(19) DANMARK

(10) **DK/EP 1974017 T4**

Patent- og Varemærkestyrelsen

(12) Oversættelse af ændret europæisk patentskrift

(51) Int.Cl.: ***C 12 N 5/0781 (2010.01)*** ***C 12 N 5/10 (2006.01)***

(45) Oversættelsen bekendtgjort den: **2023-09-25**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om opretholdelse af patentet i ændret form: **2023-06-21**

(86) Europæisk ansøgning nr.: **06824300.5**

(86) Europæisk indleveringsdag: **2006-12-08**

(87) Den europæiske ansøgnings publiceringsdag: **2008-10-01**

(86) International ansøgning nr.: **NL2006000625**

(87) Internationalt publikationsnr.: **WO2007067046**

(30) Prioritet: **2005-12-09 WO PCT/NL2005/000848** **2006-06-12 EP 06076211**

(84) Designerede stater: **AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR**

(73) Patenthaver: **Academisch Medisch Centrum bij de Universiteit van Amsterdam , Meibergdreef 9, 1105 AZ Amsterdam, Holland**
Kling Biotherapeutics B.V., Meibergdreef 59, 1105 BA Amsterdam, Holland

(72) Opfinder: **SPITS, Hergen, 318 West Poplar Avenue, San Mateo, California 94402, USA**
SCHEEREN, Ferenc Alexander, Hei-akker 9, NL-5171 WN Kaatsheuvel, Holland
BEAUMONT, Tim, Hoger Amstellaan 27, NL-1191 GM Ouderkerk a/d Amstel, Holland
DIEHL, Sean Andrew, Plataanstraat 74, NL-2023 SG Haarlem, Holland

(74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**

(54) Benævnelse: **Midler og fremgangsmåder til at påvirke stabiliteten af antistof producerende celler**

(56) Fremdragne publikationer:

EP-A- 1 627 563
WO-A-03/052083
GB-A- 2 398 783
US-A- 4 997 764
SHVARTS AVI ET AL: "A senescence rescue screen identifies BCL6 as an inhibitor of anti-proliferative p19ARF-p53 signaling" GENES AND DEVELOPMENT, vol. 16, no. 6, 15 March 2002 (2002-03-15), pages 681-686, XP002385891 ISSN: 0890-9369
YAMOCHI TADANORI ET AL: "Adenovirus-mediated high expression of BCL-6 CV-1 cells induces apoptotic cell death accompanied by down-regulation of BCL-2 and BCL-XL" ONCOGENE, vol. 18, no. 2, 14 January 1999 (1999-01-14), pages 487-494, XP009079150 ISSN: 0950-9232
OZAKI KATSUTOSHI ET AL: "Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 NOV 2004, vol. 173, no. 9, 1 November 2004 (2004-11-01), pages 5361-5371, XP002335928 ISSN: 0022-1767
MEHTA D S ET AL: "IL-21 induces the apoptosis of resting and activated primary B cells" JOURNAL OF

Fortsættes ...

IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIMORE, US, vol. 170, no. 8, 15 April 2003 (2003-04-15), pages 4111-4118, XP002335927 ISSN: 0022-1767
TRAGGIAI ELISABETTA ET AL: "An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus" NATURE MEDICINE, vol. 10, no. 8, August 2004 (2004-08), pages 871-875, XP002385893 ISSN: 1078-8956 cited in the application
SHAFFER A L ET AL: "Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program" IMMUNITY, vol. 17, no. 1, July 2002 (2002-07), pages 51-62, XP002385892 ISSN: 1074-7613
KNÖDEL M ET AL: "Blimp-1 over-expression abrogates IL-4- and CD40-mediated suppression of terminal B cell differentiation but arrests isotype switching" EUROPEAN JOURNAL OF IMMUNOLOGY, WEINHEIM, DE, vol. 31, no. 7, 2001, pages 1972-1980, XP002903679 ISSN: 0014-2980
SHAPIRO-SHELEF MIRIAM ET AL: "Blimp-1 is required for maintenance of long-lived plasma cells in the bone marrow." THE JOURNAL OF EXPERIMENTAL MEDICINE. 5 DEC 2005, vol. 202, no. 11, 5 December 2005 (2005-12-05), pages 1471-1476, XP002385969 ISSN: 0022-1007
SHEN CHUN-PYN ET AL: "B-cell-specific DNA binding by an E47 homodimer" MOLECULAR AND CELLULAR BIOLOGY, vol. 15, no. 8, 1995, pages 4518-4524, XP009079292 ISSN: 0270-7306
MATHAS STEPHAN ET AL: "Intrinsic inhibition of transcription factor E2A by HLH proteins ABF-1 and Id2 mediates reprogramming of neoplastic B cells in Hodgkin lymphoma" NATURE IMMUNOLOGY, vol. 7, no. 2, February 2006 (2006-02), pages 207-215, XP009079152 ISSN: 1529-2908
WO-A2-2005/052139
Reljic et al., 2000. J. Exp. Med, 192:1841-1847
Scheeren et al., 2005. Nat. Immunol., 6:303-313
Boise et al., 1993. Cell, 74: 597-608
Grillot et al., 1996. J. Exp. Med., 183:381-391
Zhang et al., 1996. Cellular Immunology, 173:149-154
Solvason et al., 1998. J. Exp. Med., 187:1081-1091
Charbonneau and Gauthier, 2000. Cytotechnology, 34:131-139
Jung et al., 2002. Biotechnology and Bioengineering, 79:180-187
Turner et al., 1994. Cell, 77: 297-306
Lin et al., 2002. Mol. Cell Biol., 22: 4771-4780
Sciammas and Davis, 2004. J. Immunol., 172:5427-5440
Abstract of Ettinger presentation at GARN 2005, September 15-18,Vienna, Austria
Shapiro-Shelef et al., 2003. Immunity, 19:607-620
Antibody Engineering, Borrebaeck, 1985, Ch9, p267-293
Supplementary figurs of D13

DESCRIPTION

[0001] The invention relates to the field of cell biology.

[0002] *Ex vivo* cell cultures are important tools in current biological and medical applications. One important application is culturing antibody producing cells in order to harvest antibodies, preferably monoclonal antibodies. Monoclonal antibodies (mAbs) represent multiple identical copies of a single antibody molecule which copies bind to antigens with the same affinity and promote the same effector functions. Amongst the benefits of mAbs is their specificity for the same epitope on an antigen. This specificity confers certain clinical advantages on mAbs over more conventional treatments while offering patients an effective, well tolerated therapy option with generally low side effects. Moreover mass are useful for biological and medical research.

[0003] The proliferative capacity of most primary cells in culture is limited by the induction of senescence. This state of irreversible growth-arrest is characterized by expression of a number of senescence-associated markers, such as senescence-associated beta-galactosidase, plasminogen-activator inhibitor 1 (PAI=1), $p19^{ARF}$, p53, $p21^{CIP1}$, and $p16^{INK4A}$. In order to provide a proliferating cell line, cells are often fused to cancer cells in order to produce hybridoma cells. The resulting hybridoma cells are capable of dividing indefinitely and grow well in cell culture. Individual hybridomas with a desired characteristic can then be selected for a given purpose.

[0004] In order to directly obtain human monoclonal antibodies with a desired specificity it would be convenient to isolate a B cell capable of producing such antibody and to culture the B cell *ex vivo*. However, hybridoma technology with human B cells has not been very successful because the resulting hybridomas are unstable. Many attempts for *ex vivo* culturing of B cells have been undertaken. It is well documented that human naïve and memory B cells can be cultured for a limited period following engagement of CD40 in the presence of cytokines, including IL-2, IL-4 and IL-10 (Banchereau et al., 1991) and it is believed that this system mimics the *in vivo* response of B cells towards cognate antigen primed CD40L-expressing helper T cells. In the absence of CD40 ligation, IL-10 alone or in combination with IL-2 induces differentiation into antibody-producing cells (Malisan et al., 1996). The mechanisms of regulation of survival and proliferation of mature B cells cultured under these conditions are only partly known.

[0005] Engagement of CD40 on B cells has multiple effects including protection against apoptosis, (partial) inhibition of differentiation and induction of cytokine responsiveness by B cells. Expression of a large number of cell cycle inhibitors was decreased by CD40 engagement including Rb-1 and Rb-2 (Dadgostar et al., 2002) and it is likely that downregulation of such genes release resting B cells from quiescence. Although CD40 triggering leads to a brief proliferative response, cytokines are instrumental in sustaining cell cycle progression of the triggered B cells. IL-2 and IL-4 are the most efficient cytokines that promote continued cell cycle progression of CD40 or surface Ig-stimulated B cells. Yet, B cell cultures described in the above mentioned papers are only stable during a limited period.

[0006] Another approach for immortalizing B cells is Epstein-Barr virus (EBV) transformation. However the frequency of B cells that are transformed by EBV is low and therefore attempts to generate EBV transformed B cells that produce desired antibodies have met with little success. Recently, Traggiai et al have reported a method for more efficient Epstein-Barr virus transformation of human B cells that increased the frequency of B cells that were transformed. With this method B cells obtained from a patient who recovered from severe acute respiratory syndrome coronavirus (SARS-CoV) infection were transformed with EBV and transformed B cell clones that produce monoclonal antibodies specific for SARS and other viral proteins were isolated (Traggiai et al, 2004).

[0007] Yet another approach for immortalizing B cells is described in patent application WO 03/052083. This application describes a method of stabilizing B cells wherein human B cells are transduced with constitutively active signal transducer of activation and transcription (CA-STAT). A prolonged life span of B cells was observed. Replicating B cells were however not capable of producing antibody at the same time. Antibodies could be obtained by halting the replication of the cells, thereby bringing about terminal differentiation. The terminally differentiated cells produced antibody during a restricted time, after which the differentiated cells died. However, the replicating B cells of WO 03/052083 lose their capability of developing into antibody producing cells after culturing of 1.5-2 months or longer, rendering these B cell cultures unsuitable for antibody production.

[0008] Although various approaches for culturing antibody producing cells have been described, there is still a need for means and methods for influencing the stability of antibody producing cells. It is an object of the present invention to provide such means and methods.

[0009] Accordingly the invention provides a method according to claim 1 or claim 11. The amounts of both BCL6 and Blimp-1 expression products within said antibody producing cell are regulated, since both expression products are involved in the stability of an antibody producing cell. The stability of an antibody producing cell is defined as the capability of said antibody producing cell to remain in a certain developmental stage (optionally after said cell has been brought into said stage). Different developmental stages of a cell involve at least one different characteristic of said cell. For instance, a memory B cell is known to differentiate upon stimulation into an antibody-secreting plasma cell via a stage which some researchers call a plasmablast. A memory B cell, a plasmablast and a plasma cell are different developmental stages of a B cell, wherein the B cell has different characteristics. A memory B cell exhibits low proliferation and antibody secretion. A plasmablast exhibits both higher proliferation and higher antibody secretion levels as compared to a memory B cell, whereas a plasma cell secretes high antibody levels but is not capable of proliferating. These three developmental stages are also characterised by differences in cell surface markers, as shown in Table 1.

[0010] With a method of the invention it has become possible to regulate the replicative life span of an antibody producing cell. A replicative life span of an antibody producing cell is defined herein as the time span wherein a B cell and its progeny cells are capable of replicating while maintaining their capability of producing antibody and/or developing into a cell that produces antibody. The replicative life span of an antibody producing cell is for instance shortened by forcing an antibody-producing cell to enter another developmental stage. In one embodiment the replicative life span of an antibody producing cell is shortened by forcing said cell into terminal differentiation. This is characterised by increased antibody production and cell cycle arrest. During terminal differentiation cells stop proliferating and eventually die. Preferably however the replicative life span of an antibody producing cell is prolonged, meaning that said antibody producing cell will not terminally differentiate - or only after a longer period as compared to the same kind of antibody producing cells that are currently used - and continue to proliferate in vitro. According to the invention it is possible to regulate the amount of BCL6 and Blimp-1 expression product in an antibody producing cell to such extent that the antibody producing cell is brought into, and/or kept in, a predetermined developmental state in which the cells continue to proliferate. With a method of the invention it has therefore become possible to increase the replicative life span of an antibody producing cell since it is possible to maintain a B cell in a certain developmental stage wherein replication occurs. In current *ex vivo* B cell cultures the replicative life span is only a few weeks to two months. After this time the cultured cell lose their capability of replicating, their capability of producing antibody and/or their capability of developing into a cell that produces antibody. With a method according to the current invention however it has become possible to prolong the replicative life span of antibody producing cells, so that *ex vivo* cultures are generated comprising cells that are capable of replicating and producing antibody (or developing into cells that produce antibody).

[0011] An antibody producing cell is defined as a cell which cell is capable of producing and/or secreting antibody or a functional part, derivative and/or analogue thereof, and/or which cell is capable of developing into a cell which is capable of producing and/or secreting antibody or a functional part, derivative and/or analogue thereof. Preferably, said antibody producing cell comprises a B cell and/or a B cell-derived plasma cell. A B cell is called herein an antibody producing cell, even when the B cell is in a stage wherein antibody production is low or not present at all, such as a naive B cell or a memory B cell, being activated or not, because such cells are capable of developing into cells that produce antibody, such as a plasmablast and/or plasma cell. Said antibody producing cell preferably comprises a mammalian cell. Non-limiting examples include antibody producing cells derived from a human individual, rodent, rabbit, llama, pig, cow, goat, horse, ape, gorilla. Preferably, said antibody producing cell comprises a human cell, a murine cell, a rabbit cell and/or a llama cell.

[0012] A functional part of an antibody is defined as a part which has at least one same property as said antibody in kind, not necessarily in amount. Said functional part is preferably capable of binding a same antigen as said antibody, albeit not necessarily to the same extent. A functional part of an antibody preferably comprises a single domain antibody, a single chain antibody and/or a Fab fragment. A functional derivative or analogue of an antibody is defined as an antibody which has been altered such that at least one property - preferably an antigen-binding property - of the resulting compound is essentially the same in kind, not necessarily in amount.

[0013] BCL6 encodes a transcriptional repressor which is required for normal B cell and T cell development and maturation and which is required for the formation of germinal centers. (Ye, 1997). BCL6 is highly expressed in germinal center B cells whereas it is hardly expressed in plasma cells. BCL6 inhibits differentiation of activated B cells into plasma cells. The transcriptional repressor B lymphocyte induced maturation protein-1 (Blimp-1) is required for development of a B cell into a plasma cell. The human variant of Blimp-1 is named Prdm1. As used herein, any reference to Blimp-1 includes a reference to Prdm1. Blimp-1 drives plasma cell differentiation. BCL6 and Blimp-1 repress expression of the other; thus in a natural situation when one reaches an higher expression level than the other, the stage of differentiation is enforced. In the human body, differentiation of plasma cells from activated naive or memory B cells involves downregulation of BCL6 and upregulation of Blimp-1. In germinal center cells BCL6 expression is high and Blimp-1 expression is low. In resting memory cells expression of BCL6 and Blimp-1 are low. Signals that trigger differentiation cause an upregulation of Blimp-1, and this Blimp-1 counteracts the expression of BCL6. The stage where both BCL6 and Blimp-1 are expressed is short-lived and is called a plasmablast. With progressively increasing Blimp-1 levels, BCL6 expression is extinguished, resulting in a plasma cell.

[0014] In a method according to the invention BCL6 and Blimp-1 are co-expressed in an antibody producing cell (meaning that both BCL6 and Blimp-1 are expressed in an antibody producing cell) resulting in an antibody producing cell that is capable of proliferating when an appropriate signal is provided. It has been found that co-expression of BCL6 and Blimp-1 results in an antibody producing cell which is capable of both proliferating and producing antibody. BCL6 and Blimp-1 are preferably co-expressed in a B cell, preferably a human B cell. Co-expression of BCL6 and Blimp-1 in a B cell results in stabilization of said B cell in a plasmablast-like stage. Plasmablasts, like plasma cells, are capable of secreting antibody. However, plasmablasts are still capable of proliferating, whereas plasma cells have lost their capability of proliferating. Plasma cells are therefore unsuitable for culturing antibody-producing cell lines. Although plasmablasts exert highly favourable proliferating and antibody-producing characteristics, they have not yet been used for long term antibody production since it has not been possible to stabilize plasmablasts until the present invention.

[0015] With a method of the invention it has amongst other things become possible to convert a naïve B cell or a memory B cell into a plasmablast-like cell and to stabilize said cell, so that rapid differentiation into a plasma cell does not occur. This is contrary to natural development of plasma cells, wherein expression of Blimp-1 in a memory B cell results in rapid development into a plasma cell, thereby inhibiting BCL6 expression so that the resulting plasma cell hardly expresses BCL6. The present invention thus involves co-expression of both BCL6 and Blimp-1 in a B cell, resulting in a cell that is capable of both proliferating and producing antibody. Preferably a stable culture of B cells is generated. Stable long term *ex vivo* cultures of antibody producing cells have now become possible. These antibody-producing B cells that co-express BCL6 and Blimp-1 can further be stabilized through the addition of the anti-apoptotic gene Bcl-xL. With the introduction of Bcl-xL it is now possible to grow plasmablasts under conditions of low cell density. Hence, the invention also provides a method to culture plasmablastes under conditions of low cell density comprising providing an antibody producing cell with expression levels of BCL6, Blimp-1 and Bcl-xL with any of the herein described methods.

[0016] An antibody producing cell is provided with a nucleic acid sequence encoding BCL6 or a functional part, derivative and/or analogue thereof. This way, it is possible to regulate a BCL6 concentration in an antibody producing cell independently from expression of endogenous BCL6. Hence, even if expression of endogenous BCL6 is low or absent, for instance caused by Blimp-1, an exogenous nucleic acid sequence encoding BCL6 or a functional part, derivative and/or analogue thereof is still capable of producing a concentration of BCL6 which is sufficient for influencing the stability of an antibody producing cell. Provided is a method according to the invention comprising providing said antibody producing cell with a nucleic acid sequence encoding BCL6 or a functional part, derivative and/or analogue thereof. Preferably, said antibody producing cell is provided with a constitutively active nucleic acid sequence encoding BCL6 or a functional part, derivative and/or analogue thereof, so that BCL6 expression is maintained even when endogenous BCL6 expression of said cell is inhibited by an endogenous repressor such as Blimp-1. Most preferably, expression of said nucleic acid sequence encoding BCL6 or a functional part, derivative and/or analogue thereof is regulated by an exogenous inducer of repressor, so that the extent of BCL6 expression is regulated at will. For instance, an inducible promoter system is used such as a Tet-on or Tet-off system.

[0017] Further provided is a method according to the invention comprising culturing said antibody producing cell in the presence of a compound capable of directly or indirectly influencing Blimp-1 expression. A compound is used that is capable of enhancing Blimp-1 expression in order to counteract downregulation of Blimp-1 during expression of BCL6. Said compound comprises IL21.

[0018] In a preferred embodiment an antibody producing cell, preferably a B cell, is provided with Epstein-Barr virus (EBV). Infection of an antibody producing cell of the invention with EBV results in increased stability, proliferation and/or antibody production of said cell. In one particularly preferred embodiment an antibody producing cell, preferably a B cell, is cultured in the presence of IL-21 and provided with EBV. This results in improved proliferation and/or antibody production, as compared to the same kind of antibody producing cells without IL-21 and/or EBV. Said antibody producing cell is preferably cultured in the presence of IL-21 before being infected with EBV. Provided is therefore a method for increasing the stability of an antibody producing cell, comprising culturing said cell in the presence of IL-21 and providing said cell with EBV.

[0019] According to the present invention, IL-21 is particularly suitable for improving the stability of an antibody cell. Embodiments comprise culturing antibody producing cells, preferably B cells, in the presence of IL-21, which antibody producing cells are furthermore provided with with a nucleic acid sequence encoding BCL6 or a functional part, derivative thereof. Said antibody producing cell is preferably infected with EBV. For instance, an antibody producing cell that is naturally infected with EBV is used in a method according to the invention.

Alternatively, or additionally, an antibody producing cell is provided with EBV.

[0020] In a preferred embodiment, the invention further provides a method for influencing the stability of an antibody producing cell as described herein further comprising directly or indirectly increasing the amount of Bcl-xL expression product within said antibody producing cell. This is for example accomplished by providing said antibody producing cell with a nucleic acid sequence encoding Bcl-xL or a functional part, derivative and/or analogue thereof or by nucleic acid sequences encoding other anti-apoptotic genes including but not limited to Bcl-2. In yet another embodiment this is accomplished by providing said antibody producing cell with a compound capable of directly or indirectly enhancing Bcl-xL expression, preferably said compound comprises APRIL, BAFF, CD40, BCR stimulation, cytokines, growth factors or downstream effectors like JNK and AKT (PKB).

[0021] Bcl-xL is a member of the anti-apoptotic Bcl-2 family, Bcl2-proteins interact with and counteract so-called Bcl-2 homology domain 3 (BH3)-only family members such as Bax, Bak, Bim, and Bad, which induce cytochome c release following intrinsic death stimuli (Boise, L. H., 1993). Thus, protection of mitochondrial membrane integrity through proteins like Bcl-xL is critical for cell survival.

[0022] A method according to the invention is particularly suitable for producing an antibody producing cell culture comprising antibody producing cells that are capable of proliferating and secreting antibody. In one embodiment, a memory B cell is used in order to produce an *ex vivo* B cell culture. Alternatively, or additionally, a naive B cell is used. Said memory B cell and/or naïve B cell is preferably human so that human antibodies are produced. Preferably a memory B cell is used with a desired specificity. This means that a memory B cell is used which is capable of developing into an antibody secreting cell, which antibodies have a desired specificity against an antigen of interest. Said antigen of interest for instance comprises a pathogen-derived antigen, a tumor-derived antigen and/or an autoantigen. In one embodiment B cells are isolated from a peripheral blood sample, a cord blood sample and/or a tonsil sample, using methods known in the art. Memory B cells are for instance isolated by selection for the B cell marker CD19 and (subsequent) selection for cell surface IgG and/or CD27. In a germinal center B cell, BCL6 expression is high whereas Blimp-1 expression is low. Natural development into an antibody secreting cell involves upregulation of Blimp-1 expression. Since Blimp-1 represses BCL6 expression, upregulation of Blimp-1 results in downregulation of BCL6 in a natural situation. In the present invention however, Blimp-1 expression is upregulated while BCL6 expression is at least in part maintained. This results in an antibody producing cell wherein BCL6 and Blimp-1 are co-expressed. Said antibody producing cell is capable of proliferating and secreting antibody and is therefore suitable for use in an *ex vivo* B cell culture. In a preferred embodiment, said antibody producing cell is protected by apoptosis by Bcl-xL. Said antibody producing cell is preferably infected with EBV. In one embodiment, an antibody producing cell that is naturally infected with EBV is used. Alternatively, or additionally, an antibody producing cell is provided with EBV. An antibody producing cell according to the present invention provides the advantage that it is stable and does not undergo terminal differentiation during a prolonged period. Said antibody producing cell according to the invention is stable for at least one week, preferably for at least one month, more preferably for at least three months, most preferably for at least six months. A B cell according to the invention is preferably cultured in the presence of CD40L since replication of most B cells is favoured by CD40L.

[0023] In one embodiment BCL6 expression is maintained at essentially the same level, or at a higher level, as compared to a germinal center B cell since a significant BCL6 expression, together with Blimp-1 expression, results in an antibody producing cell with preferred proliferation and antibody production properties and/or stability. In a preferred embodiment, said BCL6 expression and Blimp-1 expression are accompanied by Bcl-xL expression, resulting in even more preferred proliferation and antibody production properties and/or stability.

[0024] Also disclosed is a method for producing an antibody producing cell which is stable for at least one week, preferably for at least one month, more preferably for at least three months, more preferably for at least six months, the method comprising:

- providing a memory B cell or a naïve B cell;
- increasing an expression level of Blimp-1 in said cell; and
- increasing and/or maintaining a BCL6 expression level in said cell. An *ex vivo* method for producing an antibody producing cell comprising increasing an expression level of Blimp-1 in a memory B cell or a naïve B cell and increasing and/or maintaining a BCL6 expression level in said cell is also provided. Said BCL6 and Blimp-1 expression levels are preferably brought to, and/or maintained at, essentially the same level, or at a higher level, as compared to a plasmablast. In one embodiment said B cell is infected with EBV (naturally and/or artificially) and/or transduced with Bcl-xL. Most preferably a memory B cell is used. Said memory B cell preferably has a specificity for a pathogen-derived antigen, a tumor-derived antigen and/or an autoantigen.

[0025] Blimp-1 expression and BCL6 expression are influenced in various ways, as already described herein before. For instance, Blimp-1 expression is enhanced in a memory B cell and/or a naïve B cell by providing said B cell with a compound capable of directly or indirectly enhancing Blimp-1 expression.

[0026] IL-21 is used because this cytokine is particularly suitable for enhancing Blimp-1 expression and stabilizing an antibody producing cell with a method according to the present invention.

[0027] In a preferred embodiment Blimp-1 expression is upregulated in a B cell, preferably by culturing said B cell in the presence of IL-21. Said B cell preferably comprises a memory B cell. After this, BCL6 expression is preferably enhanced. It has been demonstrated that Blimp-1 upregulation in a first stage followed by BCL6 upregulation results in particularly stable B cells capable of replicating and producing antibody. In one embodiment of the invention Blimp-1 expression is still upregulated while BCL6 expression is enhanced. Alternatively however, Blimp-1 expression is not upregulated while BCL6 expression is enhanced. This way, the replication capacity of a B cell is particularly enhanced. Hence, an antibody producing capacity of a B cell is preferably enhanced firstly, by upregulating expression and/or activity of Blimp-1. Subsequently, a replication capacity of said B cell is preferably enhanced; by upregulating expression and/or activity of BCL6. The B cell is preferably cultured in the absence of a compound capable of enhancing Blimp-1 expression and/or activity, until replication is significantly increased. Subsequently, said B cell is preferably cultured again in the presence of an enhancer of Blimp-1 expression and/or activity, so that antibody production is maintained. As is shown in the examples, it is possible to regulate Blimp-1 and BCL6 in various ways, resulting in co-expression of both Blimp-1 and BCL6 in a B cell which B cell is capable of replicating and producing antibody. In one preferred embodiment said B cell is infected with EBV (naturally and/or artificially) and/or transduced with Bcl-xL. IL-21. According to one embodiment, said B cell is subsequently provided with a nucleic acid sequence encoding BCL6 or a functional part, derivative and/or analogue thereof. Said B cells are preferably cultured for a few days in the absence of said compound capable of activating STAT3 in order to enhance replication. Subsequently, said cells are preferably again cultured with - and/or provided with - a compound capable of activating STAT3.

[0028] In the Examples a particularly preferred embodiment is shown, wherein B cells are firstly cultured in the presence of IL-21. Subsequently the B cells are provided with a nucleic acid sequence encoding BCL6. The B cells are cultured in the absence of IL-21 and in the presence of IL-2 and IL-4 for a few days in order to allow BCL6 expression, after which IL21 is administered again to the culture in order to enhance replication and antibody production. Stable B cells are obtained wherein BCL6 and Blimp-1 are co-expressed, which B cells are capable of replicating and producing antibody in an *ex vivo* culture during at least 6 months. In one preferred embodiment said B cells are infected with EBV. A B cell culture according to the invention is preferred since the B cells are capable of replicating and producing antibody in an *ex vivo* culture during a longer period of time as compared to current B cell cultures.

[0029] Prior art attempts to use STAT5 in order to obtain a stable B cell culture capable of producing antibodies, such as described in WO 03/052083, failed because the B cells lose their capability of developing into antibody producing cells within 2 months.

[0030] Blimp-1 expression is upregulated in a B cell, by culturing said B cell in the presence of IL-21. Said B cell preferably comprises a memory B cell.

[0031] Hence, a method of the invention allows for subtle regulation of the replication capacity and antibody producing capacity of B cells cultured ex *vivo.* When upregulation of antibody production is desired, Blimp-1 expression is favored over BCL6 expression. When upregulation of replication is desired, BCL6 expression is favored over Blimp-1 expression. A method of the invention allows maintenance of an equilibrium wherein BCL6 and Blimp-1 are co-expressed, resulting in antibody producing cells which are capable of replicating and producing antibody *ex vivo.* In one embodiment said B cells are infected with EBV after said equilibrium has been established, in order to further increase and stabilize antibody production.

[0032] Moreover, the disclosure further discloses that regulation of the mentioned equilibrium is also obtained by an E protein (for example E47). Expression of E47 within B cells expressing high levels of STAT5b intervenes with differentiation and proliferation, i.e. blocking of STAT5 via E47 and SOCS results in decreased BCL6 levels and subsequently in increased Blimp-1 levels. Upregulated levels of Blimp-1 result in a decreased proliferation and in a differentiation of the involved cell towards an antibody-producing cell. In other words, expression of E47 within a B cell enhances Blimp-1 expression which results in B-cell differentiation towards an antibody producing phenotype (plasmacell).

[0033] The invention further describes the stabilization of the growth of antibody producing cells with Bcl-xL.

[0034] The invention therefore discloses a method for producing an antibody producing cell which is stable for at least one week, preferably at least one month, more preferably at least three months, more preferably at least six months, the method comprising:

- providing a B cell with a compound capable of directly or indirectly enhancing Blimp-1 expression and/or culturing a B cell in the presence of a compound capable of directly or indirectly enhancing Blimp-1 expression; and
- providing said B cell with a compound capable of directly or indirectly enhancing BCL6 expression or with a compound capable of maintaining BCL6 expression at essentially at a higher level, as compared to a germinal center B cell.

[0035] As already explained herein before, said compound capable of directly or indirectly enhancing Blimp-1 expression comprises IL-21.

[0036] In a further preferred embodiment, an antibody producing cell is provided with an immortalizing agent in order to increase the stability, proliferation an/or antibody production of said cell. As already explained before, said immortalizing agent preferably comprises a transforming agent. In a particularly preferred embodiment a B cell is infected with EBV. Said B cell is cultured in the presence of IL-21. As shown in the Examples, B cells infected with EBV and cultured in the presence of IL-21 show strong proliferation and enhanced antibody production. Said B cells are preferably cultured in the presence of IL-21 before being infected with EBV. However, it is also possible to isolate EBV infected B cells, preferably B cells that are naturally infected with EBV, and to culture them in the presence of IL-21. Further provided is therefore a method for influencing the stability of a B cell, comprising culturing said B cell in the presence of IL-21 and infecting said B cell with EBV. A method for influencing the stability of an EBV-infected B cell, comprising culturing said EBV-infected B cell in the presence of IL-21 is also herewith provided. In one embodiment a B cell is cultured in the presence of IL-21, infected with EBV, and subsequently cultured in the absence of IL-21.

[0037] One embodiment comprises influencing the amount of BCL6 expression product and Blimp-1 expression product in addition to EBV infection. Preferably, an antibody producing cell, preferably a B cell, is infected with EBV while BCL6 and Blimp-1 are co-expressed in said antibody producing cell. In one preferred embodiment, a B cell is provided with BCL6, and/or a compound capable of directly or indirectly enhancing BCL6 expression, and with EBV. Said B cells are preferably cultured in the presence of IL-21.

[0038] Further provided is therefore a method according to the invention, comprising providing a B cell; culturing said B cell in the presence of IL-21; providing said B cell with BCL6 or a functional part, derivative; providing said B cell with Epstein Barr Virus; and culturing said B cell *ex vivo.* A method comprising providing an EBV-infected B cell; culturing said B cell in the presence of Il-21; providing said B cell with BCL6, or a functional part, derivative; and culturing said B cell *ex vivo* is also herewith provided. B cells are produced which show strong proliferation and antibody production.

[0039] In one embodiment a plurality of B cells is tested for a specificity for a given antigen. This is done using any method known in the art, for instance an ELISA. Subsequently, at least one B cell with a specificity for a given antigen is selected. This is for instance performed by incubating B cells with a labelled antigen and isolating said B cells using methods known in the art. Selected B cells are cultured in the presence of IL-21. According to this embodiment, selected cells are provided with exogenous BCL6, or a functional part, derivative in order to induce, maintain and/or improve the presence and/or amount of BCL6 expression product. At least one B cell comprising exogenous BCL6, or a functional part, derivative is subsequently selected. Said selected B cell is infected with Epstein Barr Virus. This embodiment is particularly suitable for selecting and culturing B cells with a given specificity which are derived from a B cell pool. For instance, human B cells are collected by selection for CD19, which is a B cell marker, and incubated with an antigen of interest. This way, human B cells with a desired specificity are selected and further cultured *ex vivo.* Said B cells are preferably cultured in the presence of Il-21 in at least one stage of the culturing period. In one preferred embodiment said B cell is cultured in the presence of IL-21 before BCL6, or a functional part, derivative, is introduced into said B cell. In yet another preferred embodiment, said B cell is further provided with Bcl-xL or a functional part, derivative and/or analogue thereof.

[0040] Even though EBV promotes proliferation and antibody production, EBV infection of an antibody producing cell is not always preferred. For instance, if strict control of the properties and genetic characteristics of an antibody producing cell are desired, one may choose not to use EBV infection because EBV infection involves incorporation of unknown nucleic acid sequences into a cell's genome. Moreover a B cell infected by EBV loses its B cell receptor (BCR) surface expression. This may be undesired, for instance when B cells are intended to be isolated and/or screened for a desired specificity after a long period of culture. Such isolation and/or screening method usually involves binding of B cells with a desired specificity to an antigen of interest with their BCR. EBV infected B cells, with significantly reduced BCR expression, are therefore less suitable for such isolation/screening methods. Hence, in cases where the presence of a B cell receptor on B cells is desired, such as for instance in screening assays, the B cells are preferably not, or at a later stage, infected with EBV.

[0041] One embodiment provides a method according to the invention further comprising selecting and/or isolating an antibody or a functional part, derivative and/or analogue of interest. In one embodiment IgM producing cells and IgG producing cells are selected and/or isolated. Preferably an IgG producing cell is selected and/or isolated.

[0042] Antibody producing cells generated with a method according to the invention are suitable for producing antibodies against an antigen of interest. In one preferred embodiment however, the genes encoding the Ig heavy and/or light chains are isolated from said cell and expressed in a second cell, such as for instance cells of a Chinese hamster ovary (CHO) cell line. Said second cell, also called herein a producer cell, is preferably adapted to commercial antibody production. Proliferation of said producer cell results in a producer cell line capable of producing antibody. Preferably, said producer cell line is suitable for producing compounds for use in humans. Hence, said producer cell line is preferably free of pathogenic agents such as pathogenic micro-organisms.

[0043] A method according to the invention is preferably used for generating an antibody producing cell that is stable for at least one week, preferably at least one month, more preferably at least three months, more preferably at least six months so that commercial antibody production has become possible. One preferred embodiment provides a method according to the invention, wherein an antibody producing cell is produced that is capable of producing antibodies against an antigen of interest. Said antigen of interest preferably comprises a pathogen-derived antigen, a tumor-derived antigen and/or an autoantigen. Most preferably a stable cell line capable of producing monoclonal antibodies is produced. This is preferably performed by using memory B cells that have for instance been isolated from a sample by selection for CD19 (B cell marker) and cell surface IgG and/or CD27 (to mark memory cells). Furthermore, an antibody producing cell capable of specifically binding an antigen of interest is for instance selected in a binding assay using said antigen of interest. Subsequently, according to this preferred embodiment Blimp-1 and BCL6 are co-expressed in said antibody producing cell, resulting in a culture of cells capable of specifically binding said antigen of interest. Preferably said antibody producing cell is infected with EBV. In yet another preferred embodiment, said B cell is further provided with Bcl-xL or a functional part, derivative and/or analogue thereof.

[0044] If only one memory cell is used, a cell line according to the invention producing monoclonal antibodies is obtained. It is also possible to generate a monoclonal antibody producing cell line starting with various B cells capable of producing antibody against different antigens. After a stable B cell culture has been produced with a method according to the invention, a B cell capable of producing antibodies against a specific antigen of interest is isolated and at least a functional part of a gene encoding the Ig heavy chain and/or light chain from said B cell is expressed in a second cell line. Preferably at least a functional part of the gene encoding the Ig heavy chain and at least a functional part of the gene encoding the Ig light chain from said B cell are expressed in a second cell line.

[0045] In one embodiment an antibody producing cell, preferably but not necessarily a memory B cell, that has been obtained from an individual which had been previously exposed to an antigen of interest, is used in a method according to the invention. This way, it has become possible to produce human antibodies of interest *ex vivo.*

[0046] The invention furthermore provides an antibody producing cell according to the claims which is stable for at least one week, preferably for at least one month, more preferably for at least three months, more preferably for at least six months, meaning that an antibody producing cell according to the present invention is capable of both replicating and producing antibody, or capable of replicating and developing into a cell that produces antibody, during said time periods. Antibody producing cells according to the invention comprise, amongst other things, cells producing IgM and cells producing other immunoglobulin isotypes like IgG, IgA, IgE. An antibody producing cell according to the invention is particularly suitable for use in an antibody producing cell line. Antibody producing cells according to the invention are preferably cultured *ex vivo* and antibodies produced by said cells are preferably collected for further use. Alternatively, or additionally, the antibody encoding genes of said cells are isolated for further use. Antibodies or functional parts, derivatives and/or analogues thereof produced with a method according to the invention are useful for a wide variety of applications, such as for instance therapeutic, prophylactic and diagnostic applications, as well as research purposes and *ex vivo* experiments. For instance, a screening assay is performed wherein antibodies or functional parts, derivatives and/or analogues according to the invention are incubated with a sample in order to determine whether an antigen of interest is present.

[0047] An antibody producing cell according to the invention preferably comprises a mammalian cell, more preferably a human cell, a murine cell, a rabbit cell and/or a llama cell. In a particularly preferred embodiment said antibody producing cell comprises a human cell, producing human antibody, because human antibodies are particularly suitable for therapeutic and/or prophylactic applications in human individuals. an exogenous nucleic acid sequence encoding STAT3 or a functional part, thereof.

[0048] As explained herein before, BCL6 expression is enhanced in a variety of ways. BCL6 expression is preferably upregulated using a nucleic acid sequence encoding BCL6, or a functional part, derivative of BCL6 Provided is therefore an antibody producing cell according to the invention, comprising an exogenous nucleic acid sequence encoding BCL6 or a functional part, derivative and/or analogue thereof, and
an exogenous nucleic acid sequence ancoding STAT3 or a functional part, thereof.

[0049] In one embodiment said nucleic acid sequence encoding BCL6, STAT3 and/or Bcl-xL and/or a functional part, derivative and/or analogue of BCL6, and/or STAT3 and/or Bcl-xL is constitutively active, so that BCL6, STAT3 and/or a functional part, derivative and/or analogue thereof remains present in an antibody producing cell according to the invention even when endogenous BCL6, and/or STAT3 and/or Bcl-xL genes are downregulated by endogenous compounds. Most preferably, expression of said nucleic acid sequence encoding BCL6, STAT3 and/or Bcl-xL or a functional part, derivative and/or analogue of BCL6, and/or STAT3 and/or Bcl-xL is regulated by an activator and/or repressor that is inducible by an exogenous compound, so that the amount of BCL6, STAT3 and/or Bcl-xL or a functional part, derivative and/or analogue thereof is regulated at will by regulating the amount of exogenous compound that is administered. One embodiment therefore provides an antibody producing cell according to the invention, wherein expression of said nucleic acid sequence encoding BCL6, STAT3 and/or Bcl-xL or a functional part, derivative and/or analogue of BCL6, and/or STAT3 and/or Bcl-xL, is regulated by an activator and/or repressor that is inducible by an exogenous compound.

[0050] An antibody producing cell according to the present invention with an increased stability is particularly suitable for the production of an *ex vivo* cell line. The invention therefore further provides a method for producing a B cell line comprising:

- obtaining a stable antibody producing cell with a method according to the invention, and
- culturing said antibody producing cell *ex vivo.*

[0051] Most preferably a stable cell line comprising B cells capable of producing antibodies specifically directed against an antigen of interest is generated. This is preferably done by obtaining a B cell which is capable of developing into a cell which produces antibodies against an antigen of interest, such as for instance a pathogen-derived antigen, a tumor-derived antigen and/or an autoantigen. The amount of BCL6 and Blimp-1 expression in said cell is subsequently regulated. In one embodiment said B cell is infected with EBV. Said B cell is preferably obtained from an individual who has been exposed to an antigen of interest. Said individual preferably comprises a mammal, more preferably a human individual, a rabbit, a rodent and/or a llama. In a particularly preferred embodiment said individual is a human individual.

[0052] One important application is the production of antibodies that are capable of specifically binding an antigen of interest. The invention therefore can be used in a method for producing antibodies capable of specifically binding an antigen of interest, the method comprising:

- obtaining a B cell capable of differentiating into a B cell which B cell produces antibodies capable of specifically binding said antigen of interest,
- producing an antibody producing cell that is stable for at least one week, preferably at least one month, more preferably at least three months, more preferably at least six months using said B cell in a method according to the invention, and
- obtaining antibodies produced by said antibody producing cell.

[0053] Said antibody producing cell is preferably further cultured *ex vivo* in order to provide a stable cell line capable of producing antibodies which are specifically directed towards an antigen of interest. More preferably at least a functional part of a gene encoding the Ig heavy chain and/or light chain from said B cell is expressed in a second cell. Said second cell is preferably used in order to produce a commercially suitable cell line.

[0054] The invention is further explained in the following examples. These examples do not limit the scope of the invention, but merely serve to clarify the invention.

EXAMPLES

Example 1

Methods

[0055] Human memory B cells are purified from peripheral blood or tonsil by first by positive selection for B cells with CD19 MACS® beads (Miltenyi Biotech). Memory B cells are then selected by surface staining and cell sorting for IgG. IgG^+ B cells are then cultured with mouse fibroblast L cells expressing CD40L in the presence of mouse or human IL-21 for 36 to 48 hours. Cells are then transferred to Retronectin® (Takara, Shiga, Japan)- coated tissue culture plates where they are transduced with a retrovirus encoding human BCL6-IRES-GFP for 16 h at 37°C. Transduced cells are then cultured on CD40L-L cells in the presence of human IL-2 and human IL-4. After approximately 3-4 weeks the GFP^+ cells (that is, $BCL6^+$ cells) reach 100% of the culture after which $BCL6^+$ cells are cultured with IL-2 and IL-4 or with human or mouse IL-21. Using flow cytometry we monitor the expression of GFP, CD19, CD38, CD20, MHC class II, CD27 (BD Biosciences), and other markers using labeled antibodies. We monitor growth by cell counting, and Ig production is monitored by enzyme ELISA detection of Ig in the culture supernatant (Dako, Glostrup, Denmark). Gene expression is monitored by reverse transcriptase polymerase chain reaction (RT-PCR, Invitrogen, Breda, Netherlands).

Results

[0056] introduction of BCL6 into memory B cells results in a greatly extended lifespan over normal B cells in culture (months vs. ~3 weeks). These cells maintain CD19, surface Ig, MHC class II, and express intermediate levels of CD38 and CD20, suggesting a memory cell phenotype (not shown). Culture of these cells on CD40L-L cells in the presence of IL-21 results in a significant growth advantage (**Figure 1**) and acquisition of a plasmablast-like cell surface phenotype ($CD38^{hi}CD20^+$, **Figure 2**). Importantly, IL-21 cultured cells secrete 300% more IgG compared with cells cultured IL-2 and IL-4. Together these data show that IL-21 culture promotes plasmablast development in an immortalized B cell population, exhibiting enhanced growth and antibody production.

Example 2

[0057] A non-limiting model of one embodiment of the present invention is depicted in Figure 4.

[0058] In the human body, differentiation of plasma cells from memory B cells involves downregulation of BCL6 and upregulation of Blimp-1. In memory cells BCL6 is high and Blimp-1 expression is low. Signals that trigger differentiation cause an upregulation of Blimp-1, and this Blimp-1 counteracts the expression of BCL6. This stage is short-lived and is called the plasmablast. With progressively increasing Blimp-1 levels, BCL6 expression is extinguished, resulting in a plasma cell.

[0059] In one embodiment of the invention BCL6 expression is "locked", for instance because of stable expression mediated by a retroviral expression cassette integrated into the DNA of the B cells. Then, with BCL6 levels maintained, we "switch on" Blimp-1 expression, for instance by use of a cytokine that activates STAT3, such as IL-21 (figure 3). This combination, through modulation of key transcription, results in stable growth of cells that secrete antibody and have phenotype characteristics of a plasmablast.

Table 1: Cell surface markers of memory B cells, plasmablasts and plasma cells

	Memory	**Plasmablast**	**Plasma Cell**
CD38	+	++	++
CD20	+	+	-
CD27	+	+	-
CD19	++	+	-
CD138	-	-	+
proliferation	**low**	**high**	**none**
Ig secretion	**low**	**intermediate**	**high**
Table 2	**Memory**	**Plasmablast**	**Plasma Cell**
BCL6	++	+	-
Blimp-1	-	+	++

Example 3

Materials and Methods

Maintenance and isolation of human B cells

[0060] Using standard procedures, CD19 positive human B cells were isolated from bloodbank derived buffy coat (other sources can be fresh heparin or ACD blood, or a lymphoid organ for example tonsil or spleen). In brief, total peripheral blood mononuclear cells (PBMC) were isolated using ficoll density separation (Amersham, Buckinghamshire, UK). CD19 labeled beads were used to positively selected B cells by MACS cell sorting technique (Miltenyi, Auburn, CA, USA). Cells were subsequently stained with appropriate combinations of monoclonal antibodies (mAbs) to CD19, CD27, IgG, IgM, CD3 (Becton Dickinson (BD), Franklin Lakes, NJ, USA) and phycoerythrin (PE) labeled Tetanus Toxoid (provided by A. Radbruch, Berlin, Germany) or any other labeled antigen Cells were then sorted using the FACSAria (BD). Sorted cells were washed and cultured (1.5 to $2x10^5$ cells/ml) on irradiated CD40L-expressing L-cells ($5x10^4$ cells/ml; provided by DR. J. Banchereau, Schering Plough France, Dardilly France), in Iscove's Modified D Minimal Essential Medium (IMDM) containing 8% fetal calf serum (FCS) and Penicillin/Streptomycin. Unless mentioned otherwise, these CD40L-expressing L-cells are always present in the cultures.

Transduction and regulation of mouse constitutive active STAT5b in B cells

[0061] Purified B cells were primed to become transduced with the caSTAT5b gene. Two priming protocols were used: (1) purified B cells were cultured for 3 days with interleukin (IL) 2 (20 U/ml, Chiron, Emeryville, CA, USA) followed by a 24 hour culture with IL-2 and IL-4 (10 ng/ml, R&D, Minneapolis, MN, USA) or (2) purified B cells were cultured for 36 hours with recombinant mouse IL-21 (50 ng/ml, R&D). Subsequently, cells were plated on recombinant human fibronectin fragments CH-296 (Hanenberg H., Nat., Med. 1996; RetroNectin, Takara, Japan) and human serum albumine treated plates (Corning Life Sciences, Corning, NY, USA) in the absence of L-cells, with the cytokines IL-2/4 or IL-21. At last, cells were transduced with the caSTAT5b gene (described by Ariyoshi K., JBC, 2000 and obtained from T. Kitamura, IMSUT, Tokyo, Japan) fused to the estrogen receptor (ER, provided by H. Kurata, DNAX Institute, Palo Alto, CA, USA). The activity of the caSTAT5b-ER fusion product can be controlled by the hormone hydroxytamoxifen (4HT, Sigma-Aldrich, St. Louis, MO, USA). The transduction was performed using a retrovirus as described previously (Heemskerk M.H., JEM, 1997; Heemskerk M.H., Cell Immunol. 1999; Scheeren F.A., Nat Immunol, 2005). Transduction efficiency was determined by antibody staining of a truncated, signaling incompetent mutant of Nerve Growth Factor Receptor (ΔNGFR, provided by C. Bonini, St. Raphael Hospital, Milan, Italy). Thus, outgrowth of B cells that contain the caSTAT5b gene depends on the presence of 4HT and these cells can be detected by antibody staining for NGFR (Chromaprobe, Maryland Heights, MO, USA).

Development of 100% caSTAT5b positive B cell lines that secrete antibodies

[0062] We have developed a B cell line that produces monoclonal antibodies and is 100% caSTAT5b (=NGFR) positive. This was achieved by differentiating B cells from a memory into an antibody producing phenotype, and transducing with the caSTA5b-ER-IRES-NGFR construct. The action of caSTAT5b makes the differentiated B cells insensitive to cell death. Differentiation of B cells is induced in the first 2 to 3 weeks after isolation (figure 5), using a cytokine mixture (IL-2, 4, 21 or combinations of these cytokines and CD40L). The time point that caSTAT5b is activated by adding 4HT affects the overall phenotype of the cultures. This because caSTAT5b blocks the cell to change its phenotype e.g. blocks further differentiation. Thus, the longer 4HT is withheld the more B cells will differentiate into antibody producing cells or into a type of cell that preferentially grows out under these culture conditions (suggestions for cell types are: naive, follicular, memory, antibody producing, plasma blast, plasma cell, marginal zone, perisinusoidal or transitional B cells - many of those B cell subsets have only been determined in mice). When 4HT is present in the culture medium, caSTA5b-ER-IRES-NGFR positive B cells can survive for long periods (Table 2).

Table 2. Overview of caSTAT5b-ER-IRES-NGFR transduced human B cell cultures. PBMC were obtained after Ficoll gradient isolation of bloodbank derived buffy coats and subsequently sorted by CD19 MACS and CD27 or by FACSAria cell sorting. Purified B cells were then cultured in the presence of L-cells with indicated cytokines before being transduced with a retrovirus containing the caSTAT5b-ER-IRES-NGFR gene construct.

Donor	Isolation		Transduction		Culture Time
	Date	Subtype	Protocol	Time on IL-21	
B12	28-04-2005	CD19+TT+	IL-2 IL-4	4wks	18-10-2005
B15	17-05-2005	CD19+TT+	IL-21	36h	05-12-2005
B16	31-05-2005	CD 19+TT+	IL-21	20d	05-12-2005
B18	22-06-2005	CD19+CD27+ and TT+	IL-2 IL-4 and IL-21	time series (36h to 20d)	05-12-2005
B19	22-06-2005	CD19+CD27+ and TT+	IL-2 IL-4 and IL-21	time series (36h to 20d)	05-12-2005
B20	06-07-2005	CD19+TT+	IL-21	36h	05-12-2005
B21	06-07-2005	CD 19+TT+	IL-21	36h	06-09-2005
B22 / B23 / B24	06-09-2005	CD19+CD27+ IgM- and TT+	IL-21	5d	05-12-2005
B25 /B26	20-10-2005	CD19+CD27+IgM-	IL-21	7d	05-12-2005
B27 / B28	10-11-2005	CD19+CD27+	IL-21	7d	05-12-2005

Donor	Isolation		Transduction		Culture Time
	Date	Subtype	Protocol	Time on IL-21	
B29 / B30	22-11-2005	CD19+CD27+	IL-21	42h	05-12-2005

Development of single-cell derived, clonal B cell cultures

[0063] Outgrowth of caSTA5b-ER-IRES-NGFR positive B cells generally takes about 4 weeks, after which clonal cultures can be obtained by performing limiting dilution (LD) cultures or single cell sorting using flow cytometry (the FACSAria). These cultures consist of 2500 to 5000 L-cells, normal concentrations of IL-2 and IL-4 and either 1, 5 or 10 B cell/96-well when the LD is performed with 100% NGFR+ cells and 10, 100 and 1000 cell/96-well when NGFR+ cells are sorted into 96 well using the FACSAria.

Restimulation of antibody production of caSTAT5b-ER positive B cell cultures

[0064] Poly-, oligo- or monoclonal caSTAT5b-ER-IRES-NGFR positive B cell cultures that were negative or low on antibody production were washed extensively before cultures were (1) deprived of 4HT, IL-2 and IL-4 before being cultured with IL-21, then after 4-10 days of supernatants were tested for IgM and IgG production or (2) deprived of 4HT for 10 days meanwhile cultured with IL-2 and IL-4, and then at day 10 IL-2 and IL-4 are replaced by IL-21. Then at different time points supernatants are tested for IgM and IgG production.

Results

[0065] *B cell differentiation and proliferation; the IL-2 and IL-4* us. *IL-21 protocol* IL-21 treated B cell cultures showed enhanced proliferative responses within the first 2-3 weeks compared to IL-2 and IL-4 (figure 6a). However, unlike the IL-2 and IL-4 cultures, continuous IL-21 stimulation resulted in decreased proliferation and cell death, even in the presence of active STAT5b (figure 6b). Suggesting that IL-21 eventually had to be replaced by IL-2 and IL-4. To study this in more detail, time series experiment were performed with CD19+CD27+ memory B cells, in which IL-21 was replaced by IL-2 and IL-4 after 36 hours or 5, 10, 15 and 20 days. As shown in figure 7, most cultures could be maintained after IL-21 withdrawal, even cultures that received IL-21 for 20 days.

Antibody production by IL-21 boosted total human memory B cell cultures

[0066] Interestingly, in contrast to the IL-2 and IL-4 cultures, the IL-21 boosted cultures were able to produce antibodies for a relatively long period (IgG and IgM as measured by ELISA, Dako, Glostrup, Denmark) (figure 8a and 8b, respectively). Importantly, of the polyclonal memory B cell cultures of donors B18 and B19, single-cell clones were obtained by LD culture (table 3).

Table 3. Frequency of clones that were isolated from CD19+CD27+NGFR+ sorted B cells. Cells were sorted in 96 wells at either 1000, 100, 10 or 1 cell per well. Wells contained 5000 CD40L-expressing L cells, IL-2 and IL-4. ¼ to ½ of the medium was replaced twice a week with fresh cytokines and 2500 L cells.

donor	B18		B19	
	positive well	total # wells	positive well	total # well
1000 c/w	8	9	8	10
100 c/w	21	48	19	48
10 c/w	6	48	2	48
1 c/w	1	96	6	96

[0067] The majority of the clonal cultures produced IgM while only some produced IgG (figure 9). In addition, two clonal cultures produced both IgM and IgG clone 7 and clone 8). Whether these clones are indeed clonal or that class switching occurred remains to be determined. In the later case one BCR VDJ region should be found in the IgG or IgM gene fragments in this culture.

Antibody production of IL-21 boosted Tetanus Toxoid specific B cell cultures

[0068] Next, we tested whether we could isolate B cells producing Tetanus Toxoid (TT) specific antibodies. In brief, the following protocol was carried out: PART 1

1. (1) CD27+TT+ B cells were sorted (recovery was donor dependent and ranged from 10000-1000 cells),
2. (2) cultured with IL-21 for 36 h,
3. (3) transduced with caSTA5b-ER-IRES-NGFR,
4. (4) and cultured for variable times with IL-21 (36h to 3wks) after which IL-21 was replaced by IL-2, IL-4 and 4HT

PART 2

(5) when cultures were 100% NGFR+ they were cloned by limiting dilution (LD)

[0069] After 2 to 3 months of culture, 100% NGFR+, α-TT-specific polyclonal B cell cultures were obtained from at least 7 different donors (PART 1). All donors were tested positive in a α-TT-IgG specific antibody ELISA (r-biopharm, Darmstadt, Germany). As shown in figure 10a, α-TT IgG levels were relatively low. Since immortalization of memory B cells resulted in high numbers of IgM producing cultures (figure 9), that indicates that the majority of the TT cultures are IgM positive. As shown in figure 10b, five out of seven donors were producing IgM, suggesting that the anti-TT antibodies are from IgM origin and thus not detected by our α-TT IgG ELISA

[0070] This let us to develop an α-TT IgM ELISA based on the r-biopharm TT IgG ELISA. The only difference is in the final step, now a α-human IgM-HRP antibody instead of a anti-human IgG-HRP is added.

[0071] Next, from the polyclonal TT cultures, α-TT-specific B cell clones were derived by LD cultures (PART 2). These LD cultures were started with 100% NGFR+ polyclonal α-TT-specific B cells from four donors (table 4). Clones from donor B16 mainly produced IgG, while B18 and B19 produced IgM and B15 produced both IgG and IgM (not shown). Subsequently, supernatants of these clones were tested in the IgG TT or IgM TT ELISA (figure 11). Besides donor 15 all donors showed TT binding, although only 5 clones produced relatively high anti-TT antibody titers.

Table 4. Limiting dilution culture of 100% NGFR+ TT-specific B cells. Indicated is the total number of clones isolated and under what conditions they were isolated, either 1, 5 or 10 cell/well. One 96 well plate was used for each condition (1, 5 or 10 cell/well). Wells contained 2500 CD40L-expressing L cells, IL-2 and IL-4. ¼ to ½ of the medium was replaced twice a week with fresh cytokines and 2500 L cells.

donor	Number of positive clones from 96 well			
	Total #	from 1 c/w	from 5 c/w	from 10 c/w
B15	12	2	10	-
B16	14	-	7	7
B18	10	10	-	-
B19	11	1	3	7

Restimulation of antibody production of IL-21 boosted Tetanus Toxoid specific B cell cultures

[0072] We were able to generate IgM and IgG producing poly- and monoclonal B cell cultures using IL-21 as a stimulus. Nevertheless, antibody production was not stable. To our surprise, however, these IL-21 treated cultures could be restimulated to produce IgG and IgM antibodies (figure 12a and 12b, respectively). This was achieved by 4HT withdrawal and simultaneously stimulation with IL-21. Using this protocol, total antibody production increased 2- to 1000-fold for IgM, and 2- to 25- fold for IgG. Several of the supernatants of restimulated monoclonal cultures were now tested positive in the IgG and IgM Tetanus ELISA (figure 13).

[0073] Important to note is that caSTAT5b or caSTAT5b-ER B cell cultures that had not been treated with IL-21 prior to caSTAT5b transduction and subsequent expansion could not be restimulated to produce antibodies under any conditions (see patent application WO 03/052083; not shown here).

Example 4

Materials and methods

[0074] The detailed methods regarding:

- *Maintenance and isolation of human B cells;*
- *Transduction and regulation of mouse constitutive active STAT5b in B cells;*
- *Development of 100% caSTAT5b positive B cell lines that secrete antibodies;*
- *Development of single-cell derived, clonal B cell cultures;* and
- *Restimulation of antibody production of caSTA5b-ER positive B cell cultures*

are described in Example 3

Antibody production in IL-21 containing B cell cultures that are EBV infected and express BCL6-IRES-NGFR or caSTAT5-ER-IRES-NGFR

[0075] Purified primary CD19+CD27+ B cells (B cells) were stimulated for 36h with IL-21 and irradiated CD40L expressing L-cells (L-cells) before being transduced with BCL6-IRES-NGFR or caSTAT5b-ER-IRES-NGFR. After transduction, BCL6 transduced cells were cultured with IL-21, and caSTAT5b-ER transduced cells were cultured with IL-2 and IL-4. Transduced cells became NGFR positive within 2 to 3 days and were subsequently sorted on a FACSAria. NGFR sorted cells were then cultured at cell densities of 100 to 5000 cells/96 well (mini bulk cultures, MBC). The BCL6/IL-21 MBC proliferated strongly compared to caSTAT5b-ER cultures. These cultures were tested for antibody production, expanded to 24 well and frozen (viable cells, cell pellet and supernatant). The caSTAT5b-ER cultures were expanded and split in two parallel 96 wells. One well was cultured with IL-2, IL-4 and 4HT and the other well was cultured with IL-21 and without 4HT.

RT-PCR

[0076] To test if the strong proliferative response was related to the presence of EBV, an EBV RT-PCR was performed. Total RNA was isolated from thawed pellets using the RNeasy mini kit (Qiagen). RNA was reverse-transcribed in a volume of 20µl containing 5 first-strand buffer, 500µM dNTPs, 25µg/l oligo(dT) and 200 U superscript II RT (Life Technologies). A portion of the cDNA solution (1µl) was amplified by PCR in a 50µl solution containing 20mM Tris-HCl, 50mM KCl, 1.5mM MgC12, 5 mM dNTPs, 2.5 U Taq DNA polymerase (Life Technologies) and 30 pmol of each primer. PCR conditions were as follows: a 7-minute denaturing step at 94°C followed by 30 cycles of 30s at 94°C, 30 s at 62°C (HPRT1), 52°C (LMP-1) and 58°C (EBNA1/2) and 30s at 72°C, and a final 7-minute extension at 72°C. The oligonucleotides used for RT-PCR were as follows: HPRT1 forward (5'-TATGGACAGGACTGAACGTCTTGC-3') and HPRT1 reverse (5'-GACACAAACATGATTCAAATCCCTGA-3'); LMP-1 forward: (5'-GCGACTCTGCTGGAAATGAT-3') and LMP-1 reverse (5'-GACATGGTAATGCCTAGAAG-3'); EBNA1/2 forward (5'-AGCAAGAAGAGGAGGTGGTAAG-3') and EBNA1/2 reverse (5'-GGCTCAA.AGTGGTCTCTAATGC-3').

[0077] In addition to the RT-PCR we performed a PCR directly on cell pellet and supernatant DNA that was isolated using the QIAmp isolation kit (Qiagen).

EBV cultures

[0078] To study the role of EBV in our system in more detail, we set up experiments to determine proliferation and antibody production in untransduced, BCL6-IRES-NGFR and caSTAT5b-ER-IRES-NGFR transduced cells that were obtained from cultures with or without natural occurring EBV.

First experimental setup

Table 5: Experimental setup

[0079]

Table 5 in summary:

BCL6				
1		BCL6	IL-21	
2		BCL6	IL-21	EBV
3			IL-21	EBV
BCL6 MBC with IL-21 and L-cells (cultures Ce2-B9 and Ce2-G9)				
BCL6 MBC with IL-21, L-cells and EBV (cultures Be3-F3 and Be2-G9)				

BCL6				
EBV MBC with IL-21 and L-cells (cultures B28 and B29)				
Cultures with IL-2 and IL-4 were also included. At weekly intervals antibody levels and cell numbers were determined (Table 7). EBV status of the cultures is shown in figure 14.				

Second experimental setup

[0080]

Table 6: Experimental setup caSTAT5b-ER

1	4HT	IL-2 and IL-4	
2	no 4HT	IL-21	
3	no 4HT	IL-21	EBV

[0081] Table 6. caSTAT5b-ER MBC were cultured on 4HT, L-cells, IL-2 and IL-4 in 96 well at 1000 c/w. When cell density was high enough parallel cultures were created. One part was maintained on 4HT, L-cells, IL2 and IL4 while the other part was switched to IL-21, L-cells but no 4HT. Antibody production was determined by ELISA (DAKO). EBV infection was determined by LMP1 PCR (Figure 16).

Results

EBV infection of BCL6 cultures, determined by PCR

[0082] It was found that IL-21 induced strong B cell proliferation and differentiation, which we tested in two donors in combination with the transduction of BCL6 (Table 5). Indeed we found strong B cell outgrowth in culture conditions in which we cultured B cells at 1000 and 100 c/w densities (Table 7). However in one donor almost all cultures were suspected to be EBV infected based on the phenotype by light microscopy, color of the culture supernatant and enormous cell expansion. Indeed this donor (B29) turned out to be EBV infected (Figure 14). The massive outgrowth of EBV infected cells demonstrates that the combination of BCL6 and IL-21 gives a growth advantage for EBV infected cells especially since the frequency of EBV infected B cells *in vivo* is thought to be relatively low. In contrast to donor B29, donor B30 was EBV negative, except for a weak and relatively smal LMP1 band in sample Ce2-F2 (Figure 14).

Proliferation of BCL6 cultures in the presence or absence of EBV, IL-2 IL-4 and IL-21

[0083] To study the role of EBV, BCL6, IL-2, IL-4 and IL-21 on cell growth, proliferation kinetics were compared (see table 5 experimental set up). Samples were selected based on LMP-1 EBV PCR reactions (Figure 14). EBV status was confirmed by the ability of EBV infected cells to grow in the absence of L-cells. The BCL6 samples cultured with IL-2 and IL-4 displayed a low proliferative capacity and could not be maintained (Table 7). All samples cultured in medium containing IL-21 showed strong proliferation. IL-2 and IL-4 could only induce strong proliferation when cells were infected with EBV alone, not in combination with BCL6.

[0084] Table 7: proliferation of BCL6 B

Table 7. Proliferation of BCL6 transduced and non-transduced B cells with and without EBV or with and without IL-2 IL-4 or IL-21; all samples contained L cells.

cells			
Culture condition		cytokines added	
	IL-21		IL-2 IL-4
BCL6	++		-
cells			
BCL6/EBV	+++		+
EBV	+++		+++

Determine antibody production by BCL6 cultures in the absence or presence of EBV, IL-2 IL-4 and IL-21

[0085] IgG antibody production was determined of the long term cultures as described in table 5. The indicated antibody production levels are the mean antibody production of six to ten different measurements in time of two donors (figure 15). It is clearly shown that the BCL6/EBV/IL-21 cultures produce significantly more IgG than the BCL6/IL-21 cultures. Hence, EBV significantly enhances antibody production of BCL6 transduced B cells.

[0086] Thus, the combination of IL-21 and EBV results in high levels of antibody production, in the absence as well as in the presence of BCL6.

Determine the B cell receptor (BCR) expression alter long term culture of BCL6 transduced and EBV infected cells

[0087] It has been described that EBV infected cells lose their BCR expression. Therefore B cells in IL-21 containing cultures (described in table 5) were stained for NGFR, CD19, Kappa en Lambda.

[0088] The BCL6 transduced, EBV negative cells remained BCR expression positive as determined by Kappa and Lambda staining. Hence, such cells are particularly suitable for isolating and/or screening after a long period of culture for a desired specificity, for instance using labelled antigen, because such cells will bind said labelled antigen with their BCR. However, when EBV was present BCR expression was lost or diminished. Since the BCL6/IL-21 cultures produced relatively lower amounts of antibody, as compared to the EBV infected cells, but maintain BCR surface expression this demonstrates that these cells remain in a pre-plasmablast phenotype while the EBV infected cells, which produce high amounts of antibody, differentiate or have been differentiated towards a phenotype better described as plasmablast.

[0089] In conclusion, in cases where the presence of a B cell receptor on B cells is desired, such as for instance in screening assays, the B cells are preferably not, or at a later stage, infected with EBV.

EBV infection of caSTAT5b-ER cultures, determined by PCR

[0090] Parallel to the BCL6 transductions, transductions using caSTAT5b-ER were performed. Strikingly, in contrast to the BCL6 cultures, which all became EBV infected, the caSTAT5b-ER cultures of the same donor (B29) seemed to keep the natural distribution (percentage) of EBV infected cells. Several cultures had clear signs of EBV infection and were checked by LMP1 PCR and indeed were found to be positive. One of the signs caSTAT5b-ER cultures were EBV positive was the ability of the MBC to survive when treated with IL-21 in the absence of 4HT. These 4HT deprived B cells lack the active form of caSTAT5b and normally die within 2 to 3 weeks.

Proliferation and antibody production by caSTAT5b-ER-transduced B cells under different conditions

[0091] To study the role of EBV in the caSTAT5b-ER system experiments were performed as described in table 6. In table 8 a schematic overview is presented of the response of caSTAT5b-ER transduced B cells. Active caSTAT5b-ER by the presence of 4HT blocks B cell differentiation irrespective which cytokines were present or even if B cells were EBV infected. Therefore, caSTAT5b-ER cultures that are EBV infected and maintained in IL-2 IL-4 or IL-21 but with 4HT do not produce antibody. Withdrawal of 4HT results in differentiation and subsequent antibody production. Preferably, IL-21 is added during and/or after withdrawal of 4HT. However, these cells ultimately die since caSTAT5b is inactive, and thus antibodies will only be produced for a restricted period. Hence, if no EBV is present, IL-21 is eventually replaced by at least one other growth stimulating agent, such as for instance Il-2 and IL-4, as already described in Example 3.

[0092] EBV and IL-21 together in the absence of 4HT are two strong stimuli which induce long term proliferation and high levels of antibody production (Figure 18). This combination is therefore preferred.

Table 8: proliferation, survival and antibody production of caSTAT5b-ER B cells

cytokines	EBV	4HT	proliferation	survival	production
IL-2 IL-4	-	+	+	pos	neg
IL-2 IL-4	+	+	++	pos	neg
IL-21	+	+	++	pos	neg
IL-21	-	-	++	neg	intermediate
IL-21	+	-	++	pos	high

Example 5

Methods

[0093] BCL6-IRES-YFP positive cells were transduced with STAT3/ER-IRES-GFP using standard procedures and expanded on L cells with IL-2 and IL-4 as described in the Methods section of Example 1. The expression of STAT3 was regulated through the presence or absence of tamoxifen (4HT). YFP and GFP positive cell were sorted and equal numbers were cultured. BLIMP1 gene expression was monitored by RT-PCR and antibody production was determined in cultures with and without 4HT.

Results

[0094] Addition of 4HT to the cells resulted in increased cell numbers, increased Blimp-1 expression. Moreover, enhanced IgG production was measured as is shown in figure 19a-c.

Example 6

[0095] CD19 positive B cells were transduced with control YFP-IRES-YFP (cYFP); BCL6-IRES-YFP (BCL6-YFP) or Bcl-xL-GFP (Bcl-xL-GFP). Cells were then maintained on CD40L and IL-4 and the percent YFP and GFP single and double positive cells was determined over time by FACS in unsorted bulk cultures.

[0096] Cell division and cumulative expansion was determined in single and double positive cell in the presence of IL-4 or IL-21.

[0097] To check for gene expression and EBV co-infection, RT-PCR analysis of Bcl-xL, BCL6, LMP1, and EBNA1 mRNA expression was performed in cultures of single BCL6-transduced and BCL6/Bcl-xL-double transduced bulk cultures.

Results

[0098] Figures 20-23 show that the double transduced B cells containing BCL6 and Bcl-xL had a higher cumulative expansion rate and indeed also were the dominant cells to grow out in bulk memory B cell cultures. We also show that these double transduced cells divided twice as fast compared to the single transduced cells. Not shown here are the antibody production levels (IgG and IgM), which were equal between the BCL6 and BCL6/Bcl-xL cultures when cultured with IL-4 or IL-21.

Example 7

[0099] The Hodgkin cell line L591, which is positive for tyrosine-phophorylated STAT5, was cultured independent of L cells (CD40 stimulation) and cytokines. L591 cells were transduced by lentivirus containing E47-IRES-GFP or control virus with GFP only (methods are described in more detail in example 1). Transduced cells were sorted and cell growth was followed in time.

Results

[0100] Figure 24 shows that L591 cells quickly stop dividing when E47 is expressed. This is suggestive for the effect of E47, which via its downstream targets (besides others Socs1, Socs3, Id2, Eto2 and Xbpl) induces B cell differentiation toward an antibody producing B cell phenotype. The induced differentiation by E47 could also indicate that the effects of STAT5 are abolished or that E47 directly or in directly affects the amount and action of functional STAT5. Thus based on the data with the Hodgkin cell line L591 we state that STAT5b/ER positive B cell cultures that are maintained on L cells with 4HT and IL-2 and IL-4 or IL-21, can be induced to differentiate towards antibody producing cells when the E47 is active.

Brief description of the drawings

[0101]

Figure 1. Enhanced growth of BCL6^{+} cells cultured with IL-21. 100% pure BCL6^{+} memory B cells were cultured in the presence of IL-2 and IL-4 (conventional culture conditions), or with IL-21 alone. The total expansion of live cells over 17 days of culture with IL-21 is shown.

Figure 2. Plasmablast immortalization of BCL6-positive cells with IL-21. Memory B cells were transduced with a retrovirus expressing BCL6-GFP and cultured with IL-2 and IL-4 (to prevent differentiation) or with IL-21 for 14 days. The surface staining for CD38 and CD20 of GFP^+ (that is, $BCL6^+$) cells is shown. IL-21 induces an 8-fold increase in the amount of B cells with a plasmablast phenotype.

Figure 3. IL-21 upregulates BLIMP1 in $BCL6^+$ B cells. 100% pure BCL6-$\Delta NGFR^+$ were cultured with IL-2 and IL-4 or IL-21 for 24 days. cDNA was generated from total RNA and mRNA levels of BLIMP1 and HPRT (loading control) were determined by reverse transcriptase polymerase chain reaction.

Figure 4. Non-limiting model of one embodiment according to the present invention

Figure 5.
General overview of ideal culture scheme, see for more details the material and methods section of Example 3.

Figure 6
Growth dynamics of IL-2 and IL-4 vs. IL-21 stimulated B cell. (a) Peripheral blood (PB) memory B cells derived from two donors (B18 and B19) were stimulated either with IL-21 or IL-2 and IL-4. Cells were transduced with caSTAT5b-ER-IRES-NGFR at day 2 for the IL-21 and at day 5 for IL-2 and IL-4 treated cultures; 4HT was added at day 13. (b) Of 4 donors Tetanus Toxoid specific B cells were sorted from PB (cell numbers ranged from 1000-10.000). Cells were cultured in 96 well with IL-21 and transduced with caSTAT5b-ER-IRES-NGFR on day 2. 4HT was added on day 4 and IL-21 was replaced with IL-2, IL-4 and 4HT after 7 days (B14 and B15) or was replaced after 20 days (B16 and B17). Cells were counted by hand and dead cells were excluded.

Figure 7
Percentage caSTAT5b-ER-IRES-NGFR transduced cells was determined using the LSR II (BD). Of two donors (B18 and B19) IL-2 and IL-4 vs. IL-21 time series experiment were performed. Of each donor ¼ of the cells were transduced using the IL-2 and IL-4 protocol, the remaining % was transduced using IL-21. Directly after the IL-21 transduction (36h) one third of the IL-21 culture was switched to IL-2 and IL-4. This was repeated on day 5, 10 and 20 of the IL-21 culture.

Figure 8
Total human IgG and IgM antibody production by caSTAT5b-ER-IRES-NGFR transduced PB derived memory B cells, as described in figure 6 and 7. (a) Mean IgG production of donor B18 and B19 is shown. B cells were transduced using the IL-2 and IL-4 vs. the IL-21 protocol. The IgG production indicated with the open symbols represent all cultures that had been treated with IL-21, irrespective when they were switched to IL-2 and IL-4. (b) IgM production in samples as described above, note that the time scale is different.

Figure 9

Antibody production of B cell clones derived from memory B cells of donors B18 and B19 transduced with caSTAT5b-ER-IRES-NGFR. Ten-day-old cultures that were derived from IL-21 stimulated B cells (stimulated for 36h) were used for LD culture. Twelve clones were obtained; 5 from and 7 from B19. IgG production is the mean of three time points; IgM production is the mean of two time points.

Figure 10
IgG Tetanus Toxoid ELISA on supernatant of polyclonal, 100% caSTA5b-ER-IRES-NGFR positive, Tetanus Toxoid sorted human B cells. (a) Of 7 donors rapidly proliferating clonal cultures were derived. Shown is the average TT antibody production of at least 3 different measurements per donor. Each time the relative OD was determined (generally a relative increase of > 2 to 3 times the background is assumed positive). (b) To determine if TT IgG ELISA negative cultures could be producing IgM, the same 7 donor samples were tested in a total IgM ELISA.

Figure 11
Anti-Tetanus Toxoid ELISA. The binding of IgG and IgM α-TT specific antibodies by ELISA was determined. Supernatants of 100% NGFR positive clonal B cell cultures derived form donors B15, B16, B18 and B19 were tested. Two times the background was set as positive.

Figure 12
Total IgG and IgM production after restimulation of clonal B cell cultures (a) donor B16 which produces IgG and (b) donor B19 which produces IgM. Production was measured in supernatant of cultures that were either cultured with IL-2, IL-4 and in the presence or absence of 4HT or with IL-21 and in the presence or absence of 4HT. Cultures containing IL-2 and IL-4 did not show an increase in antibody secretion (not shown). Only cultures that responded to the restimulation are shown (10 out of 14 IgG and 8 out of 9 IgM clones responded)

Figure 13
Antibodies secreted by IL-21 restimulated and 4HT deprived cultures, as described in the legend of figure 8 were tested for their antigen specificity. The supernatants derived from restimulated donor B16 clonal TT cultures were tested in the α-TT IgG ELISA (a), the supernatants derived from donor B19 cultures were tested in the IgM ELISA (b). Shown is the relative increase in antibody binding compared to the negative control, samples B19-10B7 and 10E1 were cut off at 30 for visibility; values were 96 and 121, respectively.

Figure 14
LMP1 RT-PCR was performed on RNA isolated from frozen cell pellets of indicated cultures. Shown are 15 cultures which were randomly selected and tested for EBV infection. Sample coding: B indicates donor B29, C indicates donor B30 and both were cultured at 1000 c/w (e3) or 100 c/w (e2). All cultures were transduced with BCL6 except for B28 UTD (untransduced) and B29 UTD; JY cells were used as positive controls.

Figure 15
IgG antibody production (ng/ml) by BCL6 and EBV cultures as described in table 5. For each condition the average of two samples is shown each of which consist of a longitudinal follow up

of 6 to 10 time points. An asterisks indicates samples are significantly different ($p < 0.05$, unpaired student t-test). The samples cultured with IL-2 and IL-4 are a combination of EBV and BCL6 positive and negative cultures longitudinal followed.

Figure 16

Kappa-FITC and Lambda-PE staining on CD19, and NGFR positive cells. One B cell is either positive for Kappa or Lambda. Samples were measured using a LSRII (BD) and analysed using FlowJo software.

Figure 17

Shown is a representative LMP1 PCR performed on DNA isolated from frozen cell pellets of donor B25 1000c/w MBC transduced with caSTAT5b and suspected to be EBV positive based on the color of the culture medium, growth kinetics and phenotype as observed by light microscopy. All other cultures were EBV negative.

Figure 18

Average IgG production (ng/ml) in caSTAT5b-ER B cells cultured without 4HT, with IL-2 IL-4 or IL-21 and with or without EBV. The increase in antibody production in the presence of IL-21 was significant compared to cultures with IL-2 and IL-4 ($p < 0.05$). The increase in antibody production in IL-21 containing, EBV infected cultures was significant compared to cultures without EBV ($p < 0.05$), nonparametric Mann-Whitney)

Figure 19

(A-C) BCL6-IRES-YFP$^+$ cells were transduced with STAT3ER-IRES-GFP and expanded on CD40L L cells with IL-2 and IL-4. BCL6 / STAT3ER positive cells were sorted by FACS and equal numbers were cultured in the absence of cytokines, but in the presence or absence of 4HT (1 μM) for 4 days. (A) Live cell numbers after 4 days. (B) Semi-quantitative RT-PCR for *BLIMP1* and *HPRT1* expression in BCL6-YFP$^+$ / STAT3ER-GFP$^+$ cells (C) IgG production in BCL6-YFP$^+$ STAT3ER-GFP$^+$ treated for 4 days ± 4HT.

Figure 20

1. A. CD19$^+$ B cells were transduced with control YFP-IRES-YFP (cYFP); BCL6-IRES-YFP (BCL6-YFP) or BclXL-GFP (Bcl-xL-GFP). Cells were then maintained on CD40L and IL-4 and the percent YFP or GFP positive was determined over time by FACS. All data represented in A and B are derived from CD19+CD3- gating.
2. B. Unsorted bulk cultures of Bcl-xL-IRES-GFP and BCL6-IRES-YFP double transduced B cells on CD40L and IL-4. Individual GFP$^+$, YFP$^+$, and GFP/YFP double positive cells was determined by FACS

Figure 21.

IL-21 increased proliferation of B cells transduced with Bcl-xL, BCL6, or Bcl-xL+BCL6 double transduced cells.

At day 17 after transduction and maintainence on CD40L and IL-4, cultures were split and cultured on CD40L in the presence of IL-4, or IL-21. Absolute number of transduced cells was determined and the cumulative expansion was calculated in single transduced cells (A) or in double transduced cells (B).

Figure 22.
Long-term cultures are EBV-RT-PCR analysis of Bcl-xL, BCL6, LMP1, and EBNA1 mRNA expression in Day 66 cultures of BCL6-transduced cells (Lane 1) and BCL6/Bcl-xL-double transduced bulk cultures (Lane 2). 1µl of a cDNA reaction performed in the absence of reverse transcriptase (-RT) reaction was used as a negative control for genomic DNA contamination. Positive controls: Bcl-xL, STAT5-ER transduced B cells cultured with 4-HT; BCL6, LMP1, and EBNA1, human Raji B cells.

Figure 23.
Doubling time of Bcl-xL, BCL6, and Bcl-xL-BCL6 double transduced cells. Based on the number of transduced (GFP+,YFP+) B cells the doubling time between days 51-59 of culture was calculated in Bcl-xL-, and BCL6-transduced cells in single transductions as well as in Bcl-xL+BCL6 double-transduced bulk cultures.

Figure 24
L591 a Hodgkin cell line, was transduced by lentivirus containing E47-IRES-GFP. GFP positive cells were sorted and cultured independent of L cells (CD40 stimulation) and cytokines. Cell numbers were determined in time.

<u>**References**</u>

[0102]

Banchereau, J. , de Paoli, P. , Valle, A. , Garcia, E. , Rousset, F., (1991). Long term human B cell lines dependent on interleukin-4 and antibody to CD40, Science 251, 70-2.

Boise, L. H., M. Gonzalez-Garcia, C. E. Postema, L. Ding, T. Lindsten, L. A. Turka, X. Mao, G. Nunez, and C. B. Thompson. (1993). Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74:597.

Dadgostar, H. , Zarnegar, B. , Hoffmann, A. , Qin, X. F. , Truong, U. , Rao, G. , Baltimore, D. , and Cheng, G. (2002). Cooperation of multiple signaling pathways in CD40-regulated gene expression in B lymphocytes. Proc.Natl.Acad.Sci USA 99, 1497-1502.

Malisan, F. , Briere, F. , Bridon, J.M. , Harindranath, N. , Mills, F. C. , Max, E. E. , Banchereau, J. , Martinez-Valdez, H. (1996). Interleukin-10 induces immunoglobulin G isotype switch recombination in human CD40-activated naive B lymphocytes, J.Exp.Med. 183, 937-47.

Mathas S, Janz M, Hummel F, Hummel M, Wollert-Wulf B, Lusatis S, Anagnostopoulos I, Lietz A, Sigvardsson M, Jundt F, Johrens K, Bommert K, Stein H, Dorken B (2006). Intrinsic inhibition of transcription factor E2A by HLH proteins ABF-1 and Id2 mediates reprogramming of neoplastic B cells in Hodgkin lymphoma. Nat Immunol. 7, 207-215.

Traggiai, E. , Becker, S. , Subbarao, K. , Kolesnikova, L. , Uematsu, Y. , Gismondo, M.R. , Murphy, B.R. , Rappuoli, R. , Lanzavecchia, A. (2004). An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. Nature Medicine Volume 10, No. 8, 871-875.

Ye, B. H. , Cattoretti, G. , Shen, Q. , Zhang, J. , Hawe, N. , de Waard, R. , Leung, C. , Nouri-Shirazi, M. , Orazi, A., Chaganti, R. S., et al. (1997). The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. Nat Genet 16, 161-170.

REFERENCES CITED IN THE DESCRIPTION

Cited references

Patent documents cited in the description

- WO03052083A [0007] [0007] [0029] [0073]

Non-patent literature cited in the description

- **ARIYOSHI K.**JBC, 2000, [0061]
- **HEEMSKERK M.H.**JEM, 1997, [0061]
- **HEEMSKERK M.H.**Cell Immunol., 1999, [0061]
- **SCHEEREN F.A.**Nat Immunol, 2005, [0061]
- **BANCHEREAU, J.PAOLI, P.VALLE, A.GARCIA, E.ROUSSET, F.**Long term human B cell lines dependent on interleukin-4 and antibody to CD40Science, 1991, vol. 251, 70-2 [0102]
- **BOISE, L. H.M. GONZALEZ-GARCIAC. E. POSTEMAL. DINGT. LINDSTENL. A.**

TURKAX. MAOG. NUNEZC. B. THOMPSON. Bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death.Cell, 1993, vol. 74, 597- [0102]

- **DADGOSTAR, H.ZARNEGAR, B.HOFFMANN, A.QIN, X. F.TRUONG, U.RAO, G.BALTIMORE, D.CHENG, G.**Cooperation of multiple signaling pathways in CD40-regulated gene expression in B lymphocytes.Proc.Natl.Acad.Sci USA, 2002, vol. 99, 1497-1502 [0102]
- **MALISAN, F.BRIERE, F.BRIDON, J.M.HARINDRANATH, N.MILLS, F. C.MAX, E. E.BANCHEREAU, J.MARTINEZ-VALDEZ, H.**Interleukin-10 induces immunoglobulin G isotype switch recombination in human CD40-activated naive B lymphocytesJ.Exp.Med., 1996, vol. 183, 937-47 [0102]
- **MATHAS SJANZ MHUMMEL FHUMMEL MWOLLERT-WULF BLUSATIS SANAGNOSTOPOULOS ILIETZ ASIGVARDSSON MJUNDT F**Intrinsic inhibition of transcription factor E2A by HLH proteins ABF-1 and Id2 mediates reprogramming of neoplastic B cells in Hodgkin lymphoma.Nat Immunol., 2006, vol. 7, 207-215 [0102]
- **TRAGGIAI, E.BECKER, S.SUBBARAO, K.KOLESNIKOVA, L.UEMATSU, Y.GISMONDO, M.R.MURPHY, B.R.RAPPUOLI, R.LANZAVECCHIA, A.**An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus.Nature Medicine, 2004, vol. 10, 8871-875 [0102]
- **YE, B. H.CATTORETTI, G.SHEN, Q.ZHANG, J.HAWE, N.WAARD, R.LEUNG, C.NOURI-SHIRAZI, M.ORAZI, A.CHAGANTI, R. S. et al.**The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation.Nat Genet, 1997, vol. 16, 161-170 [0102]

PATENTKRAV

1. Fremgangsmåde til at øge den replikative levetid af en antistof producerende celle, omfattende at fremme Blimp-1 ekspression og at øge eller opretholde mængden af BCL6 ekspressionsprodukt sammenlignet med en hukommelse B celle eller en naiv B celle i den antistof producerende celle ved

- at tilvejebringe den antistof producerende celle med en nukleinsyresekvens, der koder for BCL6 eller en funktionel del eller funktionel derivat deraf, der er i stand til at øge den replikative levetid af en antistof producerende celle, og

- at dyrke den antistof producerende celle under tilstedeværelse af en forbindelse, der er i stand til at øge Blimp-1 ekspression,

hvor forbindelsen, der er i stand til at øge Blimp-1 ekspression, omfatter IL-21.

2. Fremgangsmåde ifølge et krav 1, omfattende at tilvejebringe den antistof producerende celle med et yderligere immortalitetsmiddel.

3. Fremgangsmåde ifølge krav 2, hvor immortalitetsmidlet omfatter et transformerende middel.

4. Fremgangsmåde ifølge krav 2 eller 3, hvor immortalitetsmidlet omfatter Epstein-Barr virus.

5. Fremgangsmåde ifølge ethvert af kravene 2-4, hvor den antistof producerende celle dyrkes under tilstedeværelse af IL-21 inden den antistof producerende celle er forsynet med en nukleinsyresekvens, der koder for BCL6 eller en funktionel del eller funktionel derivat deraf, der er i stand til at øge den replikative levetid af en antistof producerende celle.

6. Fremgangsmåde ifølge ethvert af kravene 1-5, hvor den antistof producerende celle dyrkes under tilstedeværelse af IL-21 og er forsynet med Epstein-Barr virus.

7. Fremgangsmåde ifølge ethvert af kravene 1-6, yderligere omfattende at øge mængden af Bcl-xL ekspressionsprodukt i den antistof producerende celle.

8. Fremgangsmåde ifølge krav 7, omfattende at tilvejebringe den antistof producerende celle med en nukleinsyresekvens, der koder for Bcl-xL eller en funktionel del eller funktionel

derivat deraf, der er i stand til at øge den replikative levetid af en antistof producerende celle.

9. Fremgangsmåde ifølge krav 8, omfattende at tilvejebringe den antistof producerende celle med en forbindelse, der er i stand til at fremme Bcl-xL ekspression.

10. Fremgangsmåde ifølge krav 9, hvor forbindelsen omfatter APRIL, BAFF, CD40, BCR stimulering, cytokiner, vækstfaktorer eller nedstrømseffektorer såsom JNK og AKT (PKB).

11. Fremgangsmåde til at fremstille en antistof producerende celle, der er i stand til at replikere i mindst én uge, fremgangsmåden omfatter:

- at øge et ekspressionsniveau af Blimp-1 i en B celle, sammenlignet med en hukommelse B celle eller en naiv B celle, ved at tilvejebringe B cellen med en nukleinsyresekvens, der koder for STAT3 eller en funktionel del eller funktionel derivat deraf, der er i stand til at opregulere Blimp-1 ekspression, og/eller at dyrke B cellen under tilstedeværelse af en forbindelse, der er i stand til at øge Blimp-1 ekspression, hvor forbindelsen, der er i stand til at fremme Blimp-1 ekspression omfatter IL-21, og

at tilvejebringe B cellen med en nukleinsyresekvens, der koder for BCL6 eller en funktionel del eller funktionel derivat deraf, der er i stand til at øge den replikative levetid af en antistof producerende celle.

12. Fremgangsmåde ifølge krav 11, hvor BCL6 ekspressionsniveauet i cellen er bragt til, og/eller fastholdt ved, i alt væsentlig det samme niveau eller ved et højere niveau sammenlignet med en plasmablast.

13. Fremgangsmåde ifølge ethvert af kravene 11-12, yderligere omfattende at øge et ekspressionsniveau af Bcl-xL i cellen.

14. Fremgangsmåde ifølge krav 13, omfattende at tilvejebringe B cellen med en forbindelse, der er i stand til at fremme Bcl-xL ekspression.

15. Fremgangsmåde ifølge krav 14, hvor forbindelsen, der er i stand til at fremme Bcl-xL ekspression, omfatter en nukleinsyre, der koder for Bcl-xL eller en funktionel del eller funktionel derivat deraf, der er i stand til at øge den replikative levetid af en antistof producerende celle.

16. Fremgangsmåde ifølge krav 15, hvor forbindelsen, der er i stand til at fremme Bcl-xL ekspression, omfatter APRIL, BAFF, CD40, BCR stimulering, cytokiner, vækstfaktorer eller nedstrømseffektorer såsom JNK og AKT (PKB).

17. Fremgangsmåde ifølge ethvert af kravene 1-16, hvor ekspression af nukleinsyresekvensen, der koder for BCL6, STAT3, Bcl-xL eller en funktionel del, funktionel derivat og/eller funktionel analog af BCL6 og/eller STAT3 og/eller Bcl-xL reguleres ved en aktivator og/eller repressor, der kan induceres ved en eksogen forbindelse.

18. Fremgangsmåde ifølge ethvert af kravene 1-17, hvor en antistof producerende celle dyrkes, der er i stand til at replikere i mindst tre måneder.

19. Fremgangsmåde ifølge ethvert af kravene 1-18, hvor den antistof producerende celle er i stand til at fremstille antistoffer mod et antigen af interesse.

20. Fremgangsmåde ifølge krav 19, hvor den antistof producerende celle er blevet tilvejebragt fra et individ, hvilket individ tidligere er blevet udsat for antigenet af interesse.

21. Fremgangsmåde ifølge ethvert af kravene 1-20, yderligere omfattende udtrykkende et gen af B cellen, der koder for tungkædet Ig og/eller letkædet Ig i en anden celle.

22. Antistof producerende celle, der er i stand til at replikere i mindst ni uger, hvor BCL6 og Blimp-1 er udtrykt samtidigt, og hvor cellen omfatter en eksogen nukleinsyresekvens, der koder for BCL6 eller en funktionel del eller funktionel derivat deraf, der er i stand til at øge den replikative levetid af en antistof producerende celle, og omfatter en eksogen nukleinsyresekvens, der koder for STAT3 eller en funktionel del eller funktionel derivat deraf, der er i stand til at opregulere Blimp-1 ekspression.

23. Antistof producerende celle ifølge krav 22, der omfatter en eksogen nukleinsyresekvens, der koder for Bcl-xL eller en funktionel del eller funktionel derivat deraf, der er i stand til at øge den replikative levetid af en antistof producerende celle.

24. Antistof producerende celle ifølge ethvert af kravene 22-23, hvor ekspression af nukleinsyresekvensen, der koder for BCL6, STAT3, Bcl-xL eller en funktionel del eller

funktionel derivat af BCL6 og/eller STAT3 og/eller Bcl-xL reguleres ved en aktivator og/eller repressor, der kan induceres ved en eksogen forbindelse.

25. Fremgangsmåde til at fremstille en B celleline omfattende:

- at tilvejebringe en antistof producerende celle med en fremgangsmåde ifølge ethvert af kravene 1-21, og

- at dyrke den antistof producerende celle ex vivo.

26. Fremgangsmåde ifølge krav 25, omfattende:

- at fremstille en antistof producerende celle, der er i stand til at replikere i mindst én uge ved anvendelse af en hukommelse B celle og/eller en naiv B celle fra et individ, der er blevet udsat for et antigen af interesse i en fremgangsmåde ifølge ethvert af kravene 1-21, og

- at dyrke den antistof producerende celle ex vivo.

27. Fremgangsmåde til tilvejebringelse af antistoffer, omfattende:

- at tilvejebringe en antistof producerende celle med en fremgangsmåde ifølge ethvert af kravene 1-21;

- at dyrke den antistof producerende celle *ex vivo,* og

- at høste antistoffer fremstillet af den antistof producerende celle.

28. Fremgangsmåde til at fremstille antistoffer, der er i stand til specifikt at binde et antigen af interesse, fremgangsmåden omfatter:

- af fremstille et antistof producerende celle, der er i stand til at replikere i mindst én uge ved anvendelse af en hukommelse B celle, der er i stand til at differentiere ind i en B celle, hvilken B celle fremstiller antistoffer, der er i stand til specifikt at binde antigenet af interesse, i en fremgangsmåde ifølge ethvert af kravene 1-21, og

- at tilvejebringe antistoffer fremstillet af den antistof producerende celle.

29. Fremgangsmåde ifølge krav 28, yderligere omfattende at dyrke den antistof producerende celle *ex vivo.*

DRAWINGS

Figure 1

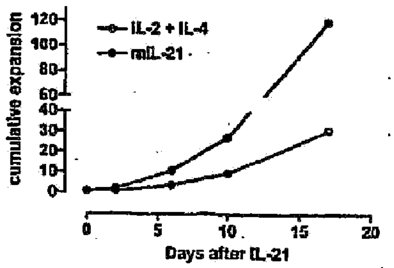


Figure 2

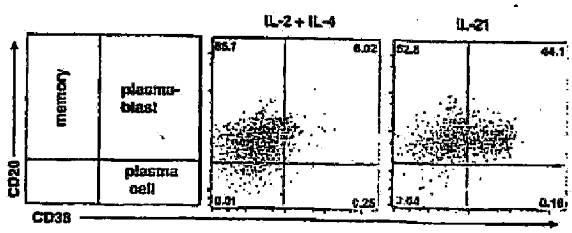


Figure 3

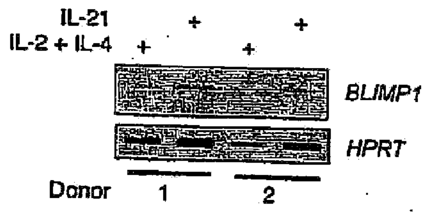


Figure 4

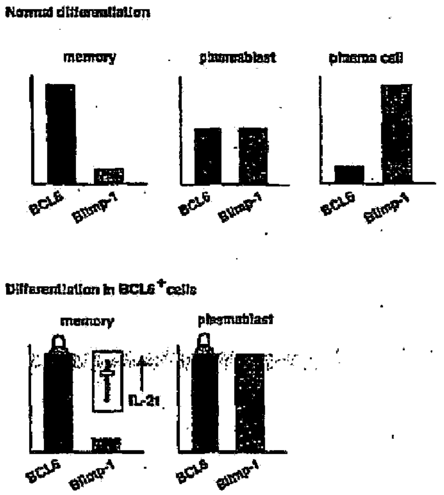
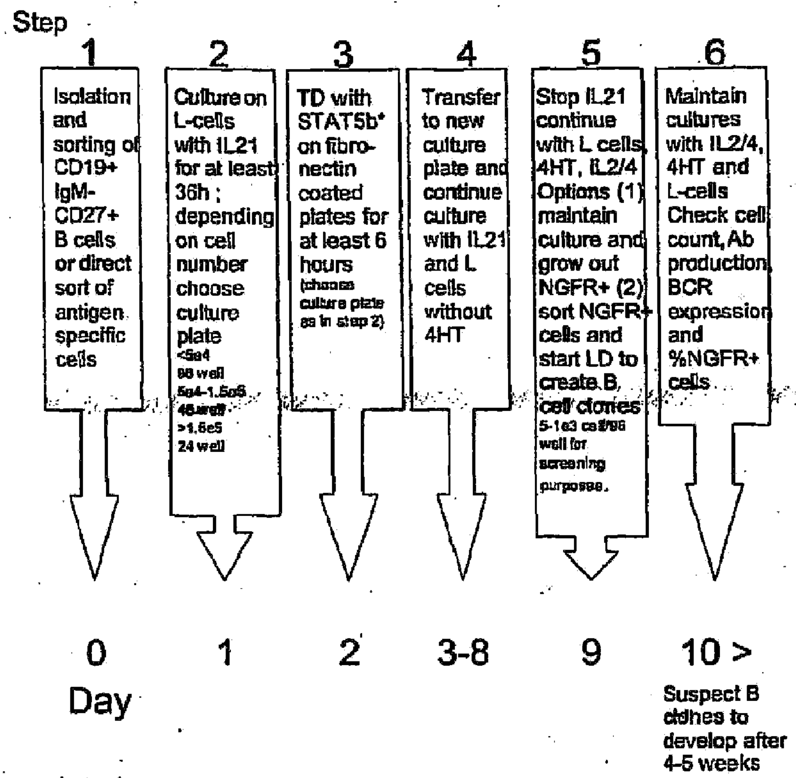

Normal differentiation
memory
plasmablast
plasma cell
BCL6
Blimp-1
BCL6
Blimp-1
BCL6
Blimp-1
Differentiation in BCL6+ cells
memory
plasmablast
IL-21
BCL6
Blimp-1
BCL6
Blimp-1

Figure 5

Culture scheme



Legend:

*	caSTAT5b/GFP or any other marker gene caSTAT5b/ER/GFP expression caSTAT5b under control of 4HT
L cell	CD40L (CD154) positive L-cell (mouse fibroblast cell line)
STAT5b	constitutive active STAT5b-ER-IRES-NGFR
TD	transduction
IL21	Interleukin 21
4HT	4-HydroxyTamoxifen
LD	Limiting dilution
BCR	B cell receptor
NGFR	nerve growth factor receptor
Ab	antibody

Figure 6a

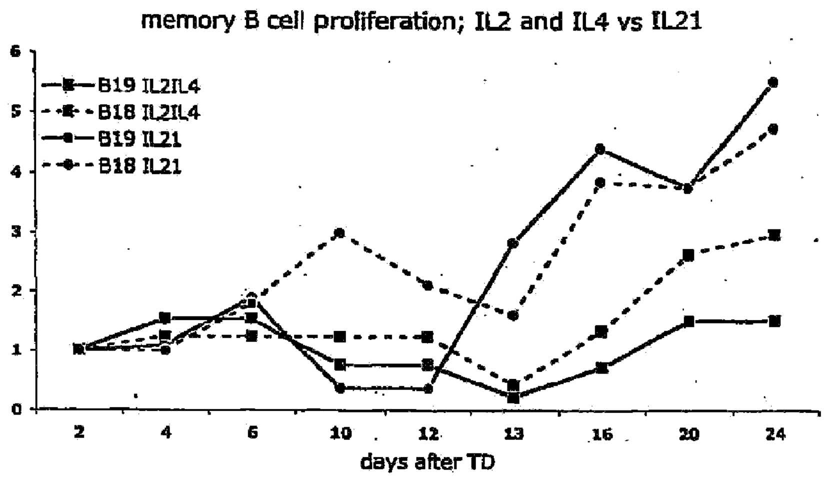


Figure 6b

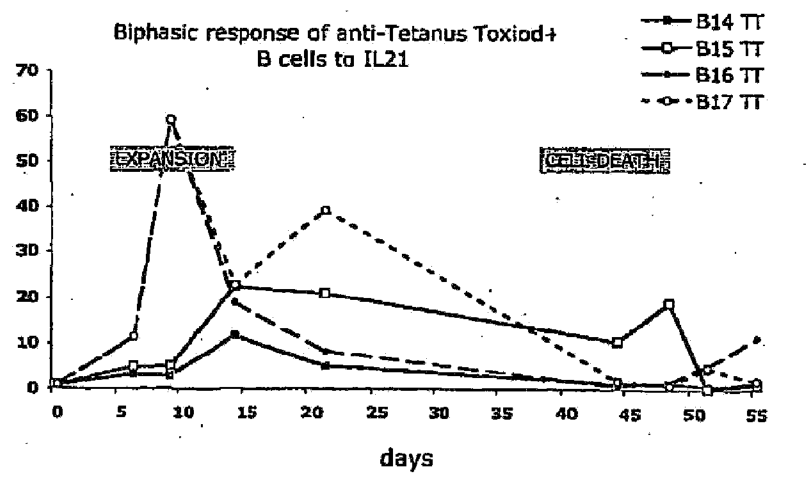
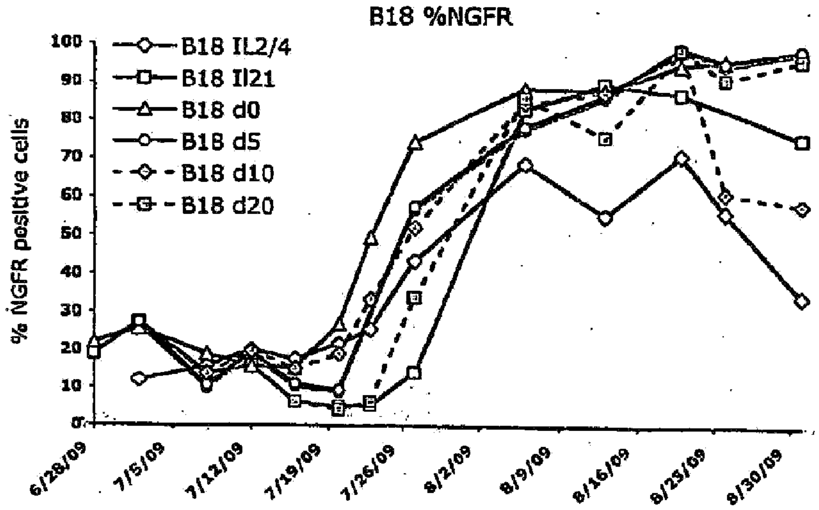


Figure 7


B18 %NGFR
% NGFR positive cells
B18 IL2/4
B18 Il21
B18 d0
B18 d5
B18 d10
B18 d20
6/28/09
7/5/09
7/12/09
7/19/09
7/26/09
8/2/09
8/9/09
8/16/09
8/23/09
8/30/09

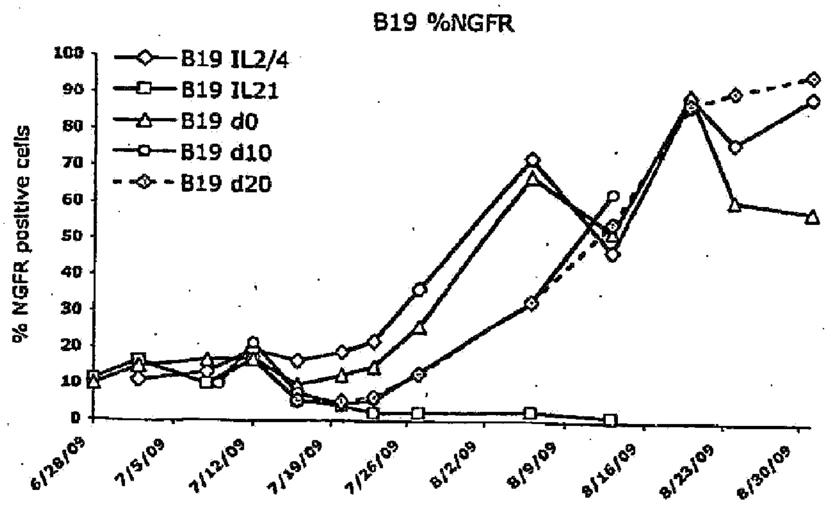

B19 %NGFR
% NGFR positive cells
B19 IL2/4
B19 IL21
B19 d0
B19 d10
B19 d20
6/28/09
7/5/09
7/12/09
7/19/09
7/26/09
8/2/09
8/9/09
8/16/09
8/23/09
8/30/09

Figure 8a

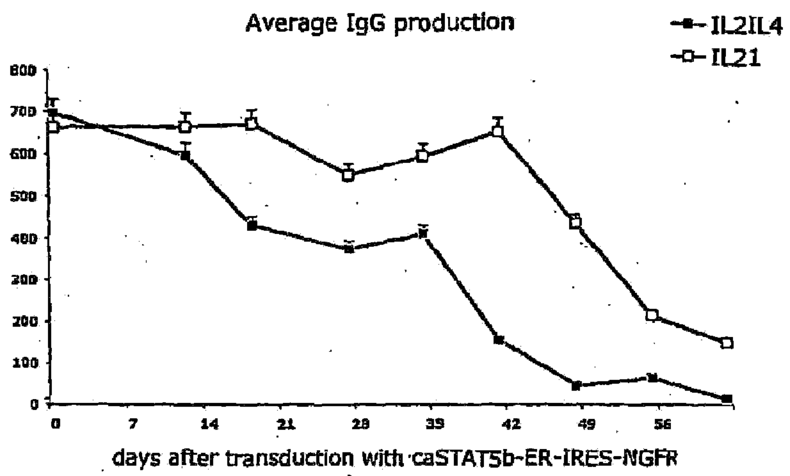


Figure 8b

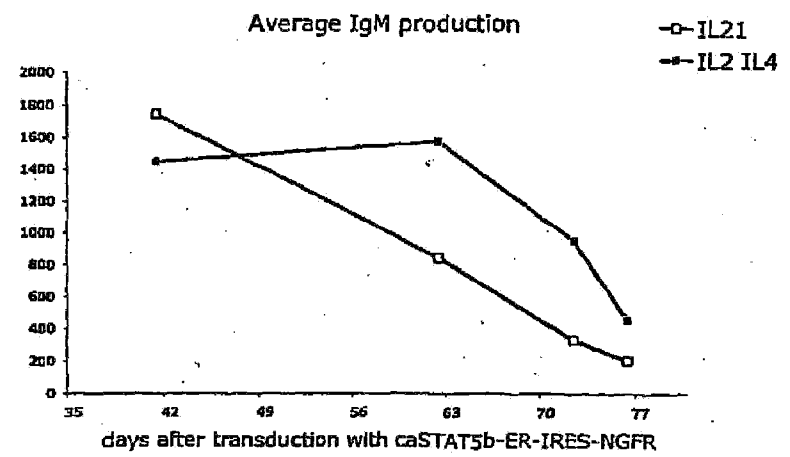


Figure 9

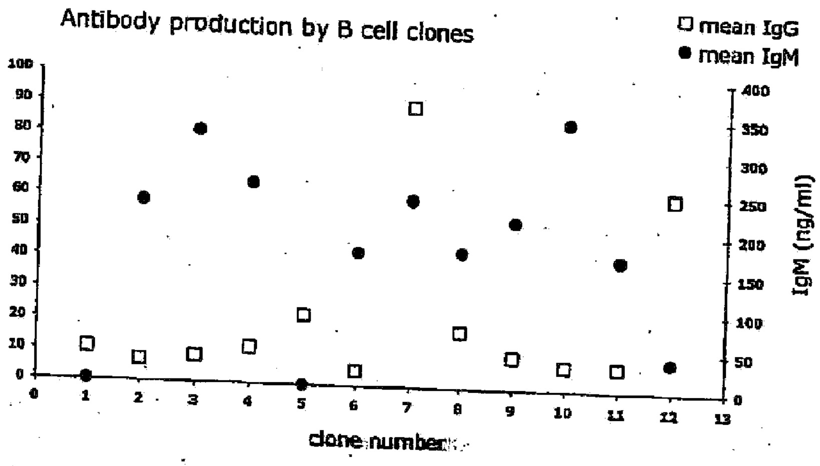

Antibody production by B cell clones
mean IgG
mean IgM
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
400
350
300
250
200
150
100
50
0
IgM (ng/ml)
clone number

Figure 10a

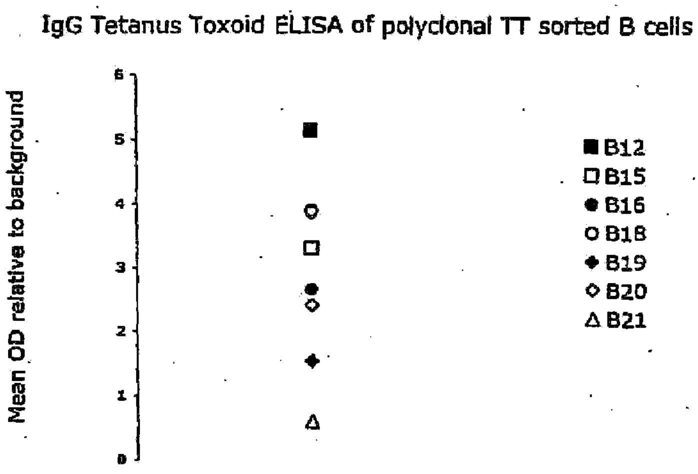


Figure 10b

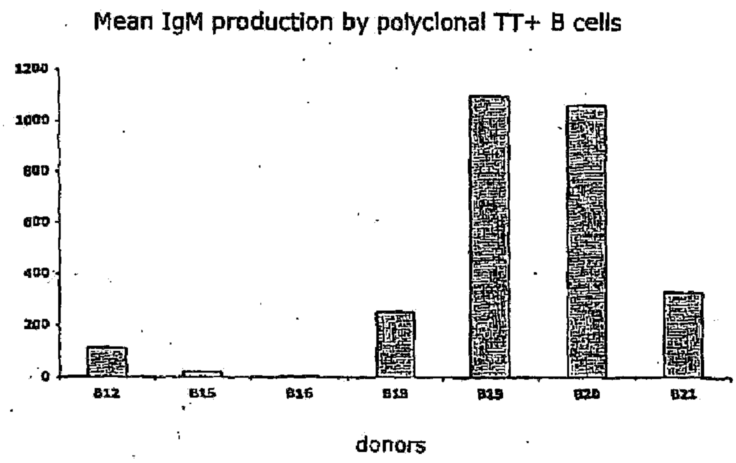


Figure 11

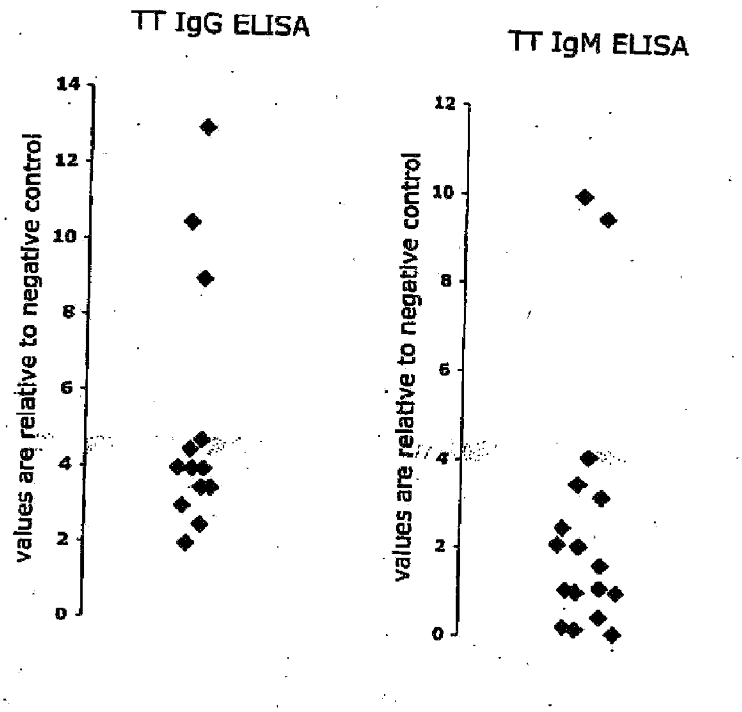

TT IgG ELISA
values are relative to negative control
14
12
10
8
6
4
2
0
TT IgM ELISA
values are relative to negative control
12
10
8
6
4
2
0

Figure 12a

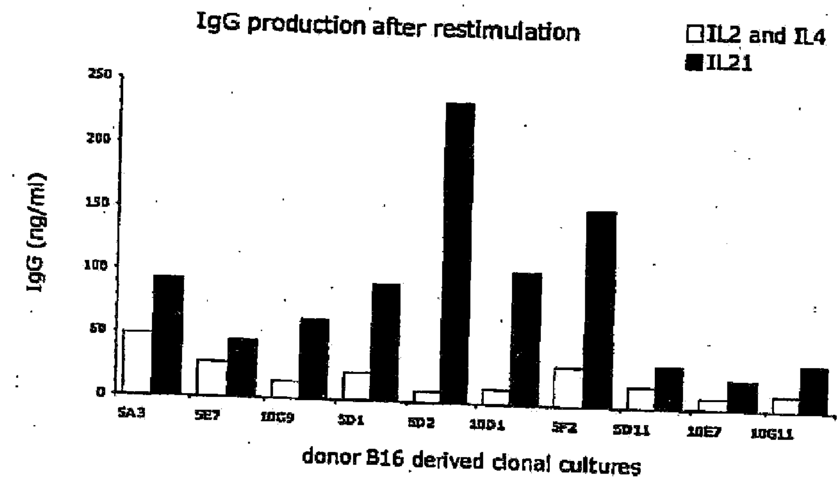


Figure 12b

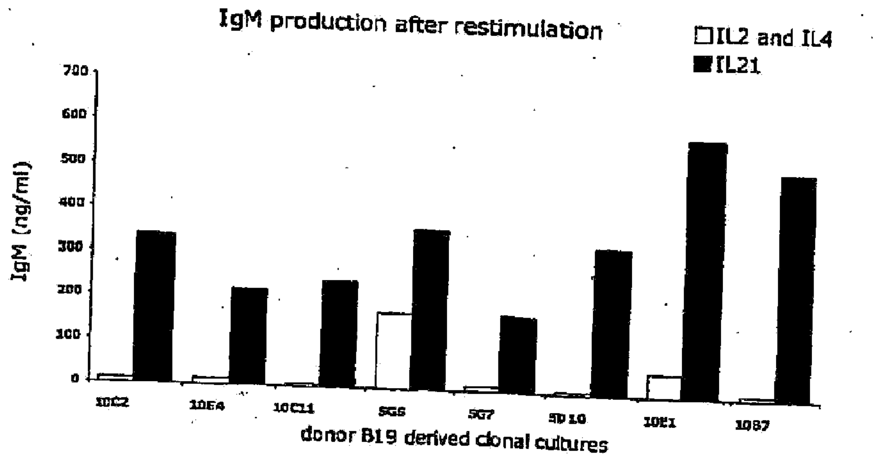


Figure 13a

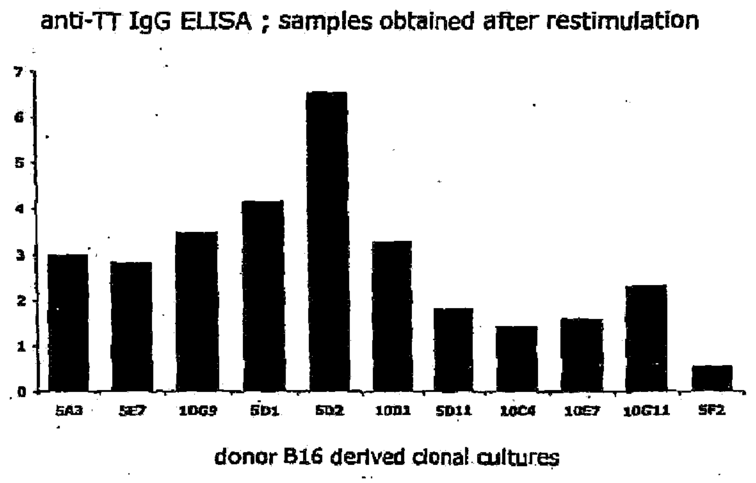


Figure 13b

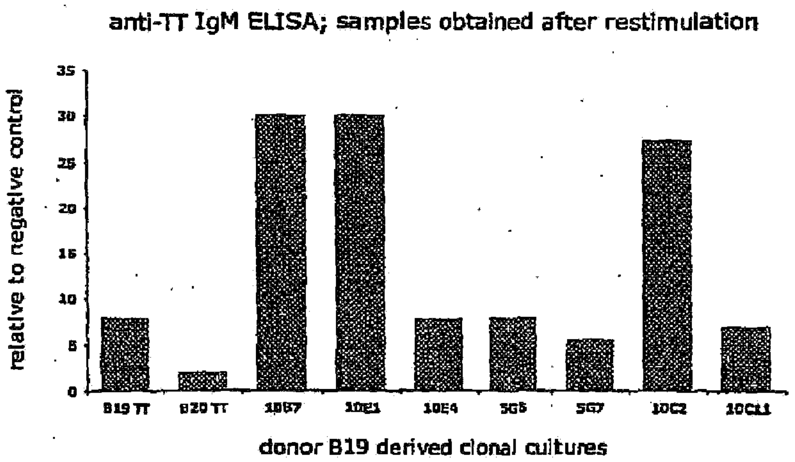


Figure 14

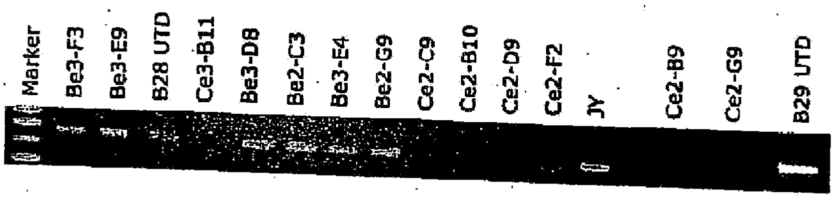

Marker
Be3-F3
Be3-E9
B28 UTD
Ce3-B11
Be3-D8
Be2-C3
Be3-E4
Be2-G9
Ce2-C9
Ce2-B10
Ce2-D9
Ce2-F2
JY
Ce2-B9
Ce2-G9
B29 UTD

Figure 15

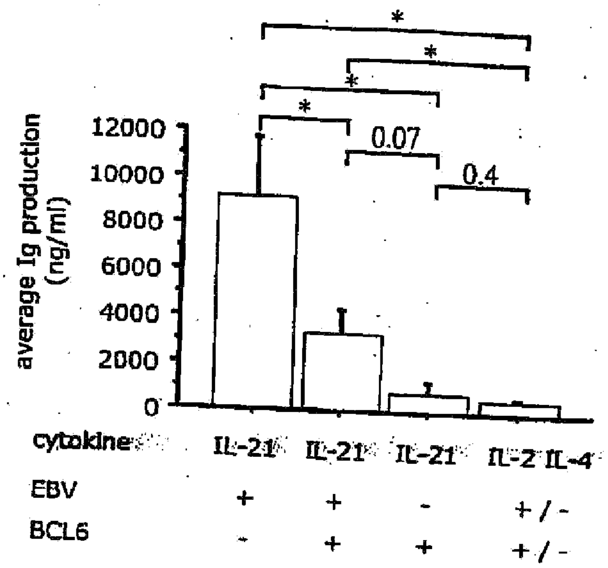

*
*
*
*
0.07
0.4
average Ig production (ng/ml)
12000
10000
8000
6000
4000
2000
0
cytokine
IL-21
IL-21
IL-21
IL-2 IL-4
EBV
+
+
-
+ / -
BCL6
-
+
+
+ / -

Figure 16

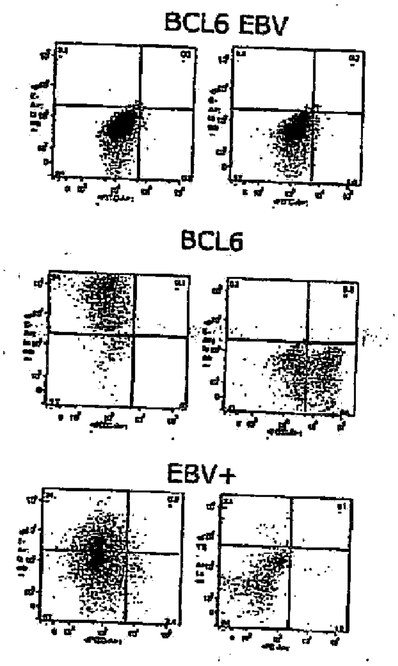


Figure 17

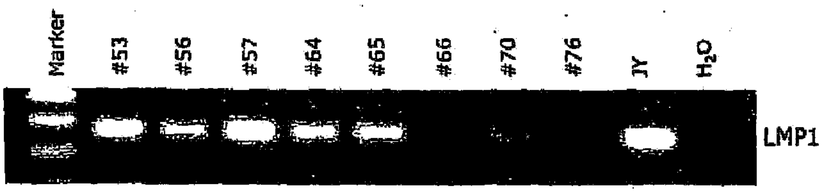
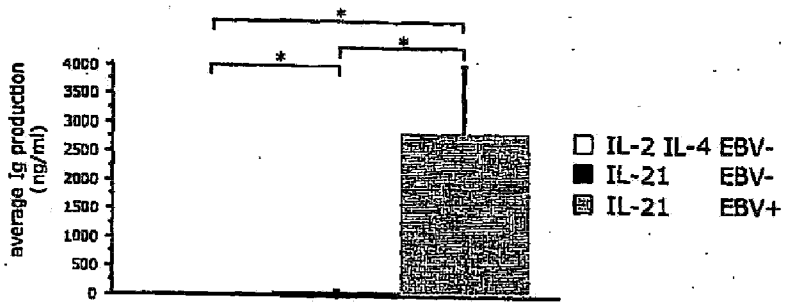


Figure 18



* indicates P < 0.05, unparallel Mann-Whitney test

Figure 19.

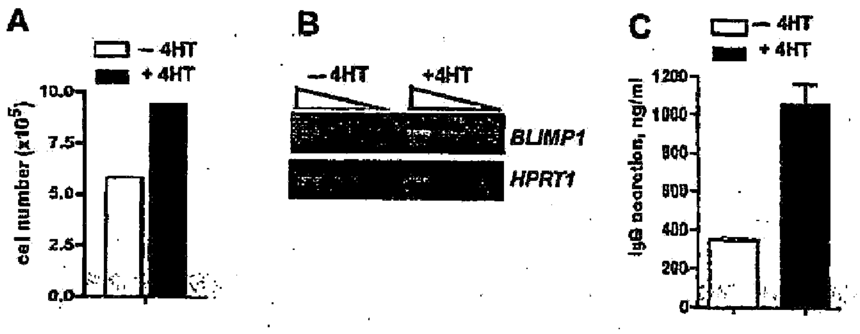

A
— 4HT
+ 4HT
cel number (x10⁵)
10.0
7.5
5.0
2.5
0.0
B
— 4HT
+4HT
BLIMP1
HPRT1
C
— 4HT
+ 4HT
IgG secretion, ng/ml
1200
1000
800
600
400
200
0

Figure 20.

Long-term growth of BCL-XL and BCL6-transduced B cells

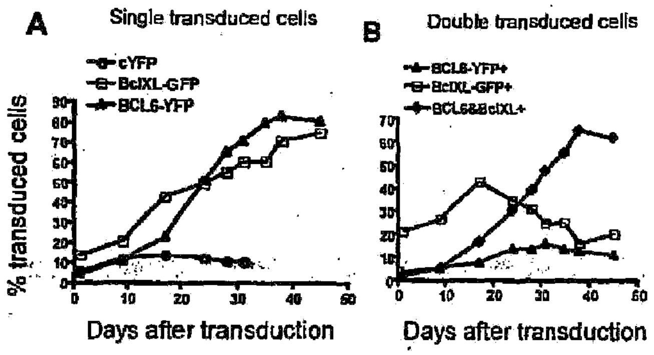


Figure 21.

IL-21 increased proliferation of B cells transduced with BCL-XL, BCL6, or BCL-XL+BCL6 double transduced cells.

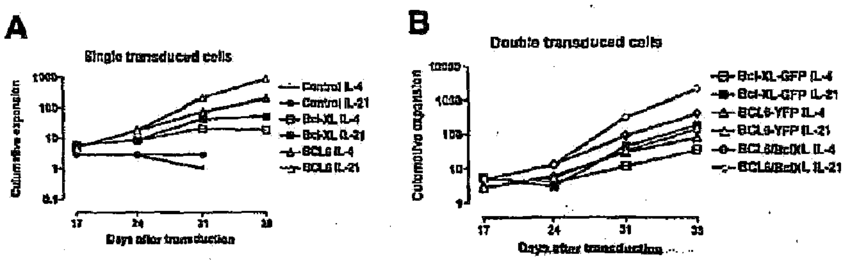


Figure 22.

Long-term cultures are EBV−

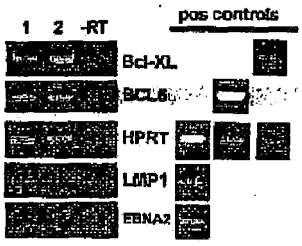


Figure 23.

Doubling time of BCL-XL, BCL6, and BCL-XL-BCL6 double transduced cells.

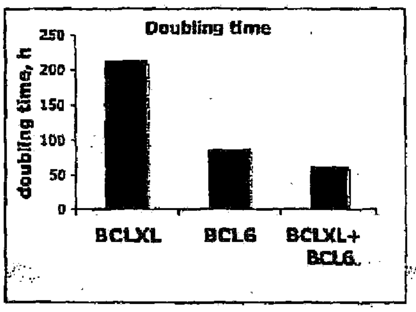
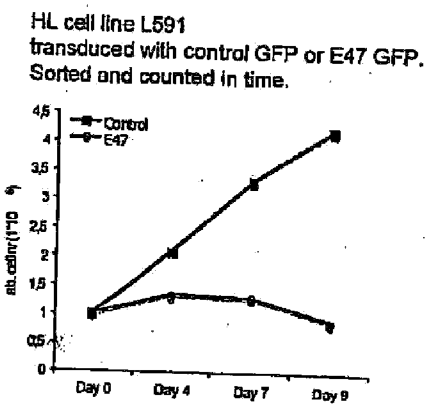


Figure 24.


HL cell line L591
transduced with control GFP or E47 GFP.
Sorted and counted in time.
Control
E47
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
Day 0
Day 4
Day 7
Day 9