Supplementary Information

Enhancement of lipopolysaccharide-induced nitric oxide and interleukin-6 production by PEGylated gold nanoparticles in RAW264.7 cells

Zhimin Liu [a, b], Wenqing Li [a, b], Feng Wang [b], Chunyang Sun [c], Lu Wang [a, b], Jun Wang [c*],

Fei Sun [a*]

1. Supplemental Materials and Methods

1.1 Syntheses of α-lipoyl-ω-methoxy poly(ethylene glycol) (LA-PEG-OCH$_3$)

The synthesis of LA-PEG-OCH$_3$ started from α-hydroxy-ω-methoxy poly(ethylene glycol) (HO-PEG-OCH$_3$, Mn = 5000). HO-PEG-OCH$_3$ (1.0 g, 0.200 mmol) was dried by azotropical distillation with toluene and dissolved in anhydrous CH$_2$Cl$_2$. The solution was added α-lipoic acid (60 mg, 0.291 mmol, Beijing Chemical Reagent Co.), N,N'-dicyclohexylcarbodiimide (DCC, 60 mg, 0.291 mmol, Beijing Chemical Reagent Co.) and 4-dimethylaminopyridine (DMAP, 28 mg, 0.228 mmol, Shanghai Medpep Co. Ltd.). The mixture was stirred at room temperature for 48 h, and then filtrated to remove dicyclohexylurea. Then it was concentrated and precipitated into ethylether twice to produce LA-PEG-OCH$_3$ with a yield of 70%. A Bruker AV300 NMR spectrometer was used for ^{1}H NMR spectra measurements and deuterium oxide (D$_2$O) was used as the solvent. ^{1}H NMR (Bruker AV300 NMR, D$_2$O, ppm): 1.43 (m, 2 H, -NHC(O)CH$_2$CH$_2$CH$_2$CH$_2$CH-), 1.63 (m, 4 H, -NHC(O)CH$_2$CH$_2$CH$_2$CH$_2$CH-), 1.66-2.04 (m, 2 H, -CHCH$_2$CH$_2$SS-), 2.39 (t, 2 H, -NHC(O)CH$_2$CH$_2$CH$_2$CH$_2$CH-), 2.46 (m, 1 H, -CHCH$_2$CH$_2$SS-), 3.16 (t, 2 H,

-CHCH$_2$CH$_2$SS-), 3.35 (s, 3 H, -OCH$_2$CH$_2$OCH$_3$), 3.35 (t, 2 H, -OCH$_2$CH$_2$NHC(O)-), 3.67 (m, ~452 H, -CH$_2$CH$_2$O-).

1.2 General Characterization of gold nanoparticles (AuNPs) and PEGylated gold nanoparticles (PEG@AuNPs)

Transmission electron microscope (TEM) measurements of AuNPs were performed on a JEOL 2010 high-resolution transmission electron microscope with an accelerating voltage of 200 KV. The sample was prepared by pipetting a drop of the aqueous solution of nanoparticles (0.1 mg ml^{-1}) onto a 230 mesh copper grid coated with carbon, and the sample was allowed to dry in air before the measurement. UV-vis absorption spectra of citric acid-stabilized AuNPs and PEG@AuNPs were obtained in a quartz cuvette using a Shimadzu UV 2501 spectrophotometer. The size and zeta potential measurements of PEG@AuNPs in aqueous solution were measured respectively by dynamic light scattering and a Malvern Zetasizer Nano ZS90 (Malvern, Worcestershire, UK) with a He-Ne laser (633 nm) and 90° collecting optics.

1.3 Endotoxin shock model

BALB∕c mice were purchased from Vital River Laboratories in Beijing, China, housed at the University of Science and Technology of China (USTC, Hefei, China) and maintained under temperature (22 °C) and light cycle (12 h light, 12 h dark)-controlled quarters. Mice were provided food and water ad libitum. This study received ethical approval from the institutional review boards of USTC.

Mice received a single injection of sterilized water or PEG@AuNP (45 mg kg^{-1}) *via* the tail vein, and an hour later, mice were administrated intraperitoneally with 0.9% NaCl or LPS (10 mg kg^{-1}). After treatment, mice were monitored for survival for 72 h. For cytokine measurements, six hours after treatment, blood samples were obtained and plasma IL-6 and TNF-α were measured by ELISA.
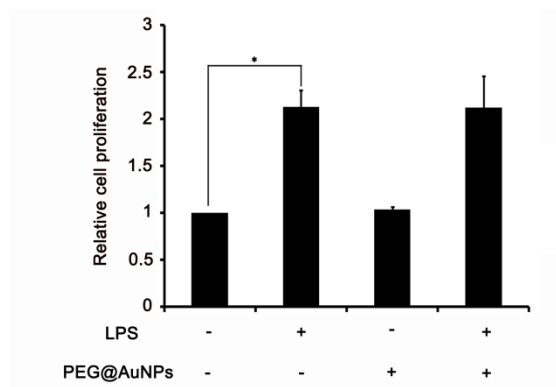
Supplemental Figures

Figure. S1 Proliferation of macrophages treated with vehicle (sterilized water and 0.9% NaCl), LPS, PEG@AuNPs, and PEG@AuNPs plus LPS for 72 h, respectively. Macrophages were pretreated with and without PEG@AuNPs (40 μg ml^{-1}) for 1 h, and then treated with and without LPS (100 ng ml^{-1}) for 72 h. The proliferation of macrophages was examined with Cell Counting Kit-8. Data shown represent the mean ± SEM of three independent experiments performed in triplicate. * $P < 0.05$. +, present; −, absent.
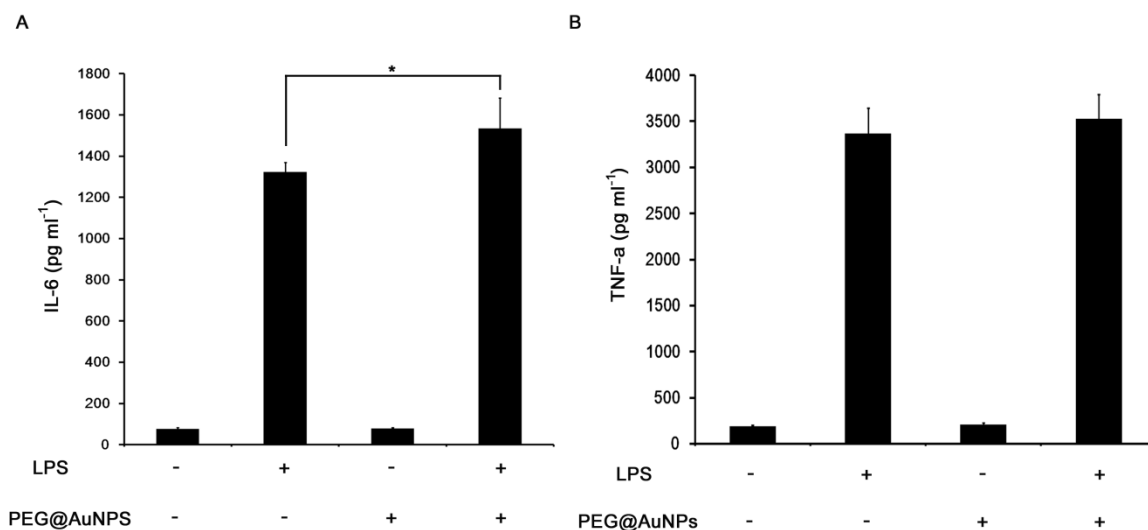
Figure. S2 Plasma IL-6 and TNF-α levels of mice (n = 5) treated with sterilized water plus 0.9% NaCl, sterilized water plus LPS, PEG@AuNPs plus 0.9% NaCl and PEG@AuNPs plus LPS. Mice were intravenously injected the mice with sterilized water or PEG@AuNPs, followed by intraperitoneal injection with 0.9% NaCl or LPS. After a 6-h treatment, blood samples were obtained and plasma IL-6 and TNF-α were measured by ELISA. Data shown represent the mean ± SEM of three independent experiments performed in triplicate. * $P <$ 0.05. +, present; −, absent.
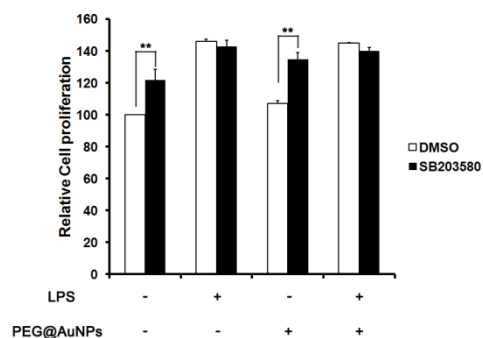
Figure. S3 Proliferation of macrophages pretreated with SB203580 (10 μM) or vehicle (DMSO), together with and without PEG@AuNPs (40 μg ml^{-1}), followed by treatment with and without LPS (100 ng ml^{-1}) for 72 h. The proliferation of macrophages was examined by CCK-8 kit. Data shown represent the mean ± SEM of three independent experiments performed in triplicate. ** $P < 0.01$. +, present; −, absent.

Group	Survival (%)						
n = 5	4 h	6 h	12 h	20 h	24 h	48 h	72 h
Au- LPS-	100%	100%	100%	100%	100%	100%	100%
Au- LPS+	100%	100%	100%	100%	100%	100%	100%
Au+ LPS-	100%	100%	100%	100%	100%	100%	100%
Au+ LPS+	100%	100%	100%	0	0	0	0

Supplementary Table 1 Survival of mice (n = 5) treated with sterilized water plus 0.9% NaCl, sterilized water plus LPS, PEG@AuNPs plus 0.9% NaCl and PEG@AuNPs plus LPS.