**SUPPLEMENT**

**MATERIALS AND METHODS**

**Isolation of umbilical cord-derived mesenchymal stem cells (UCMSCs)**

Umbilical cords were obtained from full-term infants immediately after birth. The cord blood vessels were carefully removed to retain Wharton's jelly, which was digested in 1 mg/ml collagenase (Sigma, St. Louis, MO), and then placed in α-MEM (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS; Gibco, Gaithersburg, MD) and 1% antibiotic-antimycotic solution (Gibco, Gaithersburg, MD). Cells were incubated at 37°C with 5% CO2 in a humidified atmosphere. After 48 hours, the medium with the suspension of non-adhered cells was discarded, and the medium was changed twice a week thereafter. When reaching 80-90% confluence, cultured cells were detached with trypsin-EDTA (Gibco, Carlsbad, CA) and replated at a density of 6 × 103 cells/cm2 for subculture. UCMSC processing was performed in the MSC Laboratory of HealthBanks Biotech Co., Ltd. (Taipei, Taiwan).

**Identification of UCMSCs**

To evaluate the expression of surface markers, cultured UCMSCs were detached, washed, and resuspended in phosphate-buffered saline (PBS; Gibco, Gaithersburg, MD). After fixing and blocking, the cells were immunolabeled with fluorescein isothiocyanate or phycoerythrin (PE) conjugated mouse antihuman antibodies specific to CD34, CD45, CD14, CD29, CD44, CD73, CD90, CD105, HLA-A, HLA-B, HLA-C or HLA-DR. Nonspecific mouse IgG served as isotype control. All reagents were purchased from BD Biosciences. Data were analyzed by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA) with CellQuest software.

To assess the differentiation potential, cultured UCMSCs were detached from culture dishes and replated in 60-mm dishes. To promote osteogenesis, the UCMSCs were grown in Dulbecco's modiﬁed Eagle's medium (DMEM; Gibco, Gaithersburg, MD) with 10% FBS, 10 mM β-glycerophosphate (Sigma, St Louis, MO), 0.1 μM dexamethasone (Sigma, St Louis, MO), and 0.2 mM ascorbic acid (Sigma, St Louis, MO). After 2 weeks, osteogenic differentiation was demonstrated by mineralized deposits stainable with von Kossa stain (Cedarlane, Ontario, Canada). To induce adipogenesis, the UCMSCs were incubated in DMEM with 10% FBS, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine (Sigma, St Louis, MO), 0.1 mM indomethacin (Sigma, St Louis, MO), and 10 μg/ml insulin (Novo Nordisk A/S, Bagsværd, Denmark). After 2 weeks, adipogenic differentiation was confirmed by intracellular accumulation of lipid droplets stainable with oil red O (Sigma, St Louis, MO).

**Cecal ligation and puncture (CLP) model of sepsis in mice**

Six-week-old male C57BL/6 mice were provided by the National Science Council, and weighed about 25 g at the time of operation. CLP can lead to focal inflammation which subsequently becomes systemic as a consequence of continuous dissemination of endogenous intestinal bacteria. A CLP model closely resembles the process of septic peritonitis in humans. Before surgery, the mice were anesthetized by intramuscular injections of 75 mg/kg ketamine and 5 mg/kg xylazine. The distal one half of the cecum was ligated with a 4-0 silk tie after laparotomy. A single through-and-through perforation was made in the ligated segment with a 21-gauge needle, and a 1-mm column of fecal material was extruded through the puncture site. The cecum was then replaced into the abdomen and the abdominal incision was closed in two layers with 4-0 silk sutures. Sham-operated mice underwent the same procedure, including opening the peritoneum and exposing the bowel, but without ligation and needle perforation of the cecum. Immediately after CLP or sham operation, the mice were fluid resuscitated with subcutaneous injections of 50 ml/kg pre-warmed saline.

**Quantitative real-time polymerase chain reaction (Q-PCR)**

Liver tissue samples were collected in Trizol reagent (Invitrogen, Carlsbad, CA) immediately after sacrifice. The samples were then cut into pieces, milled with a tissue grinder, and filtered. Total RNA was extracted using Trizol reagent. Following extraction, the concentration of the RNA samples was measured spectrophotometrically at an optical density of 260/280 (NanoDrop Technologies, Wilmington, DE). cDNA was then synthesized using MMLV reverse transcriptase (Promega, Madison, WI) in the presence of oligo-dT primer (Promega, Madison, WI). The sequences of primers were as follows: MyD88, sense 5'-CTACAGAGCAAGGAATGTGACT-3' and antisense 5'-ATAGTGATGAACCGCAGGATAC-3'; NFκB, sense 5'-GCGACAAGGTGCAGAAAGA-3' and antisense 5'-TGAGAAAAGGAGCCTCGTG-3'; TRAM, sense 5'-GGTCAAGCAGTACCACTTCC-3' and antisense 5'-GAGACGCCTTAGCCTCCAGT-3'; IRF3, sense 5'-CAAGAGGCTTGTGATGGTCA-3' and antisense 5'-GCAAGTCCACGGTTTTCAGT-3'. The expression of GAPDH (sense 5'-GCCAAAAGGGTCATCATCTC and antisense 5'-CACACCCATCACAAACATGG) was used as an internal control to normalize the specific gene expression in each sample. According to the manufacturer’s instructions, Q-PCR was performed using cDNA samples with SYBR Green PCR Master Mix on an ABI 7300 Real-time PCR system (Applied Biosystems, Foster City, CA).

**Enzyme-linked immunosorbent assay for NFκB phosphorylation**

Liver tissue samples were collected and stored at -80°C until assayed. To determine NFκB phosphorylation, the samples were cut into pieces, milled with a tissue grinder, and filtered. Phosphorylated NFκB and total NFκB were measured using an EnzyFluoTM NFκB Phosphorylation Assay Kit (ENFκB-100) according to the manufacturer's instructions (BioAssay Systems, Hayward, CA).

**Western blot analysis**

Liver tissue samples were collected and stored at -80°C until assayed. After lysis, the samples (50 μg) were run on 12.5% SDS PAGE gels and blotted to membranes. The membranes were blocked with 5% BSA in TBS, and then incubated overnight at 4°C with anti-MyD88 or anti-TRAM antibodies. Anti-rabbit HRP antibody was used as a secondary antibody for 1 hour at room temperature. As a loading control, the same blots were re-probed with anti-α-tubulin and anti-mouse HRP antibodies. All reagents were purchased from GeneTex (Irvine, CA).

**Histopathologic and immunohistochemical analysis**

Immediately after sacrifice, liver tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5-μm thickness. After deparaffinization and rehydration, the sections were stained with hematoxylin-eosin. Immunohistochemical staining specific to mouse MyD88 and TRAM (Thermo, Rockfore, IL) was performed according to the manufacturer's protocol.

**Cytometric bead array immunoassay to determine serum cytokine levels**

Blood was obtained by cardiac puncture immediately after death. To determine circulating cytokine levels, serum was separated by centrifugation at 10,000 g for 10 minutes at 4°C, aliquoted, and stored at -80°C until assayed. The concentrations of monocyte chemotactic protein (MCP)-1, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin (IL)-6, and IL-10 were measured by cytometric bead array immunoassay (BD CBA Mouse Soluble Protein Flex Set System; BD Biosciences, San Jose, CA), according to the manufacturer's instructions. Briefly, a mixture of capture bead reagent (50 μl) was added to a serum sample (50 μl) and incubated for 1 hour at room temperature. Then 50 μl of the mixed PE detection reagent was added and further incubated for 1 hour. After washing, the samples were analyzed using flow cytometry (FACSCanto; BD Biosciences, San Jose, CA) with FCAP Array™ software.