hydrochloride, pH 8.5. This typically resulted in the immobilization of between 500 and 4000 resonance units (RU). Human and murine FcRn were buffer exchanged against 50 mM PBS buffer pH 6.0

containing 0.05% Tween 20. Dilutions were made in the same buffer. All binding experiments were performed at 25°C with concentrations ranging from 120 to 1 μg/ml at a flow rate of 5 to 10 μl/min; data were collected for 25 to 50 minutes and three 1-minute pulses of PBS buffer pH 7.2 were used to regenerate the surfaces. FcRn was also flowed over an uncoated cell and the sensorgrams from these blank runs subtracted from those obtained with IgG1-coupled chips. Runs were analyzed using the software BIAevaluation 3.1 (Pharmacia). Association constants (K_As) were determined from Scatchard analysis by measuring the concentration of free reactants and complex at equilibrium after correction for nonspecific binding. In equilibrium binding BIAcore experiments (Karlsson *et al.*, 1991, *supra*; van der Merwe *et al.*, 1993, *supra*; van der Merwe *et al.*, 1994, *supra*; Raghavan *et al.*, *Immunity*, 1:303-315, 1994; Malchiodi *et al.*, *J. Exp. Med.*, 182:1833-1845, 1995), the concentration of the complex can be assessed directly as the steady-state response. The concentration of free analyte (human or murine FcRn) is equal to the bulk analyte concentration since analyte is constantly replenished during sample injection. The concentration of free ligand on the surface of the sensor chip can be derived from the concentration of the complex and from the total binding capacity of the surface as $K_A = R_{eq}/C(R_{max} - R_{eq})$ where C is the free analyte concentration, R_{eq} is the steady-state response, and R_{max} is the total surface binding capacity. Rearranging, the equation reads: $R_{eq}/C = K_A R_{max} - K_A R_{eq}$.

A plot of R_{eq}/C versus R_{eq} at different analyte concentrations thus gives a straight line from which K_A can be calculated (see Table IX). Errors were estimated as the standard deviation for two or three independent determinations and were <20%.

Representative mutations identified after panning libraries 1 through 4 (FIG. 6, Table VIII) were introduced into the Fc portion of a human IgG1. Injection of different concentrations of human or murine FcRn over the immobilized IgG1 variants gave concentration-dependent binding. Typical resonance profiles for equilibrium binding of the mutant M252Y/S254T/T256E to murine and human FcRn are shown in FIGs. 7A and B. To estimate apparent K_As, concentrations of FcRn ranging from 120 to 1 μg/ml were used. In all cases, equilibrium (or near-equilibrium) binding levels were reached within 50 minutes. To estimate the increase in RU resulting from the non specific effect of protein on the bulk refractive index, binding of FcRn to an uncoated cell was measured and the sensorgrams from these blank runs subtracted from those obtained with IgG1-coupled chips. The scatchard plots for the binding of the mutant M252Y/S254T/T256E to murine and human FcRn are shown in FIGs. 7C and D. The plots were all linear, and apparent K_As were

calculated from the relevant slopes. Measurements were carried out in duplicate or triplicate and confirmed that the immobilized IgGs retained their original binding activity.

Since there are two non-equivalent binding sites on mouse IgG1 for murine FcRn with affinities of < 130 nM and 6 μM (Sanchez *et al.*, *Biochemistry*, 38:9471-9476, 1999; Schuck *et al.*, *Mol. Immunol.*, 36:1117-1125, 1999; Ghetie and Ward, *Ann. Rev. Immunol.*, 18:739-766, 2000), the receptor was used in solution to avoid avidity effects that arise when IgG1 binds to immobilized FcRn. Consistent with this, systematically higher affinities are observed when FcRn, rather than IgG, immobilized on the biosensor chip (Popov *et al.*, 1996, *supra*; Vaughn and Bjorkman, *Biochemistry*, 36:9374-9380, 1997; Martin and Bjorkman, *Biochemistry*, 38:12639-12647; West and Bjorkman, *Biochemistry*, 39:9698-9708, 2000). Under our experimental BIAcore conditions, mainly interactions corresponding to the higher-affinity association (*i.e.* single liganded-recptor) are measured, according for the linearity of the scatchard plots (FIGs. 7C and D).

BIAcore analysis was also used to compare the affinity of wild type IgG1 and IgG1 mutants. Phage-derived IgG1 mutants exhibiting a significant increase in affinity towards murine FcRn at pH 6.0 ($\Delta\Delta G \geq 1.0$ kcal/mol) also shoed significant binding to the mouse receptor at pH 7.2 with SPR $signal_{pH7.4}$/SPR $signal_{pH6.0} > 0.6$ at saturation. IgG1 mutants with moderate increase in affinity towards murine FcRn at pH 6.0 ($\Delta\Delta G < 0.4$ kcal/mol) bound very poorly to the mouse receptor at pH 7.2. In contrast, IgG1 mutants exhibiting large affinity increase towards human FcRn at pH 6.0 ($\Delta\Delta G \geq 1.0$ kcal/mol) only showed minimal binding to the human receptor at pH 7.4 with SPR $signal_{pH7.4}$/SPR $signal_{pH6.0} < 0.15$ at saturation.

Those skilled in the art will recognize, or be able to ascertain using no more routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

What is claimed is:

1. A modified IgG comprising an IgG constant domain comprising one or more amino acid modifications relative to a wild-type IgG constant domain, wherein the modified IgG has an increased half-life compared to the half-life of an IgG having the wild-type IgG constant domain, and wherein the one or more amino acid modifications are at one or more of positions 251, 253, 255, 285-290, 308-314, 385-389, and 428-435.

2. A modified non-human IgG comprising a non-human IgG constant domain comprising one or more amino acid modifications relative to a wild-type non-human IgG constant domain, wherein the modified IgG has an increased half-life compared to the half-life of an IgG having the wild-type non-human IgG constant domain, and wherein the one or more amino acid modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, with the proviso that the one or more amino acid modifications do not include substitution with leucine at position 252, serine at position 254, and phenylalanine at position 256.

3. A modified human or humanized IgG comprising a human IgG constant domain comprising one or more amino acid modifications relative to a wild-type human IgG constant domain, wherein the modified human or humanized IgG has an increased half-life compared to the half-life of a human or humanized IgG having the wild-type human IgG constant domain, and wherein the one or more amino acid modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436.

4. The modified IgG according to claim 1, 2, or 3, wherein at least one of the amino acid modifications is an amino acid substitution.

5. The modified IgG according to claim 1, 2, or 3, wherein at least one of the amino acid modifications is an amino acid deletion.

6. The modified IgG according to claim 1, 2, or 3, wherein at least one of the amino acid modifications is an amino acid insertion.

7. The modified IgG according to claim 1, 2 or 3 which has a higher affinity for FcRn than the IgG having the wild-type IgG constant domain.

8. The modified IgG according to claim 7 which has a higher affinity for the FcRn at pH 6.0 than at pH 7.4.

9. The modified IgG according to claim 1, wherein said one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 255, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, or 436.

10. The modified IgG according to claim 2 or 3, wherein said one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434 or 336.

11. The modified IgG according to claim 1 or 3 wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine or serine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

12. The modified IgG according to claim 11, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

13. The modified IgG according to claim 11, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

14. The modified IgG according to claim 11, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

15. The modified IgG according to claim 2 wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylaline at position 252, substitution with threonine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

16. The modified IgG according to claim 15, wherein said one or more amino acid substitutions are substitution with tyrosine at position 252, threonine at position 254 and glutamate at 256.

17. The modified IgG according to claim 15, wherein said one or more amino acid substitutions are substitution with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

18. The modified IgG according to claim 15, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

19. The modified IgG according to claim 3 or 15 which has the heavy chain variable domain and light chain variable domain of SYNAGIS®.

20. The modified IgG according to claim 3 or 15 which has the heavy chain variable domain and light chain variable domain of AFFF, p12f2, p12f4, p11d4, Ale109, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L215B10, A13A11, A1H5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

21. A fusion protein comprising a non-IgG polypeptide covalently linked to a modified IgG constant domain, or a fragment thereof that binds to FcRn, said modified IgG

constant domain or fragment comprising one or more amino acid modifications relative to a wild-type IgG constant domain, wherein the one or more modifications are at one or more of positions 251, 253, 255, 285-290, 308-314, 385-389, and 428-436, and wherein said fusion protein has a longer half life than the non-IgG polypeptide alone.

22. A fusion protein comprising a non-IgG polypeptide covalently linked to a modified non-human IgG constant domain, or a fragment thereof that binds to FcRn, said modified non-human IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type non-human IgG constant domain, wherein the one or more modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, with the proviso that the modified amino acid sequence does not have leucine at position 252, serine at position 254, and phenylalanine at position 256, and wherein said fusion protein has a longer half life than the non-IgG polypeptide alone.

23. A fusion protein comprising a non-IgG polypeptide covalently linked to a modified human IgG constant domain, or a fragment thereof that binds to FcRn, said modified human IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type human IgG constant domain, wherein the one or more modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, and wherein said fusion protein has a longer half life than the non-IgG polypeptide alone.

24. The fusion protein according to claim 21, 22, or 23, wherein at least one of the amino acid modifications is an amino acid substitution.

25. The fusion protein according to claim 21, 22, or 23, wherein at least one of the amino acid modifications is an amino acid deletion.

26. The fusion protein according to claim 21, 22, or 23, wherein at least one of the amino acid modifications is an amino acid insertion.

27. The fusion protein according to claim 21, 22, or 23, wherein the modified IgG constant domain or fragment has an increased affinity for FcRn relative to the wild-type IgG constant domain.

28. The fusion protein according to claim 27, wherein the modified IgG constant domain or fragment has a higher affinity for the FcRn at pH 6.0 than at pH 7.4.

29. The fusion protein according to claim 21, 22, or 23, wherein the non-IgG polypeptide is an immunoglobulin.

30. The fusion protein according to claim 21, wherein the one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 255, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, or 436.

31. The fusion protein according to claim 22 or 23, wherein the one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434 or 336.

32. The fusion protein according to claim 21 or 23, wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine or serine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

33. The fusion protein according to claim 32, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

34. The fusion protein according to claim 32, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

35. The fusion protein according to claim 32, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

36. The fusion protein according to claim 32, wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

37. The fusion protein according to claim 36, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

38. The fusion protein according to claim 36, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

39. The fusion protein according to claim 36, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

40. A molecule comprising a non-protein agent conjugated to a modified IgG constant domain, or a fragment thereof that binds to FcRn, said modified IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type IgG constant domain, wherein the one or more modifications are at one or more of positions 251, 253, 255, 285-290, 308-314, 385-389, and 428-436, and wherein said molecule has a longer half life than the non-protein agent alone.

41. A molecule comprising a non-protein agent conjugated to a modified non-human IgG constant domain, or a fragment thereof that binds to FcRn, said modified non-human IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type non-human IgG constant domain, wherein the one or more modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, with the proviso that the modified amino acid sequence does not have leucine at position 252, serine at position 254, and phenylalanine at position 256, and wherein said molecule has a longer half life than the non-protein agent alone.

42. A molecule comprising a non-protein agent conjugated to a modified human IgG constant domain, or a fragment thereof that binds to FcRn, said modified human IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type human IgG constant domain, wherein the one or more modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, and wherein said molecule has a longer half life than the non-protein agent alone.

43. The molecule according to claim 40, 41, or 42 wherein at least one of the amino acid modifications is an amino acid substitution.

44. The molecule according to claim 40, 41, or 42 wherein at least one of the amino acid modifications is an amino acid deletion.

45. The molecule according to claim 40, 41 or 42, wherein at least one of the amino acid modifications is an amino acid insertion.

46. The molecule according to claim 40, 41 or 42, wherein the modified IgG constant domain or fragment has an increased affinity for FcRn relative to the wild-type constant domain.

47. The molecule according to claim 46, wherein the modified IgG constant domain or fragment has a higher affinity for the FcRn at pH 6.0 than at pH 7.4.

48. The molecule according to claim 40, wherein the one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 255, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, or 436.

49. The molecule according to claim 41 or 42, wherein the one or more amino acid modifications are substitution at one or more of positions 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434 or 336.

50. The molecule according to claim 40 or 42, wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine or serine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

51. The molecule according to claim 50, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

52. The molecule according to claim 50, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

53. The molecule according to claim 50, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

54. The molecule according to claim 41, wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution

with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

55. The molecule according to claim 54, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

56. The molecule according to claim 54, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

57. The molecule according to claim 54, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

58. A pharmaceutical composition comprising the modified human or humanized IgG according to claim 3 and a pharmaceutically acceptable carrier.

59. A pharmaceutical composition comprising the fusion protein according to claim 23 and a pharmaceutically acceptable carrier.

60. A pharmaceutical composition comprising the molecule according to claim 42 and a pharmaceutically acceptable carrier.

61. A method of treating a disease or disorder comprising administering to a patient in need thereof a therapeutically effective amount of the modified human or humanized IgG according to claim 3.

62. The method according to claim 61 which comprises passive immunotherapy.

63. A method of treating a disease or disorder comprising administering to a patient in need thereof a therapeutically effective amount of the fusion protein according to claim 23.

64. A method of treating a disease or disorder comprising administering to a patient in need thereof a therapeutically effective amount of the molecule according to claim 42.

65. A nucleic acid comprising a nucleotide sequence encoding the modified IgG constant domain according to claim 1, 2 or 3, or an FcRn binding fragment thereof.

66. A nucleic acid comprising a nucleotide sequence encoding the fusion protein according to claim 21, 22, or 23.

67. A host cell comprising the nucleic acid according to claim 65.

68. A host cell comprising the nucleic acid according to claim 66.

69. A kit comprising the modified human or humanized IgG according to claim 3.

70. A kit comprising the fusion protein according to claim 23.

71. A kit comprising the molecule according to claim 42.

72. A method of preventing a disease or disorder comprising administering to a subject a prophylactically effective amount of the modified human or humanized IgG according to claim 3.

73. The method according to claim 72 which is passive immunotherapy.

74. The method according to claim 72 wherein the disease is RSV infection.

75. The method according to claim 74, wherein said modified human or humanized IgG is a modified SYNAGIS® antibody.

76. The method according to claim 74, wherein said modified human or humanized IgG is a modified AFFF, p12f2, p12f4, p11d4, Ale109, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L215B10, A13A11, A1H5, A4B4(1), A4B4L1FR-S28R or A4B4-F52S antibody.

77. A method of vaccinating a subject comprising administering to said subject an amount of the modified human or humanized IgG according to claim 3 effective to elicit an immune response.

78. A method of vaccinating a subject comprising administering to said subject an amount of the fusion protein according to claim 23 effective to elicit an immune response.

79. The method according to 61 which comprises administrating a dose of said modified human or humanized IgG that is lower than the lowest therapeutically effective dose of a second IgG identical to said modified human or humanized IgG except that said second IgG lacks said one or more amino acid modifications.

80. The method according to claim 79 which results in fewer or less severe side effects than administration of the therapeutically effective dose of the second IgG.

81. The method according to claim 61 which comprises administering a therapeutically effective dosing schedule having less frequent doses of said modified human or humanized IgG than the therapeutically effective dosing schedule having the least frequent dosing of a second IgG identical to said modified human or humanized IgG except that said second IgG lacks said one or more amino acid modifications.

82. The method according to claim 72 which comprises administrating a prophylactically effective dose of said modified human or humanized IgG that is lower than the lowest prophylactically effective dose of a second IgG identical to said modified human or humanized IgG except that said second IgG lacks said one or more amino acid modifications.

83. The method according to claim 82 which results in fewer or less severe side effects than administration of the prophylactically effective dose of the second IgG.

84. The method according to claim 72 which comprises administering a prophylactically effective dosing schedule having less frequent doses of said modified human or humanized IgG than the prophylactically effective dosing schedule with the least frequent dosing of a second IgG identical to said humani or humanized IgG except that said second IgG lacks said one or more amino acid modifications.

85. A method of *in vivo* diagnosis in a subject comprising:

(a) administering to a subject an effective amount of the modified human or humanized IgG according to claim 3 labeled with a detectable marker, said modified human or humanized IgG specifically binding to an antigen associated with a disease or disorder;

(b) allowing the modified human or humanized IgG to concentrate at sites in said subject where said antigen is found; and

(c) detecting said detectable marker,

whereby detection of said detectable marker above a background or standard level indicates that the subject has said disease or disorder.

86. The modified human or humanized IgG according to claim 3 which immunospecifically binds to an RSV antigen.

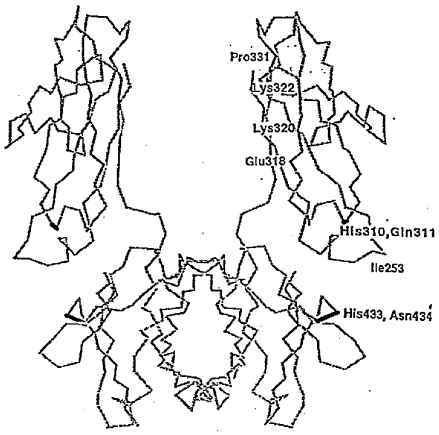
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FIG. 1

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                  10                  15
|-----------------Hinge-----------------------------------|----

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30
-------------------------------------------------------------

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45
-------------------------------------------------------------

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60
-----------------------------------------------------CH2-----

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80
-------------------------------------------------------------

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95
-------------------------------------------------------------

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110
-------------------------------------------------------------

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125
-----------------------------------------|------------------

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140
-------------------------------------------------------------

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160
-----------------------------------------------------------CH3-

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175
-------------------------------------------------------------

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190
-------------------------------------------------------------

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205
-------------------------------------------------------------

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220
-------------------------------------------------------------

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
-------------------------------|
```

FIG. 2

```
Met Gly Val Pro Arg Pro Gln Pro Trp Ala Leu Gly Leu Leu Leu Phe
1               5                  10                  15

Leu Leu Pro Gly Ser Leu Gly Ala Glu Ser His Leu Ser Leu Leu Tyr
            20                  25                  30

His Leu Thr Ala Val Ser Ser Pro Ala Pro Gly Thr Pro Ala Phe Trp
        35                  40                  45

Val Ser Gly Trp Leu Gly Pro Gln Gln Tyr Leu Ser Tyr Asn Ser Leu
    50                  55                  60

Arg Gly Glu Ala Glu Pro Cys Gly Ala Trp Val Trp Glu Asn Gln Val
65                  70                  75                  80

Ser Trp Tyr Trp Glu Lys Glu Thr Thr Asp Leu Arg Ile Lys Glu Lys
                85                  90                  95

Leu Phe Leu Glu Ala Phe Lys Ala Leu Gly Gly Lys Gly Pro Tyr Thr
            100                 105                 110

Leu Gln Gly Leu Leu Gly Cys Glu Leu Gly Pro Asp Asn Thr Ser Val
        115                 120                 125

Pro Thr Ala Lys Phe Ala Leu Asn Gly Glu Glu Phe Met Asn Phe Asp
    130                 135                 140

Leu Lys Gln Gly Thr Trp Gly Gly Asp Trp Pro Glu Ala Leu Ala Ile
145                 150                 155                 160

Ser Gln Arg Trp Gln Gln Gln Asp Lys Ala Ala Asn Lys Glu Leu Thr
                165                 170                 175

Phe Leu Leu Phe Ser Cys Pro His Arg Leu Arg Glu His Leu Glu Arg
            180                 185                 190

Gly Arg Gly Asn Leu Glu Trp Lys Glu Pro Pro Ser Met Arg Leu Lys
        195                 200                 205

Ala Arg Pro Ser Ser Pro Gly Phe Ser Val Leu Thr Cys Ser Ala Phe
    210                 215                 220

Ser Phe Tyr Pro Pro Glu Leu Gln Leu Arg Phe Leu Arg Asn Gly Leu
225                 230                 235                 240

Ala Ala Gly Thr Gly Gln Gly Asp Phe Gly Pro Asn Ser Asp Gly Ser
                245                 250                 255

Phe His Ala Ser Ser Ser Leu Thr Val Lys Ser Gly Asp Glu His His
            260                 265                 270

Tyr Cys Cys Ile Val Gln His Ala Gly Leu Ala Gln Pro Leu Arg Val
        275                 280                 285

Glu Leu Glu Ser Pro Ala Lys Ser Ser Val Leu Val Val Gly Ile Val
    290                 295                 300

Ile Gly Val Leu Leu Leu Thr Ala Ala Ala Val Gly Gly Ala Leu Leu
305                 310                 315                 320

Trp Arg Arg Met Arg Ser Gly Leu Pro Ala Pro Trp Ile Ser Leu Arg
                325                 330                 335

Gly Asp Asp Thr Gly Val Leu Leu Pro Thr Pro Gly Glu Ala Gln Asp
            340                 345                 350

Ala Asp Leu Lys Asp Val Asn Val Ile Pro Ala Thr Ala
        355                 360                 365
```

FIG. 3A

```
Met Gly Met Pro Leu Pro Trp Ala Leu Ser Leu Leu Leu Val Leu Leu
1               5                  10                  15

Pro Gln Thr Trp Gly Ser Glu Thr Arg Pro Pro Leu Met Tyr His Leu
            20                  25                  30

Thr Ala Val Ser Asn Pro Ser Thr Gly Leu Pro Ser Phe Trp Ala Thr
        35                  40                  45

Gly Trp Leu Gly Pro Gln Gln Tyr Leu Thr Tyr Asn Ser Leu Arg Gln
    50                  55                  60

Glu Ala Asp Pro Cys Gly Ala Trp Val Trp Glu Asn Gln Val Ser Trp
65                  70                  75                  80

Tyr Trp Glu Lys Glu Thr Thr Asp Leu Lys Ser Lys Glu Gln Leu Phe
                85                  90                  95

Leu Glu Ala Leu Lys Thr Leu Glu Lys Ile Leu Asn Gly Thr Tyr Thr
            100                 105                 110

Leu Gln Gly Leu Leu Gly Cys Glu Leu Ala Ser Asp Asn Ser Ser Val
        115                 120                 125

Pro Thr Ala Val Phe Ala Leu Asn Gly Glu Glu Phe Met Lys Phe Asn
    130                 135                 140

Pro Arg Ile Gly Asn Trp Thr Gly Glu Trp Pro Glu Thr Glu Ile Val
145                 150                 155                 160

Ala Asn Leu Trp Met Lys Gln Pro Asp Ala Ala Arg Lys Glu Ser Glu
                165                 170                 175

Phe Leu Leu Asn Ser Cys Pro Glu Arg Leu Leu Gly His Leu Glu Arg
            180                 185                 190

Gly Arg Arg Asn Leu Glu Trp Lys Glu Pro Pro Ser Met Arg Leu Lys
        195                 200                 205

Ala Arg Pro Gly Asn Ser Gly Ser Ser Val Leu Thr Cys Ala Ala Phe
    210                 215                 220

Ser Phe Tyr Pro Pro Glu Leu Lys Phe Arg Phe Leu Arg Asn Gly Leu
225                 230                 235                 240

Ala Ser Gly Ser Gly Asn Cys Ser Thr Gly Pro Asn Gly Asp Gly Ser
                245                 250                 255

Phe His Ala Trp Ser Leu Leu Glu Val Lys Arg Gly Asp Glu His His
            260                 265                 270

Tyr Gln Cys Gln Val Glu His Glu Gly Leu Ala Gln Pro Leu Thr Val
        275                 280                 285

Asp Leu Asp Ser Ser Ala Arg Ser Ser Val Pro Val Val Gly Ile Val
    290                 295                 300

Leu Gly Leu Leu Leu Val Val Val Ala Ile Ala Gly Gly Val Leu Leu
305                 310                 315                 320

Trp Gly Arg Met Arg Ser Gly Leu Pro Ala Pro Trp Leu Ser Leu Ser
                325                 330                 335

Gly Asp Asp Ser Gly Asp Leu Leu Pro Gly Gly Asn Leu Pro Pro Glu
            340                 345                 350

Ala Glu Pro Gln Gly Ala Asn Ala Phe Pro Ala Thr Ser
        355                 360                 365
```

FIG. 3B

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                  10                  15
|-----------------Hinge-----------------------------------|----

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30
-------------------------------------------------------------

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45
-------------------------------------------------------------

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60
-----------------------------------------------------CH2-----

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80
-------------------------------------------------------------

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95
-------------------------------------------------------------

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110
-------------------------------------------------------------

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125
-----------------------------------------------|-------------
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140
-------------------------------------------------------------

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160
-----------------------------------------------------------CH3-

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175
-------------------------------------------------------------

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190
-------------------------------------------------------------

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205
-------------------------------------------------------------

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220
-------------------------------------------------------------

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
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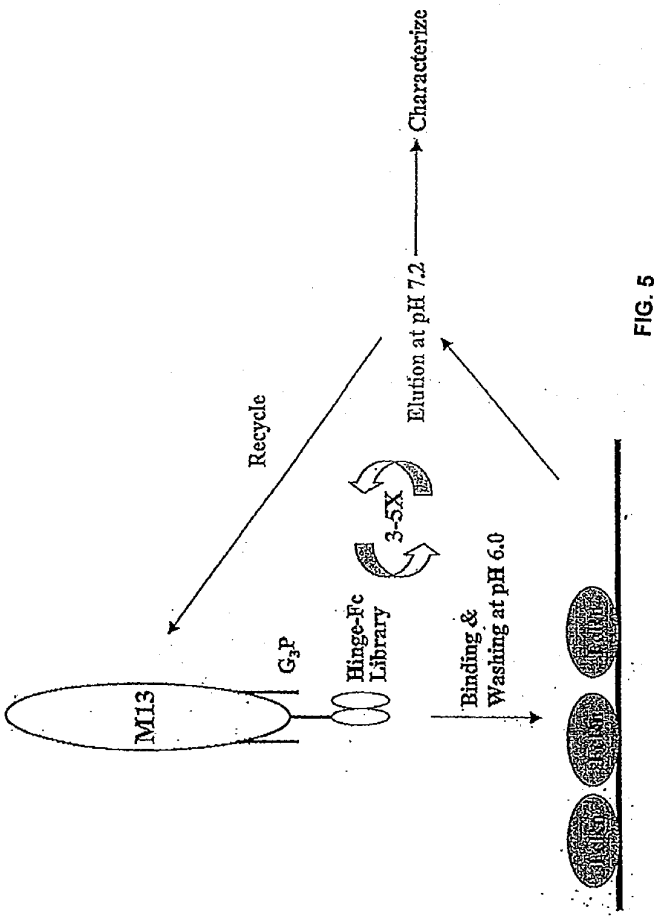
FIG. 4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Recycle
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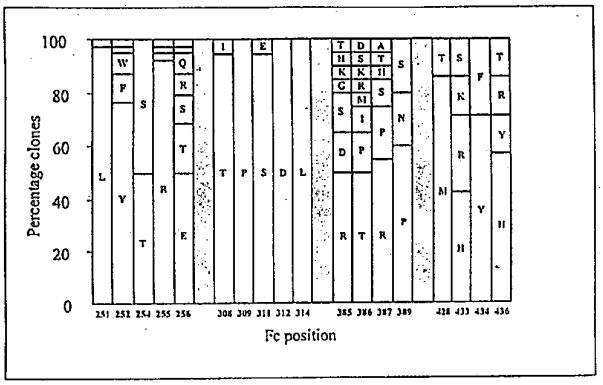
Binding &
Washing at pH 6.0
Hinge-Fc
Library
G3P
M13

FIG. 5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Percentage clones
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Fc position

FIG. 6

FIG. 7B

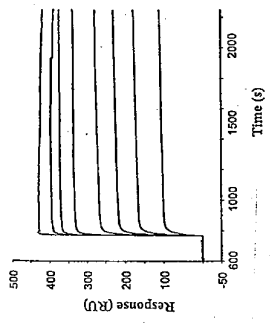


FIG. 7D

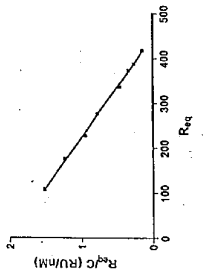


FIG. 7A

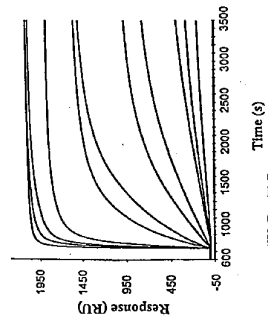
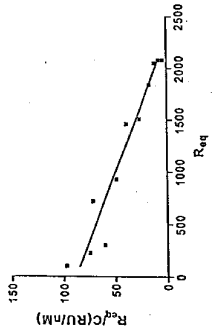


FIG. 7C



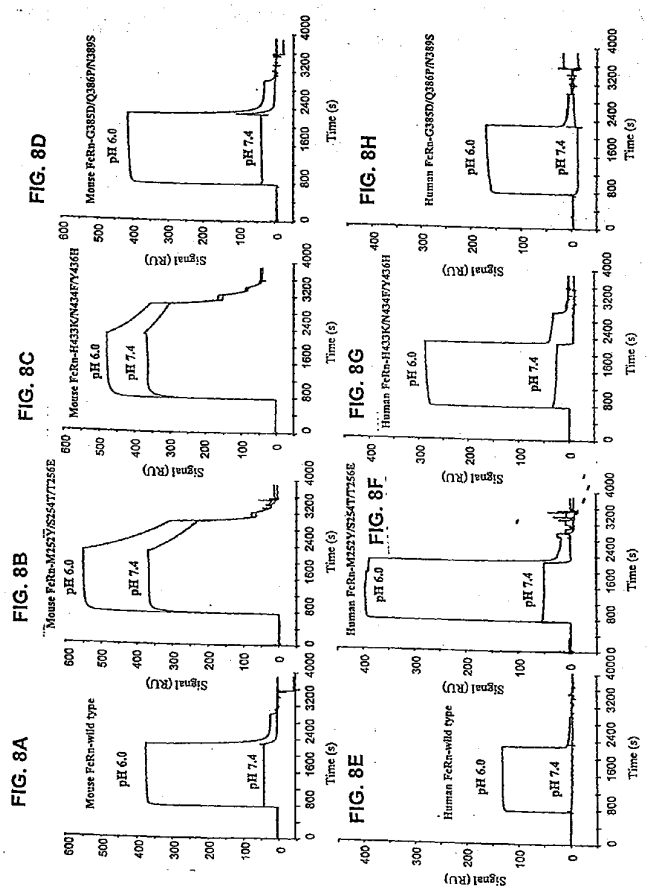


FIG. 8A
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pH 7.4
FIG. 8B
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pH 6.0
pH 7.4
FIG. 8C
Mouse FcRn-I1433K/N434F/Y436H
pH 6.0
pH 7.4
FIG. 8D
Mouse FcRn-G385D/Q386P/N389S
pH 6.0
pH 7.4
FIG. 8E
Human FcRn-wild type
pH 6.0
pH 7.4
FIG. 8F
Human FcRn-M252Y/S254T/T256E
pH 6.0
pH 7.4
FIG. 8G
Human FcRn-I1433K/N434F/Y436H
pH 6.0
pH 7.4
FIG. 8H
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pH 6.0
pH 7.4
Signal (RU)
Time (s)

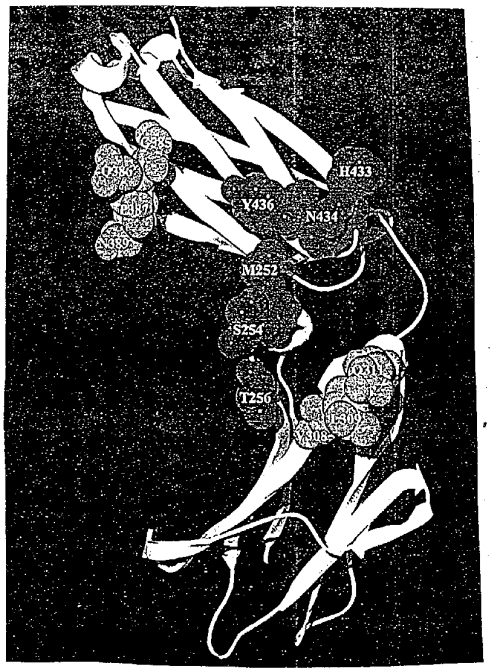
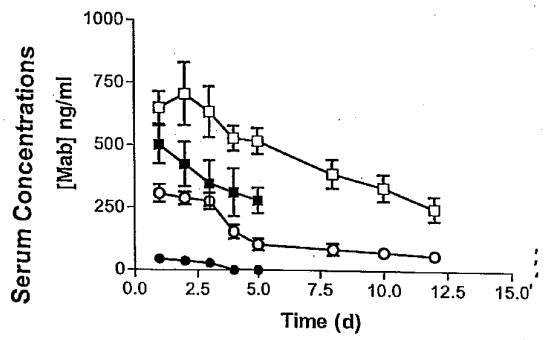


FIG. 9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[Mab] ng/ml
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7.5
10.0
12.5
15.0
Time (d)

FIG. 10

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    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
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Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
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Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
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Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
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<400> 22
Ser Leu Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 23
<211> 7
<212> PRT
<213> Homo sapiens

<400> 23
Asp Thr Phe Tyr Leu Ser Ser
1 5

<210> 24
<211> 120
<212> PRT
<213> Homo sapiens

<220>
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<223> VH Domain

<400> 24
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 25
<211> 16
<212> PRT
<213> Homo sapiens

<400> 25
Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 26
<211> 106
<212> PRT
<213> Homo sapiens

<400> 26
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Arg Gly Leu Pro Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 27
<211> 7
<212> PRT
<213> Homo sapiens

<400> 27
Asp Thr Arg Gly Leu Pro Ser
1 5

<210> 28
<211> 120
<212> PRT
<213> Homo sapiens

<400> 28
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 29
<211> 10
<212> PRT
<213> Homo sapiens

<400> 29
Asp Met Ile Phe Asn Trp Tyr Phe Asp Val
1 5 10

<210> 30
<211> 106

<212> PRT
<213> Homo sapiens

<400> 30
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Pro Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Met Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 31
<211> 10
<212> PRT
<213> Homo sapiens

<400> 31
Ser Pro Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 32
<211> 7
<212> PRT
<213> Homo sapiens

<400> 32
Asp Thr Met Arg Leu Ala Ser
1 5

<210> 33
<211> 120
<212> PRT
<213> Homo sapiens

<400> 33
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 34
<211> 106
<212> PRT
<213> Homo sapiens

<400> 34
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 35
<211> 7
<212> PRT
<213> Homo sapiens

<400> 35
Asp Thr Phe Lys Leu Ser Ser
1 5

<210> 36
<211> 120
<212> PRT
<213> Homo sapiens

<400> 36
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 37
<211> 16
<212> PRT
<213> Homo sapiens

<400> 37
Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 38
<211> 106
<212> PRT
<213> Homo sapiens

<400> 38
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 39
<211> 10
<212> PRT
<213> Homo sapiens

<400> 39
Ser Ala Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 40
<211> 120

<212> PRT
<213> Homo sapiens

<400> 40
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 41
<211> 16
<212> PRT
<213> Homo sapiens

<400> 41
Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 42
<211> 106
<212> PRT
<213> Homo sapiens

<400> 42
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 43
<211> 7
<212> PRT
<213> Homo sapiens

<400> 43
Asp Thr Met Tyr Gln Ser Ser
1 5

<210> 44
<211> 120
<212> PRT
<213> Homo sapiens

<400> 44
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 45
<211> 16
<212> PRT
<213> Homo sapiens

<400> 45
Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser Leu Lys Asp
1 5 10 15

<210> 46
<211> 106
<212> PRT
<213> Homo sapiens

<400> 46
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Pro Ser Ser Arg Val Gly Tyr Met
20 25 30

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His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Met Tyr Gln Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Phe Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
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<210> 47
<211> 10
<212> PRT
<213> Homo sapiens

<400> 47
Leu Pro Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 48
<211> 120
<212> PRT
<213> Homo sapiens

<400> 48
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
            20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser
    50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
            100 105 110

Gly Thr Thr Val Thr Val Ser Ser
        115 120

<210> 49
<211> 106
<212> PRT
<213> Homo sapiens

<400> 49
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

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Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Phe Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105

<210>  50
<211>  7
<212>  PRT
<213>  Homo sapiens

<400>  50
Asp Thr Phe Phe Leu Asp Ser
1               5

<210>  51
<211>  120
<212>  PRT
<213>  Homo sapiens

<400>  51
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1               5                   10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
            20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser
        115                 120

<210>  52
<211>  106
<212>  PRT
<213>  Homo sapiens
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<400> 52
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Arg Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 53
<211> 7
<212> PRT
<213> Homo sapiens

<400> 53
Asp Thr Arg Tyr Gln Ser Ser
1 5

<210> 54
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 54
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 55
<211> 120
<212> PRT
<213> Homo sapiens

<400> 55
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Ala
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 56
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 56
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 57

<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 57
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 58
<211> 7
<212> PRT
<213> Homo sapiens

<400> 58
Asp Thr Tyr Lys Gln Thr Ser
1 5

<210> 59
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 59
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Arg Tyr Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 60
<211> 106
<212> PRT
<213> Homo sapiens

<400> 60
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Phe Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 61
<211> 9
<212> PRT
<213> Homo sapiens

<400> 61
Phe Gln Gly Ser Phe Tyr Pro Phe Thr
1 5

<210> 62
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 62
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

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His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Phe Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105

<210>  63
<211>  7
<212>  PRT
<213>  Homo sapiens

<400>  63
Asp Thr Phe Lys Leu Thr Ser
1               5

<210>  64
<211>  106
<212>  PRT
<213>  Homo sapiens

<220>
<221>  misc_feature
<223>  VL Domain

<400>  64
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105

<210>  65
<211>  106
<212>  PRT
<213>  Homo sapiens
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<400> 65
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 66
<211> 7
<212> PRT
<213> Homo sapiens

<400> 66
Asp Thr Phe Arg Leu Ala Ser
1 5

<210> 67
<211> 120
<212> PRT
<213> Homo sapiens

<400> 67
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Ala
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 68

<211> 106
<212> PRT
<213> Homo sapiens

<400> 68
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Tyr Arg His Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 69
<211> 7
<212> PRT
<213> Homo sapiens

<400> 69
Asp Thr Tyr Arg His Ser Ser
1 5

<210> 70
<211> 106
<212> PRT
<213> Homo sapiens

<400> 70
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 71
<211> 106
<212> PRT
<213> Homo sapiens

<400> 71
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Phe His Arg Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 72
<211> 10
<212> PRT
<213> Homo sapiens

<400> 72
Ser Leu Ser Ser Ser Val Gly Tyr Met His
1 5 10

<210> 73
<211> 7
<212> PRT
<213> Homo sapiens

<400> 73
Asp Thr Phe Phe His Arg Ser
1 5

<210> 74
<211> 106
<212> PRT
<213> Homo sapiens

<400> 74
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
20 25 30

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(54) Title: MOLECULES WITH EXTENDED HALF-LIVES, COMPOSITIONS AND USES THEREOF

(57) Abstract: The present invention provides molecules, including IgGs, non-IgG immunoglobulins, proteins and non-protein agents, that have increased *in vivo* half-lives due to the presence of an IgG constant domain, or a portion thereof that binds the FcRn, having one or more amino acid modifications that increase the affinity of the constant domain or fragment for FcRn. Such proteins and molecules with increased half-lives have the advantage that smaller amounts and or less frequent dosing is required in the therapeutic, prophylactic or diagnostic use of such molecules.

MOLECULES WITH EXTENDED HALF-LIVES, COMPOSITIONS AND USES THEREOF

This application claims the benefit of United States provisional application Serial Nos. 60/254,884, filed December 12, 2000, and 60/289,760, filed May 9, 2001, both of which are incorporated by reference herein in their entireties. This invention was made, in part, with United States Government support under award number A139167 from the National Institute of Health. The United States Government may have certain rights in the invention.

1. INTRODUCTION

The present invention relates to molecules whose *in vivo* half-lives are increased by modification of an IgG constant domain, or FcRn (Fc Receptor-neonate) binding domain thereof. Specifically, these molecules have amino acid modifications that increase the affinity of the constant domain or fragment thereof for the FcRn. Increasing the half-life of therapeutic and diagnostic IgGs and other bioactive molecules using methods of the invention has many benefits including reducing the amount and/or frequency of dosing of these molecules, for example, in vaccines, passive immunotherapy and other therapeutic and prophylactic methods. The invention further relates to fusion proteins containing all or a portion (a FcRn binding portion) of an IgG constant domain having one or more of these amino acid modifications and a non-IgG protein or non-protein molecule conjugated to such a modified IgG constant domain, where the presence of the modified IgG constant domain increases the *in vivo* half-life of the non-IgG protein or molecule.

2. BACKGROUND OF THE INVENTION

The use of immunoglobulins as therapeutic agents has increased dramatically in recent years and have expanded to different areas of medical treatments. Such uses include treatment of agammaglobulinemia and hypogammaglobulinemia, as immunosuppressive agents for treating autoimmune diseases and graft-vs.-host (GVH) diseases, the treatment of lymphoid malignancies, and passive immunotherapies for the treatment of various systemic and infectious diseases. Also, immunoglobulins are useful as *in vivo* diagnostic tools, for example, in diagnostic imaging procedures.

One critical issue in these therapies is the persistence of immunoglobulins in the circulation. The rate of immunoglobulin clearance directly affects the amount and frequency of dosage of the immunoglobulin. Increased dosage and frequency of dosage may cause adverse effects in the patient and also increase medical costs.

IgG is the most prevalent immunoglobulin class in humans and other mammals and is utilized in various types of immunotherapies and diagnostic procedures. The mechanism of IgG catabolism in the circulation has been elucidated through studies related to the transfer of passive immunity from mother to fetus/neonate through the placenta or yolk sac or through colostrum (maternofetal transfer of IgG via transcytosis) in rodents (Brambell, *Lancet*, ii:1087-1093, 1966; Rodewald, *J. Cell Biol.*, 71:666-670, 1976; Morris *et al.*, In: *Antigen Absorption by the Gut*, pp. 3-22, 1978, University Park Press, Baltimore; Jones *et al.*, *J. Clin. Invest.*, 51:2916-2927, 1972).

The involvement of certain receptors in the maternofetal transmission of maternal IgGs was first suggested by Brambell's group in their study on the intestinal absorption of maternal antibodies from ingested milk in newborn rats (Halliday, *Proc. R. Soc. B.*, 143:408-413, 1955; Halliday, *Proc. R. Soc. B.*, 144:427-430, 1955; Halliday, *Proc. R. Soc. B.*, 148:92-103, 1957; Morris, *Proc. R. Soc. B.*, 148:84-91, 1957; Brambell *et al.*, *Proc. R. Soc. B.*, 149:1-11, 1958; Morris, *Proc. R. Soc. B.*, 160:276-292, 1964). Brambell *et al.* suggested, based on the observation that heterologous IgGs interfered with the transmission of a specific antibody, that IgG molecules from various species might have sufficiently similar structures or sequences that bind to common receptors (Brambell *et al.*, *Proc. R. Soc. B.*, 149:1-11, 1958).

A high-affinity Fc receptor, FcRn, has been implicated in this transfer mechanism. The FcRn receptor has been isolated from duodenal epithelial brush borders of suckling rats (Rodewald *et al.*, *J. Cell Biol.*, 99:154s-164s, 1984; Simister *et al.*, *Eur. J. Immunol.*, 15:733-738, 1985) and the corresponding gene has been cloned (Simister *et al.*, *Nature*, 337:184, 1989 and *Cold Spring Harbor Symp. Quant. Biol., LIV*, 571-580, 1989). The later clonings of FcRn-encoding genes from mice (Ahouse *et al.*, *J. Immunol.*, 151:6076-6088, 1993) and humans (Story *et al.*, *J. Exp. Med.*, 180:2377-2381, 1994) demonstrate high homology of these sequences to the rat FcRn, suggesting a similar mechanism of maternofetal transmission of IgGs involving FcRn in these species.

Meanwhile, a mechanism for IgG catabolism was also proposed by Brambell's group (Brambell *et al.*, *Nature*, 203:1352-1355, 1964; Brambell, *Lancet*, ii:1087-1093, 1966). They proposed that a proportion of IgG molecules in the circulation are bound by certain cellular receptors (*i.e.*, FcRn), which are saturable, whereby the IgGs

are protected from degradation and eventually recycled into the circulation; on the other hand, IgGs which are not bound by the receptors are degraded. The proposed mechanism was consistent with the IgG catabolism observed in hypergammaglobulinemic or hypogammaglobulinemic patients. Furthermore, based on his studies as well as others (see, *e.g.,* Spiegelberg *et al.*, *J. Exp. Med.*, 121:323-338, 1965; Edelman *et al.*, *Proc. Natl. Acad. Sci. USA*, 63:78-85, 1969), Brambell also suggested that the mechanisms involved in maternofetal transfer of IgG and catabolism of IgG may be either the same or, at least, very closely related (Brambell, *Lancet*, ii:1087-1093, 1966). Indeed, it was later reported that a mutation in the Fc-hinge fragment caused concomitant changes in catabolism, maternofetal transfer, neonatal transcytosis, and, particularly, binding to FcRn (Ghetie *et al.*, *Immunology Today*, 18(12):592-598, 1997).

These observations suggested that portions of the IgG constant domain control IgG metabolism, including the rate of IgG degradation in the serum through interactions with FcRn. Indeed, increased binding affinity for FcRn increased the serum half-life of the molecule (Kim *et al.*, *Eur. J. Immunol.*, 24:2429-2434, 1994; Popov *et al.*, *Mol. Immunol.*, 33:493-502, 1996; Ghetie *et al.*, *Eur. J. Immunol.*, 26:690-696, 1996; Junghans *et al.*, *Proc. Natl. Acad. Sci. USA*, 93:5512-5516, 1996; Israel *et al.*, *Immunol.*, 89:573-578, 1996).

Various site-specific mutagenesis experiments in the Fc region of mouse IgGs have led to identification of certain critical amino acid residues involved in the interaction between IgG and FcRn (Kim *et al.*, *Eur. J. Immunol.*, 24:2429-2434, 1994; Medesan *et al.*, *Eur. J. Immunol.*, 26:2533, 1996; Medesan *et al.*, *J. Immunol.*, 158:2211-2217, 1997). These studies and sequence comparison studies found that isoleucine at position 253, histidine at position 310, and histidine at position 435 (according to Kabat numbering, Kabat *et al.*, In: *Sequences of Proteins of Immunological Interest*, US Department of Health and Human Services, 1991, which is hereby incorporated by reference in its entirety), are highly conserved in human and rodent IgGs, suggesting their importance in IgG-FcRn binding.

Additionally, various publications describe methods for obtaining physiologically active molecules whose half-lives are modified either by introducing an FcRn-binding polypeptide into the molecules (WO 97/43316; U.S. Patent No. 5,869,046; U.S. Patent No. 5,747,035; WO 96/32478; WO 91/14438) or by fusing the molecules with antibodies whose FcRn-binding affinities are preserved but affinities for other Fc receptors have been greatly reduced (WO 99/43713) or fusing with FcRn binding domains of

antibodies (WO 00/09560; U.S. Patent No. 4,703,039). However, none of these publications disclose specific mutants in the IgG constant domain that affect half-life.

Prior studies have demonstrated that certain constant domain mutations actually reduce binding to FcRn and, thereby, reduce the IgG *in vivo* half-life. PCT publication WO 93/22332 (by Ward *et al.*) discloses various recombinant mouse IgGs whose *in vivo* half-lives are reduced by mutations between about residue 253 and about residue 434. Particularly, substitutions of isoleucine at position 253; histidine at position 310; glutamine at position 311; His at position 433; and asparagine at position 434 were found to reduce IgG half-life.

Modulation of IgG molecules by amino acid substitution, addition, or deletion to increase or reduce affinity for FcRn is also disclosed in WO 98/23289; however, the publication does not list any specific mutants that exhibit either longer or shorter *in vivo* half-lives.

In fact, only one mutant of mouse IgG1 that actually exhibited increased half-life, the triple mutation Thr252 to Ala, Thr254 to Ser, and Thr256 to Phe, has been identified (WO 97/34631).

In view of the pharmaceutical importance of increasing the *in vivo* half-lives of immunoglobulins and other bioactive molecules, there is a need to develop modified IgGs and FcRn-binding fragments thereof, (particularly modified human IgGs) that confer increased *in vivo* half-life on immunoglobulins and other bioactive molecules.

3. SUMMARY OF THE INVENTION

The present invention is based upon the inventors' identification of several mutations in the constant domain of a human IgG molecule that increase the affinity of the IgG molecule for the FcRn. In particular, the present inventors have screened libraries of human IgG1 constant domains with random amino acid mutations introduced into particular regions of the constant domain for increased affinity for FcRn. Such random mutations were made in the regions of residues 251-256, 285-290, and 308-314, all of which are in CH2 domain, and 385-389 and 428-436, which are in CH3 domain, of human IgG1 hinge-Fc regions (residues as depicted in Figure 2 (SEQ ID NO:83 or analogous residues in hinge-Fc regions of other IgG molecules as determined by sequence alignment). As used herein, all residues of the IgG constant domain are numbered according to Kabat *et al.* (Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1991, which is incorporated by reference herein in its entirety) and as presented in Figure 2 (SEQ ID NO:83), and include corresponding residues in other IgG constant domains as

determined by sequence alignment. The *in vivo* half-life, or persistence in serum or other tissues of a subject, of antibodies, and other therapeutic agents and other bioactive molecules is an important clinical parameter which determines the amount and frequency of antibody (or any other pharmaceutical molecule) administration. Accordingly, such molecules, including antibodies, with increased half-life are of significant pharmaceutical importance.

Thus, the present invention relates to a modified molecule (preferably a protein, but may be a non-protein agent) that has an increased *in vivo* half-life by virtue of the presence of a modified IgG constant domain, or FcRn-binding portion thereof (preferably the Fc or hinge-Fc domain) (preferably from a human IgG) wherein the IgG constant domain, or fragment thereof, is modified (*e.g.*, by amino acid substitution, deletion or insertion) to increase the affinity for the FcRn. In a particular embodiment, the present invention relates to modified IgGs, whose *in vivo* half-lives are extended by the modification of amino acid residues identified to be involved in the interaction of the hinge-Fc domain with the FcRn receptor. Preferably, the constant domain or fragment thereof has higher affinity for FcRn at pH 6.0 than at pH 7.4. Such modifications may also alter (*i.e.*, increase or decrease) the bioavailability (*e.g.*, transport to mucosal surfaces, or other target tissues) of the molecules. The invention also relates to other types of immunoglobulins or fragments thereof (*i.e.*, non-IgG immunoglobulins), non-immunoglobulin proteins and non-protein agents that are fused or conjugated to, or engineered to contain, an IgG constant domain, or FcRn-binding fragment thereof, having one or more such amino acid modifications.

In preferred embodiments, the present invention provides molecules, particularly, immunoglobulins whose *in vivo* half-lives are extended by the presence of an IgG constant domain, or FcRn binding fragment thereof (preferably, Fc or hinge-Fc domain), that has modifications of one or more of amino acid residues 251-256, 285-290, 308-314, 385-389, and 428-436 that increase the affinity of the constant domains or fragments thereof for FcRn. In certain embodiments, these modifications preferably exclude residues 252, 254, and 256, in particular when the IgG constant domain or fragment thereof, is murine. In particular embodiments, the modification is at one or more surface-exposed residues, and the modification is a substitution with a residue of similar charge, polarity or hydrophobicity to the residue being substituted. In preferred embodiments, the modified IgG constant domain, or fragment thereof, binds with higher affinity to FcRn at pH 6.0 than at pH 7.4. In a preferred embodiment, the constant domain, or fragment thereof, is modified by substitution of one or more of amino acid residues 251-256, 285-

290, 308-314, 385-389, and 428-436 that increase the affinity of the constant domain or FcRn-binding fragments thereof for FcRn. In certain embodiments, substitutions of residue 252 with leucine, residue 254 with serine, and/or residue 256 with phenylalanine are excluded, particularly when the constant domain or fragment thereof is derived from a mouse IgG.

In specific embodiments, the invention provides immunoglobulins or other bioactive molecules that contain an IgG1 constant domain, or FcRn-binding fragment thereof (preferably Fc or hinge-Fc domain) (preferably human), having amino acid modifications at one or more of position 308, 309, 311, 312, and 314, more specifically, having substitutions at one or more of positions 308, 309, 311, 312 and 314 with threonine, proline, serine, aspartic acid and leucine respectively. In another embodiment, residues at one or more of positions 308, 309, and 311 are substituted with isoleucine, proline, and glutamic acid, respectively. In yet another embodiment, residues at one or more of positions 308, 309, 311, 312, and 314, are substituted with threonine, proline, serine, aspartic acid, and leucine, respectively. The invention further relates to combinations of these amino acid substitutions.

Furthermore, the invention provides immunoglobulins or other bioactive molecules that contain an IgG1 constant domain, or FcRn-binding fragment thereof (preferably, Fc or hinge-Fc domain) (preferably human), having amino acid modifications at one or more of positions 251, 252, 254, 255, and 256, more specifically, having substitutions at one or more of these positions. In specific embodiments, residue 251 is substituted with leucine or arginine, residue 252 is substituted with tyrosine, phenylalanine, serine, tryptophan or threonine, residue 254 is substituted with threonine or serine, residue 255 is substituted with leucine, glycine, isoleucine or arginine, and/or residue 256 is substituted with serine, arginine, glutamine, glutamic acid, aspartic acid, alanine, asparagine or threonine. In a more specific embodiment, residue 251 is substituted with leucine, residue 252 is substituted with tyrosine, residue 254 is substituted with threonine or serine, and/or residue 255 is substituted with arginine. In yet another specific embodiment, residue 252 is substituted with phenylalanine and/or residue 256 is substituted with aspartic acid. In a preferred embodiment, residue 251 is substituted with leucine, residue 252 is substituted with tyrosine, residue 254 is substituted with threonine or serine, and/or residue 255 is substituted with arginine. The invention further relates to any combination of these substitutions.

Furthermore, the invention provides immunoglobulins or other bioactive molecules that contain an IgG1 constant domain, or FcRn-binding fragment thereof

(preferably, Fc or hinge-Fc domain) (preferably human), having amino acid modifications at one or more of positions 428, 433, 434, and 436, more specifically, having substitutions at one or more of these positions. In specific embodiments, residue 428 is substituted with methionine, threonine, leucine, phenylalanine, or serine, residue 433 is substituted with lysine, arginine, serine, isoleucine, proline, glutamine, or histidine, residue 434 is substituted with phenylalanine, tyrosine, or histidine, and/or residue 436 is substituted with histidine, asparagine, arginine, threonine, lysine, methionine, or threonine. In a more specific embodiment, residues at one or more positions 433, 434, and 436 are substituted with lysine, phenylalanine, and histidine, respectively. In a preferred embodiment, residue 428 is substituted with methionine and/or residue 434 is substituted with tyrosine.

Furthermore, the invention provides immunoglobulins or other bioactive molecules that contain an IgG1 constant domain, or FcRn-binding fragment thereof (preferably, Fc or hinge-Fc domain) (preferably human), having amino acid modifications at one or more positions 385, 386, 387, and 389, more specifically, having substitutions at one or more of these positions. In specific embodiments, residue 385 is substituted with arginine, aspartic acid, serine, threonine, histidine, lysine, or alanine, residue 386 is substituted with threonine, proline, aspartic acid, serine, lysine, arginine, isoleucine, or methionine, residue 387 is substituted with arginine, histidine, serine, threonine, alanine, or proline and/or residue 389 is substituted with proline or serine. In more specific embodiments, residues at one or more positions 385, 386, 387, and 389 are substituted with arginine, threonine, arginine, and proline, respectively. In yet another specific embodiment, residues at one or more positions 385, 386, and 389 are substituted with aspartic acid, proline, and serine, respectively.

Molecules of the invention include any combination of the above-described substitutions at one or more of residues 251, 252, 254, 255, 256, 308, 309, 311, 312, 385, 386, 387, 389, 428, 433, 434, and/or 436. In a preferred embodiment, the molecule of the invention contains a Fc region, or FcRn-binding domain thereof, having one or more of the following substitutions: leucine at residue 251, tyrosine at residue 252, threonine or serine at residue 254, arginine at residue 255, threonine at residue 308, proline at residue 309, serine at residue 311, aspartic acid at residue 312, leucine at residue 314, arginine at residue 385, threonine at residue 386, arginine at residue 387, proline at residue 389, methionine at residue 428, and/or tyrosine at residue 434.

Included within the invention are pharmaceutical compositions and methods of prophylaxis and therapy using modified immunoglobulins, proteins and other bioactive molecules of the invention having extended half-lives. Also included are methods of

diagnosis using modified immunoglobulins, proteins and other bioactive molecules of the invention having extended half-lives. In a specific embodiment, the invention provides an anti-respiratory syncytial virus (RSV) antibody useful to treat or prevent RSV infection, such as SYNAGIS® (see U.S. Patent No. 5,824,307 and Johnson *et al.*, *J. Infectious Disease* 176:1215-1224, 1997, both of which are incorporated by reference in their entireties), and other anti-RSV antibodies, including variants of SYNAGIS® (see United States patent Application Serial No., 09/724,396, filed November 28, 2000, United States patent Application Serial No. 09/724,531, filed November 28, 2000, United States patent Application Serial No. ___, filed November 28, 2001 (attorney docket no. *10271-047*), and United States patent Application Serial No. ___, filed November 28, 2001 (attorney docket no. *10271-048*), all entitled "Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment," all by Young *et al.*, all of which are incorporated by reference herein in their entireties, particularly the sequences of heavy and light chain variable domains and CDRs of anti-RSV antibodies disclosed therein), which has one or more amino acid modifications in the constant domain that increase the affinity of the antibody for FcRn and that has an increased *in vivo* half-life (see also, Section 5.1 *infra*).

3.1 DEFINITIONS

The term "IgG Fc region" as used herein refers the portion of an IgG molecule that correlates to a crystallizable fragment obtained by papain digestion of an IgG molecule. The Fc region consists of the C-terminal half of the two heavy chains of an IgG molecule that are linked by disulfide bonds. It has no antigen binding activity but contains the carbohydrate moiety and the binding sites for complement and Fc receptors, including the FcRn receptor (see below). The Fc fragment contains the entire second constant domain CH2 (residues 231-340 of human IgG1, according to the Kabat numbering system) (*e.g.*, SEQ ID NO:80) and the third constant domain CH3 (residues 341-447) (*e.g.*, SEQ ID NO:81).

The term "IgG hinge-Fc region" or "hinge-Fc fragment" as used herein refers to a region of an IgG molecule consisting of the Fc region (residues 231-447) and a hinge region (residues 216-230; *e.g.*, SEQ ID NO:82) extending from the N-terminus of the Fc region. An example of the amino acid sequence of the human IgG1 hinge-Fc region is SEQ ID NO:83.

The term "constant domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The

constant domain contains the CH1, CH2 and CH3 domains of the heavy chain and the CHL domain of the light chain.

The term "FcRn receptor" or "FcRn" as used herein refers to an Fc receptor ("n" indicates neonatal) which is known to be involved in transfer of maternal IgGs to a fetus through the human or primate placenta, or yolk sac (rabbits) and to a neonate from the colostrum through the small intestine. It is also known that FcRn is involved in the maintenance of constant serum IgG levels by binding the IgG molecules and recycling them into the serum. The binding of FcRn to IgG molecules is strictly pH-dependent with optimum binding at pH 6.0. FcRn comprises a heterodimer of two polypeptides, whose molecular weights are approximately 50 kD and 15 kD, respectively. The extracellular domains of the 50 kD polypeptide are related to major histocompatibility complex (MHC) class I α-chains and the 15 kD polypeptide was shown to be the non-polymorphic β_2-microglobulin (β_2-m). In addition to placenta and neonatal intestine, FcRn is also expressed in various tissues across species as well as various types of endothelial cell lines. It is also expressed in human adult vascular endothelium, muscle vasculature and hepatic sinusoids and it is suggested that the endothelial cells may be most responsible for the maintenance of serum IgG levels in humans and mice. The amino acid sequences of human FcRn and murine FcRn are indicated by SEQ ID NO:84 and SEQ ID NO:85, respectively. Homologs of these sequences having FcRn activity are also included.

The term "*in vivo* half-life" as used herein refers to a biological half-life of a particular type of IgG molecule or its fragments containing FcRn-binding sites in the circulation of a given animal and is represented by a time required for half the quantity administered in the animal to be cleared from the circulation and/or other tissues in the animal. When a clearance curve of a given IgG is constructed as a function of time, the curve is usually biphasic with a rapid α-phase which represents an equilibration of the injected IgG molecules between the intra- and extra-vascular space and which is, in part, determined by the size of molecules, and a longer β-phase which represents the catabolism of the IgG molecules in the intravascular space. The term "*in vivo* half-life" practically corresponds to the half life of the IgG molecules in the β-phase.

An "isolated" or "purified" antibody or fusion protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of an antibody or a fusion protein in which the antibody or the fusion protein is separated from cellular components of the cells from which it is isolated or recombinantly

produced. Thus, an antibody or a fusion protein that is substantially free of cellular material includes preparations of antibody or fusion protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of contaminating protein. When the antibody or the fusion protein is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the antibody or the fusion protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly such preparations of the antibody or the fusion protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the antibody or antibody fragment of interest. In a preferred embodiment of the present invention, antibodies are isolated or purified. In another preferred embodiment of the invention, fusion proteins are isolated or purified.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. An "isolated" nucleic acid molecule does not include cDNA molecules within a cDNA library. In a preferred embodiment of the invention, nucleic acid molecules encoding antibodies are isolated or purified. In another preferred embodiment of the invention, nucleic acid molecules encoding fusion proteins are isolated or purified.

The term "host cell" as used herein refers to the particular subject cell transfected with a nucleic acid molecule or infected with phagemid or bacteriophage and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

The names of amino acids referred to herein are abbreviated either with three-letter or one-letter symbols.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are

then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, *Proc. Natl. Acad. Sci. U.S.A.* 87:2264-2268, modified as in Karlin and Altschul, 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score-50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, http://www.ncbi.nlm.nih.gov). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, *CABIOS* 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

4. DESCRIPTION OF THE FIGURES

FIG. 1 shows the structure of the IgG hinge-Fc region indicating the locations of the residues identified to be involved in the interaction with the FcRn receptor (Ghetie *et al.*, *Immunology Today*, 18(12):592-598, 1997).

FIG. 2 shows the amino acid sequence of the human IgG1 hinge-Fc region (SEQ ID NO:83) containing a hinge region (SEQ ID NO:82), CH2 domain (SEQ ID NO:80), and CH3 domain (SEQ ID NO:81).

FIGS. 3 (A and B) show the amino acid sequences of (A) human FcRn (SEQ ID NO:84) and (B) mouse FcRn (SEQ ID NO:85), respectively.

FIG. 4 shows the amino acid sequence of the human IgG1 hinge-Fc region (SEQ ID NO:83), in which wild-type residues which are mutated by amino acid substitutions are indicated in underlined bold-face.

FIG. 5 shows a schematic diagram of panning process for the phage-displayed modified hinge-Fc library.

FIG. 6 shows a summary of the occurrence of selected mutant residues at the variant positions in the libraries screened.

FIGS. 7 (A-D). (A) shows the binding of murine FcRn to immmobilized IgG1 having M252Y/S254T/T256E substitutions. Murine FcRn was injected at 10 different concentrations ranging from 1nM to 556 nM over a surface on which 4000 resonance units (RU) of IgG1 had been coupled. After equilibrium was reached, residual bound protein was eluted with a pulse of PBS, pH 7.4. (B) shows the binding of human FcRn to immmobilized IgG1/M252Y/S254T/T256E. Murine FcRn was injected at 8 different concentrations ranging from 71 nM to 2.86 μM over a surface on which 1000 RU of IgG1 had been coupled. After equilibrium was reached, residual bound protein was eluted with a pulse of PBS, pH 7.4. (C) and (D) show scatchard analyses of the data in (A) and (B), respectively, after correction for nonspecific binding. R_{eq} is the corrected equilibrium response at a given concentration C. The plots are linear with correlation coefficients of 0.97 and 0.998, respectively. The apparent K_d are 24 nM and 225 nM, respectively.

FIGS. 8 (A-H). (A)-(D) show the results from BIAcore analysis of the binding of murine FcRn at pH 6.0 and pH 7.4 to (A) wild type human IgG1, (B) M252Y/S254T/T256E, (C) H433K/N434F/Y436H, and (D) G385D/G386P/N389S, respectively, after correction for nonspecific binding. Murine FcRn was injected at a concentration of 1.1 μm over a surface on which 1000 RU of wild type IgG1, 1000 RU of M252Y/S254T/T256E, 955 RU of H433K/N434F/Y436H, and 939 RU of G385D/Q386P/N389S had been coupled. (E)-(H) show the results from BIAcore analysis

of the binding of human FcRn at pH 6.0 and pH 7.4 to (E) wild type human IgG1, (F) M252Y/S254T/T256E, (G) H433K/N434F/Y436H, and (H) G385D/Q386P/N389S, respectively, after correction for nonspecific binding. Human FcRn was injected at a concentration of 1.4 μm over a surface on which 1000 RU of wild type IgG1, 1000 RU of M252Y/S254T/T256E, 955 RU of H433K/N434F/Y436H, and 939 RU of G385D/Q386P/N389S had been coupled.

FIG. 9 shows the space-filling model of the surface of the Fc fragment of a human IgG1 based upon the human IgG1 structure of Deisenhofer, 1981, *Biochemistry* 20:2361-2370. Residues are color-coded according to the gain of free energy of stabilization of the Fc-FcRn complex: red, substitutions at these positions were found to increase affinity b a factor of at least 2.5 times in the Fc/human FcRn interaction and of at least 5 time in the Fc/mouse FcRn interaction; blue, substitutions at those positions were found to increase affinity by a factor of less than 2 times in both the Fc-human FcRn and Fc-mouse FcRn interaction. The figure was drawn using Swiss pdb viewer (Guex and Peitsch, 1997, *Electrophoresis* 18:2714-2723).

FIG. 10 shows the changes in serum concentration ([Mab] ng/ml) over time (in days) of antibody having a wild type constant domain (SYNAGIS®) (open squares), or constant domains with the following mutations: M252Y/S254T/T256E (open circles), G385D/Q386P/N389S (solid squares), and H433K/N434F/Y436H (solid circles). Antibody concentration was determined using anti-human IgG ELISA.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to molecules, particularly proteins, more particularly immunoglobulins, that have an increased *in vivo* half-life and comprise an IgG constant domain, or fragment thereof that binds to an FcRn (preferably a Fc or hinge-Fc domain), that contains one or more amino acid modifications relative to a wild type IgG constant domain which modifications increase the affinity of the IgG constant domain, or fragment thereof, for the FcRn. In a preferred embodiment, the invention particularly relates to the modification of human or humanized IgGs and other bioactive molecules containing FcRn-binding portions of human IgGs, which have particular use in human therapy, prophylaxis and diagnosis.

5.1 MOLECULES WITH INCREASED *IN VIVO* HALF-LIVES

The present invention is based upon identification of amino acid modifications in particular portions of the IgG constant domain that interact with the FcRn, which modifications increase the affinity of the IgG, or fragment thereof, for the FcRn. Accordingly, the invention relates to molecules, preferably proteins, more preferably immunoglobulins, that comprise an IgG constant domain, or FcRn binding fragment thereof (preferably a Fc or hinge-Fc domain fragment), having one or more amino acid modifications (*i.e.*, substitutions, insertions or deletions) in one or more regions that interact with the FcRn, which modifications increase the affinity of the IgG or fragment thereof, for the FcRn, and also increase the *in vivo* half-life of the molecule. In preferred embodiments, the one or more amino acid modifications are made in one or more of residues 251-256, 285-290, 308-314, 385-389, and 428-436 of the IgG hinge-Fc region (for example, as in the human IgG1 hinge-Fc region depicted in Figure 4, SEQ ID NO:83), or analogous residues thereof, as determined by amino acid sequence alignment, in other IgG hinge-Fc regions. In a preferred embodiment, the amino acid modifications are made in a human IgG constant domain, or FcRn-binding domain thereof. In a certain embodiment, the modifications are not made at residues 252, 254, or 256 (*i.e.*, all are made at one or more of residues 251, 253, 255, 285-290, 308-314, 385-389, or 428-436) of the IgG constant domain. In a more preferred embodiment, the amino acid modifications are not the substitution with leucine at residue 252, with serine at 254, and/or with phenylalanine at position 256. In particular, in preferred embodiments, such modifications are not made when the IgG constant domain, hinge-Fc domain, hinge-Fc domain or other FcRn-binding fragment thereof is derived from a mouse.

The amino acid modifications may be any modification, preferably at one or more of residues 251-256, 285-290, 308-314, 385-389, and 428-436, that increases the *in vivo* half-life of the IgG constant domain, or FcRn-binding fragment thereof (*e.g.*, Fc or hinge-Fc domain), and any molecule attached thereto, and increases the affinity of the IgG, or fragment thereof, for FcRn. Preferably, the one or more modifications also result in a higher binding affinity of the constant domain, or FcRn-binding fragment thereof, for FcRn at pH 6.0 than at pH 7.4. In other embodiments, the modifications alter (*i.e.*, increase or decrease) bioavailability of the molecule, in particular, alters (*i.e.*, increases or decreases) transport (or concentration or half-life) of the molecule to mucosal surfaces (*e.g.*, of the lungs) or other portions of a target tissue. In a preferred embodiment, the amino acid modifications alter (preferably, increase) transport or concentration or half-life of the

molecule to the lungs. In other embodiments, the amino acid modifications alter (preferably, increase) transport (or concentration or half-life) of the molecule to the heart, pancreas, liver, kidney, bladder, stomach, large or small intestine, respiratory tract, lymph nodes, nervous tissue (central and/or peripheral nervous tissue), muscle, epidermis, bone, cartilage, joints, blood vessels, bone marrow, prostate, ovary, uterine, tumor or cancer tissue, etc. In a preferred embodiment, the amino acid modifications do not abolish, or, more preferably, do not alter, other immune effector or receptor binding functions of the constant domain, for example, but not limited to complement fixation, ADCC and binding to FcγRI, FcγRII, and FcγRIII, as can be determined by methods well-known and routine in the art. In another preferred embodiment, the modified FcRn binding fragment of the constant domain does not contain sequences that mediate immune effector functions or other receptor binding. Such fragments may be particularly useful for conjugation to a non-IgG or non-immunoglobulin molecule to increase the *in vivo* half-life thereof. In yet another embodiment, the effector functions are selectively altered (*e.g.*, to reduce or increase effector functions).

In preferred embodiments, the amino acid modifications are substitutions at one or more of residues 308, 309, 311, 312 and 314, particularly a substitution with threonine at position 308, proline at position 309, serine at position 311, aspartic acid at position 312, and/or leucine at position 314. Alternatively, the modification is the substitution with an isoleucine at position 308, proline at position 309, and/or a glutamic acid at position 311. In yet another embodiment, residues at one or more of positions 308, 309, 311, 312, and 314, are substituted with threonine, proline, leucine, alanine, and alanine, respectively. Accordingly, in certain embodiments the residue at position 308 is substituted with threonine or isoleucine, the residue at position 309 is substituted with proline, the residue at position 311 is substituted with serine, glutamic acid or leucine, the residue at position 312 is substituted with alanine, and/or the residue at position 314 is substituted with leucine or alanine. In a preferred embodiment, the substitution is a threonine at position 308, a proline at position 309, a serine at position 311, an aspartic acid at position 312, and/or a leucine at position 314.

In preferred embodiments, the amino acid modifications are substitutions at one or more of residues 251, 252, 254, 255, and 256. In specific embodiments, residue 251 is substituted with leucine or arginine, residue 252 is substituted with tyrosine, phenylalanine, serine, tryptophan or threonine, residue 254 is substituted with threonine or serine, residue 255 is substituted with arginine, leucine, glycine, or isoleucine, and/or residue 256 is substituted with serine, arginine, glutamine, glutamic acid, aspartic acid,

alanine, asparagine or threonine. In a more specific embodiment, residue 251 is substituted with leucine, residue 252 is substituted with tyrosine, residue 254 is substituted with threonine or serine, residue 255 is substituted with arginine, and/or residue 256 is substituted with glutamic acid.

In preferred embodiments, the amino acid modifications are substitutions at one or more of residues 428, 433, 434, and 436. In specific embodiments, residue 428 is substituted with threonine, methionine, leucine, phenylalanine, or serine, residue 433 is substituted with lysine, arginine, serine, isoleucine, proline, glutamine or histidine, residue 434 is substituted with phenylalanine, tyrosine, or histidine, and/or residue 436 is substituted with histidine, asparagine, arginine, threonine, lysine, or methionine. In a more specific embodiment, residues at position 428 and/or 434 are substituted with methionine, and/or histidine respectively.

In preferred embodiments, the amino acid modifications are substitutions at one or more of residues 385, 386, 387, and 389, more specifically, having substitutions at one or more of these positions. In specific embodiments, residue 385 is substituted with arginine, aspartic acid, serine, threonine, histidine, lysine, alanine or glycine, residue 386 is substituted with threonine, proline, aspartic acid, serine, lysine, arginine, isoleucine, or methionine, residue 387 is substituted with arginine, proline, histidine, serine, threonine, or alanine, and/or residue 389 is substituted with proline, serine or asparagine. In more specific embodiments, residues at one or more positions 385, 386, 387, and 389 are substituted with arginine, threonine, arginine, and proline, respectively. In yet another specific embodiment, residues at one or more positions 385, 386, and 389 are substituted with aspartic acid, proline, and serine, respectively.

In particular embodiments, amino acid modifications are made at one or a combination of residues 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, and/or 436, particularly where the modifications are one or more of the amino acid substitutions described immediately above for these residues.

In a preferred embodiment, the molecule of the invention contains a Fc region, or FcRn-binding domain thereof, having one or more of the following substitutions: leucine at residue 251, tyrosine at residue 252, threonine or serine at residue 254, arginine at residue 255, threonine at residue 308, proline at residue 309, serine at residue 311, aspartic acid at residue 312, leucine at residue 314, arginine at residue 385, threonine at residue 386, arginine at residue 387, proline at residue 389, methionine at residue 428, and/or tyrosine at residue 434.

In a preferred embodiment, the FcRn binding domain has a substitution at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16 or all 18 of residues 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, and/or 436.

Amino acid modifications can be made by any method known in the art and many such methods are well known and routine for the skilled artisan. For example, but not by way of limitation, amino acid substitutions, deletions and insertions may be accomplished using any well-known PCR-based technique. Amino acid substitutions may be made by site-directed mutagenesis (see, for example, Zoller and Smith, *Nucl. Acids Res.* 10:6487-6500, 1982; Kunkel, *Proc. Natl. Acad. Sci USA* 82:488, 1985, which are hereby incorporated by reference in their entireties). Mutants that result in increased affinity for FcRn and increased *in vivo* half-life may readily be screened using well-known and routine assays, such as those described in Section 5.11, *infra*. In a preferred method, amino acid substitutions are introduced at one or more residues in the IgG constant domain or FcRn-binding fragment thereof and the mutated constant domains or fragments are expressed on the surface of bacteriophage which are then screened for increased FcRn binding affinity (see, in particular, Section 5.2 and 5.11, *infra)*.

Preferably, the amino acid residues to be modified are surface exposed residues. Additionally, in making amino acid substitutions, preferably the amino acid residue to be substituted is a conservative amino acid substitution, for example, a polar residue is substituted with a polar residue, a hydrophilic residue with a hydrophilic residue, hydrophobic residue with a hydrophobic residue, a positively charged residue with a positively charged residue, or a negatively charged residue with a negatively charged residue. Moreover, preferably, the amino acid residue to be modified is not highly or completely conserved across species and/or is critical to maintain the constant domain tertiary structure or to FcRn binding. For example, but not by way of limitation, modification of the histidine at residue 310 is not preferred.

Specific mutants of the Fc domain that have increased affinity for FcRn were isolated after the third-round panning (as described in Section 6) from a library of mutant human IgG1 molecules having mutations at residues 308-314 (histidine at position 310 and tryptophan at position 313 are fixed), those isolated after the fifth-round panning of the library for residues 251-256 (isoleucine at position 253 is fixed), those isolated after fourth-round panning of the library for residues 428-436 (histidine at position 429, glutamic acid at position 430, alanine at position 431, leucine at position 432, and histidine at position 435 are fixed), and those isolated after sixth-round panning of the library for residues 385-389 (glutamic acid at position 388 is fixed) are listed in Table I. The wild type human IgG1 has

a sequence Val-Leu-His-Gln-Asp-Trp-Leu (SEQ ID NO:86) at positions 308-314, Leu-Met-Ile-Ser-Arg-Thr (SEQ ID NO:87) at positions 251-256, Met-His-Glu-Ala-Leu-His-Asn-His-Tyr (SEQ ID NO:88) at positions 428-436, and Gly-Gln-Pro-Glu-Asn (SEQ ID NO:89) at positions 385-389.

Table I

MUTANTS ISOLATED BY PANNING

LIBRARY	MUTANTS*
251-256	Leu **Tyr** Ile **Thr** Arg **Glu** (SEQ ID NO:90)
	*Leu **Tyr** Ile Ser Arg Thr* (SEQ ID NO:91)
	*Leu **Tyr** Ile Ser Arg **Ser*** (SEQ ID NO:92)
	*Leu **Tyr** Ile Ser Arg **Arg*** (SEQ ID NO:93)
	*Leu **Tyr** Ile Ser Arg **Gln*** (SEQ ID NO:94)
	*Leu **Trp** Ile Ser Arg Thr* (SEQ ID NO:95)
	Leu **Tyr** Ile Ser **Leu Gln** (SEQ ID NO:96)
	Leu **Phe** Ile Ser Arg **Asp** (SEQ ID NO:97)
	Leu **Phe** Ile Ser Arg **Thr** (SEQ ID NO:98)
	Leu **Phe** Ile Ser Arg **Arg** (SEQ ID NO:99)
	Leu **Phe** Ile **Thr Gly Ala** (SEQ ID NO:100)
	Leu **Ser** Ile Ser Arg **Glu** (SEQ ID NO:101)
	Arg Thr Ile Ser **Ile Ser** (SEQ ID NO:102)
308-314	**Thr Pro** His **Ser** Asp Trp Leu (SEQ ID NO:103)
	Ile Pro His **Glu** Asp Trp Leu (SEQ ID NO:104)
385-389	**Arg Thr Arg** Glu **Pro** (SEQ ID NO:105)
	Asp Pro** Pro Glu **Ser (SEQ ID NO:106)
	Ser Asp Pro Glu **Pro** (SEQ ID NO:107)
	Thr Ser His Glu Asn (SEQ ID NO:108)
	Ser Lys Ser Glu Asn (SEQ ID NO:109)
	His Arg Ser Glu Asn (SEQ ID NO:110)
	Lys Ile Arg Glu Asn (SEQ ID NO:111)

LIBRARY	MUTANTS*
	Gly **Ile Thr** Glu **Ser** (SEQ ID NO:112)
	Ser Met Ala Glu **Pro** (SEQ ID NO:113)
428-436	*Met His Glu Ala Leu Arg Tyr His* ***His*** (SEQ ID NO:114)
	Met His Glu Ala Leu His **Phe** His **His** (SEQ ID NO:115)
	Met His Glu Ala Leu **Lys Phe** His **His** (SEQ ID NO:116)
	Met His Glu Ala Leu **Ser Tyr** His **Arg** (SEQ ID NO:117)
	Thr His Glu Ala Leu His **Tyr** His **Thr** (SEQ ID NO:118)
	Met His Glu Ala Leu His **Tyr** His **Tyr** (SEQ ID NO:119)

* Substituting residues are indicated in bold face

The underlined sequences in Table I correspond to sequences that occurred 10 to 20 times in the final round of panning and the sequences in italics correspond to sequences that occurred 2 to 5 times in the final round of panning. Those sequences that are neither underlined nor italicized occurred once in the final round of panning.

In one preferred embodiment, the invention provides modified immunoglobulin molecules (*e.g.*, various antibodies) that have increased *in vivo* half-life and affinity for FcRn relative to unmodified molecules (and, in preferred embodiments, altered bioavailabilty such as increased or decreased transport to mucosal surfaces or other target tissues). Such immunoglobulin molecules include IgG molecules that naturally contain an FcRn binding domain and other non-IgG immunoglobulins (*e.g.*, IgE, IgM, IgD, IgA and IgY) or fragments of immunoglobulins that have been engineered to contain an FcRn-binding fragment (*i.e.*, fusion proteins comprising non-IgG immunoglobulin or a portion thereof and an FcRn binding domain). In both cases the FcRn-binding domain has one or more amino acid modifications that increase the affinity of the constant domain fragment for FcRn.

The modified immunoglobulins include any immunoglobulin molecule that binds (preferably, immunospecifically, *i.e.*, competes off non-specific binding), as determined by immunoassays well known in the art for assaying specific antigen-antibody binding) an antigen and contains an FcRn-binding fragment. Such antibodies include, but are not limited to, polyclonal, monoclonal, bi-specific, multi-specific, human, humanized, chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, disulfide-linked Fvs, and fragments containing either a VL or VH domain or even a complementary

determining region (CDR) that specifically binds an antigen, in certain cases, engineered to contain or fused to an FcRn binding domain.

The IgG molecules of the invention, and FcRn-binding fragments thereof, are preferably IgG1 subclass of IgGs, but may also be any other IgG subclasses of given animals. For example, in humans, the IgG class includes IgG1, IgG2, IgG3, and IgG4; and mouse IgG includes IgG1, IgG2a, IgG2b, IgG2c and IgG3. It is known that certain IgG subclasses, for example, mouse IgG2b and IgG2c, have higher clearance rates than, for example, IgG1 (Medesan *et al.*, *Eur. J. Immunol.*, 28:2092-2100, 1998). Thus, when using IgG subclasses other than IgG1, it may be advantageous to substitute one or more of the residues, particularly in the CH2 and CH3 domains, that differ from the IgG1 sequence with those of IgG1, thereby increasing the *in vivo* half-life of the other types of IgG.

The immunoglobulins (and other proteins used herein) may be from any animal origin including birds and mammals. Preferably, the antibodies are human, rodent (*e.g.*, mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described *infra* and, for example, in U.S. Patent No. 5,939,598 by Kucherlapati *et al.*

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide or may be specific for heterologous epitopes, such as a heterologous polypeptide or solid support material. See, *e.g.*, PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, *et al.*, *J. Immunol.*, 147:60-69, 1991; U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny *et al.*, *J. Immunol.*, 148:1547-1553, 1992.

The antibodies of the invention include derivatives that are otherwise modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding antigen and/or generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of

tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas, pp. 563-681 (Elsevier, N.Y., 1981) (both of which are incorporated herein by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with an antigen of interest or a cell expressing such an antigen. Once an immune response is detected, *e.g.*, antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells. Hybridomas are selected and cloned by limiting dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding the antigen. Ascites fluid, which generally contains high levels of antibodies, can be generated by inoculating mice intraperitoneally with positive hybridoma clones.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and $F(ab')_2$ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). $F(ab')_2$ fragments contain the complete light chain, and the variable region, the CH1 region and the hinge region of the heavy chain.

For example, antibodies can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains, such as Fab and Fv or disulfide-bond stabilized Fv, expressed from a repertoire or combinatorial antibody library (*e.g.*, human or murine). Phage expressing an

antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage, including fd and M13. The antigen binding domains are expressed as a recombinantly fused protein to either the phage gene III or gene VIII protein. Alternatively, the modified FcRn binding portion of immunoglobulins of the present invention can be also expressed in a phage display system. Examples of phage display methods that can be used to make the immunoglobulins, or fragments thereof, of the present invention include those disclosed in Brinkman *et al.*, *J. Immunol. Methods*, 182:41-50, 1995; Ames *et al.*, *J. Immunol. Methods*, 184:177-186, 1995; Kettleborough *et al.*, *Eur. J. Immunol.*, 24:952-958, 1994; Persic *et al.*, *Gene*, 187:9-18, 1997; Burton *et al.*, *Advances in Immunology*, 57:191-280, 1994; PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/1 1236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired fragments, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax *et al.*, *BioTechniques*, 12(6):864-869, 1992; and Sawai *et al.*, *AJRI*, 34:26-34, 1995; and Better *et al.*, *Science*, 240:1041-1043, 1988 (each of which is incorporated by reference in its entirety). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston *et al.*, *Methods in Enzymology*, 203:46-88, 1991; Shu *et al.*, *PNAS*, 90:7995-7999, 1993; and Skerra *et al.*, *Science*, 240:1038-1040, 1988.

For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a constant region derived from a human immunoglobulin. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison,

Science, 229:1202, 1985; Oi *et al.*, *BioTechniques*, 4:214 1986; Gillies *et al.*, *J. Immunol. Methods*, 125:191-202, 1989; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entireties. Humanized antibodies are antibody molecules from non-human species that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. See, *e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; Riechmann *et al.*, *Nature*, 332:323, 1988, which are incorporated herein by reference in their entireties. Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101 and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology*, 28(4/5):489-498, 1991; Studnicka *et al.*, *Protein Engineering*, 7(6):805-814, 1994; Roguska *et al.*, *Proc Natl. Acad. Sci. USA*, 91:969-973, 1994), and chain shuffling (U.S. Patent No. 5,565,332), all of which are hereby incorporated by reference in their entireties.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645; WO 98/50433; WO 98/24893; WO 98/16654; WO 96/34096; WO 96/33735; and WO 91/10741, each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.*, 13:65-93, 1995. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.*, PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806;

5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entireties. In addition, companies such as Abgenix, Inc. (Freemont, CA), Medarex (NJ) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers *et al.*, *Bio/technology*, 12:899-903, 1988).

In particular embodiments, the modified antibodies have *in vivo* therapeutic and/or prophylactic uses. Examples of therapeutic and prophylactic antibodies which may be so modified include, but are not limited to, SYNAGIS® (MedImmune, MD) which is a humanized anti-respiratory syncytial virus (RSV) monoclonal antibody for the treatment of patients with RSV infection; HERCEPTIN® (Trastuzumab) (Genentech, CA) which is a humanized anti-HER2 monoclonal antibody for the treatment of patients with metastatic breast cancer; REMICADE® (infliximab) (Centocor, PA) which is a chimeric anti-TNFα monoclonal antibody for the treatment of patients with Crone's disease; REOPRO® (abciximab) (Centocor) which is an anti-glycoprotein IIb/IIIa receptor on the platelets for the prevention of clot formation; ZENAPAX® (daclizumab) (Roche Pharmaceuticals, Switzerland) which is an immunosuppressive, humanized anti-CD25 monoclonal antibody for the prevention of acute renal allograft rejection. Other examples are a humanized anti-CD18 F(ab')$_2$ (Genentech); CDP860 which is a humanized anti-CD18 F(ab')$_2$ (Celltech, UK); PRO542 which is an anti-HIV gp120 antibody fused with CD4 (Progenics/Genzyme Transgenics); Ostavir which is a human anti Hepatitis B virus antibody (Protein Design Lab/Novartis); PROTOVIR™ which is a humanized anti-CMV IgG1 antibody (Protein Design Lab/Novartis); MAK-195 (SEGARD) which is a murine anti-TNF-α F(ab')$_2$ (Knoll Pharma/BASF); IC14 which is an anti-CD14 antibody (ICOS Pharm); a humanized anti-VEGF IgG1 antibody (Genentech); OVAREX™ which is a murine anti-CA 125 antibody (Altarex); PANOREX™ which is a murine anti-17-IA cell surface antigen IgG2a antibody (Glaxo Wellcome/Centocor); BEC2 which is a murine anti-idiotype (GD3 epitope) IgG antibody (ImClone System); IMC-C225 which is a chimeric anti-EGFR IgG antibody (ImClone System); VITAXIN™ which is a humanized anti-αVβ3 integrin antibody (Applied Molecular Evolution/MedImmune); Campath 1H/LDP-03 which is a humanized anti CD52 IgG1 antibody (Leukosite); Smart M195 which is a humanized anti-CD33 IgG antibody (Protein Design Lab/Kanebo); RITUXAN™ which is a chimeric anti-CD20 IgG1

antibody (IDEC Pharm/Genentech, Roche/Zettyaku); LYMPHOCIDE™ which is a humanized anti-CD22 IgG antibody (Immunomedics); Smart ID10 which is a humanized anti-HLA antibody (Protein Design Lab); ONCOLYM™ (Lym-1) is a radiolabelled murine anti-HLA DIAGNOSTIC REAGENT antibody (Techniclone); ABX-IL8 is a human anti-IL8 antibody (Abgenix); anti-CD11a is a humanized IgG1 antibody (Genetech/Xoma); ICM3 is a humanized anti-ICAM3 antibody (ICOS Pharm); IDEC-114 is a primatied anti-CD80 antibody (IDEC Pharm/Mitsubishi); ZEVALIN™ is a radiolabelled murine anti-CD20 antibody (IDEC/Schering AG); IDEC-131 is a humanized anti-CD40L antibody (IDEC/Eisai); IDEC-151 is a primatized anti-CD4 antibody (IDEC); IDEC-152 is a primatized anti-CD23 antibody (IDEC/Seikagaku); SMART anti-CD3 is a humanized anti-CD3 IgG (Protein Design Lab); 5G1.1 is a humanized anti-complement factor 5 (C5) antibody (Alexion Pharm); D2E7 is a humanized anti-TNF-α antibody (CAT/BASF); CDP870 is a humanized anti-TNF-α Fab fragment (Celltech); IDEC-151 is a primatized anti-CD4 IgG1 antibody (IDEC Pharm/SmithKline Beecham); MDX-CD4 is a human anti-CD4 IgG antibody (Medarex/Eisai/Genmab); CDP571 is a humanized anti-TNF-α IgG4 antibody (Celltech); LDP-02 is a humanized anti-α4β7 antibody (LeukoSite/Genentech); OrthoClone OKT4A is a humanized anti-CD4 IgG antibody (Ortho Biotech); ANTOVA™ is a humanized anti-CD40L IgG antibody (Biogen); ANTEGREN™ is a humanized anti-VLA-4 IgG antibody (Elan); MDX-33 is a human anti-CD64 (FcγR) antibody (Medarex/Centeon); SCH55700 is a humanized anti-IL-5 IgG4 antibody (Celltech/Schering); SB-240563 and SB-240683 are humanized anti-IL-5 and IL-4 antibodies, respectively, (SmithKline Beecham); rhuMab-E25 is a humanized anti-IgE IgG1 antibody (Genentech/Norvartis/Tanox Biosystems); IDEC-152 is a primatized anti-CD23 antibody (IDEC Pharm); ABX-CBL is a murine anti CD-147 IgM antibody (Abgenix); BTI-322 is a rat anti-CD2 IgG antibody (Medimmune/Bio Transplant); Orthoclone/OKT3 is a murine anti-CD3 IgG2a antibody (ortho Biotech); SIMULECT™ is a chimeric anti-CD25 IgG1 antibody (Novartis Pharm); LDP-01 is a humanized anti-β_2-integrin IgG antibody (LeukoSite); Anti-LFA-1 is a murine anti CD18 F(ab')$_2$ (Pasteur-Merieux/Immunotech); CAT-152 is a human anti-TGF-β_2 antibody (Cambridge Ab Tech); and Corsevin M is a chimeric anti-Factor VII antibody (Centocor).

In specific embodiments, the invention provides modified antibodies having one or more of the mutations described herein and that immunospecifically bind RSV, *e.g.*, SYNAGIS®. The present invention also provides modified antibodies having one or more of the mutations described herein and that comprise a variable heavy (VH) and/or variable light (VL) domain having the amino acid sequence of any VH and/or VL domain listed in

Table III. The present invention further encompasses anti-RSV antibodies comprising one or more VH complementarity determining regions (CDRs) and/or one or more VL CDRs having the amino acid sequence of one or more VH CDRs and/or VL CDRS listed in Table III or one or more of the CDRs listed in Table II wherein one or more of the bolded and underlined residues has an amino acid substitution, preferably that increases the affinity of the antibody for RSV. In specific embodiments, the antibody to be modified is AFFF, p12f2, p12f4, p11d4, Ale109, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L215B10, A13A11, A1H5, A4B4(1), A4B4L1FR-S28R, A4B4-F52S.

Table II.

CDR Sequences of SYNAGIS®

CDR	Sequence	SEQ ID NO:
VH1	T**S**GMSVG	1
VH2	DIWWD**D**K**KD**YNPSLK**S**	2
VH3	**S**MI**T**N**W**YFDV	3
VL1	**KCQLS**VGYMH	4
VL2	DT**SKL**AS	5
VL3	FQGS**G**YP**F**T	6

Table III

ANTI-RSV ANTIBODIES

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
SYNAGIS	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMFTNWYFDV (SEQ ID NO:3)	SEQ ID NO:8	KCQLSVGYMH (SEQ ID NO:4)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
AFFF	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMFTNFYFDV (SEQ ID NO:12)	SEQ ID NO:13	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQFSGYPFT (SEQ ID NO:16)
p12f2	SEQ ID NO:17	TPGMSVG (SEQ ID NO:18)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMFFNFYFDV (SEQ ID NO:20)	SEQ ID NO:21	SLSSRVGYMH (SEQ ID NO:22)	DTEYLSS (SEQ ID NO:23)	FQGSGYPFT (SEQ ID NO:6)
p12f4	SEQ ID NO:24	TPGMSVG (SEQ ID NO:18)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMFFNFYFDV (SEQ ID NO:20)	SEQ ID NO:26	SLSSRVGYMH (SEQ ID NO:22)	DTRGLPS (SEQ ID NO:27)	FQGSGYPFT (SEQ ID NO:6)
p11f4	SEQ ID NO:28	TPGMSVG (SEQ ID NO:18)	DIWFDGKKHYNPSLKD (SEQ ID NO:25)	DMFFNWYFDV (SEQ ID NO:29)	SEQ ID NO:30	SPSSRVGYMH (SEQ ID NO:31)	DTMRLAS (SEQ ID NO:32)	FQGSGYPFT (SEQ ID NO:6)
Ale109	SEQ ID NO:33	TAGMSVG (SEQ ID NO:10)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMFFNWYFDV (SEQ ID NO:29)	SEQ ID NO:34	SLSSRVGYMH (SEQ ID NO:22)	DTFKLSS (SEQ ID NO:35)	FQGSGYPFT (SEQ ID NO:6)
A12a6	SEQ ID NO:36	TAGMSVG (SEQ ID NO:10)	DIWWDGKKDYNPSLKD (SEQ ID NO:37)	DMFFNFYFDV (SEQ ID NO:20)	SEQ ID NO:38	SASSRVGYMH (SEQ ID NO:39)	DTFKLSS (SEQ ID NO:35)	FQGSGYPFT (SEQ ID NO:6)
A13e4	SEQ ID NO:40	TAGMSVG (SEQ ID NO:10)	DIWWDGKKSYNPSLKD (SEQ ID NO:41)	DMFFNFYFDV (SEQ ID NO:20)	SEQ ID NO:42	SLSSRVGYMH (SEQ ID NO:22)	DTMYQSS (SEQ ID NO:43)	FQGSGYPFT (SEQ ID NO:6)
A17d4	SEQ ID NO:44	TAGMSVG (SEQ ID NO:10)	DIWWDDKKSYNPSLKD (SEQ ID NO:45)	DMFFNFYFDV (SEQ ID NO:20)	SEQ ID NO:46	LPSSRVGYMH (SEQ ID NO:47)	DTMYQSS (SEQ ID NO:43)	FQGSGYPFT (SEQ ID NO:6)

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VH Domain	VL CDR1	VL CDR2	VL CDR3
A4B4	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO:49	SASSHVGYMH (SEQ ID NO:39)	DTFFLDS (SEQ ID NO:50)	FQGSGYPFT (SEQ ID NO:6)
A8C7	SEQ ID NO:51	TAGMSVG (SEQ ID NO:10)	DIWWDDEKSYNPSLKD (SEQ ID NO:45)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:52	SPSSHVGYMH (SEQ ID NO:51)	DTRYQSS (SEQ ID NO:53)	FQGSGYPFT (SEQ ID NO:6)
IX-499LHFR	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNWYFDV (SEQ ID NO:3)	SEQ ID NO:54	SASSSVGYMH (SEQ ID NO:14)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
H2-3F4	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
M35B	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTYKQTS (SEQ ID NO:57)	FQGSGYPFT (SEQ ID NO:6)
Y10H6	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMIFNWYFDV (SEQ ID NO:29)	SEQ ID NO:58	SASSSVGYMH (SEQ ID NO:14)	DTRYLSS (SEQ ID NO:59)	FQGSGYPFT (SEQ ID NO:6)
DG	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
AFFF(1)	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	SMITNFYFDV (SEQ ID NO:12)	SEQ ID NO:60	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQGSFYPFT (SEQ ID NO:61)
6H8	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:62	SASSSVGYMH (SEQ ID NO:14)	DTFKLTS (SEQ ID NO:63)	FQGSGYPFT (SEQ ID NO:6)
L1-7E5	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:64	SASSHVGYMH (SEQ ID NO:39)	DTFKLAS (SEQ ID NO:15)	FQGSGYPFT (SEQ ID NO:6)
L215B10	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDDKKDYNPSLKS (SEQ ID NO:2)	DMITNFYFDV (SEQ ID NO:79)	SEQ ID NO:65	SASSSVGYMH (SEQ ID NO:14)	DTFRLAS (SEQ ID NO:66)	FQGSGYPFT (SEQ ID NO:6)
A13A11	SEQ ID NO:67	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:29)	SEQ ID NO:68	SPSSHVGYMH (SEQ ID NO:51)	DTYRHSS (SEQ ID NO:69)	FQGSGYPFT (SEQ ID NO:6)

Antibody Name	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Domain	VL CDR1	VL CDR2	VL CDR3
A1H5	SEQ ID NO:70	TAGMSVG (SEQ ID NO:10)	DIWWDGKKHYNPSLKD (SEQ ID NO:25)	DMFNWYFDV (SEQ ID NO:29)	SEQ ID NO:71	SLSSSVGYMH (SEQ ID NO:72)	DTFHHS (SEQ ID NO:73)	FQGSGYPFT (SEQ ID NO:6)
A4B4(1)	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMFNFYFDV (SEQ ID NO:20)	SEQ ID NO:74	SASSRVGYMH (SEQ ID NO:39)	DTLLLDS (SEQ ID NO:75)	FQGSGYPFT (SEQ ID NO:6)
A4B4-1F R-52BR	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMFNFYFDV (SEQ ID NO:20)	SEQ ID NO:11	SASSRVGYMH (SEQ ID NO:39)	DTSKLAS (SEQ ID NO:5)	FQGSGYPFT (SEQ ID NO:6)
A4B4-F52S	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDDKKHYNPSLKD (SEQ ID NO:19)	DMFNFYFDV (SEQ ID NO:20)	SEQ ID NO:76	SASSRVGYMH (SEQ ID NO:39)	DTSDLDS (SEQ ID NO:77)	FQGSGYPFT (SEQ ID NO:6)

In other embodiments, the antibody is a modified anti-$\alpha_v\beta_3$ antibody, preferably a Vitaxin antibody (see, PCT publications WO 98/33919 and WO 00/78815, both by Huse *et al.*, and both of which are incorporated by reference herein in their entireties).

Modified IgGs of the present invention having longer half-lives than wild type may also include IgGs whose bioactive sites, such as antigen-binding sites, Fc-receptor binding sites, or complement-binding sites, are modified by genetic engineering to increase or reduce such activities compared to the wild type.

Modification of these and other therapeutic antibodies to increase the *in vivo* half-life permits administration of lower effective dosages and/or less frequent dosing of the therapeutic antibody. Such modification to increase *in vivo* half-life can also be useful to improve diagnostic immunoglobulins as well, for example, permitting administration of lower doses to achieve sufficient diagnostic sensitivity.

The present invention also provides fusion proteins comprising a bioactive molecule and an hinge-Fc region or a fragment thereof (preferably human) having one or more modifications (*i.e.*, substitutions, deletions, or insertions) in amino acid residues identified to be involved in the interaction between the hinge-Fc region and the FcRn receptor. In particular, the present invention provides fusion proteins comprising a bioactive molecule recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a CH2 domain having one or more modifications in amino acid residues 251-256, 285-290, and/or amino acid residues 308-314, and/or to a CH3 domain having one or more modifications in amino acid residues 385-389 and/or 428-436, in particular, one or more of the amino acid substitutions discussed above. The fusion of a bioactive molecule to a constant domain or a fragment thereof with one or more of such modifications increases the *in vivo* half-life of the bioactive molecule.

In a preferred embodiment, fusion proteins of the invention comprise a bioactive molecule recombinantly fused or chemically conjugated to a CH2 domain having one or more amino acid residue substitutions in amino acid residues 251-256, 285-290, and/or amino acid residues 308-314, and/or to a CH3 domain having one or more modifications in amino acid residues 385-389 and/or 428-436. In certain embodiments, a fusion protein comprises a CH2 domain of IgG molecule in which amino acid residues 253, 310, and 313 are not modified. In another embodiments, a fusion protein comprises a CH3 domain of IgG molecule in which amino acid residues 388, 429, 430, 431, 432, and 435 are not modified.

A bioactive molecule can be any polypeptide or synthetic drug known to one of skill in the art. Preferably, a bioactive molecule is a polypeptide consisting of at least 5,

preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acid residues. Examples of bioactive polypeptides include, but are not limited to, various types of antibodies, cytokines (*e.g.*, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-15, IFN-γ, IFN-α, and IFN-β), cell adhesion molecules (*e.g.*, CTLA4, CD2, and CD28), ligands (*e.g.*, TNF-α,, TNF-β, and an anti-angiogenic factor such as endostatin), receptors, antibodies and growth factors (*e.g.*, PDGF, EGF, NGF, and KGF).

A bioactive molecule can also be a therapeutic moiety such as a cytotoxin (*e.g.*, a cytostatic or cytocidal agent), a therapeutic agent or a radioactive element (*e.g.*, alpha-emitters, gamma-emitters, etc.). Examples of cytostatic or cytocidal agents include, but are not limited to, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The present invention also provides polynucleotides comprising a nucleotide sequence encoding a modified IgG of the invention and fragments thereof which contain the modified FcRn binding sites with increased affinity and vectors comprising said polynucleotides. Furthermore, the invention includes polynucleotides that hybridize under stringent or lower stringent hybridization conditions to polynucleotides encoding modified IgGs of the present invention.

The nucleotide sequence of modified IgGs and the polynucleotides encoding the same may be obtained by any methods known in the art, including general DNA sequencing method, such as dideoxy chain termination method (Sanger sequencing), and oligonucleotide priming in combination with PCR, respectively.

5.2. IDENTIFICATION OF MUTATIONS WITHIN THE HINGE-FC REGION OF IMMUNOGLOBULIN MOLECULES

One or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and 428-436 of the constant domain may be introduced utilizing any technique known to those of skill in the art. The constant domain or fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and 428-436 may be screened by, for example, a binding assay to identify the constant domain or fragment thereof with increased affinity for the FcRn receptor (*e.g.*, as described in section 5.11, *infra*). Those modifications in the hinge-Fc domain or the fragments thereof which increase the affinity of the constant domain or fragment thereof for the FcRn receptor can be introduced into antibodies to increase the *in vivo* half-lives of said antibodies. Further, those modifications in the constant domain or the fragment thereof which increase the affinity of the constant domain or fragment thereof for the FcRn can be fused to bioactive molecules to increase the *in vivo* half-lives of said bioactive molecules (and, preferably alter (increase or decrease) the bioavailability of the molecule, for example, to increase or decrease transport to mucosal surfaces (or other target tissue) (*e.g.*, the lungs).

5.2.1. MUTAGENESIS

Mutagenesis may be performed in accordance with any of the techniques known in the art including, but not limited to, synthesizing an oligonucleotide having one or more modifications within the sequence of the constant domain of an antibody or a fragment thereof (*e.g.*, the CH2 or CH3 domain) to be modified. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to about 75 nucleotides or more in length is preferred, with about 10 to about 25 or more residues on both sides of the junction of the sequence being altered. A number of such primers introducing a variety of different mutations at one or more positions may be used to generated a library of mutants.

The technique of site-specific mutagenesis is well known in the art, as exemplified by various publications (see, *e.g.*,. Kunkel *et al.*, *Methods Enzymol.*, 154:367-82, 1987, which is hereby incorporated by reference in its entirety). In general, site-directed mutagenesis is performed by first obtaining a single-stranded vector or melting

apart of two strands of a double stranded vector which includes within its sequence a DNA sequence which encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as T7 DNA polymerase, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform or transfect appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement. As will be appreciated, the technique typically employs a phage vector which exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily commercially available and their use is generally well known to those skilled in the art. Double stranded plasmids are also routinely employed in site directed mutagenesis which eliminates the step of transferring the gene of interest from a plasmid to a phage.

Alternatively, the use of PCR™ with commercially available thermostable enzymes such as *Taq* DNA polymerase may be used to incorporate a mutagenic oligonucleotide primer into an amplified DNA fragment that can then be cloned into an appropriate cloning or expression vector. See, *e.g.*, Tomic *et al.*, *Nucleic Acids Res.*, 18(6):1656, 1987, and Upender *et al.*, *Biotechniques*, 18(1):29-30, 32, 1995, for PCR™ - mediated mutagenesis procedures, which are hereby incorporated in their entireties. PCR™ employing a thermostable ligase in addition to a thermostable polymerase may also be used to incorporate a phosphorylated mutagenic oligonucleotide into an amplified DNA fragment that may then be cloned into an appropriate cloning or expression vector (see *e.g.*, Michael, *Biotechniques*, 16(3):410-2, 1994, which is hereby incorporated by reference in its entirety).

Other methods known to those of skill in art of producing sequence variants of the Fc domain of an antibody or a fragment thereof can be used. For example, recombinant vectors encoding the amino acid sequence of the constant domain of an antibody or a fragment thereof may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants.

5.2.2. PANNING

Vectors, in particular, phage, expressing constant domains or fragments thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436 can be screened to identify constant domains or fragments thereof

having increased affinity for FcRn to select out the highest affinity binders from a population of phage. Immunoassays which can be used to analyze binding of the constant domain or fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436 to the FcRn include, but are not limited to, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, and fluorescent immunoassays. Such assays are routine and well known in the art (see, *e.g.*, Ausubel *et al.*, eds, 1994, *Current Protocols in Molecular Biology*, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly herein below (but are not intended by way of limitation). BIAcore kinetic analysis can also be used to determine the binding on and off rates of a constant domain or a fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436 to the FcRn. BIAcore kinetic analysis comprises analyzing the binding and dissociation of a constant domain or a fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436 from chips with immobilized FcRn on their surface (see section 5.1 and the Example section *infra*).

5.2.3. SEQUENCING

Any of a variety of sequencing reactions known in the art can be used to directly sequence the nucleotide sequence encoding constant domains or fragments thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert (*Proc. Natl. Acad. Sci. USA*, 74:560, 1977) or Sanger (*Proc. Natl. Acad. Sci. USA*, 74:5463, 1977). It is also contemplated that any of a variety of automated sequencing procedures can be utilized (*Bio/Techniques*, 19:448, 1995), including sequencing by mass spectrometry (see, *e.g.*, PCT Publication No. WO 94/16101, Cohen *et al.*, *Adv. Chromatogr.*, 36:127-162, 1996, and Griffin *et al.*, *Appl. Biochem. Biotechnol.*, 38:147-159, 1993).

5.3. RECOMBINANT METHODS OF PRODUCING ANTIBODIES

The antibodies of the invention or fragments thereof can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

The nucleotide sequence encoding an antibody may be obtained from any information available to those of skill in the art (*i.e.*, from Genbank, the literature, or by routine cloning). If a clone containing a nucleic acid encoding a particular antibody or an epitope-binding fragment thereof is not available, but the sequence of the antibody molecule or epitope-binding fragment thereof is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A^+ RNA, isolated from any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5 'ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et al.*, 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and Ausubel *et al.*, eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence by, for example, introducing amino acid substitutions, deletions, and/or insertions into the epitope-binding domain regions of the antibodies and preferably, into the hinge-Fc regions of the antibodies which are involved in the interaction with the FcRn. In a preferred embodiment, antibodies having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and 428-436 are generated.

Recombinant expression of an antibody requires construction of an expression vector containing a nucleotide sequence that encodes the antibody. Once a nucleotide sequence encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably, but not necessarily, containing the heavy or light chain variable region) has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and

appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding the constant region of the antibody molecule with one or more modifications in the amino acid residues involved in the interaction with the FcRn (see, *e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464). The nucleotide sequence encoding the heavy-chain variable region, light-chain variable region, both the heavy-chain and light-chain variable regions, an epitope-binding fragment of the heavy- and/or light-chain variable region, or one or more complementarity determining regions (CDRs) of an antibody may be cloned into such a vector for expression.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody having an increased affinity for the FcRn and an increased *in vivo* half-life. Thus, the invention includes host cells containing a polynucleotide encoding an antibody, a constant domain or a FcRn binding fragment thereof having one or more modifications in amino acid residues 251-256, 285-290, 308-314, 385-389, and/or 428-436, preferably, operably linked to a heterologous promoter.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include, but are not limited to, microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; and tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; and mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 3T3 and NSO cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for

the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, *Gene*, 45:101, 1986, and Cockett *et al.*, *Bio/Technology*, 8:2, 1990).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther *et al.*, *EMBO*, 12:1791, 1983), in which the antibody coding sequence may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; and pIN vectors (Inouye & Inouye, *Nucleic Acids Res.*, 13:3101-3109, 1985 and Van Heeke & Schuster, *J. Biol. Chem.*, 24:5503-5509, 1989).

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized to express an antibody molecule of the invention. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region El or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, *Proc. Natl. Acad. Sci. USA*, 81:355-359, 1984). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of

appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bitter *et al.*, *Methods in Enzymol.*, 153:516-544, 1987).

In addition, a host cell strain may be chosen which modulates the expression of the antibody sequences, or modifies and processes the antibody in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the antibody. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the antibody expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, W138, and in particular, myeloma cells such as NS0 cells, and related cell lines, see, for example, Morrison *et al.*, U.S. Patent No. 5,807,715, which is hereby incorporated by reference in its entirety.

For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell*, 11:223, 1977), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA*, 48:202, 1992), and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell*, 22:8-17, 1980) genes can be employed in tk$^-$, hgprt$^-$ or aprt$^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler *et al.*, *Natl. Acad. Sci. USA*, 77:357, 1980 and O'Hare *et*

al., *Proc. Natl. Acad. Sci. USA*, 78:1527, 1981); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, *Biotherapy*, 3:87-95, 1991; Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.*, 32:573-596, 1993; Mulligan, *Science*, 260:926-932, 1993; and Morgan and Anderson, *Ann. Rev. Biochem.*, 62: 191-217, 1993; and May, *TIB TECH*, 11(5):155-2 15, 1993); and *hygro*, which confers resistance to hygromycin (Santerre *et al.*, *Gene*, 30:147, 1984). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.* (eds.), 1993, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; in Chapters 12 and 13, Dracopoli *et al.* (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY; and Colberre-Garapin *et al.*, *J. Mol. Biol.*, 150:1, 1981, which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, 1987, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. Academic Press, New York). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse *et al.*, *Mol., Cell. Biol.*, 3:257, 1983).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides or different selectable markers to ensure maintenance of both plasmids. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature*, 322:52, 1986; and Kohler, *Proc. Natl. Acad. Sci. USA*, 77:2 197, 1980). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by recombinant expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange,

affinity, particularly by affinity for the specific antigen after Protein A purification, and sizing column chromatography), centrifugation, differential solubility, or by any other standard techniques for the purification of proteins. Further, the antibodies of the present invention or fragments thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

5.3.1. ANTIBODY CONJUGATES

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to heterologous polypeptides (*i.e.*, an unrelated polypeptide; or portion thereof, preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids of the polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. Antibodies fused or conjugated to heterologous polypeptides may also be used in *in vitro* immunoassays and purification methods using methods known in the art. See *e.g.*, PCT Publication No. WO 93/21232; EP 439,095; Naramura *et al.*, *Immunol. Lett.*, 39:91-99, 1994; U.S. Patent 5,474,981; Gillies *et al.*, *PNAS*, 89:1428-1432, 1992; and Fell *et al.*, *J. Immunol.*, 146:2446-2452, 1991, which are incorporated herein by reference in their entireties.

Antibodies can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:821-824, 1989, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, *Cell*, 37:767 1984) and the "flag" tag (Knappik *et al.*, *Biotechniques*, 17(4):754-761, 1994).

The present invention also encompasses antibodies conjugated to a diagnostic or therapeutic agent or any other molecule for which *in vivo* half-life is desired to be increased. The antibodies can be used diagnostically to, for example, monitor the development or progression of a disease, disorder or infection as part of a clinical testing procedure to, *e.g.*, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent

materials, bioluminescent materials, radioactive materials, positron emitting metals, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{111}In or ^{99m}Tc.

An antibody may be conjugated to a therapeutic moiety such as a cytotoxin (*e.g.*, a cytostatic or cytocidal agent), a therapeutic agent or a radioactive element (*e.g.*, alpha-emitters, gamma-emitters, etc.). Cytotoxins or cytotoxic agents include any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Further, an antibody may be conjugated to a therapeutic agent or drug moiety that modifies a given biological response. Therapeutic agents or drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α-interferon (IFN-α), β-interferon (IFN-β),

nerve growth factor (NGF), platelet derived growth factor (PDGF), tissue plasminogen activator (TPA), an apoptotic agent (*e.g.*, TNF-α, TNF-β, AIM I as disclosed in PCT Publication No. WO 97/33899), AIM II (see, PCT Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *J. Iminunol.*, 6:1567-1574, 1994), and VEGI (PCT Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent (*e.g.*, angiostatin or endostatin); or a biological response modifier such as, for example, a lymphokine (*e.g.*, interleukin-1 ("IL- 1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), and granulocyte colony stimulating factor ("G-CSF")), or a growth factor (*e.g.*, growth hormone ("GH")).

Techniques for conjugating such therapeutic moieties to antibodies are well known; see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), 1985, pp. 243-56, Alan R. Liss, Inc.); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery (2nd Ed.)*, Robinson *et al.* (eds.), 1987, pp. 623-53, Marcel Dekker, Inc.); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), 1985, pp. 475-506); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.),1985, pp. 303-16, Academic Press; and Thorpe *et al.*, *Immunol. Recombinant expression vector.*, 62:119-58, 1982.

An antibody or fragment thereof, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

5.4 METHODS OF PRODUCING FUSION PROTEINS

Fusion proteins can be produced by standard recombinant DNA techniques or by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer. For example, a nucleic acid molecule encoding a fusion protein can be synthesized by conventional techniques

including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g., Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, 1992). Moreover, a nucleic acid encoding a bioactive molecule can be cloned into an expression vector containing the Fc domain or a fragment thereof such that the bioactive molecule is linked in-frame to the constant domain or fragment thereof.

Methods for fusing or conjugating polypeptides to the constant regions of antibodies are known in the art. See, *e.g.*, U.S. Patent Nos. 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, 5,723,125, 5,783,181, 5,908,626, 5,844,095, and 5,112,946; EP 307,434; EP 367,166; EP 394,827; PCT publications WO 91/06570, WO 96/04388, WO 96/22024, WO 97/34631, and WO 99/04813; Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA*, 88: 10535-10539, 1991; Traunecker *et al.*, *Nature*, 331:84-86, 1988; Zheng *et al.*, *J. Immunol.*, 154:5590-5600, 1995; and Vil *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:11337-11341, 1992, which are incorporated herein by reference in their entireties.

The nucleotide sequence encoding a bioactive molecule may be obtained from any information available to those of skill in the art (*e.g.*, from Genbank, the literature, or by routine cloning), and the nucleotide sequence encoding a constant domain or a fragment thereof with increased affinity for the FcRn may be determined by sequence analysis of mutants produced using techniques described herein, or may be obtained from Genbank or the literature. The nucleotide sequence coding for a fusion protein can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. A variety of host-vector systems may be utilized in the present invention to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

The expression of a fusion protein may be controlled by any promoter or enhancer element known in the art. Promoters which may be used to control the expression of the gene encoding fusion protein include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 290:304-310, 1981), the promoter contained in the 3'

long terminal repeat of Rous sarcoma virus (Yamamoto, *et al.*, *Cell*, 22:787-797, 1980), the herpes thymidine kinase promoter (Wagner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445, 1981), the regulatory sequences of the metallothionein gene (Brinster *et al.*, *Nature*, 296:39-42, 1982), the tetracycline (Tet) promoter (Gossen *et al.*, *Proc. Nat. Acad. Sci. USA*, 89:5547-5551, 1995); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 75:3727-3731, 1978), or the *tac* promoter (DeBoer, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 80:21-25, 1983; see also "Useful proteins from recombinant bacteria" in *Scientific American*, 242:74-94, 1980); plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella *et al.*, *Nature*, 303:209-213, 1983) or the cauliflower mosaic virus 35S RNA promoter (Gardner, *et al.*, *Nucl. Acids Res.*, 9:2871, 1981), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella *et al.*, *Nature*, 310:115-120, 1984); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, *Cell* 38:639-646, 1984; Ornitz *et al.*, 50:399-409, *Cold Spring Harbor Symp. Quant. Biol.*, 1986; MacDonald, *Hepatology* 7:425-515, 1987); insulin gene control region which is active in pancreatic beta cells (Hanahan, *Nature* 315:115-122, 1985), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, *Cell*, 38:647-658, 1984; Adames *et al.*, *Nature* 318:533-538, 1985; Alexander *et al.*, *Mol. Cell. Biol.*, 7:1436-1444, 1987), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, *Cell*, 45:485-495, 1986), albumin gene control region which is active in liver (Pinkert *et al.*, *Genes and Devel.*, 1:268-276, 1987), α-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, *Mol. Cell. Biol.*, 5:1639-1648, 1985; Hammer *et al.*, *Science*, 235:53-58, 1987; α 1-antitrypsin gene control region which is active in the liver (Kelsey *et al.*, *Genes and Devel.*, 1:161-171, 1987), beta-globin gene control region which is active in myeloid cells (Mogram *et al.*, *Nature*, 315:338-340, 1985; Kollias *et al.*, *Cell*, 46:89-94, 1986; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead *et al.*, *Cell*, 48:703-712, 1987); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, *Nature*, 314:283-286, 1985); neuronal-specific enolase (NSE) which is active in neuronal cells (Morelli *et al.*, *Gen. Virol.*, 80:571-83, 1999); brain-derived neurotrophic factor (BDNF) gene control region which is active in neuronal cells (Tabuchi *et al.*, *Biochem. Biophysic. Res. Comprising.*, 253:818-823,

1998); glial fibrillary acidic protein (GFAP) promoter which is active in astrocytes (Gomes *et al.*, Braz. J. Med. Biol. Res., 32(5):619-631, 1999; Morelli *et al.*, *Gen. Virol.*, 80:571-83, 1999) and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason *et al.*, *Science*, 234:1372-1378, 1986).

In a specific embodiment, the expression of a fusion protein is regulated by a constitutive promoter. In another embodiment, the expression of a fusion protein is regulated by an inducible promoter. In accordance with these embodiments, the promoter may be a tissue-specific promoter.

In a specific embodiment, a vector is used that comprises a promoter operably linked to a fusion protein-encoding nucleic acid, one or more origins of replication, and, optionally, one or more selectable markers (*e.g.*, an antibiotic resistance gene).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the fusion protein coding sequence may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan & Shenk, *Proc. Natl. Acad. Sci. USA*, 81:355-359, 1984). Specific initiation signals may also be required for efficient translation of inserted fusion protein coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bitter *et al.*, *Methods in Enzymol.*, 153:516-544, 1987).

Expression vectors containing inserts of a gene encoding a fusion protein can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a gene encoding a fusion protein in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted gene encoding the fusion protein. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (*e.g.*, thymidine kinase activity, resistance to

antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of a nucleotide sequence encoding a fusion protein in the vector. For example, if the nucleotide sequence encoding the fusion protein is inserted within the marker gene sequence of the vector, recombinants containing the gene encoding the fusion protein insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the gene product (*i.e.*, fusion protein) expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties of the fusion protein in *in vitro* assay systems, *e.g.*, binding with anti-bioactive molecule antibody.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered fusion protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, phosphorylation of proteins). Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system will produce an unglycosylated product and expression in yeast will produce a glycosylated product. Eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, neuronal cell lines such as, for example, SK-N-AS, SK-N-FI, SK-N-DZ human neuroblastomas (Sugimoto *et al.*, *J. Natl. Cancer Inst.*, 73: 51-57, 1984), SK-N-SH human neuroblastoma (*Biochim. Biophys. Acta*, 704: 450-460, 1982), Daoy human cerebellar medulloblastoma (He *et al.*, *Cancer Res.*, 52: 1144-1148, 1992) DBTRG-05MG glioblastoma cells (Kruse *et al.*, 1992, *In Vitro Cell. Dev. Biol.*, 28A:609-614, 1992), IMR-32 human neuroblastoma (*Cancer Res.*, 30: 2110-2118, 1970), 1321N1 human astrocytoma (*Proc. Natl Acad. Sci. USA*, 74: 4816, 1997), MOG-G-CCM human astrocytoma (Br. J. Cancer, 49: 269, 1984), U87MG human glioblastoma-astrocytoma (*Acta Pathol. Microbiol. Scand.*, 74: 465-486, 1968), A172 human glioblastoma (Olopade *et al.*, *Cancer Res.*, 52: 2523-2529, 1992), C6 rat glioma cells (Benda *et al.*, Science, 161: 370-371, 1968), Neuro-2a mouse neuroblastoma (*Proc. Natl. Acad. Sci. USA*, 65: 129-136, 1970), NB41A3 mouse neuroblastoma (*Proc. Natl. Acad. Sci. USA*, 48: 1184-1190, 1962), SCP sheep choroid plexus (Bolin *et al.*, *J. Virol. Methods*, 48: 211-221, 1994), G355-5, PG-4 Cat normal astrocyte (Haapala *et al.*, *J. Virol.*, 53: 827-833, 1985), Mpf

ferret brain (Trowbridge *et al.*, *In Vitro,* 18: 952-960, 1982), and normal cell lines such as, for example, CTX TNA2 rat normal cortex brain (Radany *et al.*, *Proc. Natl. Acad. Sci. USA,* 89: 6467-6471, 1992) such as, for example, CRL7030 and Hs578Bst. Furthermore, different vector/host expression systems may effect processing reactions to different degrees.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the fusion protein may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched medium, and then are switched to a selective medium. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines that express the differentially expressed or pathway gene protein. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the differentially expressed or pathway gene protein.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, *et al.*, *Cell,* 11:223, 1997), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA,* 48:2026, 1962), and adenine phosphoribosyltransferase (Lowy, *et al.*, 1980, *Cell,* 22:817, 1980) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler, *et al.*, *Natl. Acad. Sci.USA*, 77:3567, 1980; O'Hare, *et al.*, *Proc. Natl. Acad. Sci. USA,* 78:1527, 1981); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA,* 78:2072, 1981); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, *et al.*, *J. Mol. Biol.,* 150:1, 1981); and hygro, which confers resistance to hygromycin (Santerre, *et al.*, *Gene*, 30:147, 1984) genes.

Once a fusion protein of the invention has been produced by recombinant expression, it may be purified by any method known in the art for purification of a protein, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

5.5. PROPHYLACTIC AND THERAPEUTIC USES OF ANTIBODIES

The present invention encompasses antibody-based therapies which involve administering antibodies to an animal, preferably a mammal, and most preferably a human, for preventing, treating, or ameliorating symptoms associated with a disease, disorder, or infection. Prophylactic and therapeutic compounds of the invention include, but are not limited to, antibodies and nucleic acids encoding antibodies. Antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

Antibodies of the present invention that function as antagonists of a disease, disorder, or infection can be administered to an animal, preferably a mammal and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with the disease, disorder, or infection. For example, antibodies which disrupt or prevent the interaction between a viral antigen and its host cell receptor may be administered to an animal, preferably a mammal and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with a viral infection.

In a specific embodiment, an antibody or fragment thereof prevents a viral or bacterial antigen from binding to its host cell receptor by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to antigen binding to its host cell receptor in the absence of said antibodies. In another embodiment, a combination of antibodies prevent a viral or bacterial antigen from binding to its host cell receptor by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to antigen binding to its host cell receptor in the absence of said antibodies. In a preferred embodiment, the antibody is used to treat or prevent RSV infection

Antibodies which do not prevent a viral or bacterial antigen from binding its host cell receptor but inhibit or downregulate viral or bacterial replication can also be administered to an animal to treat, prevent or ameliorate one or more symptoms associated with a viral or bacterial infection. The ability of an antibody to inhibit or downregulate viral or bacterial replication may be determined by techniques described herein or otherwise known in the art. For example, the inhibition or downregulation of viral replication can be determined by detecting the viral titer in the animal.

In a specific embodiment, an antibody inhibits or downregulates viral or bacterial replication by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at

least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to viral or bacterial replication in absence of said antibody. In another embodiment, a combination of antibodies inhibit or downregulate viral or bacterial replication by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to viral or bacterial replication in absence of said antibodies.

Antibodies can also be used to prevent, inhibit or reduce the growth or metastasis of cancerous cells. In a specific embodiment, an antibody inhibits or reduces the growth or metastasis of cancerous cells by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the growth or metastasis in absence of said antibody. In another embodiment, a combination of antibodies inhibits or reduces the growth or metastasis of cancer by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the growth or metastasis in absence of said antibodies. Examples of cancers include, but are not limited to, leukemia (*e.g.*, acute leukemia such as acute lymphocytic leukemia and acute myelocytic leukemia), neoplasms, tumors (*e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma), heavy chain disease, metastases, or any disease or disorder characterized by uncontrolled cell growth.

Antibodies can also be used to reduce the inflammation experienced by animals, particularly mammals, with inflammatory disorders. In a specific embodiment, an antibody reduces the inflammation in an animal by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the inflammation in an animal in the not administered said antibody. In another embodiment, a combination of antibodies reduce the inflammation in an animal by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the inflammation in an animal in not administered said antibodies. Examples of inflammatory disorders include, but are not limited to, rheumatoid arthritis, spondyloarthropathies, inflammatory bowel disease and asthma.

In certain embodiments, the antibody used for treatment of inflammation (or cancer) is a modified anti-$\alpha_v\beta_3$ antibody, preferably a Vitaxin antibody (see, PCT publications WO 98/33919 and WO 00/78815, both by Huse *et al.*, and both of which are incorporated by reference herein in their entireties).

Antibodies can also be used to prevent the rejection of transplants. Antibodies can also be used to prevent clot formation. Further, antibodies that function as agonists of the immune response can also be administered to an animal, preferably a mammal, and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with the disease, disorder, or infection.

One or more antibodies that immunospecifically bind to one or more antigens may be used locally or systemically in the body as a therapeutic. The antibodies of this invention may also be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the number or activity of effector cells which interact with the antibodies. The antibodies of this invention may also be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the immune response. The antibodies of this invention may also be advantageously utilized in combination with one or more drugs used to treat a disease, disorder, or infection such as, for example anti-cancer agents, anti-inflammatory agents or anti-viral agents. Examples of anti-cancer agents include, but are not limited to, isplatin, ifosfamide, paclitaxel, taxanes, topoisomerase I inhibitors (*e.g.*, CPT-11, topotecan,

9-AC, and GG-211), gemcitabine, vinorelbine, oxaliplatin, 5-fluorouracil (5-FU), leucovorin, vinorelbine, temodal, and taxol. Examples of anti-viral agents include, but are not limited to, cytokines (*e.g.*, IFN-α, IFN-β, IFN-γ), inhibitors of reverse transcriptase (*e.g.*, AZT, 3TC, D4T, ddC, ddI, d4T, 3TC, adefovir, efavirenz, delavirdine, nevirapine, abacavir, and other dideoxynucleosides or dideoxyfluoronucleosides), inhibitors of viral mRNA capping, such as ribavirin, inhibitors of proteases such HIV protease inhibitors (*e.g.*, amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir,), amphotericin B, castanospermine as an inhibitor of glycoprotein processing, inhibitors of neuraminidase such as influenza virus neuraminidase inhibitors (*e.g.*, zanamivir and oseltamivir), topoisomerase I inhibitors (*e.g.*, camptothecins and analogs thereof), amantadine, and rimantadine. Examples of anti-inflammatory agents include, but are not limited to, nonsteroidal anti-inflammatory drugs such as COX-2 inhibitors (*e.g.*, meloxicam, celecoxib, rofecoxib, flosulide, and SC-58635, and MK-966), ibuprofen and indomethacin, and steroids (*e.g.*, deflazacort, dexamethasone and methylprednisolone).

In a specific embodiment, antibodies administered to an animal are of a species origin or species reactivity that is the same species as that of the animal. Thus, in a preferred embodiment, human or humanized antibodies, or nucleic acids encoding human or human, are administered to a human patient for therapy or prophylaxis.

In preferred embodiments, immunoglobulins having extended *in vivo* half-lives are used in passive immunotherapy (for either therapy or prophylaxis). Because of the extended half-life, passive immunotherapy or prophylaxis can be accomplished using lower doses and/or less frequent administration of the therapeutic resulting in fewer side effects, better patient compliance, less costly therapy/prophylaxis, etc. In a preferred embodiment, the therapeutic/prophylactic is an antibody that binds RSV, for example, SYNAGIS® or other anti-RSV antibody. Such anti-RSV antibodies, and methods of administration are disclosed in U.S. patent application Serial Nos. 09/724,396 and 09/724,531, both entitled "Methods of Administering/Dosing Anti-RSV Antibodies For Prophylaxis and Treatment," both by Young *et al.*, both filed November 28, 2000, and continuation-in-part applications of these applications, Serial Nos. _____ and _____, respectively (attorney docket Nos. 10271-048 and 10271-047, respectively), also entitled "Methods of Administering/Dosing Anti-RSV Antibodies for Prophylaxis and Treatment," by Young *et al.*, all which are incorporated by reference herein in their entireties. Also included are the anti-RSV antibodies described in Section 5.1, *supra*.

In a specific embodiment, fusion proteins administered to an animal are of a species origin or species reactivity that is the same species as that of the animal. Thus, in a

preferred embodiment, human fusion proteins or nucleic acids encoding human fusion proteins, are administered to a human subject for therapy or prophylaxis.

5.6. PROPHYLACTIC AND THERAPEUTIC USES OF FUSION PROTEINS AND CONJUGATED MOLECULES

The present invention encompasses fusion protein-based and conjugated molecule-based therapies which involve administering fusion proteins or conjugated molecules to an animal, preferably a mammal and most preferably a human, for preventing, treating, or ameliorating symptoms associated with a disease, disorder, or infection. Prophylactic and therapeutic compounds of the invention include, but are not limited to, fusion proteins and nucleic acids encoding fusion proteins and conjugated molecules. Fusion proteins and conjugated molecules may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

Fusion proteins and conjugated molecules of the present invention that function as antagonists of a disease, disorder, or infection can be administered to an animal, preferably a mammal, and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with the disease, disorder, or infection. Further, fusion proteins and conjugated molecules of the present invention that function as agonists of the immune response may be administered to an animal, preferably a mammal, and most preferably a human, to treat, prevent or ameliorate one or more symptoms associated with the disease, disorder, or infection.

One or more fusion proteins and conjugated molecules may be used locally or systemically in the body as a therapeutic. The fusion proteins and conjugated molecules of this invention may also be advantageously utilized in combination with monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the number or activity of effector cells which interact with the antibodies. The fusion proteins and conjugated molecules of this invention may also be advantageously utilized in combination with monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the immune response. The fusion proteins and conjugated molecules of this invention may also be advantageously utilized in combination with one or more drugs used to treat a disease, disorder, or infection such as, for example anti-cancer agents, anti-inflammatory agents or anti-viral agents. Examples of anti-cancer agents include, but are not limited to, isplatin, ifosfamide, paclitaxel, taxanes,

topoisomerase I inhibitors (*e.g.*, CPT-11, topotecan, 9-AC, and GG-211), gemcitabine, vinorelbine, oxaliplatin, 5-fluorouracil (5-FU), leucovorin, vinorelbine, temodal, and taxol. Examples of anti-viral agents include, but are not limited to, cytokines (*e.g.*, IFN-α, IFN-β, IFN-γ), inhibitors of reverse transcriptase (*e.g.*, AZT, 3TC, D4T, ddC, ddI, d4T, 3TC, adefovir, efavirenz, delavirdine, nevirapine, abacavir, and other dideoxynucleosides or dideoxyfluoronucleosides), inhibitors of viral mRNA capping, such as ribavirin, inhibitors of proteases such HIV protease inhibitors (*e.g.*, amprenavir, indinavir, nelfinavir, ritonavir, and saquinavir,), amphotericin B, castanospermine as an inhibitor of glycoprotein processing, inhibitors of neuraminidase such as influenza virus neuraminidase inhibitors (*e.g.*, zanamivir and oseltamivir), topoisomerase I inhibitors (*e.g.*, camptothecins and analogs thereof), amantadine, and rimantadine. Examples of anti-inflammatory agents include, but are not limted to, nonsteroidal anti-inflammatory drugs such as COX-2 inhibitors (*e.g.*, meloxicam, celecoxib, rofecoxib, flosulide, and SC-58635, and MK-966), ibuprofen and indomethacin, and steroids (*e.g.*, deflazacort, dexamethasone and methylprednisolone).

5.7. ADMINISTRATION OF ANTIBODIES OR FUSION PROTEINS

The invention provides methods of treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administrating to a subject of an effective amount of an antibody of the invention, or pharmaceutical composition comprising an antibody of the invention. The invention also provides methods of treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of a fusion protein or conjugated molecule of the invention, or a pharmaceutical composition comprising a fusion protein or conjugated molecules of the invention. In a preferred aspect, an antibody or fusion protein or conjugated molecule, is substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side-effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats etc.) and a primate (*e.g.*, monkey such as a cynomolgous monkey and a human). In a preferred embodiment, the subject is a human.

Various delivery systems are known and can be used to administer an antibody or fusion protein or conjugated molecule of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the

antibody or fusion protein, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, *J. Biol. Chem.*, 262:4429-4432, 1987), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering an antibody, a fusion protein or conjugated molecule, or pharmaceutical composition include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, antibodies, fusion proteins, conjugated molecules, or pharmaceutical compositions are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety. In a preferred embodiment, an antibody, a fusion protein, conjugated molecules, or a pharmaceutical composition is administered using Alkermes AIR™ pulmonary drug delivery technology (Alkermes, Inc., Cambridge, MA).

The invention also provides that an antibody, a fusion protein, or conjugated molecule is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of antibody, fusion protein, or conjugated molecule. In one embodiment, the antibody, fusion protein, or conjugated molecule is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the antibody, fusion protein, or conjugated molecule is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, or at least 75 mg. The lyophilized antibody, fusion protein, or conjugated molecule should be stored at between 2 and 8°C in its original container and the antibody, fusion protein, or conjugated molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, an antibody, fusion protein, or conjugated molecule is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the antibody, fusion protein, or conjugated molecule.

Preferably, the liquid form of the antibody, fusion protein, or conjugated molecule is supplied in a hermetically sealed container at least 1 mg/ml, more preferably at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, or at least 25 mg/ml.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering an antibody or a fusion protein, care must be taken to use materials to which the antibody or the fusion protein does not absorb.

In another embodiment, the composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science*, 249:1527-1533, 1990; Treat *et al.*, in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327; see generally ibid.).

In yet another embodiment, the composition can be delivered in a controlled release or sustained release system. Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more antibodies, or one or more fusion proteins. See, *e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.*, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," *Radiotherapy & Oncology*, 39:179-189, 1996; Song *et al.*, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," *PDA Journal of Pharmaceutical Science & Technology*, 50:372-397, 1995; Cleek *et al.*, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," *Pro. Intl. Symp. Control. Rel. Bioact. Mater.*, 24:853-854, 1997; and Lam *et al.*, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," *Proc. Int'l. Symp. Control Rel. Bioact. Mater.*, 24:759-760, 1997, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled release system (see Langer, supra; Sefton, *CRC Crit. Ref. Biomed. Eng.*, 14:20, 1987; Buchwald *et al.*, *Surgery*, 88:507, 1980; and Saudek *et al.*, *N. Engl. J. Med.*, 321:574, 1989). In another embodiment, polymeric materials can be used to achieve controlled release of antibodies or fusion proteins (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J., Macromol. Sci. Rev. Macromol. Chem.*, 23:61, 1983; see also Levy *et al.*, *Science*, 228:190, 1985; During *et al.*, *Ann. Neurol.*, 25:351, 1989;

Howard *et al.*, *J. Neurosurg.*, 7 1:105, 1989); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer, *Science*, 249:1527-1533, 1990).

In a specific embodiment where the composition of the invention is a nucleic acid encoding an antibody or fusion protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded antibody or fusion protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:1864-1868, 1991), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a prophylactically or therapeutically effective amount of an antibody, fusion protein or conjugated molecule, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's complete and incomplete, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful adjuvants for humans such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and

aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a prophylactically or therapeutically effective amount of the antibody or fragment thereof, or fusion protein or conjugated molecule, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the composition of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disease, disorder, or infection can be determined by standard clinical techniques. The precise dose to be employed in the formulation will depend on the route of administration, the age of the subject, and the seriousness of the disease, disorder, or infection, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model (*e.g.*, the cotton rat or Cynomolgous monkey) test systems.

For fusion proteins, the therapeutically or prophylactically effective dosage administered to a subject ranges from about 0.001 to 50 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. For antibodies, the therapeutically or prophylactically effective dosage administered to a subject is typically 0.1 mg/kg to 200 mg/kg of the subject's body weight. Preferably, the dosage administered to a subject is between 0.1 mg/kg and 20 mg/kg of the subject's body weight and more preferably the dosage administered to a subject is between 1 mg/kg to 10 mg/kg of the subject's body weight. The dosage will, however, depend upon the extent to which the *in vivo* half-life of the molecule has been increased Generally, human antibodies and human fusion proteins have longer half-lives within the human body than antibodies of fusion proteins from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies or human fusion proteins and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies, fusion proteins, or conjugated molecules may be reduced also by enhancing uptake and tissue penetration (*e.g.*, into the lung) of the antibodies or fusion proteins by modifications such as, for example, lipidation.

Treatment of a subject with a therapeutically or prophylactically effective amount of an antibody, fusion protein, or conjugated molecule can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with an antibody, fusion protein, or conjugated molecule in the range of between about 0.1 to 30 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. In other embodiments, the pharmaceutical composition of the invention is administered once a day, twice a day, or three times a day. In other embodiments, the pharmaceutical composition is administered once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a

year or once per year. It will also be appreciated that the effective dosage of the antibody, fusion protein, or conjugated molecule used for treatment may increase or decrease over the course of a particular treatment.

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5.7.1. GENE THERAPY

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or fusion proteins, are administered to treat, prevent or ameliorate one or more symptoms associated with a disease, disorder, or infection, by way of gene therapy. Gene 10 therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded antibody or fusion protein that mediates a therapeutic or prophylactic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

15 For general reviews of the methods of gene therapy, see Goldspiel *et al.*, *Clinical Pharmacy,* 12:488-505, 1993; Wu and Wu, *Biotherapy,* 3:87-95, 1991; Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.,* 32:573-596, 1993; Mulligan, *Science,* 260:926-932, 1993; and Morgan and Anderson, *Ann. Rev. biochem.* 62:191-217, 1993; *TIBTECH* 11(5):155-215, 1993. Methods commonly known in the art of recombinant DNA technology which can be 20 used are described in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, a composition of the invention comprises nucleic acids encoding an antibody, said nucleic acids being part of an expression vector that expresses the 25 antibody in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a 30 desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA,* 86:8932-8935, 1989; and Zijlstra *et al.*, *Nature,* 342:435-438, 1989).

In another preferred aspect, a composition of the invention comprises nucleic acids encoding a fusion protein, said nucleic acids being a part of an expression vector that 35 expression the fusion protein in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the coding region of a

fusion protein, said promoter being inducible or constitutive, and optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the coding sequence of the fusion protein and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the fusion protein encoding nucleic acids.

Delivery of the nucleic acids into a subject may be either direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the subject. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, *e.g.*, by infection using defective or attenuated retroviral or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, *J. Biol. Chem.*, 262:4429-4432, 1987) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180; WO 92/22635; WO 92/20316; WO 93/14188; WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, *Proc. Natl. Acad. Sci. USA,* 86:8932-8935, 1989; and Zijlstra *et al.*, *Nature,* 342:435-438, 1989).

In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an antibody or a fusion protein are used. For example, a retroviral vector can be used (see Miller *et al.*, *Meth. Enzymol.*, 217:581-599, 1993). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody or a fusion protein to

be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the nucleotide sequence into a subject. More detail about retroviral vectors can be found in Boesen *et al.*, *Biotherapy,* 6:291-302, 1994, which describes the use of a retroviral vector to deliver the mdr 1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, *J. Clin. Invest.*, 93:644-651, 1994; Klein *et al.*, Blood 83:1467-1473, 1994; Salmons and Gunzberg, *Human Gene Therapy,* 4:129-141, 1993; and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.,* 3:110-114, 1993.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development,* 3:499-503, 1993, present a review of adenovirus-based gene therapy. Bout *et al.*, *Human Gene Therapy,* 5:3-10, 1994, demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, *Science,* 252:431-434, 1991; Rosenfeld *et al.*, *Cell,* 68:143-155, 1992; Mastrangeli *et al.*, *J. Clin. Invest.,* 91:225-234, 1993; PCT Publication WO 94/12649; and Wang *et al.*, *Gene Therapy,* 2:775-783, 1995. In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (see, *e.g.*,Walsh *et al.*, *Proc. Soc. Exp. Biol. Med.,* 204:289-300, 1993, and U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcellmediated gene transfer,

spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, *Meth. Enzymol.*, 217:599-618, 1993; Cohen *et al.*, *Meth. Enzymol.*, 217:618-644, 1993; and *Clin. Pharma. Ther.*, 29:69-92, 1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the subject.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody or a fusion protein are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see *e.g.*, PCT Publication WO 94/08598; Stemple and Anderson, *Cell*, 7 1:973-985, 1992; Rheinwald, *Meth. Cell Bio.*, 21A:229, 1980; and Pittelkow and Scott, *Mayo Clinic Proc.*, 61:771, 1986).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

5.8. CHARACTERIZATION AND DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

Antibodies, fusion proteins, and conjugated molecules of the present invention may be characterized in a variety of ways. In particular, antibodies of the invention may be assayed for the ability to immunospecifically bind to an antigen. Such an assay may be performed in solution (*e.g.*, Houghten, *Bio/Techniques,* 13:412-421, 1992), on beads (Lam, *Nature,* 354:82-84, 1991, on chips (Fodor, *Nature,* 364:555-556, 1993), on bacteria (U.S. Patent No. 5,223,409), on spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. USA,* 89:1865-1869, 1992) or on phage (Scott and Smith, *Science,* 249:386-390, 1990; Devlin, *Science,* 249:404-406, 1990; Cwirla *et al.*, *Proc. Natl. Acad. Sci. USA,* 87:6378-6382, 1990; and Felici, *J. Mol. Biol.,* 222:301-310, 1991) (each of these references is incorporated herein in its entirety by reference). Antibodies that have been identified to immunospecifically bind to an antigen or a fragment thereof can then be assayed for their specificity affinity for the antigen.

The antibodies of the invention or fragments thereof may be assayed for immunospecific binding to an antigen and cross-reactivity with other antigens by any method known in the art. Immunoassays which can be used to analyze immunospecific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40°C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40°C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to

immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, *e.g.*, an anti-human antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*, ^{32}P or ^{125}I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*, ^{3}H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of the present invention or a fragment thereof for the antigen and the binding off-rates can be determined from the saturation data by scatchard analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with an antibody of the present invention or a fragment thereof conjugated to a labeled compound (*e.g.*, ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibodies to an antigen. BIAcore kinetic analysis comprises analyzing the binding and dissociation of an antigen from chips with immobilized antibodies on their surface (see the Example section *infra*).

The antibodies of the invention as well as fusion proteins and conjugated molecules can also be assayed for their ability to inhibit the binding of an antigen to its host cell receptor using techniques known to those of skill in the art. For example, cells expressing the receptor for a viral antigen can be contacted with virus in the presence or absence of an antibody and the ability of the antibody to inhibit viral antigen's binding can measured by, for example, flow cytometry or a scintillation counter. The antigen or the antibody can be labeled with a detectable compound such as a radioactive label (*e.g.*, ^{32}P, ^{35}S, and ^{125}I) or a fluorescent label (*e.g.*, fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine) to enable detection of an interaction between the antigen and its host cell receptor. Alternatively, the ability of antibodies to inhibit an antigen from binding to its receptor can be determined in cell-free assays. For example, virus or a viral antigen (*e.g.*, RSV F glycoprotein) can be contacted in a cell-free assay with an antibody and the ability of the antibody to inhibit the virus or the viral antigen from binding to its host cell receptor can be determined. Preferably, the antibody is immobilized on a solid support and the antigen is labeled with a detectable compound. Alternatively, the antigen is immobilized on a solid support and the antibody is labeled with a detectable compound. The antigen may be partially or completely purified (*e.g.*, partially or completely free of other polypeptides) or part of a cell lysate. Further, the antigen may be a fusion protein comprising the viral antigen and a domain such as glutathionine-S-transferase. Alternatively, an antigen can be biotinylated using techniques

well known to those of skill in the art (*e.g.*, biotinylation kit, Pierce Chemicals; Rockford, IL).

The antibodies, fusion proteins, and conjugated molecules of the invention can also be assayed for their ability to inhibit or downregulate viral or bacterial replication using techniques known to those of skill in the art. For example, viral replication can be assayed by a plaque assay such as described, *e.g.*, by Johnson *et al.*, *Journal of Infectious Diseases*, 176:1215-1224, 1997. The antibodies, fusion proteins, and conjugated molecules of the invention of the invention can also be assayed for their ability to inhibit or downregulate the expression of viral or bacterial polypeptides. Techniques known to those of skill in the art, including, but not limited to, Western blot analysis, Northern blot analysis, and RT-PCR, can be used to measure the expression of viral or bacterial polypeptides. Further, the antibodies, fusion proteins, and conjugated molecules of the invention of the invention can be assayed for their ability to prevent the formation of syncytia.

The antibodies, fusion proteins, conjugated molecules, and compositions of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays which can be used to determine whether administration of a specific antibody, a specific fusion protein, a specific conjugated molecule, or a composition of the present invention is indicated, include *in vitro* cell culture assays in which a subject tissue sample is grown in culture, and exposed to or otherwise administered an antibody, a fusion protein, conjugated molecule, or composition of the present invention, and the effect of such an antibody, a fusion protein, conjugated molecule, or a composition of the present invention upon the tissue sample is observed. In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a disease or disorder, to determine if an antibody, a fusion protein, conjugated molecule, or composition of the present invention has a desired effect upon such cell types. Preferably, the antibodies, the fusion proteins, the conjugated molecules, or compositions of the invention are also tested in *in vitro* assays and animal model systems prior to administration to humans.

Antibodies, fusion proteins, conjugated molecules, or compositions of the present invention for use in therapy can be tested for their toxicity in suitable animal model systems, including but not limited to rats, mice, cows, monkeys, and rabbits. For *in vivo* testing for the toxicity of an antibody, a fusion protein, a conjugated molecule, or a composition, any animal model system known in the art may be used.

Efficacy in treating or preventing viral infection may be demonstrated by detecting the ability of an antibody, a fusion protein, a conjugated molecule, or a composition

of the invention to inhibit the replication of the virus, to inhibit transmission or prevent the virus from establishing itself in its host, or to prevent, ameliorate or alleviate one or more symptoms associated with viral infection. The treatment is considered therapeutic if there is, for example, a reduction is viral load, amelioration of one or more symptoms or a decrease in mortality and/or morbidity following administration of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention. Antibodies, fusion proteins, conjugated molecules, or compositions of the invention can also be tested for their ability to inhibit viral replication or reduce viral load in *in vitro* and *in vivo* assays.

Efficacy in treating or preventing bacterial infection may be demonstrated by detecting the ability of an antibody, a fusion protein or a composition of the invention to inhibit the bacterial replication, or to prevent, ameliorate or alleviate one or more symptoms associated with bacterial infection. The treatment is considered therapeutic if there is, for example, a reduction is bacterial numbers, amelioration of one or more symptoms or a decrease in mortality and/or morbidity following administration of an antibody, a fusion protein or a composition of the invention.

Efficacy in treating cancer may be demonstrated by detecting the ability of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention to inhibit or reduce the growth or metastasis of cancerous cells or to ameliorate or alleviate one or more symptoms associated with cancer. The treatment is considered therapeutic if there is, for example, a reduction in the growth or metastasis of cancerous cells, amelioration of one or more symptoms associated with cancer, or a decrease in mortality and/or morbidity following administration of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention. Antibodies, fusion proteins or compositions of the invention can be tested for their ability to reduce tumor formation in *in vitro*, *ex vivo*, and *in vivo* assays.

Efficacy in treating inflammatory disorders may be demonstrated by detecting the ability of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention to reduce or inhibit the inflammation in an animal or to ameliorate or alleviate one or more symptoms associated with an inflammatory disorder. The treatment is considered therapeutic if there is, for example, a reduction is in inflammation or amelioration of one or more symptoms following administration of an antibody, a fusion proteins, a conjugated molecule, or a composition of the invention.

Antibodies, fusion proteins, conjugated molecules, or compositions of the invention can be tested *in vitro* and *in vivo* for the ability to induce the expression of cytokines (*e.g.*, IFN-α, IFN-β, IFN-γ, IL-2, IL-3, IL-4, IL-5, IL-6, IL10, IL-12, and IL-15) and

activation markers (*e.g.*, CD28, ICOS, and SLAM). Techniques known to those of skill in the art can be used to measure the level of expression of cytokines and activation markers. For example, the level of expression of cytokines can be measured by analyzing the level of RNA of cytokines by, for example, RT-PCR and Northern blot analysis, and by analyzing the level of cytokines by, for example, immunoprecipitation followed by Western blot analysis or ELISA.

Antibodies, fusion proteins, conjugated molecules, or compositions of the invention can be tested *in vitro* and *in vivo* for their ability to modulate the biological activity of immune cells, preferably human immune cells (*e.g.*, T-cells, B-cells, and Natural Killer cells). The ability of an antibody, a fusion protein, a conjugated molecule, or a composition of the invention to modulate the biological activity of immune cells can be assessed by detecting the expression of antigens, detecting the proliferation of immune cells, detecting the activation of signaling molecules, detecting the effector function of immune cells, or detecting the differentiation of immune cells. Techniques known to those of skill in the art can be used for measuring these activities. For example, cellular proliferation can be assayed by ^{3}H-thymidine incorporation assays and trypan blue cell counts. Antigen expression can be assayed, for example, by immunoassays including, but are not limited to, competitive and non-competitive assay systems using techniques such as Western blots, immunohistochemistry, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and FACS analysis. The activation of signaling molecules can be assayed, for example, by kinase assays and electrophoretic shift assays (EMSAs).

Antibodies, fusion proteins, conjugated molecules, or compositions of the invention can also be tested for their ability to increase the survival period of animals, preferably mammals and most preferably humans, suffering from a disease, disorder, or infection by at least 25%, preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99%. Further, antibodies, fusion proteins, conjugated molecules, or compositions of the invention can be tested for their ability reduce the hospitalization period of animals, preferably mammals and most preferably humans, suffering from a disease, disorder, or infection by at least 60%, preferably at least 75%, at least 85%, at least 95%, or at least 99%. Techniques known to those of skill in the art can be used to analyze the function of the antibodies or compositions of the invention *in vivo*.

5.9. DIAGNOSTIC USES OF ANTIBODIES AND FUSION PROTEINS

Labeled antibodies, fusion proteins, and conjugated molecules of the invention can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders or infections. The invention provides for the detection or diagnosis of a disease, disorder or infection, comprising: (a) assaying the expression of an antigen in cells or a tissue sample of a subject using one or more antibodies that immunospecifically bind to the antigen; and (b) comparing the level of the antigen with a control level, *e.g.*, levels in normal tissue samples, whereby an increase in the assayed level of antigen compared to the control level of the antigen is indicative of the disease, disorder or infection. The invention also provides for the detection or diagnosis of a disease, disorder or infection, comprising (a) assaying the expression of an antigen in cells or a tissue sample of a subject using one or fusion proteins or conjugated molecules of the invention that bind to the antigen; and (b) comparing the level of the antigen with a control level, e.g., levels in normal tissue samples, whereby an increase of antigen compared to the control level of the antigen is indicative of the disease, disorder or infection. Accordingly, the fusion protein or conjugated molecule comprises a bioactive molecule such as a ligand, cytokine or growth factor and the hinge-Fc region or fragments thereof, wherein the fusion protein or conjugated molecule is capable of binding to an antigen being detected.

Antibodies of the invention can be used to assay antigen levels in a biological sample using classical immunohistological methods as described herein or as known to those of skill in the art (*e.g.*, see Jalkanen *et al.*, *J. Cell. Biol.*, 101:976-985, 1985; Jalkanen *et al.*, *J. Cell . Biol.*, 105:3087-3096, 1987). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, alkaline phosphatase, glucose oxidase; radioisotopes, such as iodine (^{125}I, ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{121}In), and technetium (^{99m}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine.

Fusion proteins can be used to assay antigen levels in a biological sample using, for example, SDS-PAGE and immunoassays known to those of skill in the art.

One aspect of the invention is the detection and diagnosis of a disease, disorder, or infection in a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled antibody that immunospecifically binds to an antigen; b) waiting for a

time interval following the administration for permitting the labeled antibody to preferentially concentrate at sites in the subject where the antigen is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled antibody in the subject, such that detection of labeled antibody above the background level indicates that the subject has the disease, disorder, or infection. In accordance with this embodiment, the antibody is labeled with an imaging moiety which is detectable using an imaging system known to one of skill in the art. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

In another embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled fusion protein or conjugated molecule that binds to an antigen or some other molecule; b) waiting for a time interval following the administration for permitting the labeled fusion protein or conjugated molecule to preferentially concentrate at sites in the subject where the antigen or other molecule is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled fusion protein or conjugated molecule in the subject, such that detection of labeled fusion protein above the background level indicates that the subject has the disease, disorder, or infection. In accordance with this embodiment, the fusion protein or conjugated molecule comprises a bioactive molecule such as a ligand, cytokine or growth factor and a hinge-Fc region or a fragment thereof, wherein said fusion protein or conjugated molecule is labeled with an imaging moiety and is capable of binding to the antigen being detected.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments," Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours.

In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In one embodiment, monitoring of a disease, disorder or infection is carried out by repeating the method for diagnosing the disease, disorder or infection, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the subject using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston *et al.*, U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

5.10. KITS

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody, fusion protein, or conjugated molecule, of the invention, preferably in a purified form, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated antigen as a control. Preferably, the kits of the present invention further comprise a control antibody, fusion protein, or conjugated molecule which does not react with the antigen included in the kit. In another specific embodiment, the kits of the present invention contain a means for

detecting the binding of an antibody, fusion protein, or conjugated molecule, to an antigen (*e.g.*, the antibody, fusion protein, or conjugated molecule, may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized antigen. The antigen provided in the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above-described kit includes a solid support to which antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the antigen can be detected by binding of the said reporter-labeled antibody.

5.11 *IN VITRO* AND *IN VIVO* ASSAYS FOR EXTENDED HALF-LIFE OF MODIFIED IGG HINGE-FC FRAGMENTS

The binding ability of modified IgGs and molecules comprising an IgG constant domain of FcRn fragment thereof to FcRn can be characterized by various *in vitro* assays. PCT publication WO 97/34631 by Ward discloses various methods in detail and is incorporated herein in its entirety by reference.

For example, in order to compare the ability of the modified IgG or fragments thereof to bind to FcRn with that of the wild type IgG, the modified IgG or fragments thereof and the wild type IgG can be radio-labeled and reacted with FcRn-expressing cells *in vitro*. The radioactivity of the cell-bound fractions can be then counted and compared. The cells expressing FcRn to be used for this assay are preferably endothelial cell lines including mouse pulmonary capillary endothelial cells (B10, D2.PCE) derived from lungs of B10.DBA/2 mice and SV40 transformed endothelial cells (SVEC) (Kim *et al.*, *J. Immunol.*, 40:457-465, 1994) derived from C3H/HeJ mice. However, other types of cells, such as intestinal brush borders isolated from 10- to 14-day old suckling mice, which express sufficient number of FcRn can be also used. Alternatively, mammalian cells which express recombinant FcRn of a species of choice can be also utilized. After counting the radioactivity of the bound fraction of modified IgG or that of wild type, the bound molecules can be then extracted with the detergent, and the percent release per unit number of cells can be calculated and compared.

Affinity of modified IgGs for FcRn can be measured by surface plasmon resonance (SPR) measurement using, for example, a BIAcore 2000 (BIAcore Inc.) as

described previously (Popov *et al.*, *Mol. Immunol.*, 33:493-502, 1996; Karlsson *et al.*, *J. Immunol. Methods*, 145:229-240, 1991, both of which are incorporated by reference in their entireties). In this method, FcRn molecules are coupled to a BIAcore sensor chip (*e.g.*, CM5 chip by Pharmacia) and the binding of modified IgG to the immobilized FcRn is measured at a certain flow rate to obtain sensorgrams using BIA evaluation 2.1 software, based on which on- and off-rates of the modified IgG, constant domains, or fragments thereof, to FcRn can be calculated.

Relative affinities of modified IgGs or fragments thereof, and the wild type IgG for FcRn can be also measured by a simple competition binding assay. Unlabeled modified IgG or wild type IgG is added in different amounts to the wells of a 96-well plate in which FcRn is immobilize. A constant amount of radio-labeled wild type IgG is then added to each well. Percent radioactivity of the bound fraction is plotted against the amount of unlabeled modified IgG or wild type IgG and the relative affinity of the modified hinge-Fc can be calculated from the slope of the curve.

Furthermore, affinities of modified IgGs or fragments thereof, and the wild type IgG for FcRn can be also measured by a saturation study and the Scatchard analysis.

Transfer of modified IgG or fragments thereof across the cell by FcRn can be measured by *in vitro* transfer assay using radiolabeled IgG or fragments thereof and FcRn-expressing cells and comparing the radioactivity of the one side of the cell monolayer with that of the other side. Alternatively, such transfer can be measured *in vivo* by feeding 10- to 14-day old suckling mice with radiolabeled, modified IgG and periodically counting the radioactivity in blood samples which indicates the transfer of the IgG through the intestine to the circulation (or any other target tissue, *e.g.*, the lungs). To test the dose-dependent inhibition of the IgG transfer through the gut, a mixture of radiolabeled and unlabeled IgG at certain ratio is given to the mice and the radioactivity of the plasma can be periodically measured (Kim *et al.*, *Eur. J. Immunol.*, 24:2429-2434, 1994).

The half-life of modified IgG or fragments thereof can be measure by pharmacokinetic studies according to the method described by Kim *et al.* (*Eur. J. of Immuno.* 24:542, 1994), which is incorporated by reference herein in its entirety. According to this method, radiolabeled modified IgG or fragments thereof is injected intravenously into mice and its plasma concentration is periodically measured as a function of time, for example, at 3 minutes to 72 hours after the injection. The clearance curve thus obtained should be biphasic, that is, α-phase and β-phase. For the determination of the *in vivo* half-life of the modified IgGs or fragments thereof, the clearance rate in β-phase is calculated and compared with that of the wild type IgG.

6. EXAMPLES

The following examples illustrate the production, isolation, and characterization of modified hinge-Fc fragments that have longer *in vivo* half-lives.

6.1 LIBRARY CONSTRUCTION

6.1.1 REAGENTS

All chemicals were of analytical grade. Restriction enzymes and DNA-modifying enzymes were purchased from New England Biolabs, Inc. (Beverly, MA). Oligonucleotides were synthesized by MWG Biotech, Inc. (High Point, NC). pCANTAB5E phagemid vector, anti-E-tag-horseradish peroxydase conjugate, TG1 *E. Coli* strain, IgG Sepharose 6 Fast Flow and HiTrap protein A columns were purchased from APBiotech, Inc. (Piscataway, NJ). VCSM13 helper phage and the Quick change mutagenesis kit were obtained from Stratagene (La Jolla, CA). CJ236 *E. coli* strain was purchased from Bio-Rad (Richmond, CA). BCA Protein Assay Reagent Kit was obtained from Pierce (Rockford, IL). Lipofectamine 2000 was purchased from Invitrogen, Inc. (Carlsbad, CA).

6.1.2 EXPRESSION AND PURIFICATION OF MURINE AND HUMAN FCRN

The amino acid sequences of human and mouse FcRn are SEQ ID NOs. 84 and 85, respectively (see also Firan *et al.*, *Intern. Immunol.*, 13:993-1002, 2001 and Popov *et al.*, *Mol. Immunol.*, 33:521-530, 1996, both of which are incorporated herein by reference in their entireties). Human FcRn was also obtained following isolation from human placenta cDNA (Clontech, Palo Alto, CA) of the genes for human β2-microglobulin (Kabat *et al.*, 1991, Sequences of Proteins of Immunological Interest, U.S. Public Health Service, National Institutes of Health, Washington, DC) and codons -23 to 267 of the human α chain (Story *et al.*, *J. Exp. Med.*, 180:2377-2381, 1994) using standard PCR protocols. Light and heavy chains along with their native signal sequence (Kabat *et al.*, 1991, supra; Story *et al.*, *supra*) were cloned in pFastBac DUAL and pFastBac1 bacmids, respectively, and viral stocks produced in *Spodoptera frugiperda* cells (Sf9) according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). High-Five cells were infected at a multiplicity of infection of 3 with the baculoviruses encoding α and β2 chains using commercially available protocols (Invitrogen). Recombinant human FcRn was purified as follows: supernatant of infected insect cells was dialyzed into 50 mM MES (2-N-[Morpholino]ethansulfonic acid) pH 6.0 and

applied to a 10 ml human IgG Sepharose 6 Fast Flow column (APBiotech, Piscataway, NJ). Resin was washed with 200 ml 50 mM MES pH 6.0 and FcRn eluted with 0.1 M Tris-Cl pH 8.0. Purified FcRn was dialyzed against 50 mM MES pH 6.0, flash frozen and stored at -70°C. The purity of proteins was checked by SDS-PAGE and HPLC.

6.1.3 PREPARATION OF TAA-CONTAINING ssDNA URACIL TEMPLATE

Construction of the libraries was based on a site directed mutagenesis strategy derived from the Kunkel method (Kunkel *et al.*, *Methods Enzymol.* 154:367-382, 1987). A human hinge-Fc gene spanning amino acid residues 226-478 (Kabat numbering, Kabat *et al.*, 1991, *supra*) derived from MEDI-493 human IgG1 (Johnson *et al.*, *J. Infect. Disease*, 176:1215-1224, 1997), was cloned into the pCANTAB5E phagemid vector as an SfiI/NotI fragment. Four libraries were generated by introducing random mutations at positions 251, 252, 254, 255, 256 (library 1), 308, 309, 311, 312, 314 (library 2), 385, 386, 387, 389 (library 3) and 428, 433, 434, 436 (library 4). Briefly, four distinct hinge-Fc templates were generated using PCR by overlap extension (Ho *et al.*, *Gene*, 15:51-59, 1989), each containing one TAA stop codon at position 252 (library 1), 310 (library 2), 384 (library 3) or 429 (library 4), so that only mutagenized phagemids will give rise to Fc-displaying phage.

Each TAA-containing single-stranded DNA (TAAssDNA) was then prepared as follows: a single CJ236 *E. coli* colony harboring one of the four relevant TAA-containing phagemids was grown in 10 ml 2 x YT medium supplemented with 10 μg/ml chloramphenicol and 100 μg/ml ampicillin. At OD_{600} = 1, VCSM13 helper phage was added to a final concentration of 10^{10} pfu/ml. After 2 hours, the culture was transferred to 500 ml of 2 x YT medium supplemented with 0.25 μg/ml uridine, 10 μg/ml chloramphenicol, 30 μg/ml kanamycin, 100 μg/ml ampicillin and grown overnight at 37°C. Phage were precipitated with PEG6000 using standard protocols (Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York, Vols. 1-3) and purified using the Qiaprep Spin M13 Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. 10 to 30 μg of each uracil-containing TAAssDNA template was then combined with 0.6 μg of the following phosphorylated oligonucleotides (randomized regions underlined) in 50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.5 in a final volume of 250 μl:

Library 1:

5'-CATGTGACCTCAGGSNNSNNSNNGATSNNSNNGGTGTCCTTGGGTTTT

GGGGGG-3' (SEQ ID NO:120)
Library 2:
5'-GCACTTGTACTCCTTGCCATTSNNCCASNNSNNGTGSNNSNNGGTGA
GGACGC-3' (SEQ ID NO:121)
Library 3:
5'-GGTCTTGTAGTTSNNCTCSNNSNNSNNATTGCTCTCCC-3' (SEQ ID NO:122)
Library 4:
5'-GGCTCTTCTGCGTSNNGTGSNNSNNCAGAGCCTCATGSNNCACGGAGC
ATGAG-3' (SEQ ID NO:123)
where N = A, C, T or G and S = G or C.

6.1.4 SYNTHESIS OF HETERODUPLEX DNA

Appropriate, degenerate oligonucleotides were phosphorylated in the presence of T4 polynucleotide kinase using the standard protocol. Ten to 30 µg of ssDNA U template and 0.6 µg of phosphorylated oligonucleotide were combined in 50 mM Tris-HCl containing 10 mM $MgCl_2$, pH 7.5, to a final volume of 250 µl and incubated at 90°C for 2 minutes, 50°C for 3 minutes, and 20°C for 5 minutes. Synthesis of the heteroduplex DNA was carried out by adding 30 units of both T4 DNA ligase and T7 DNA polymerase in the presence of 0.4 mM ATP, 1 mM dNTPs and 6 mM DTT and the mixture was incubated for 4 hours at 20°C. The heteroduplex DNA thus produced was then purified and desalted using Qiagen Qiaquick DNA purification Kit (Qiagen, CA).

6.1.5 ELECTROPORATION

300 µl electrocompetent TG1 *E. coli* cells were electroporated with 1 to 5 µg of the heteroduplex DNA in a 2.5 kV field using 200 Ω and 25 µF capacitance until a library size of 1 x 10^8 (library 1 and 2) or 1 x 10^7 (library 3 and 4) was reached. The cells were resuspended in 2 ml SOC medium and the procedure was repeated 6 to 10 times. The diversity was assessed by titration of recombinant *E. coli*. The pulsed cells were incubated in 50 ml SOC medium for 30 minutes at 37°C under agitation, centrifuged, and resuspended in 500 ml 2xYT containing 100 µg/ml ampicillin and 10^{10} pfu/ml of VCSM13 helper phage. The culture was incubated overnight at 37°C and the cells were pelleted by centrifugation. The phage in the supernatant which express mutated hinge-Fc portion on its GIII-coat protein were precipitated with PEG6000 as previously described (Sambrook *et al.*, 1989, *supra*) and resuspended in 5 ml of 20 mM MES, pH 6.0.

6.2 PANNING OF THE LIBRARY

Phage were panned using an ELISA-based approach. A 96-well ELISA plate was coated with 100 µl/well of 0.01 mg/ml murine FcRn in sodium carbonate buffer, pH 9.0, at 4°C overnight and then blocked with 4% skimmed milk at 37°C for 2 hours. In each well of the coated plate, 100-150 µl of the phage suspension (about 10^{13} phage in total) in 20 mM MES, pH 6.0, containing 5% milk and 0.05% Tween 20, were placed and incubated at 37°C for two to three hours with agitation.

After the incubation, the wells were washed with 20 mM MES, pH 6.0, containing 0.2% Tween 20 and 0.3 M NaCl about thirty times at room temperature. The bound phage were eluted with 100 µl/well of PBS, pH 7.4, at 37°C for 30 minutes.

The eluted phage were then added to the culture of exponentially growing *E. coli* cells and propagation was carried out overnight at 37°C in 250 ml 2xYT supplemented with 100 µg/ml ampicillin and 10^{10} pfu/ml of VCSM13 helper phage. Propagated phage were collected by centrifugation followed by precipitation with PEG and the panning process was repeated up to a total of six times.

For the phage library containing mutations in residues 308-314 (H310 and W313 fixed), the phage expressing hinge-Fc region with higher affinities for FcRn were enriched by each panning process as shown in Table IV. The panning results of the library for the mutations in the residues 251-256 (I253 fixed) and that of the library for the mutations in the residues 428-436 (H429, E430, A431, L432, and H435 fixed), are shown in Tables V and VI, respectively. Furthermore, the panning results of the library for the mutations in the residues 385-389 (E388 fixed) is shown in Table VII.

Table IV

PANNING OF LIBRARY (RESIDUES 308-314; H310 AND W313 FIXED) pCANTAB5E-KUNKEL-muFcRn (MURINE FcRn)

PANNING	OUTPUT		ENRICHMENT RATIO
	+ FcRn	- FcRn	
1st Round	1.1×10^5	0.5×10^5	2
2nd Round	1×10^4	0.2×10^4	5
3rd Round	9×10^4	0.3×10^4	30
4th Round	3×10^5	2×10^4	15

Table V

PANNING OF LIBRARY (RESIDUES 251-256; I253 FIXED)
pCANTAB5E-KUNKEL-muFcRn

PANNING	OUTPUT		ENRICHMENT RATIO
	+ FcRn	- FcRn	
1st Round	2.5×10^5	1×10^5	2.5
2nd Round	6×10^4	2×10^4	3.0
3rd Round	8×10^5	4×10^4	20
4th Round	1.2×10^6	5×10^4	24
5th Round	3.0×10^6	6×10^4	50

Table VI

PANNING OF LIBRARY (RESIDUES 428-436; H429, E430, A431, L432, AND H435 FIXED) pCANTAB5E-KUNKEL-muFcRn

PANNING	OUTPUT		ENRICHMENT RATIO
	+ FcRn	- FcRn	
1st Round	2.3×10^5	0.9×10^5	2.5
2nd Round	3×10^4	1×10^4	3
3rd Round	2×10^5	2×10^4	10
4th Round	8×10^5	5×10^4	16

Table VII

PANNING OF LIBRARY (RESIDUES 385-389; E388 FIXED)
pCANTAB5E-KUNKEL-muFcRn

PANNING	OUTPUT		ENRICHMENT RATIO
	+ FcRn	- FcRn	
1st Round	4.2×10^4	3.8×10^5	1.1
2nd Round	5×10^4	0.3×10^4	17
3rd Round	3.5×10^5	1×10^4	35
4th Round	5.5×10^5	4×10^4	14
5th Round	7.5×10^5	5×10^4	15
6th Round	2×10^6	1×10^5	20

6.3 IDENTIFICATION OF ISOLATED CLONES FROM PANNING

After each panning process, phage were isolated and the nucleic acids encoding the expressed peptides which bound to FcRn were sequenced by a standard sequencing method such as by dideoxynucleotide sequencing (Sanger *et al., Proc. Natl. Acad. Sci USA,* 74:5463-5467, 1977) using a ABI3000 genomic analyzer (Applied Biosystems, Foster City, CA).

As a result of panning, two mutants were isolated from the phage library containing mutations in residues 308-314 (H310 and W313 fixed), thirteen mutants from the library for residues 251-256 (I253 fixed), six mutants from the library for residues 428-436 (H429, E430, A431, L432, and H435 fixed), and nine mutants from the library for residues 385-389 (E388 fixed). The mutants isolated from the libraries are listed in Table VIII.

Table VIII

MUTANTS ISOLATED BY PANNING

LIBRARY	MUTANTS*
251-256	Leu **Tyr** Ile **Thr** Arg **Glu** (SEQ ID NO:90)
	*Leu **Tyr** Ile Ser Arg Thr* (SEQ ID NO:91)
	*Leu **Tyr** Ile Ser Arg **Ser*** (SEQ ID NO:92)
	*Leu **Tyr** Ile Ser Arg **Arg*** (SEQ ID NO:93)

LIBRARY	MUTANTS*
	*Leu **Tyr** Ile Ser Arg **Gln*** (SEQ ID NO:94)
	*Leu **Trp** Ile Ser Arg Thr* (SEQ ID NO:95)
	Leu **Tyr** Ile Ser **Leu Gln** (SEQ ID NO:96)
	Leu **Phe** Ile Ser Arg **Asp** (SEQ ID NO:97)
	Leu **Phe** Ile Ser Arg **Thr** (SEQ ID NO:98)
	Leu **Phe** Ile Ser Arg **Arg** (SEQ ID NO:99)
	Leu **Phe** Ile **Thr Gly Ala** (SEQ ID NO:100)
	Leu **Ser** Ile Ser Arg **Glu** (SEQ ID NO:101)
	Arg Thr Ile Ser **Ile Ser** (SEQ ID NO:102)
308-314	**Thr Pro** His **Ser** Asp Trp Leu (SEQ ID NO:103)
	Ile Pro His **Glu** Asp Trp Leu (SEQ ID NO:104)
385-389	**Arg Thr Arg** Glu **Pro** (SEQ ID NO:105)
	Asp Pro** Pro Glu **Ser (SEQ ID NO:106)
	Ser Asp Pro Glu **Pro** (SEQ ID NO:107)
	Thr Ser His Glu Asn (SEQ ID NO:108)
	Ser Lys Ser Glu Asn (SEQ ID NO:109)
	His Arg Ser Glu Asn (SEQ ID NO:110)
	Lys Ile Arg Glu Asn (SEQ ID NO:111)
	Gly **Ile Thr** Glu **Ser** (SEQ ID NO:112)
	Ser Met Ala Glu **Pro** (SEQ ID NO:113)
428-436	*Met His Glu Ala Leu **Arg** **Tyr** His **His*** (SEQ ID NO:114)
	Met His Glu Ala Leu His **Phe** His **His** (SEQ ID NO:115)
	Met His Glu Ala Leu **Lys Phe** His **His** (SEQ ID NO:116)
	Met His Glu Ala Leu **Ser Tyr** His **Arg** (SEQ ID NO:117)
	Thr His Glu Ala Leu His **Tyr** His **Thr** (SEQ ID NO:118)
	Met His Glu Ala Leu His **Tyr** His **Tyr** (SEQ ID NO:119)

* Substituting residues are indicated in bold face

The underlined sequences in Table VIII correspond to sequences that occurred 10 to 20 times in the final round of panning and the sequences in italics correspond to sequences that occurred 2 to 5 times in the final round of panning. Those sequences that are neither underlined nor italicized occurred once in the final round of panning.

6.4 EXPRESSION AND PURIFICATION OF SOLUBLE MUTANT HINGE-FC REGION

The genes encoding mutated hinge-Fc fragments are excised with appropriate restriction enzymes and recloned into an expression vector, for example, VβpelBhis (Ward, *J. Mol. Biol.*, 224:885-890, 1992). Vectors containing any other type of tag sequence, such as *c-myc* tag, decapeptide tag (Huse *et al.*, *Science*, 246:1275-1281, 1989), Flag™ (Immunex) tags, can be used. Recombinant clones, such as *E. coli*, are grown and induced to express soluble hinge-Fc fragments, which can be isolated from the culture media or cell lysate after osmotic shock, based on the tag used, or by any other purification methods well known to those skilled in the art and characterized by the methods as listed below.

6.5 CONSTRUCTION, PRODUCTION AND PURIFICATION OF IgG1 VARIANTS

Representative Fc mutations such as I253A, M252Y/S254T/T256E, M252W, M252Y, M252Y/T256Q, M252F/T256D, V308T/L309P/Q311S, G385D/Q386P/N389S, G385R/Q386T/P387R/N389P, H433K/N434F/Y436H, and N434F/Y436 were incorporated into the human IgG1 MEDI-493 (SYNAGIS®) (Johnson *et al.*, 1997, *supra*). The heavy chain was subjected to site-directed mutagenesis using a Quick Change Mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions and sequences were verified by didoxynucleotide sequencing using a ABI3000 (Applied Biosystems, Foster City, CA) sequencer. The different constructions were expressed transiently in human embryonic kidney 293 cells using a CMV immediate-early promoter and dicistronic operon in which IgG1/V_H is cosecreted with IgG1/V_L (Johnson *et al.*, 1997, *supra*). Transfection was carried out using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and standard protocols. IgGs were purified from the conditioned media directly on 1 ml HiTrap protein A columns according to the manufacterer's instructions (APBiotech).

6.6 CHARACTERIZATION OF MUTATED HINGE-FC REGION

6.6.1 *IN VITRO* CHARACTERIZATION HPLC AND SDS-PAGE

Following the purification, general characteristics such as molecular weight and bonding characteristics of the modified hinge-Fc fragments may be studied by various methods well known to those skilled in the art, including SDS-PAGE and HPLC.

FcRn binding assay

Binding activity of modified hinge-Fc fragments can be measured by incubating radio-labeled wild-type hinge-Fc or modified hinge-Fc with the cells expressing either mouse or human FcRn. Typically, endothelial cell lines such as SV40 transformed endothelial cells (SVEC) (Kim *et al.*, *J. Immunol.*, 40:457-465, 1994) are used. After incubation with the hinge-Fc fragments at 37°C for 16-18 hours, the cells are washed with medium and then detached by incubation with 5 mM Na_2EDTA in 50 mM phosphate buffer, pH 7.5, for 5 minutes. The radioactivity per 10^7 cells is measured.

Then, the cells are resuspended in 2 ml of 2.5 mg/ml CHAPS, 0.1 M Tris-HCl pH 8.0 containing 0.3 mg/ml PMSF, 25 mg/ml pepstatin and 0.1 mg/ml aprotinin and incubated for 30 minutes at room temperature. The cell suspension is then centrifuged and the supernatant separated. The radioactivity of the supernatant is measured and used to calculate the amount of the hinge-Fc fragments extracted per 10^7 cells.

The K_d for the interaction of wild type human IgG1 with murine and human FcRn (269 and 2527 nM, respectively) agree well with the values determined by others (265 and 2350 nM, respectively, Firan *et al.*, 2001, *supra*). The I253A mutation virtually abolishes binding to human and murine FcRn, as reported by others (Kim *et al.*, *Eur. J. Immunol.*, 29:2819-2825, 1991; Shields *et al.*, *J. Biol. Chem.*, 276:6591-6604, 2001). This is not the result of misfolding of the antibody as this mutant retains the same specific activity than the wild type molecule (SYNAGIS®) in a microneutralization assay (Johnson *et al.*, 1997, *supra*; data not shown).

Human IgG1 mutants with increased binding affinity towards both murine and human FcRn were generated (Table VIII). Improvements in complex stability were overall less marked for the human IgG1-human FcRn pair than for the human IgG1-murine FcRn compared to wild type IgG1 were 30-($\Delta\Delta G$ = 2.0 kcal/mol for N434F/Y436H) and 11-($\Delta\Delta G$ = 1.4 kcal/mol for M252Y/S254Y/S254T/T256E) fold, respectively. However, ranking of the most critical positions remain unchanged when comparing human and murine FcRn: the largest increases in IgG1-murine FcRn complex stability ($\Delta\Delta G$ > 1.3 kcal/mol) occurred on

mutations at positions 252, 254, 256 (M252Y/S254T/T256E and M252W) and 433, 434, 436 (H433K/N434F/Y436H and N434F/Y436H). Likewise, the same mutations were found to have the most profound impact on the IgG1-human FcRn interaction and also resulted in the largest increases in complex stability ($\Delta\Delta G > 1.0$ kcal/mol). Substitutions at positions 308, 309, 311, 385, 386, 387 and 389 had little or no effect on the stability of the complexes involving human or murine FcRn ($\Delta\Delta G < 0.5$ kcal/mol). Residues at the center of the Fc-FcRn combining site contribute significantly more to improvement in complex stability than residues at the periphery (FIG. 9).

Efficient binding of human Fc to murine FcRn apparently requires the presence of several wild type Fc residues. For example, leucine is very conserved at 251, arginine at 255, aspartic acid at 310, leucine at 314 and methionine at 428 (FIG. 6). Another specificity trend is observed when one considers positions 308, 309, and 311 where threonine, proline, and serine, respectively, are very strongly favored over the corresponding wild type residues (FIG. 6). However, generation of this strong consensus sequences does not correlate with the magnitude of increase in affinity as V308T/L309P/Q311S binds less than 2-fold better than the wild type IgG1 to both human and murine FcRn (Table IX).

Increases in affinity can be strongly dependent upon residue substitution at one 'hot spot' position. For example, the single mutation M252Y causes an increase in binding to murine FcRn by 9-fold, whereas additional mutations bring little (M252Y/S254T/T256E) or no (M252Y/T256Q) added benefit. The same trend is observed for the human receptor, although to a lesser extent. Indeed, M252Y/S254T/T256E shows a marked improvement of 2.5-fold in affinity compared to M252Y. This probably reflects the differences between the binding site of human and murine FcRn (West and Bjorkman, *Biochemistry*, 39:9698-9708, 2000).

Phage-derived IgG1 mutants exhibiting a significant increase in affinity towards murine FcRn ($\Delta\Delta G > 1.3$ kcal/mol) also showed significant binding activity to the receptor at pH 7.2 when compared to wild type IgG1 (FIGs. 8A-H). IgG1 mutants with moderate increase in affinity ($\Delta\Delta G < 0.3$ kcal/mol) bound very poorly at pH 7.2 (data not shown). In contrast, IgG1 mutants with large ($\Delta\Delta G > 1.0$ kcal/mol) increase in affinity towards human FcRn exhibited only minimal binding at pH 7.4 when compared to wild type IgG1 (FIGs. 8A-H).

Table IX

DISSOCIATION CONSTANTS AND RELATIVE FREE ENERGY CHANGES FOR THE BINDING OF IgG1/FC MUTANTS TO MURINE AND HUMAN FcRn*

MUTANT	Dissociation Constant Fc/Murine FcRn (nM)	ΔΔG (kcal/mol)	Dissociation Constant Fc/Human FcRn (mM)	ΔΔG (kcal/mol)
wild type	269 ± 1		2527 ± 117	
I253A	NB	NA	NB	NA
M252Y/S254T/T256E	27 ± 6	1.4	225 ± 10	1.4
M252W	30 ± 1	1.3	408 ± 24	1.1
M252Y	41 ± 7	1.1	532 ± 37	0.9
M252Y/T256Q	39 ± 8	1.1	560 ± 102	0.9
M252F/T256D	52 ± 9	1.0	933 ± 170	0.6
V308T/L309P/Q311S	153 ± 23	0.3	1964 ± 84	0.1
G385D/Q386P/N389S	187 ± 10	0.2	2164 ± 331	0.1
G385R/Q386T/P387R/N389P	147 ± 24	0.4	1620 ± 61	0.3
H433K/N434F/Y436H	14 ± 2	1.8	399 ± 47	1.1
N434F/Y436H	9 ± 1	2.0	493 ± 7	1.0

*Affinity measurements were carried out by BIAcore as described above. Residue numbering is according to EU (Kabat *et al.*, 1991, *supra*). Differences in free energy changes are calculated as the differences between the Δgs of wild type and mutant reactions ($\Delta\Delta G = \Delta G_{wild\ type} - \Delta G_{mutant}$). NB, no binding. NA, not-applicable.

FcRn-mediated transfer assay

This assay follows the protocol disclosed in PCT publication WO 97/34631. Radiolabeled modified hinge-Fc fragments at various concentration (1 μg/ml-1 mg/ml) are added to the one side of the transwell and the transfer of the fragments mediated by FcRn-expressing monolayer of the cells can be quantitated by measuring the radioactivity on the other side of the transwell.

6.6.2 *IN VIVO* PHARMACOKINETIC STUDY

In order to determine the half-life of the modified IgG hinge-Fc, modified hinge-Fc fragments are radiolabelled with ^{125}I (approximate specific activity of 10^7 cpm/μg) and dissolved in saline (pH 7.2). The solution is injected intravenously into BALB/c mice (Harlan, Indianapolis, IN), which have been given NaI-containing water previously to block the thyroid, in a volume not more than 150 μl and with a radioactivity of 10 x 10^6-50 x 10^6 cpm. The mice are bled from the retro-orbital sinus at various time points, for example, at 3 minutes to 72 hours after the injection, into heparinized capillary tubes and the plasma collected from each sample is counted for radioactivity.

To generate the data provided in FIG. 10, 10 animals were used for each molecule assayed with 2.5 μg of antibody injected per animal. Antibody serum levels were determined using an anti-human IgG ELISA (FIG. 10). There seems to be an inverse correlation between affinity to mouse FcRn and persistence in serum. This might be due to the significant amount of binding of the mutants observed at pH 7.2, which leads to the sequestration (i.e., lack of release in the serum) of the molecules. Preliminary data (not shown) suggests increased transport of the mutants to the lung. Additionally, since the mutants exhibit lower levels of binding to human FcRn than murine FcRn (see FIGS. 8A-H), antibody serum levels are expected to be higher in primates and humans.

6.6.3 SURFACE PLASMON RESONANCE ANALYSES

The interaction of soluble murine and human FcRn with immobilized human IgG1 variants was monitored by surface plasmon resonance detection using a BIAcore 3000 instrument (Pharmacia Biosensor, Uppsala, Sweden). No aggregated material which could interfere with affinity measurements (van der Merwe *et al.*, *EMBO J.*, 12:4945-4954, 1993; van der Merwe *et al.*, *Biochemistry*, 33:10149-10160, 1994) was detected by gel filtration. Protein concentrations were calculated by the bicinchoninic acid (BCA) method for both human and murine FcRn or using the 1% extinction coefficient at 280 nm of 1.5 for IgG1 wild type and variants. The latter were coupled to the dextran matrix of a CM5 sensor chip (Pharmacia Biosensor) using an Amine Coupling Kit as described (Johnson *et al.*, *supra*). The protein concentrations ranged from 3-5 μg/ml in 10 mM sodium acetate, pH 5.0. The activation period was set for 7 minutes at a flow rate of 10 μl/min and the immobilization period was set to between 10 and 20 minutes at a flow rate of 10 μl/min. Excess reactive esters were quenched by injection of 70 μl of 1.0 methanolamine hydrochloride, pH 8.5. This typically resulted in the immobilization of between 500 and 4000 resonance units (RU). Human and murine FcRn were buffer exchanged against 50 mM PBS buffer pH 6.0

containing 0.05% Tween 20. Dilutions were made in the same buffer. All binding experiments were performed at 25°C with concentrations ranging from 120 to 1 μg/ml at a flow rate of 5 to 10 μl/min; data were collected for 25 to 50 minutes and three 1-minute pulses of PBS buffer pH 7.2 were used to regenerate the surfaces. FcRn was also flowed over an uncoated cell and the sensorgrams from these blank runs subtracted from those obtained with IgG1-coupled chips. Runs were analyzed using the software BIAevaluation 3.1 (Pharmacia). Association constants (K_As) were determined from Scatchard analysis by measuring the concentration of free reactants and complex at equilibrium after correction for nonspecific binding. In equilibrium binding BIAcore experiments (Karlsson *et al.*, 1991, *supra*; van der Merwe *et al.*, 1993, *supra*; van der Merwe *et al.*, 1994, *supra*; Raghavan *et al.*, *Immunity*, 1:303-315, 1994; Malchiodi *et al.*, *J. Exp. Med.*, 182:1833-1845, 1995), the concentration of the complex can be assessed directly as the steady-state response. The concentration of free analyte (human or murine FcRn) is equal to the bulk analyte concentration since analyte is constantly replenished during sample injection. The concentration of free ligand on the surface of the sensor chip can be derived from the concentration of the complex and from the total binding capacity of the surface as $K_A = R_{eq}/C(R_{max} - R_{eq})$ where C is the free analyte concentration, R_{eq} is the steady-state response, and R_{max} is the total surface binding capacity. Rearranging, the equation reads: $R_{eq}/C = K_A R_{max} - K_A R_{eq}$.

A plot of R_{eq}/C versus R_{eq} at different analyte concentrations thus gives a straight line from which K_A can be calculated (see Table IX). Errors were estimated as the standard deviation for two or three independent determinations and were <20%.

Representative mutations identified after panning libraries 1 through 4 (FIG. 6, Table VIII) were introduced into the Fc portion of a human IgG1. Injection of different concentrations of human or murine FcRn over the immobilized IgG1 variants gave concentration-dependent binding. Typical resonance profiles for equilibrium binding of the mutant M252Y/S254T/T256E to murine and human FcRn are shown in FIGs. 7A and B. To estimate apparent K_As, concentrations of FcRn ranging from 120 to 1 μg/ml were used. In all cases, equilibrium (or near-equilibrium) binding levels were reached within 50 minutes. To estimate the increase in RU resulting from the non specific effect of protein on the bulk refractive index, binding of FcRn to an uncoated cell was measured and the sensorgrams from these blank runs subtracted from those obtained with IgG1-coupled chips. The scatchard plots for the binding of the mutant M252Y/S254T/T256E to murine and human FcRn are shown in FIGs. 7C and D. The plots were all linear, and apparent K_As were

calculated from the relevant slopes. Measurements were carried out in duplicate or triplicate and confirmed that the immobilized IgGs retained their original binding activity.

Since there are two non-equivalent binding sites on mouse IgG1 for murine FcRn with affinities of < 130 nM and 6 μM (Sanchez *et al.*, *Biochemistry*, 38:9471-9476, 1999; Schuck *et al.*, *Mol. Immunol.*, 36:1117-1125, 1999; Ghetie and Ward, *Ann. Rev. Immunol.*, 18:739-766, 2000), the receptor was used in solution to avoid avidity effects that arise when IgG1 binds to immobilized FcRn. Consistent with this, systematically higher affinities are observed when FcRn, rather than IgG, immobilized on the biosensor chip (Popov *et al.*, 1996, *supra*; Vaughn and Bjorkman, *Biochemistry*, 36:9374-9380, 1997; Martin and Bjorkman, *Biochemistry*, 38:12639-12647; West and Bjorkman, *Biochemistry*, 39:9698-9708, 2000). Under our experimental BIAcore conditions, mainly interactions corresponding to the higher-affinity association (*i.e.* single liganded-receptor) are measured, according for the linearity of the scatchard plots (FIGs. 7C and D).

BIAcore analysis was also used to compare the affinity of wild type IgG1 and IgG1 mutants. Phage-derived IgG1 mutants exhibiting a significant increase in affinity towards murine FcRn at pH 6.0 ($\Delta\Delta G \geq 1.0$ kcal/mol) also shoed significant binding to the mouse receptor at pH 7.2 with SPR signal$_{pH7.4}$/SPR signal$_{pH6.0} > 0.6$ at saturation. IgG1 mutants with moderate increase in affinity towards murine FcRn at pH 6.0 ($\Delta\Delta G < 0.4$ kcal/mol) bound very poorly to the mouse receptor at pH 7.2. In contrast, IgG1 mutants exhibiting large affinity increase towards human FcRn at pH 6.0 ($\Delta\Delta G \geq 1.0$ kcal/mol) only showed minimal binding to the human receptor at pH 7.4 with SPR signal$_{pH7.4}$/SPR signal$_{pH6.0}$ < 0.15 at saturation.

Those skilled in the art will recognize, or be able to ascertain using no more routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

What is claimed is:

1. A modified IgG comprising an IgG constant domain comprising one or more amino acid modifications relative to a wild-type IgG constant domain, wherein the modified IgG has an increased half-life compared to the half-life of an IgG having the wild-type IgG constant domain, and wherein the one or more amino acid modifications are at one or more of positions 251, 253, 255, 285-290, 308-314, 385-389, and 428-435.

2. A modified non-human IgG comprising a non-human IgG constant domain comprising one or more amino acid modifications relative to a wild-type non-human IgG constant domain, wherein the modified IgG has an increased half-life compared to the half-life of an IgG having the wild-type non-human IgG constant domain, and wherein the one or more amino acid modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, with the proviso that the one or more amino acid modifications do not include substitution with leucine at position 252, serine at position 254, and phenylalanine at position 256.

3. A modified human or humanized IgG comprising a human IgG constant domain comprising one or more amino acid modifications relative to a wild-type human IgG constant domain, wherein the modified human or humanized IgG has an increased half-life compared to the half-life of a human or humanized IgG having the wild-type human IgG constant domain, and wherein the one or more amino acid modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436.

4. The modified IgG according to claim 1, 2, or 3, wherein at least one of the amino acid modifications is an amino acid substitution.

5. The modified IgG according to claim 1, 2, or 3, wherein at least one of the amino acid modifications is an amino acid deletion.

6. The modified IgG according to claim 1, 2, or 3, wherein at least one of the amino acid modifications is an amino acid insertion.

7. The modified IgG according to claim 1, 2 or 3 which has a higher affinity for FcRn than the IgG having the wild-type IgG constant domain.

8. The modified IgG according to claim 7 which has a higher affinity for the FcRn at pH 6.0 than at pH 7.4.

9. The modified IgG according to claim 1, wherein said one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 255, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, or 436.

10. The modified IgG according to claim 2 or 3, wherein said one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434 or 336.

11. The modified IgG according to claim 1 or 3 wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine or serine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

12. The modified IgG according to claim 11, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

13. The modified IgG according to claim 11, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

14. The modified IgG according to claim 11, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

15. The modified IgG according to claim 2 wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylaline at position 252, substitution with threonine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

16. The modified IgG according to claim 15, wherein said one or more amino acid substitutions are substitution with tyrosine at position 252, threonine at position 254 and glutamate at 256.

17. The modified IgG according to claim 15, wherein said one or more amino acid substitutions are substitution with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

18. The modified IgG according to claim 15, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

19. The modified IgG according to claim 3 or 15 which has the heavy chain variable domain and light chain variable domain of SYNAGIS®.

20. The modified IgG according to claim 3 or 15 which has the heavy chain variable domain and light chain variable domain of AFFF, p12f2, p12f4, p11d4, Ale109, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L215B10, A13A11, A1H5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

21. A fusion protein comprising a non-IgG polypeptide covalently linked to a modified IgG constant domain, or a fragment thereof that binds to FcRn, said modified IgG

constant domain or fragment comprising one or more amino acid modifications relative to a wild-type IgG constant domain, wherein the one or more modifications are at one or more of positions 251, 253, 255, 285-290, 308-314, 385-389, and 428-436, and wherein said fusion protein has a longer half life than the non-IgG polypeptide alone.

22. A fusion protein comprising a non-IgG polypeptide covalently linked to a modified non-human IgG constant domain, or a fragment thereof that binds to FcRn, said modified non-human IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type non-human IgG constant domain, wherein the one or more modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, with the proviso that the modified amino acid sequence does not have leucine at position 252, serine at position 254, and phenylalanine at position 256, and wherein said fusion protein has a longer half life than the non-IgG polypeptide alone.

23. A fusion protein comprising a non-IgG polypeptide covalently linked to a modified human IgG constant domain, or a fragment thereof that binds to FcRn, said modified human IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type human IgG constant domain, wherein the one or more modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, and wherein said fusion protein has a longer half life than the non-IgG polypeptide alone.

24. The fusion protein according to claim 21, 22, or 23, wherein at least one of the amino acid modifications is an amino acid substitution.

25. The fusion protein according to claim 21, 22, or 23, wherein at least one of the amino acid modifications is an amino acid deletion.

26. The fusion protein according to claim 21, 22, or 23, wherein at least one of the amino acid modifications is an amino acid insertion.

27. The fusion protein according to claim 21, 22, or 23, wherein the modified IgG constant domain or fragment has an increased affinity for FcRn relative to the wild-type IgG constant domain.

28. The fusion protein according to claim 27, wherein the modified IgG constant domain or fragment has a higher affinity for the FcRn at pH 6.0 than at pH 7.4.

29. The fusion protein according to claim 21, 22, or 23, wherein the non-IgG polypeptide is an immunoglobulin.

30. The fusion protein according to claim 21, wherein the one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 255, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, or 436.

31. The fusion protein according to claim 22 or 23, wherein the one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434 or 336.

32. The fusion protein according to claim 21 or 23, wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine or serine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

33. The fusion protein according to claim 32, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

34. The fusion protein according to claim 32, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

35. The fusion protein according to claim 32, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

36. The fusion protein according to claim 32, wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

37. The fusion protein according to claim 36, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

38. The fusion protein according to claim 36, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

39. The fusion protein according to claim 36, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

40. A molecule comprising a non-protein agent conjugated to a modified IgG constant domain, or a fragment thereof that binds to FcRn, said modified IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type IgG constant domain, wherein the one or more modifications are at one or more of positions 251, 253, 255, 285-290, 308-314, 385-389, and 428-436, and wherein said molecule has a longer half life than the non-protein agent alone.

41. A molecule comprising a non-protein agent conjugated to a modified non-human IgG constant domain, or a fragment thereof that binds to FcRn, said modified non-human IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type non-human IgG constant domain, wherein the one or more modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, with the proviso that the modified amino acid sequence does not have leucine at position 252, serine at position 254, and phenylalanine at position 256, and wherein said molecule has a longer half life than the non-protein agent alone.

42. A molecule comprising a non-protein agent conjugated to a modified human IgG constant domain, or a fragment thereof that binds to FcRn, said modified human IgG constant domain or fragment comprising one or more amino acid modifications relative to a wild-type human IgG constant domain, wherein the one or more modifications are at one or more of positions 251-256, 285-290, 308-314, 385-389, and 428-436, and wherein said molecule has a longer half life than the non-protein agent alone.

43. The molecule according to claim 40, 41, or 42 wherein at least one of the amino acid modifications is an amino acid substitution.

44. The molecule according to claim 40, 41, or 42 wherein at least one of the amino acid modifications is an amino acid deletion.

45. The molecule according to claim 40, 41 or 42, wherein at least one of the amino acid modifications is an amino acid insertion.

46. The molecule according to claim 40, 41 or 42, wherein the modified IgG constant domain or fragment has an increased affinity for FcRn relative to the wild-type constant domain.

47. The molecule according to claim 46, wherein the modified IgG constant domain or fragment has a higher affinity for the FcRn at pH 6.0 than at pH 7.4.

48. The molecule according to claim 40, wherein the one or more amino acid modifications are amino acid substitutions at one or more of positions 251, 255, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434, or 436.

49. The molecule according to claim 41 or 42, wherein the one or more amino acid modifications are substitution at one or more of positions 251, 252, 254, 255, 256, 308, 309, 311, 312, 314, 385, 386, 387, 389, 428, 433, 434 or 336.

50. The molecule according to claim 40 or 42, wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine or serine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

51. The molecule according to claim 50, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

52. The molecule according to claim 50, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

53. The molecule according to claim 50, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

54. The molecule according to claim 41, wherein said one or more amino acid modifications are substitution with leucine at position 251, substitution with tyrosine, tryptophan or phenylalanine at position 252, substitution with threonine at position 254, substitution with arginine at position 255, substitution with glutamine, arginine, serine, threonine, or glutamate at position 256, substitution with threonine at position 308, substitution with proline at position 309, substitution with serine at position 311, substitution

with aspartate at position 312, substitution with leucine at position 314, substitution with arginine, aspartate or serine at position 385, substitution with threonine or proline at position 386, substitution with arginine or proline at position 387, substitution with proline, asparagine or serine at position 389, substitution with methionine or threonine at position 428, substitution with tyrosine or phenylalanine at position 434, substitution with histidine, arginine, lysine or serine at position 433, or substitution with histidine, tyrosine, arginine or threonine at position 436.

55. The molecule according to claim 54, wherein said one or more amino acid substitutions are substitutions with tyrosine at position 252, threonine at position 254 and glutamate at 256.

56. The molecule according to claim 54, wherein said one or more amino acid substitutions are substitutions with lysine at position 433, phenylalanine at position 434 and histidine at position 436.

57. The molecule according to claim 54, wherein said amino acid substitution is a substitution with tyrosine or tryptophan at position 252.

58. A pharmaceutical composition comprising the modified human or humanized IgG according to claim 3 and a pharmaceutically acceptable carrier.

59. A pharmaceutical composition comprising the fusion protein according to claim 23 and a pharmaceutically acceptable carrier.

60. A pharmaceutical composition comprising the molecule according to claim 42 and a pharmaceutically acceptable carrier.

61. A method of treating a disease or disorder comprising administering to a patient in need thereof a therapeutically effective amount of the modified human or humanized IgG according to claim 3.

62. The method according to claim 61 which comprises passive immunotherapy.

63. A method of treating a disease or disorder comprising administering to a patient in need thereof a therapeutically effective amount of the fusion protein according to claim 23.

64. A method of treating a disease or disorder comprising administering to a patient in need thereof a therapeutically effective amount of the molecule according to claim 42.

65. A nucleic acid comprising a nucleotide sequence encoding the modified IgG constant domain according to claim 1, 2 or 3, or an FcRn binding fragment thereof.

66. A nucleic acid comprising a nucleotide sequence encoding the fusion protein according to claim 21, 22, or 23.

67. A host cell comprising the nucleic acid according to claim 65.

68. A host cell comprising the nucleic acid according to claim 66.

69. A kit comprising the modified human or humanized IgG according to claim 3.

70. A kit comprising the fusion protein according to claim 23.

71. A kit comprising the molecule according to claim 42.

72. A method of preventing a disease or disorder comprising administering to a subject a prophylactically effective amount of the modified human or humanized IgG according to claim 3.

73. The method according to claim 72 which is passive immunotherapy.

74. The method according to claim 72 wherein the disease is RSV infection.

75. The method according to claim 74, wherein said modified human or humanized IgG is a modified SYNAGIS® antibody.

76. The method according to claim 74, wherein said modified human or humanized IgG is a modified AFFF, p12f2, p12f4, p11d4, Ale109, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L215B10, A13A11, A1H5, A4B4(1), A4B4L1FR-S28R or A4B4-F52S antibody.

77. A method of vaccinating a subject comprising administering to said subject an amount of the modified human or humanized IgG according to claim 3 effective to elicit an immune response.

78. A method of vaccinating a subject comprising administering to said subject an amount of the fusion protein according to claim 23 effective to elicit an immune response.

79. The method according to 61 which comprises administrating a dose of said modified human or humanized IgG that is lower than the lowest therapeutically effective dose of a second IgG identical to said modified human or humanized IgG except that said second IgG lacks said one or more amino acid modifications.

80. The method according to claim 79 which results in fewer or less severe side effects than administration of the therapeutically effective dose of the second IgG.

81. The method according to claim 61 which comprises administering a therapeutically effective dosing schedule having less frequent doses of said modified human or humanized IgG than the therapeutically effective dosing schedule having the least frequent dosing of a second IgG identical to said modified human or humanized IgG except that said second IgG lacks said one or more amino acid modifications.

82. The method according to claim 72 which comprises administrating a prophylactically effective dose of said modified human or humanized IgG that is lower than the lowest prophylactically effective dose of a second IgG identical to said modified human or humanized IgG except that said second IgG lacks said one or more amino acid modifications.

83. The method according to claim 82 which results in fewer or less severe side effects than administration of the prophylactically effective dose of the second IgG.

84. The method according to claim 72 which comprises administering a prophylactically effective dosing schedule having less frequent doses of said modified human or humanized IgG than the prophylactically effective dosing schedule with the least frequent dosing of a second IgG identical to said humani or humanized IgG except that said second IgG lacks said one or more amino acid modifications.

85. A method of *in vivo* diagnosis in a subject comprising:

(a) administering to a subject an effective amount of the modified human or humanized IgG according to claim 3 labeled with a detectable marker, said modified human or humanized IgG specifically binding to an antigen associated with a disease or disorder;

(b) allowing the modified human or humanized IgG to concentrate at sites in said subject where said antigen is found; and

(c) detecting said detectable marker,

whereby detection of said detectable marker above a background or standard level indicates that the subject has said disease or disorder.

86. The modified human or humanized IgG according to claim 3 which immunospecifically binds to an RSV antigen.

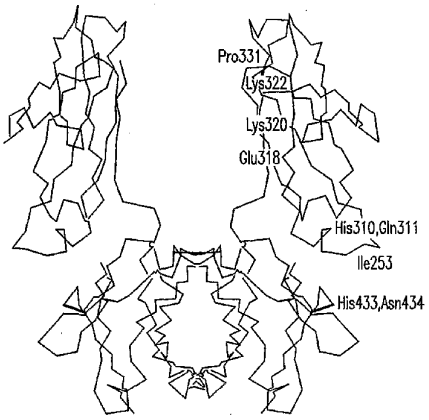

Pro331
Lys322
Lys320
Glu318
His310,Gln311
Ile253
His433,Asn434

FIG.1

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                  10                  15
|----------------Hinge---------------------------------|----
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30
-------------------------------------------------------------
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45
-------------------------------------------------------------
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60
-----------------------------------------------------CH2-----
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80
-------------------------------------------------------------
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95
-------------------------------------------------------------
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110
-------------------------------------------------------------
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125
---------------------------------------------|---------------
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140
-------------------------------------------------------------
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160
-----------------------------------------------------------CH3-
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175
-------------------------------------------------------------
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190
-------------------------------------------------------------
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205
-------------------------------------------------------------
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220
-------------------------------------------------------------
Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
------------------------------|
```

FIG.2

```
Met Gly Val Pro Arg Pro Gln Pro Trp Ala Leu Gly Leu Leu Leu Phe
1               5                  10                  15
Leu Leu Pro Gly Ser Leu Gly Ala Glu Ser His Leu Ser Leu Leu Tyr
            20                  25                  30
His Leu Thr Ala Val Ser Ser Pro Ala Pro Gly Thr Pro Ala Phe Trp
        35                  40                  45
Val Ser Gly Trp Leu Gly Pro Gln Gln Tyr Leu Ser Tyr Asn Ser Leu
    50                  55                  60
Arg Gly Glu Ala Glu Pro Cys Gly Ala Trp Val Trp Glu Asn Gln Val
65                  70                  75                  80
Ser Trp Tyr Trp Glu Lys Glu Thr Thr Asp Leu Arg Ile Lys Glu Lys
                85                  90                  95
Leu Phe Leu Glu Ala Phe Lys Ala Leu Gly Gly Lys Gly Pro Tyr Thr
            100                 105                 110
Leu Gln Gly Leu Leu Gly Cys Glu Leu Gly Pro Asp Asn Thr Ser Val
        115                 120                 125
Pro Thr Ala Lys Phe Ala Leu Asn Gly Glu Glu Phe Met Asn Phe Asp
    130                 135                 140
Leu Lys Gln Gly Thr Trp Gly Gly Asp Trp Pro Glu Ala Leu Ala Ile
145                 150                 155                 160
Ser Gln Arg Trp Gln Gln Gln Asp Lys Ala Ala Asn Lys Glu Leu Thr
                165                 170                 175
Phe Leu Leu Phe Ser Cys Pro His Arg Leu Arg Glu His Leu Glu Arg
            180                 185                 190
Gly Arg Gly Asn Leu Glu Trp Lys Glu Pro Pro Ser Met Atg Leu Lys
        195                 200                 205
Ala Arg Pro Ser Ser Pro Gly Phe Ser Val Leu Thr Cys Ser Ala Phe
    210                 215                 220
Ser Phe Tyr Pro Pro Glu Leu Gln Leu Arg Phe Leu Arg Asn Gly Leu
225                 230                 235                 240
Ala Ala Gly Thr Gly Gln Gly Asp Phe Gly Pro Asn Ser Asp Gly Ser
                245                 250                 255
Phe His Ala Ser Ser Ser Leu Thr Val Lys Ser Gly Asp Glu His His
            260                 265                 270
Tyr Cys Cys Ile Val Gln His Ala Gly Leu Ala Gln Pro Leu Arg Val
        275                 280                 285
Glu Leu Glu Ser Pro Ala Lys Ser Ser Val Leu Val Val Gly Ile Val
    290                 295                 300
Ile Gly Val Leu Leu Leu Thr Ala Ala Ala Val Gly Gly Ala Leu Leu
305                 310                 315                 320
Trp Arg Arg Met Arg Ser Gly Leu Pro Ala Pro Trp Ile Ser Leu Arg
                325                 330                 335
Gly Asp Asp Thr Gly Val Leu Leu Pro Thr Pro Gly Glu Ala Gln Asp
            340                 345                 350
Ala Asp Leu Lys Asp Val Asn Val Ile Pro Ala Thr Ala
        355                 360                 365
```

FIG.3A

```
Met Gly Met Pro Leu Pro Trp Ala Leu Ser Leu Leu Leu Val Leu Leu
1               5                  10                  15
Pro Gln Thr Trp Gly Ser Glu Thr Arg Pro Pro Leu Met Tyr His Leu
            20                  25                  30
Thr Ala Val Ser Asn Pro Ser Thr Gly Leu Pro Ser Phe Trp Ala Thr
        35                  40                  45
Gly Trp Leu Gly Pro Gln Gln Tyr Leu Thr Tyr Asn Ser Leu Arg Gln
    50                  55                  60
Glu Ala Asp Pro Cys Gly Ala Trp Val Trp Glu Asn Gln Val Ser Trp
65                  70                  75                  80
Tyr Trp Glu Lys Glu Thr Thr Asp Leu Lys Ser Lys Glu Gln Leu Phe
                85                  90                  95
Leu Glu Ala Leu Lys Thr Leu Glu Lys Ile Leu Asn Gly Thr Tyr Thr
            100                 105                 110
Leu Gln Gly Leu Leu Gly Cys Glu Leu Ala Ser Asp Asn Ser Ser Val
        115                 120                 125
Pro Thr Ala Val Phe Ala Leu Asn Gly Glu Glu Phe Met Lys Phe Asn
    130                 135                 140
Pro Arg Ile Gly Asn Trp Thr Gly Glu Trp Pro Glu Thr Glu Ile Val
145                 150                 155                 160
Ala Asn Leu Trp Met Lys Gln Pro Asp Ala Ala Arg Lys Glu Ser Glu
                165                 170                 175
Phe Leu Leu Asn Ser Cys Pro Glu Arg Leu Leu Gly His Leu Glu Arg
            180                 185                 190
Gly Arg Arg Asn Leu Glu Trp Lys Glu Pro Pro Ser Met Arg Leu Lys
        195                 200                 205
Ala Arg Pro Gly Asn Ser Gly Ser Ser Val Leu Thr Cys Ala Ala Phe
    210                 215                 220
Ser Phe Tyr Pro Pro Glu Leu Lys Phe Arg Phe Leu Arg Asn Gly Leu
225                 230                 235                 240
Ala Ser Gly Ser Gly Asn Cys Ser Thr Gly Pro Asn Gly Asp Gly Ser
                245                 250                 255
Phe His Ala Trp Ser Leu Leu Glu Val Lys Arg Gly Asp Glu His His
            260                 265                 270
Tyr Gln Cys Gln Val Glu His Glu Gly Leu Ala Gln Pro Leu Thr Val
        275                 280                 285
Asp Leu Asp Ser Ser Ala Arg Ser Ser Val Pro Val Val Gly Ile Val
    290                 295                 300
Leu Gly Leu Leu Leu Val Val Val Ala Ile Ala Gly Gly Val Leu Leu
305                 310                 315                 320
Trp Gly Arg Met Arg Ser Gly Leu Pro Ala Pro Trp Leu Ser Leu Ser
                325                 330                 335
Gly Asp Asp Ser Gly Asp Leu Leu Pro Gly Gly Asn Leu Pro Pro Glu
            340                 345                 350
Ala Glu Pro Gln Gly Ala Asn Ala Phe Pro Ala Thr Ser
        355                 360                 365
```

FIG.3B

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1               5                   10                  15
-------------------Hinge-------------------------------|----
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30
-----------------------------------------------------------------
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        40                  45                  50
-----------------------------------------------------------------
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60
-----------------------------------------------------CH2------
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80
-----------------------------------------------------------------
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95
-----------------------------------------------------------------
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110
-----------------------------------------------------------------
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125
-----------------------------------------------|-----------------
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140
-----------------------------------------------------------------
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160
-----------------------------------------------------------CH3-
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175
-----------------------------------------------------------------
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190
-----------------------------------------------------------------
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205
-----------------------------------------------------------------
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220
-----------------------------------------------------------------
Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
-------------------------------|
```

FIG.4

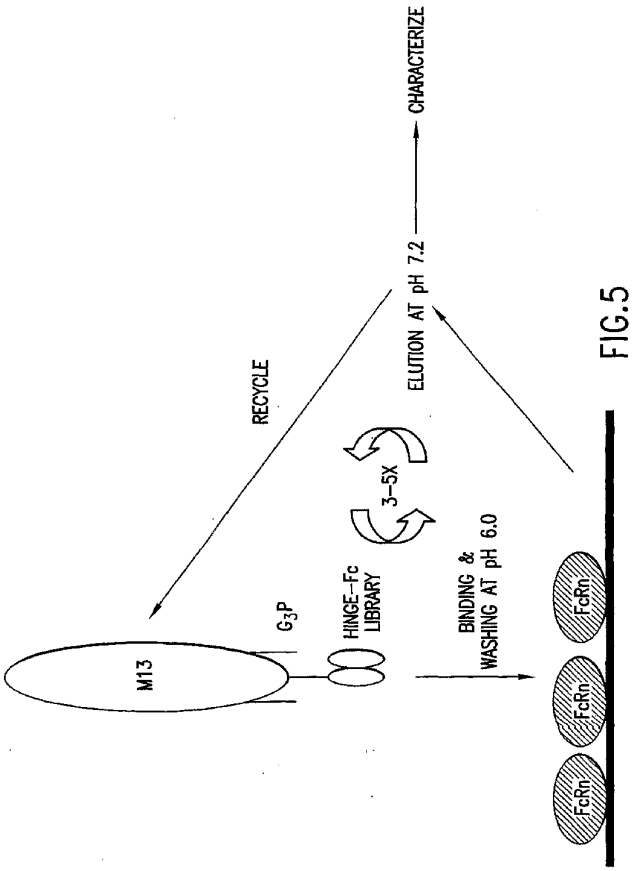

CHARACTERIZE
ELUTION AT pH 7.2
RECYCLE
3-5X
BINDING & WASHING AT pH 6.0
HINGE-Fc LIBRARY
G3P
M13
FcRn
FcRn
FcRn

FIG.5

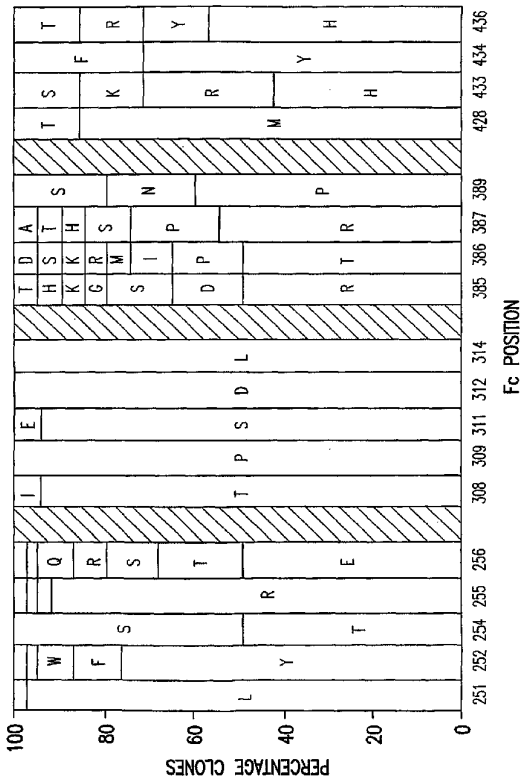

PERCENTAGE CLONES
100
80
60
40
20
0
Fc POSITION
251 252 254 255 256 308 309 311 312 314 385 386 387 389 428 433 434 436

FIG.6

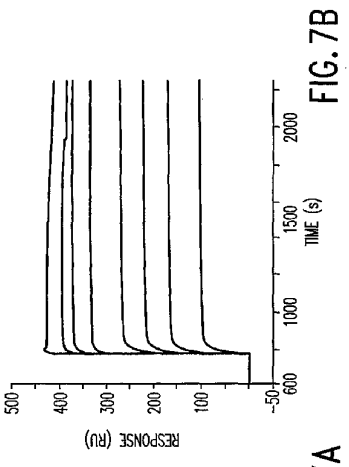


FIG. 7B

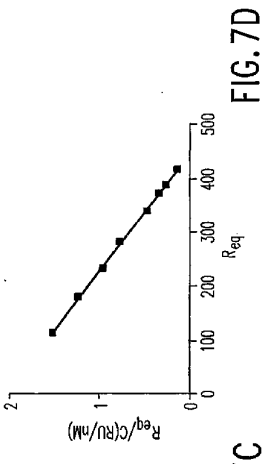
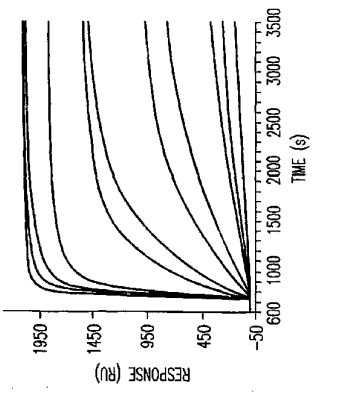


FIG. 7D

FIG. 7A



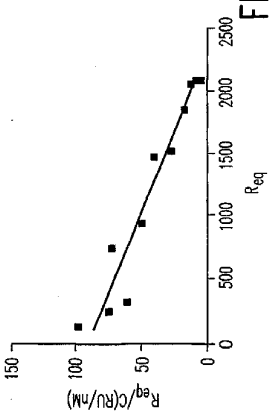


FIG. 7C

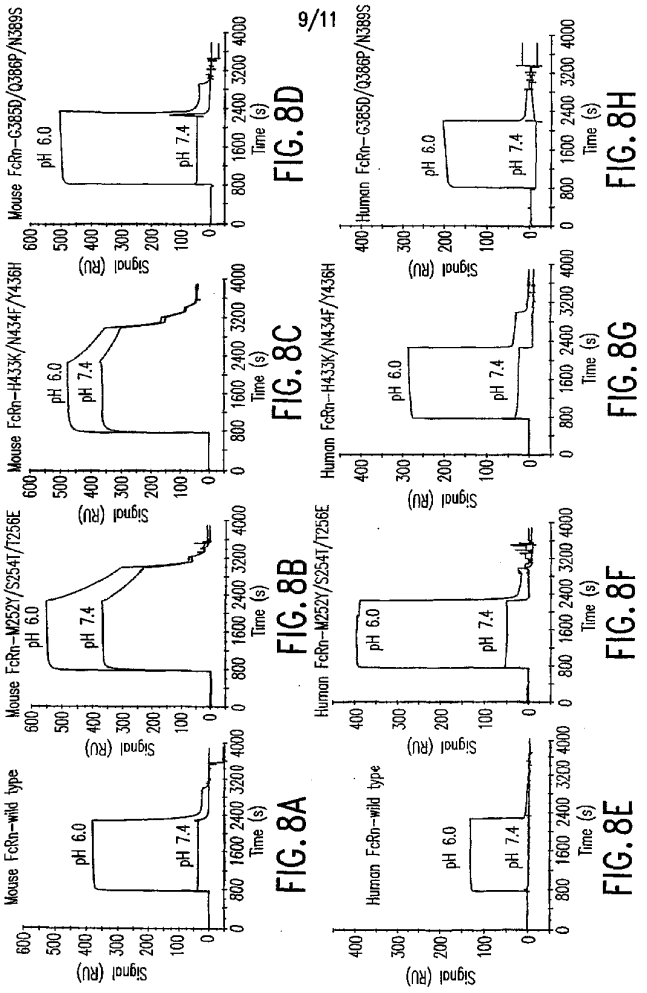
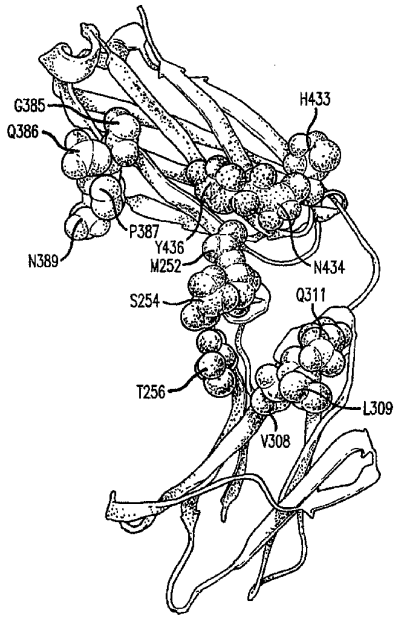


FIG. 8A FIG. 8B FIG. 8C FIG. 8D

FIG. 8E FIG. 8F FIG. 8G FIG. 8H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Q386
P387
N389
Y436
M252
S254
T256
H433
N434
Q311
L309
V308

FIG.9

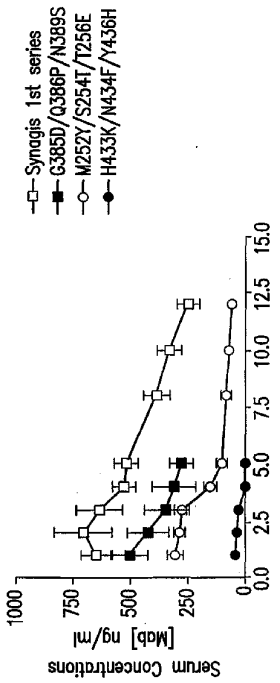
Synagis 1st series
G385D/Q386P/N389S
M252Y/S254T/T256E
H433K/N434F/Y436H
Serum Concentrations
[Mab] ng/ml
1000
750
500
250
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
Time (d)

FIG.10

SEQUENCE LISTING

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<140>
<141>

<150> 60/254,884
<151> 2000-12-12

<150> 60/238,760
<151> 2001-05-09

<160> 118

<170> PatentIn version 3.1

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1 5 10

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1 5

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1 5

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Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Ser Met Ile Thr Asn Trp Tyr Phe Asp Val Trp Gly Ala
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

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35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

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Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Ser Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Ala
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

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<213> Homo sapiens

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20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
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1 5 10

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20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Phe Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

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1 5 10

<210> 15
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<212> PRT
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Asp Thr Phe Lys Leu Ala Ser
1 5

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Phe Gln Phe Ser Gly Tyr Pro Phe Thr
1 5

<210> 17
<211> 120
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20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Ala
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

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<400> 18
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<210> 19

<211> 16
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<210> 20
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1 5 10

<210> 21
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<213> Homo sapiens

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His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Tyr Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

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Ser Leu Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 23
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<213> Homo sapiens

<400> 23
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1 5

<210> 24
<211> 120
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<213> Homo sapiens

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1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

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<210> 26
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Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Arg Gly Leu Pro Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

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Asp Thr Arg Gly Leu Pro Ser
1 5

<210> 28
<211> 120
<212> PRT
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Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
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Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

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1 5 10

<210> 30
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<212> PRT
<213> Homo sapiens

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1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Pro Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Met Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

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Ser Pro Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 32
<211> 7
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<400> 32
Asp Thr Met Arg Leu Ala Ser
1 5

<210> 33
<211> 120
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Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

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<213> Homo sapiens

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Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 35
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<400> 35
Asp Thr Phe Lys Leu Ser Ser
1 5

<210> 36
<211> 120
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Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

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Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser
        115                 120

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<213>  Homo sapiens

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<212>  PRT
<213>  Homo sapiens

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Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105

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1               5                   10

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20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

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<210> 42
<211> 106
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1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

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Asp Thr Met Tyr Gln Ser Ser
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<210> 44
<211> 120
<212> PRT
<213> Homo sapiens

<400> 44
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1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

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1 5 10 15

<210> 46
<211> 106
<212> PRT
<213> Homo sapiens

<400> 46
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Pro Ser Ser Arg Val Gly Tyr Met
20 25 30

```
His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Met Tyr Gln Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Phe Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
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Leu Pro Ser Ser Arg Val Gly Tyr Met His
1 5 10

<210> 48
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Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
            20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser
    50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln
            100 105 110

Gly Thr Thr Val Thr Val Ser Ser
        115 120

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1 5 10 15

```
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            20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
        35                  40                  45

Asp Thr Phe Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
    50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65                  70                  75                  80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
                85                  90                  95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105

<210>  50
<211>  7
<212>  PRT
<213>  Homo sapiens

<400>  50
Asp Thr Phe Phe Leu Asp Ser
1               5

<210>  51
<211>  120
<212>  PRT
<213>  Homo sapiens

<400>  51
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1               5                   10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
            20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser
        115                 120

<210>  52
<211>  106
<212>  PRT
<213>  Homo sapiens
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<400> 52
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Arg Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 53
<211> 7
<212> PRT
<213> Homo sapiens

<400> 53
Asp Thr Arg Tyr Gln Ser Ser
1 5

<210> 54
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 54
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 55
<211> 120
<212> PRT
<213> Homo sapiens

<400> 55
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Ala
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 56
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 56
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 57

<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 57
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 58
<211> 7
<212> PRT
<213> Homo sapiens

<400> 58
Asp Thr Tyr Lys Gln Thr Ser
1 5

<210> 59
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 59
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Arg Tyr Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 60
<211> 106
<212> PRT
<213> Homo sapiens

<400> 60
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Phe Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 61
<211> 9
<212> PRT
<213> Homo sapiens

<400> 61
Phe Gln Gly Ser Phe Tyr Pro Phe Thr
1 5

<210> 62
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 62
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 63
<211> 7
<212> PRT
<213> Homo sapiens

<400> 63
Asp Thr Phe Lys Leu Thr Ser
1 5

<210> 64
<211> 106
<212> PRT
<213> Homo sapiens

<220>
<221> misc_feature
<223> VL Domain

<400> 64
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 65
<211> 106
<212> PRT
<213> Homo sapiens

<400> 65
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 66
<211> 7
<212> PRT
<213> Homo sapiens

<400> 66
Asp Thr Phe Arg Leu Ala Ser
1 5

<210> 67
<211> 120
<212> PRT
<213> Homo sapiens

<400> 67
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Ala
100 105 110

Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 68

<211> 106
<212> PRT
<213> Homo sapiens

<400> 68
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Tyr Arg His Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 69
<211> 7
<212> PRT
<213> Homo sapiens

<400> 69
Asp Thr Tyr Arg His Ser Ser
1 5

<210> 70
<211> 106
<212> PRT
<213> Homo sapiens

<400> 70
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 71
<211> 106
<212> PRT
<213> Homo sapiens

<400> 71
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Ser Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Phe Phe His Arg Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 72
<211> 10
<212> PRT
<213> Homo sapiens

<400> 72
Ser Leu Ser Ser Ser Val Gly Tyr Met His
1 5 10

<210> 73
<211> 7
<212> PRT
<213> Homo sapiens

<400> 73
Asp Thr Phe Phe His Arg Ser
1 5

<210> 74
<211> 106
<212> PRT
<213> Homo sapiens

<400> 74
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Leu Leu Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 75
<211> 7
<212> PRT
<213> Homo sapiens

<400> 75
Asp Thr Leu Leu Leu Asp Ser
1 5

<210> 76
<211> 106
<212> PRT
<213> Homo sapiens

<400> 76
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
35 40 45

Asp Thr Ser Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 77
<211> 7
<212> PRT
<213> Homo sapiens

<400> 77
Asp Thr Ser Phe Leu Asp Ser
1 5

<210> 78
<211> 120
<212> PRT
<213> Homo sapiens

<400> 78
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1 5 10 15

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Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
            20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
        35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser
    50                  55                  60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr
                85                  90                  95

Cys Ala Arg Asp Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Ala
            100                 105                 110

Gly Thr Thr Val Thr Val Ser Ser
        115                 120

<210>  79
<211>  10
<212>  PRT
<213>  Homo sapiens

<400>  79
Asp Met Ile Thr Asn Phe Tyr Phe Asp Val
1               5                   10

<210>  80
<211>  109
<212>  PRT
<213>  Homo sapiens

<400>  80
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1               5                   10                  15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            20                  25                  30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        35                  40                  45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    50                  55                  60

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65                  70                  75                  80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                85                  90                  95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
            100                 105

<210>  81
<211>  108
<212>  PRT
<213>  Homo sapiens
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<400> 81
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
1 5 10 15

Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
20 25 30

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
35 40 45

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
50 55 60

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
65 70 75 80

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
85 90 95

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
100 105

<210> 82
<211> 15
<212> PRT
<213> Homo sapiens

<400> 82
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

<210> 83
<211> 232
<212> PRT
<213> Homo sapiens

<400> 83
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
1 5 10 15

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
85 90 95

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
100 105 110

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

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Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
145                 150                 155                 160

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230

<210>  84
<211>  365
<212>  PRT
<213>  Homo sapiens

<400>  84
Met Gly Val Pro Arg Pro Gln Pro Trp Ala Leu Gly Leu Leu Leu Phe
1               5                   10                  15

Leu Leu Pro Gly Ser Leu Gly Ala Glu Ser His Leu Ser Leu Leu Tyr
            20                  25                  30

His Leu Thr Ala Val Ser Ser Pro Ala Pro Gly Thr Pro Ala Phe Trp
        35                  40                  45

Val Ser Gly Trp Leu Gly Pro Gln Gln Tyr Leu Ser Tyr Asn Ser Leu
    50                  55                  60

Arg Gly Glu Ala Glu Pro Cys Gly Ala Trp Val Trp Glu Asn Gln Val
65                  70                  75                  80

Ser Trp Tyr Trp Glu Lys Glu Thr Thr Asp Leu Arg Ile Lys Glu Lys
                85                  90                  95

Leu Phe Leu Glu Ala Phe Lys Ala Leu Gly Gly Lys Gly Pro Tyr Thr
            100                 105                 110

Leu Gln Gly Leu Leu Gly Cys Glu Leu Gly Pro Asp Asn Thr Ser Val
        115                 120                 125

Pro Thr Ala Lys Phe Ala Leu Asn Gly Glu Glu Phe Met Asn Phe Asp
    130                 135                 140

Leu Lys Gln Gly Thr Trp Gly Gly Asp Trp Pro Glu Ala Leu Ala Ile
145                 150                 155                 160

Ser Gln Arg Trp Gln Gln Gln Asp Lys Ala Ala Asn Lys Glu Leu Thr
                165                 170                 175

Phe Leu Leu Phe Ser Cys Pro His Arg Leu Arg Glu His Leu Glu Arg
            180                 185                 190
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Gly Arg Gly Asn Leu Glu Trp Lys Glu Pro Pro Ser Met Arg Leu Lys
195 200 205

Ala Arg Pro Ser Ser Pro Gly Phe Ser Val Leu Thr Cys Ser Ala Phe
210 215 220

Ser Phe Tyr Pro Pro Glu Leu Gln Leu Arg Phe Leu Arg Asn Gly Leu
225 230 235 240

Ala Ala Gly Thr Gly Gln Gly Asp Phe Gly Pro Asn Ser Asp Gly Ser
245 250 255

Phe His Ala Ser Ser Ser Leu Thr Val Lys Ser Gly Asp Glu His His
260 265 270

Tyr Cys Cys Ile Val Gln His Ala Gly Leu Ala Gln Pro Leu Arg Val
275 280 285

Glu Leu Glu Ser Pro Ala Lys Ser Ser Val Leu Val Val Gly Ile Val
290 295 300

Ile Gly Val Leu Leu Leu Thr Ala Ala Ala Val Gly Gly Ala Leu Leu
305 310 315 320

Trp Arg Arg Met Arg Ser Gly Leu Pro Ala Pro Trp Ile Ser Leu Arg
325 330 335

Gly Asp Asp Thr Gly Val Leu Leu Pro Thr Pro Gly Glu Ala Gln Asp
340 345 350

Ala Asp Leu Lys Asp Val Asn Val Ile Pro Ala Thr Ala
355 360 365

<210> 85
<211> 365
<212> PRT
<213> Mus sp.

<400> 85
Met Gly Met Pro Leu Pro Trp Ala Leu Ser Leu Leu Leu Val Leu Leu
1 5 10 15

Pro Gln Thr Trp Gly Ser Glu Thr Arg Pro Pro Leu Met Tyr His Leu
20 25 30

Thr Ala Val Ser Asn Pro Ser Thr Gly Leu Pro Ser Phe Trp Ala Thr
35 40 45

Gly Trp Leu Gly Pro Gln Gln Tyr Leu Thr Tyr Asn Ser Leu Arg Gln
50 55 60

Glu Ala Asp Pro Cys Gly Ala Trp Val Trp Glu Asn Gln Val Ser Trp
65 70 75 80

Tyr Trp Glu Lys Glu Thr Thr Asp Leu Lys Ser Lys Glu Gln Leu Phe
85 90 95

Leu Glu Ala Leu Lys Thr Leu Glu Lys Ile Leu Asn Gly Thr Tyr Thr
100 105 110

Leu Gln Gly Leu Leu Gly Cys Glu Leu Ala Ser Asp Asn Ser Ser Val
115 120 125

```
Pro Thr Ala Val Phe Ala Leu Asn Gly Glu Glu Phe Met Lys Phe Asn
    130                 135                 140

Pro Arg Ile Gly Asn Trp Thr Gly Glu Trp Pro Glu Thr Glu Ile Val
145                 150                 155                 160

Ala Asn Leu Trp Met Lys Gln Pro Asp Ala Ala Arg Lys Glu Ser Glu
                165                 170                 175

Phe Leu Leu Asn Ser Cys Pro Glu Arg Leu Leu Gly His Leu Glu Arg
            180                 185                 190

Gly Arg Arg Asn Leu Glu Trp Lys Glu Pro Pro Ser Met Arg Leu Lys
        195                 200                 205

Ala Arg Pro Gly Asn Ser Gly Ser Ser Val Leu Thr Cys Ala Ala Phe
    210                 215                 220

Ser Phe Tyr Pro Pro Glu Leu Lys Phe Arg Phe Leu Arg Asn Gly Leu
225                 230                 235                 240

Ala Ser Gly Ser Gly Asn Cys Ser Thr Gly Pro Asn Gly Asp Gly Ser
                245                 250                 255

Phe His Ala Trp Ser Leu Leu Glu Val Lys Arg Gly Asp Glu His His
            260                 265                 270

Tyr Gln Cys Gln Val Glu His Glu Gly Leu Ala Gln Pro Leu Thr Val
        275                 280                 285

Asp Leu Asp Ser Ser Ala Arg Ser Ser Val Pro Val Val Gly Ile Val
    290                 295                 300

Leu Gly Leu Leu Leu Val Val Val Ala Ile Ala Gly Gly Val Leu Leu
305                 310                 315                 320

Trp Gly Arg Met Arg Ser Gly Leu Pro Ala Pro Trp Leu Ser Leu Ser
                325                 330                 335

Gly Asp Asp Ser Gly Asp Leu Leu Pro Gly Gly Asn Leu Pro Pro Glu
            340                 345                 350

Ala Glu Pro Gln Gly Ala Asn Ala Phe Pro Ala Thr Ser
        355                 360                 365

<210>  86
<211>  7
<212>  PRT
<213>  Homo sapiens

<400>  86
Val Leu His Gln Asp Trp Leu
1               5

<210>  87
<211>  6
<212>  PRT
<213>  Homo sapiens

<400>  87
Leu Met Ile Ser Arg Thr
1               5
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<210> 88
<211> 9
<212> PRT
<213> Homo sapiens

<400> 88
Met His Glu Ala Leu His Asn His Tyr
1 5

<210> 89
<211> 5
<212> PRT
<213> Homo sapiens

<400> 89
Gly Gln Pro Glu Asn
1 5

<210> 90
<211> 6
<212> PRT
<213> Homo sapiens

<400> 90
Leu Tyr Ile Thr Arg Glu
1 5

<210> 91
<211> 6
<212> PRT
<213> Homo sapiens

<400> 91
Leu Tyr Ile Ser Arg Thr
1 5

<210> 92
<211> 6
<212> PRT
<213> Homo sapiens

<400> 92
Leu Tyr Ile Ser Arg Ser
1 5

<210> 93
<211> 6
<212> PRT
<213> Homo sapiens

<400> 93
Leu Tyr Ile Ser Arg Arg
1 5

<210> 94
<211> 6
<212> PRT
<213> Homo sapiens

<400> 94
Leu Tyr Ile Ser Arg Gln
1 5

<210> 95
<211> 6
<212> PRT
<213> Homo sapiens

<400> 95
Leu Trp Ile Ser Arg Thr
1 5

<210> 96
<211> 6
<212> PRT
<213> Homo sapiens

<400> 96
Leu Tyr Ile Ser Leu Gln
1 5

<210> 97
<211> 6
<212> PRT
<213> Homo sapiens

<400> 97
Leu Phe Ile Ser Arg Asp
1 5

<210> 98
<211> 6
<212> PRT
<213> Homo sapiens

<400> 98
Leu Phe Ile Ser Arg Thr
1 5

<210> 99
<211> 6
<212> PRT
<213> Homo sapiens

<400> 99
Leu Phe Ile Ser Arg Arg
1 5

<210> 100
<211> 6
<212> PRT
<213> Homo sapiens

<400> 100
Leu Phe Ile Thr Gly Ala
1 5

<210> 101
<211> 6
<212> PRT
<213> Homo sapiens

<400> 101
Leu Ser Ile Ser Arg Glu
1 5

<210> 102
<211> 6
<212> PRT
<213> Homo sapiens

<400> 102
Arg Thr Ile Ser Ile Ser
1 5

<210> 103
<211> 7
<212> PRT
<213> Homo sapiens

<400> 103
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WO 02/060919 A3

(54) Title: MOLECULES WITH EXTENDED HALF-LIVES, COMPOSITIONS AND USES THEREOF

(57) Abstract: The present invention provides molecules, including IgGs, non-IgG immunoglobulins, proteins and non-protein agents, that have increased *in vivo* half-lives due to the presence of an IgG constant domain, or a portion thereof that binds the FcRn, having one or more amino acid modifications that increase the affinity of the constant domain or fragment for FcRn. Such proteins and molecules with increased half-lives have the advantage that smaller amounts and or less frequent dosing is required in the therapeutic, prophylactic or diagnostic use of such molecules.

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/48432

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07H 21/04; A61K 48/00
US CL : 536/23.1; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.1; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/43713 A1 (LEXIGEN PHARAMACEUTICALS CORPORATION) 02 September 1999(02.09.1999), see entire document, page 3, lines 5-7 in particular.	1-20, 58 and 86

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 October 2002 (23.10.2002)	30 JUN 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Michael A. Belyavskyi Telephone No. 703/308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/48432

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: 1-20,58 and 86

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

INTERNATIONAL SEARCH REPORT

PCT/US01/48432

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claims 1-20, 58, 86 drawn to modified IgG and a pharmaceutical composition.

Group II, claims 21-39, 59 drawn to fusion protein, comprising non-IgG polypeptide and pharmaceutical composition.

Group III, claims 40-57, 60, 71 drawn to a molecule comprising non-protein agent, pharmaceutical composition and a kit.

Group IV, claims 61,62, 72-77, 79-84 drawn to a method of treating , method of preventing a disease and method of vaccinating, comprising administrating modified IgG.

Group V, claims 63, 78 drawn to a method of treating a disease and method of vaccinating comprising administering a fusion protein.

Group VI, claim 64 drawn to a method of treating disease, comprising administrating a molecule.

Group VII, claims 65, 67, 69 drawn to a nucleic acid, encoding modified IgG, host cell and a kit.

Group VIII, claims 66, 68, 70 drawn to a nucleic acid encoding the fusion protein, host cell and a kit.

Group IX, claim 85 drawn to a method of in vivo diagnosis

The inventions listed as Groups I-IX do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The invention of Group I was found to have no special technical feature that defined contribution over the prior art of Gillies et al. (WO 9943713) (see entire document)

Gillies et al. teaches a modified IgG with an enhanced circulating half-life, wherein modification of amino acid at position 331 (page 3, lines 5 -7).

Since Applicant's inventions do not contribute a special technical feature when viewed over the prior art they do not have a single general inventive concept and so lack unity of invention.

フロントページの続き

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