Supporting material

Cytometric Bead Array for cytokines measurement

Cytometric Bead Array (CBA) allows for simultaneous measurement of multiple analytes in single sample (46). Hepatic cytokines were measure by following manufacturer's instructions (BD Bioscience). Cytokines analyzed were TNF-α, IL-6, IL-1β, MCP-1, IL-12 and IL-10. After flow cytometry, the concentrations of cytokines were calculated based on standard curve using CBA analysis software.

Nitric oxide assay

NO-derived nitrite (NO_2^-) was measured using Greiss reagent system (Promega Corporation, USA) according to the two-step assay protocol. Briefly, nitrate from supernatant of liver homogenates were converted into nitrite using nitrate reductase. Nitrite were then converted into a deep purple azo compound that can be recorded at 540 nm. Following luminescence, production of NO_2^- was quantitated according to Nitrite Standard curve by following instructions.

Phagocytosis

Single cell suspension of BMDMs were incubated in ice bath for 10 min followed by adding FITC-labeled E.coli suspension using phagotest reagent kit (Glycotope Biotechnology GmbH, Germany). The mixture was moved to a warm water bath at 37.0°C for 5 to 30 min for phagocytosis and removed on ice again to stop the reaction. Cells washed and re-suspended with PBS were subjected to flow cytometry. Mean fluorescence intensity of FITC were calculated using BD FACS DIVA software.

Quantitative Real-time PCR

RNA was extracted from Cell pellets using Tri-reagent (Thermo) according to the manufacturer's instructions. cDNA was synthesized with reverse transcription kit (Toyobo, Japan). qRT-PCR was performed using SYBR green Real-time PCR master kit (Toyobo, Japan). Relative expression levels of target genes were calculated by normalized to levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with the $2^{-\triangle\triangle Ct}$ methodology.

Western Blot

Cells were lysed in RIPA buffer For Western blotting. Briefly, equal amounts of protein (20 μg) were separated by 20% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) blotting membranes. The membranes with blocked with a 5% (w/v) skim milk solution made with Tris-buffered saline containing 0.1% Tween-20 (Sigma) (TBST) for 1.5 hours, incubated with primary antibodies at 4℃ overnight, and then washed and incubated with peroxidase-coupled goat anti-rabbit or anti-mouse secondary antibody (1:5000; CST). Membranes were immersed in enhanced hemiluminescence (ECL) solution and exposed under a Chemi Doc XRS+ Molecular Imager (Bio-Rad,

USA). Autoradiograms densities were evaluated using Image-J software.

Antibodies purchased from either Cell Signaling Technology (CST) or Abcam were used with suggested dilutions. Antibodies were: Anti-MyD88 (1:1000, CST), anti-TRIF (1:1000, CST), anti-phospho-IkBα (1:1000, CST), anti-phospho-s468 p65 (1:1000, CST), anti-phospho-s276 p65 (1:1000, Abcam) and anti-p65 (1:1000, CST), anti-acetyl p65 (lys310) (1:1000, CST), anti-phospho-ERK 1/2 (1:2000, CST), anti-ERK 1/2(1:1000, CST), anti-phospho-JNK (1:2000, CST), anti-JNK (1:1000, CST), anti-phospho-c-raf1 (1:1000, CST), anti-phospho-P38 (1:1000, CST), anti-P38 (1:1000), anti-phospho-IRF3 (1:1000), anti-IRF3 (1:1000, CST), anti-IRF7 (1:1000), anti-phospho-Y550 BTK (1:1000, abcam), anti-phospho-Y223 BTK (1:1000, abcam), anti-BTK (1:1000, abcam), anti-β-actin (1:2000, CST).

Immunohistochemistry and immunofluorescence

Liver tissue were fixed with 4% paraformaldehyde and embedded in paraffin. Tissues were sectioned consecutively and imposed to hematoxylin - eosin staining. For immunostaining, continuous sections were incubated with primary antibodies overnight at 4℃. Sections were washed with PBS and followed by incubation with fluorescent labelled or HRP linked secondary antibody for 90 min. Images were taken under Nikon microscope. Primary antibodies were: anti-human CD68 (Abcam, 1:1000), anti-human S1009A (Abcam, 1:500), anti-human Fgl2 (Abnova, 1:200), anti-mouse F4/80 (Abcam, 1:1000), anti-mouse MPO (Abcam, 1:500), anti-mouse Fgl2 (Abnova, 1:500). Omission of the primary antibody was used as a control.

Antibodies for Flow cytometry

Antibodies used were: anti-CD45 (clone I3/2.3, Biolegend), anti-Ly6G (clone 1A8, Biolegend), anti-F4/80 (clone BM8, Biolegend), anti-CD11b (clone M1/70, Biolegend), anti-Ly6C (clone HK 1.4, Biolegend), anti-iNOS/NOS2 (clone 961-1144, BD Bioscience), anti-CD206 (clone C068C2, Biolegend), anti-IL6 (clone MP5-20F3, Biolegend),anti-TNFα(clone MP6-XT22, Biolegend)，anti-TGFβ(clone TW7-16B4, Biolegend)，anti-fgl2 (clone 6D9, Abnova), anti-MHC II (clone 28-8-6, Biolegend), anti-CD80 (clone 16-10A1, Biolegend),anti-CD86 (clone GL-1, Biolegend), anti-MHC I (clone 15-5-5, Biolegend).