Supplementary Information

Bone marrow stromal cells attenuate sepsis via PGE2 dependent reprogramming of host macrophages to increase their IL-10 production

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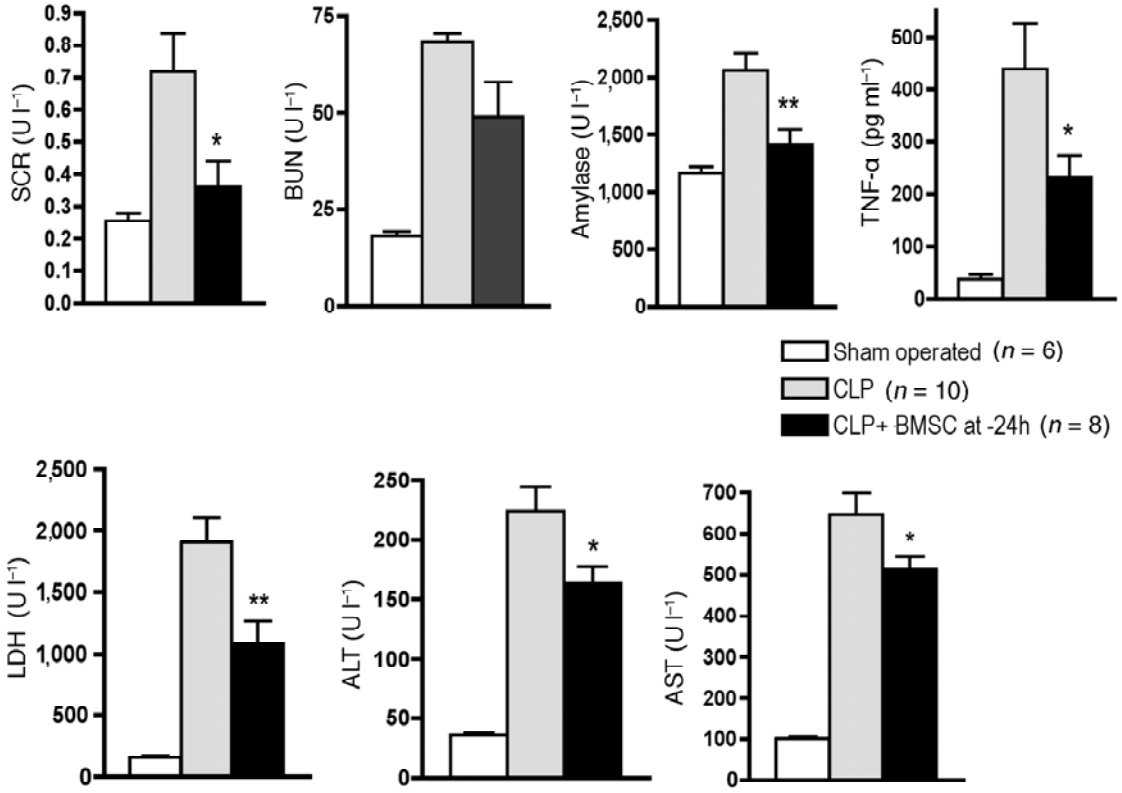
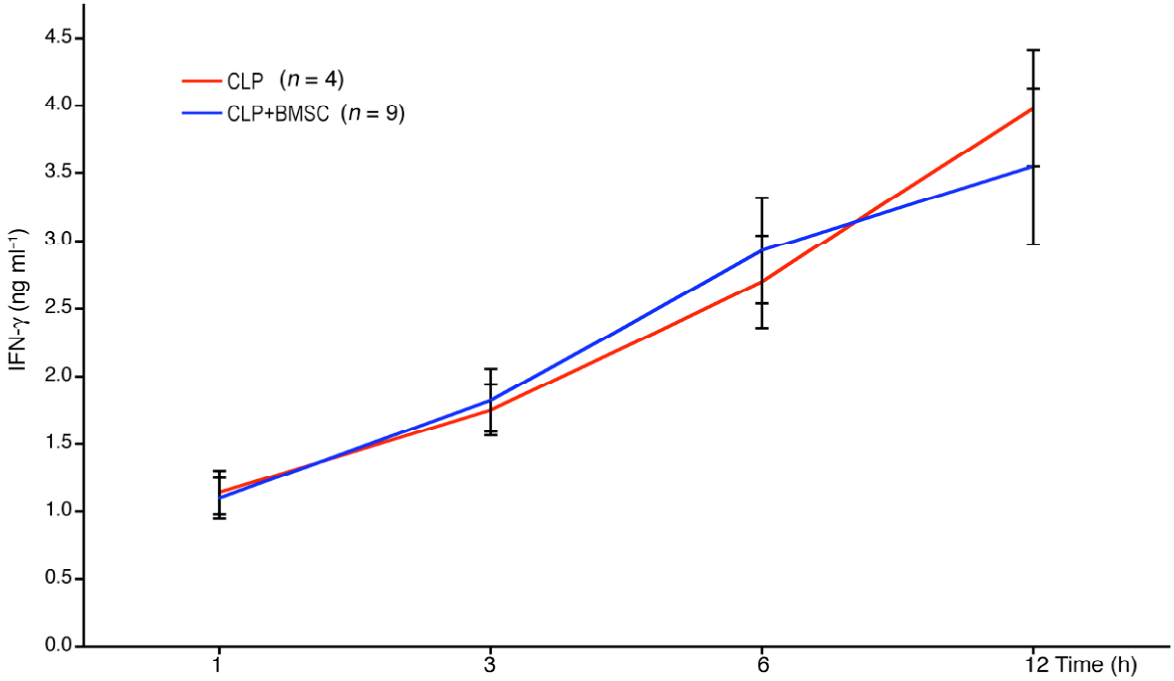
Supplementary Fig.1.



Preventive effect of BMSCs when injected 24 hours before inducing CLP.
Serum levels of creatinine (SCR) and blood urea nitrogen (BUN) reflect improved kidney function. Decreased amylase levels suggest improved pancreatic function. The levels of pro-inflammatory TNF-α are also decreased in the serum following treatment. Liver enzymes (LDH, ALT and AST) are also decreased suggesting improved liver function. Bars represent SEM, * $P < 0.05$; ** $P < 0.01$

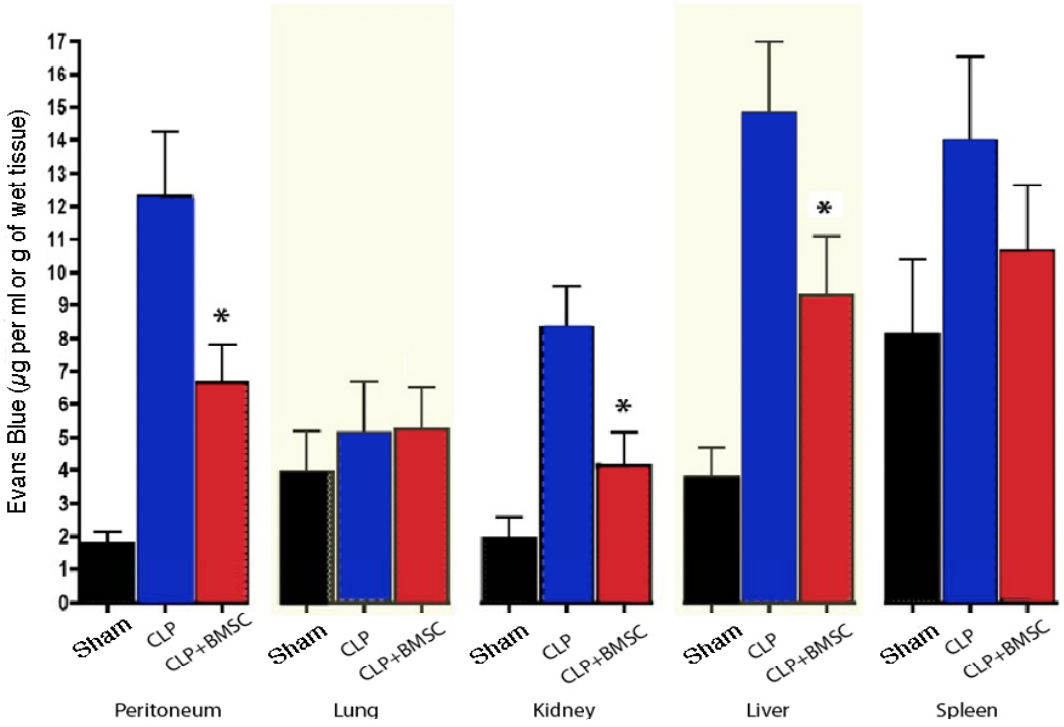
Supplementary Fig.2.



Serum levels of interferon gamma at a variety of time-points following the induction of CLP.

Following CLP induction the blood of 9 untreated and 10 BMSCs treated mice were sampled at each time point to determine the concentrations of IFN-γ. There was no difference at either time point between the two groups.

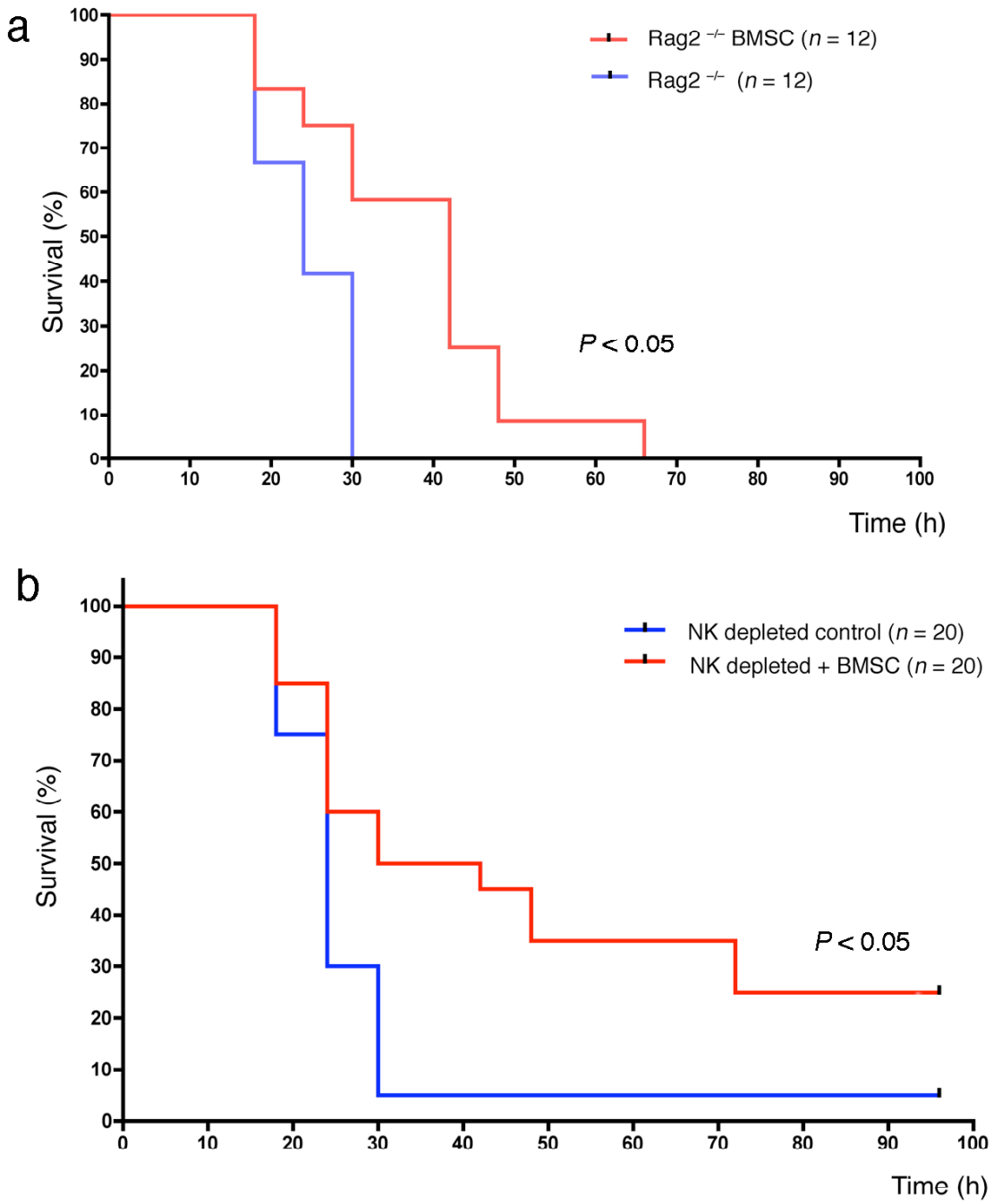
Supplementary Fig. 3.



Vascular permeability measurements in BMSCs treated and untreated CLP mice using Evans Blue dye (EB).

Thirty minutes before sacrifice the mice were injected intravenously with EB via the tail vein. Thirty min later - under anesthesia - the mice were infused with phosphate-buffered saline (PBS) through the right ventricle until blood was totally eliminated. The organs were then weighed and snap-frozen in liquid nitrogen. Following homogenization and centrifugation the supernatants were collected and EB was analyzed by measuring absorbance at 620 nm. Results were calculated from a standard curve of EB and expressed as micrograms of EB per 1 ml of peritoneal fluid or gram of organ (wet weight) for solid organ vascular permeability. Bars represent SEM, * $P < 0.05$

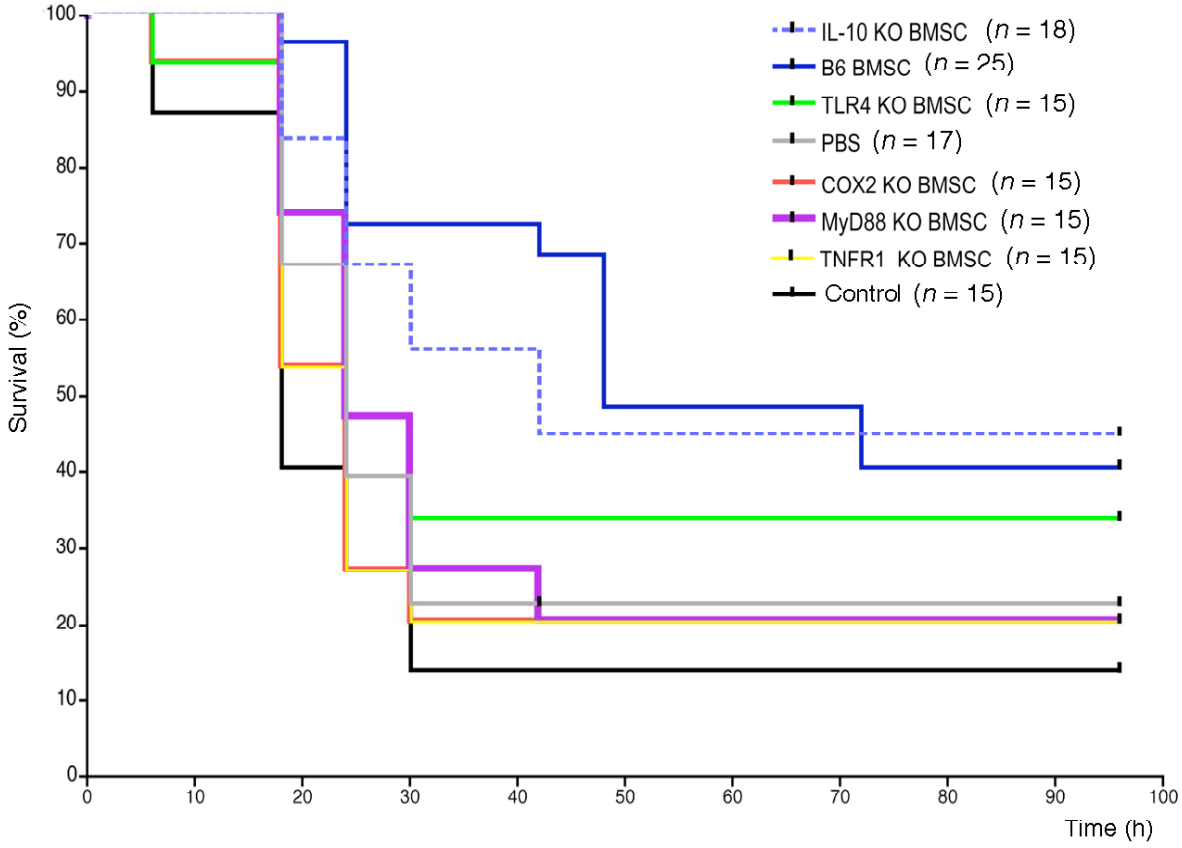
Supplementary Fig. 4.



Survival curves of mice deficient in B, T and NK cells.

Survival curve of BMSC treated septic $Rag2^{-/-}$ mice that genetically lack B and T cells and (b) mice with depletion of NK cells using asialoGM1 antibody. The BMSC treatment is still beneficial in both models, suggesting that B, T and NK cells are not necessary for the effect.

Supplementary Fig. 5.



Survival curves of CLP mice treated with BMSCs derived from a variety of transgenic mice.

Survival of mice with CLP that received iv injection of BMSCs derived from mice that genetically lack interleukin 10 (IL-10), toll-like receptor 4 (TLR4), cyclo-oxygenase 2 (COX2), myeloid differentiation primary response gene (88) (MyD88) and tumor necrosis factor receptor 1 (TNFR1). If any one of these factors - except for IL-10 - is lacking from the injected BMSCs the beneficial effect on survival is eliminated.

Supplementary Materials and Methods

Animals:

Mouse strain	Original Source	Our Source
C57/B6	JAX	JAX
Balb/C	JAX	JAX
FVB/NJ	JAX	JAX
Nos2$^{-/-}$	JAX	JAX
Rag2$^{-/-}$	JAX	JAX
Il10$^{-/-}$	JAX	A. Keane-Myers NIAID
Ifng$^{-/-}$	JAX	J. Weiss NCI
Ptgs1$^{-/-}$	JAX	J. Brown NIAID
Ptgs2$^{-/-}$	JAX	J. Brown NIAID
Ptger1$^{-/-}$		B. H.Koller UNC
Ptger2$^{-/-}$		B. H.Koller UNC
Ptger3$^{-/-}$		B. H.Koller UNC
Ptger4$^{-/-}$		B. H.Koller UNC
Tnfrsf1a$^{-/-}$	JAX	C. Cataisson NCI
Tnfrsf1b$^{-/-}$	JAX	C. Cataisson NCI
Tlr4$^{-/-}$	JAX	T. Merkel FDA
Myd88$^{-/-}$	JAX	T. Merkel FDA

Tissue harvest:

24 h after surgery blood was collected by cardiac puncture for measurement of serum markers of organ injury and cytokine response. Kidneys, livers, lungs and spleens were fixed in 10% formalin or snap-frozen in liquid nitrogen before storage at –80 °C until further study.

Macrophage depletion:

In the macrophage depletion experiment, liposomal clodronate (L-α- Phosphatidyl-cho line/cholesterol clodronate; Encapsula NanoSciences, Nashville, TN, USA) 50 mg kg^{-1} and 30 mg kg^{-1} was injected intravenously at 48 and 24 h before CLP. In the pilot study different doses were used to assess the depletion of blood monocytes, liver Kupffer cells and splenic machrophages. Plain liposome was injected in a group of septic (CLP) animals as control. This method is specific for depletion of monocytes and macrophages,

which undergo apoptosis following phagocytosis of the liposomal clodronate. Neither the clodronate filled nor the empty liposomes are considered toxic to the organs[1-3].

NK cell depletion:

In the NK cell depletion experiment, 0.5 ml rabbit anti-asialo-GM1 antibody (Wako Chemicals, Richmond, VA, USA) at a 1:10 dilution was injected intravenously 24 h before CLP. Rabbit nonspecific IgG antibody (Sigma Chemical, St. Louis, USA) was used instead of anti-asialo-GM1 in control CLP animal. The injection of anti-asialo-GM1 antibody results in nearly complete elimination of NK cell activity[4-6].

Pretreatment with anti IL-10 and anti IL-10 receptor antibody:

In anti IL-10 depletion experiment, anti IL-10 purified monoclonal antibody (Pierce Endogen, Rockford, IL, USA), anti IL-10 receptor antibody (BD Biosciences Pharmingen, CA, USA) or 200-300 µg purified rat anti mouse IgG monoclonal antibody (BD Biosciences Pharmingen, CA, USA) as isotype control were injected 30 minutes before CLP and BMSC injection.

Isolation of cells:

a. BMSCs

The bone marrow was flushed out of femurs and tibiae of mice in aseptic conditions, and cells obtained were cultured in Alpha-MEM supplemented with 20% heat inactivated FBS, 1% glutamine, and 1% Pen/Strep. To get rid of macrophage contamination we used magnetic cell sorting (Miltenyi) with the macrophage marker CD11b. Using FACS analysis we showed that all BMSCs were negative for the hematopoietic linage markers CD45, CD11b, and Gr-1. Cells were also shown to be able to differentiate into osteogenic, and adipogenic linages in vitro.

b. Macrophages

Macrophages were cultured from the bone marrow according to a protocol on the Invitrogen website (https://catalog.invitrogen.com/index.cfm?Fuse_action=iProtocol.unitSectionTree&objectId=66757375BD42FAA7D7D869E7BB3E9FFF&treeNodeId=E55125C5B15952216E54B782A4F0CC80) based on a description by Fortier and Falk[7]. Briefly: isolated bone marrow was plated in a plastic flask. Bone marrow stromal cells and resident macrophages were removed based on their plastic adherence, and non-

adherent cells were cultured for 7 days in the presence of 10 ng ml^{-1} recombinant M-CSF (Peprotech).

Peritoneal macrophages were harvested after 5 days of intraperitoneal injection with 1 ml of sterile 4% thioglycollate. Peritoneal cavity was flushed out by complete medium, and cells were washed and plated afterwards.

RAW 264.7 cells were purchased from ATCC. Cells were grown in the same medium as bone marrow and peritoneal macrophages.

c. Skin fibroblasts

Skin fibroblasts were isolated by explant culture of biopsies taken from the backs of adult mice (8-12 weeks old). After removal of hair, specimens were cut into 1-2 mm^3 fragments and allowed to adhere to the surface of 100-mm^2 tissue culture dishes. The same medium used for BMSCs was added subsequently to plates. Cells were passaged when the explant-derived cells had become confluent. After 2-3 passages, based on cell morphology, the cell population was composed of exclusively fibroblasts. (This is the only cell type that can grow out from the dermis in these conditions).

Blood chemistry and cytokine measurements:

Aspartate transaminase (AST), alanine transaminase (ALT), amylase, creatine kinase (CK), and lactate dehydrogenase (LDH) were measured using an autoanalyzer (Hitachi 917, Boehringer Mannheim, Indianapolis, IN, USA). Serum creatinine was measured by high-performance liquid chromatography (HPLC)[8]. Tumor necrosis factor-α (TNF-α), IL-6, IL-10 and IFN-γ were measured by ELISA (Quantikine mouse, R&D systems, Inc., Minneapolis, MN, USA). The number of animals in each group were between 8-16.

Assessment of microvascular permeability:

The microvascular leakage was studied using Evans Blue dye (EB) as described previously[9-11] with slight modifications. Thirty min before sacrifice the mice were injected intravenously with EB (Sigma-Aldrich, MO, USA) 2 ml kg^{-1} at 1% in 0.9% sodium chloride via the tail vein. Thirty min later under anesthesia the mice were infused with phosphate-buffered saline (PBS) through the right ventricle until blood was totally eliminated. The organs were weighed, snap-frozen in liquid nitrogen, and stored at -80 ºC. The kidneys were homogenized in 1 ml formamide and incubated at 55 ºC for 18 h. The supernatant were collected after centrifugation at 10,000 g for 30 min. For peritoneal

vascular permeability, peritoneal fluid was collected after centrifugation at 3,000 g for 10 min. The amount of EB in the supernatant was analyzed by measuring absorbance at 620 nm. Results were calculated from a standard curve of EB and expressed as micrograms of EB per gram of organ (wet weight) for solid organ vascular permeability or 1 ml of peritoneal fluid for peritoneal vascular permeability.

Bacterial numbers in blood and peritoneal cavity:

Peritoneal fluid or blood was analyzed for the presence of bacteria by dilution plating onto blood agar (Remel, Lenexa, KS, USA) and colony counting after 24 hr incubation at 37 ˚C. Bacterial counts were log normalized.

Macrophage-BMSC coculture experiments:

When PGE2 ELISA was performed cells were cultured in 24-well plates (1 million BMSCs/1 million macrophages/500 μl). For COX activity assay cells were grown in T-75 flasks, and 30 million cells were assayed for each condition. For NFkB assay cells were grown in T-75 flasks, and 10 million cells were assayed for each condition.

Before performing Western blots, COX activity assay, or NFkB assay BMSCs were separated from macrophages using CD11b magnetic beads (Miltenyi).

When performing inihibitor/neutralizing antibody experiments compounds or antibodies were added at the initiation of the coculture. In case of ODQ inhibitor experiments BMSCs or macrophages were pretreated for 4 hours before addition of LPS. Supernatants were assayed for IL-10 after 7 hours of LPS treatment.

Used agents: IL-10R neutralizing antibody (clone 1B1.3a) 5 μg ml^{-1} (BD Biosciences), TGF-beta neutralizing antibody (clone 1D11) 10 μg ml^{-1} (kind gift from Richard Dipaolo, NIAID, NIH), IFN-gamma neutralizing antibody (clone XMG1.2) 5 μg ml^{-1} (eBioscience), TNF-alpha neutralizing antibody (clone MP6-XT22) 5 μg ml^{-1} (R&D Systems).

Indomethacin 5 μM, NS398 1 μM, SC560 1 μM, L-NAME 1 mM, 1-MT 1 mM, PTX 0.5 μg ml^{-1}. A6809 (EP2 receptor antagonist) 10 μM, GW627368X (EP4 receptor antagonist) 10 μM ODQ (guanylyl cyclase inhibitor) 50 μM SC51089 10 μM, SNAP 10 μM.

Measurements of myeloperoxidase (MPO) in organs:

MPO was measured using the Mouse MPO ELISA kit (HK210; Hycult biotechnology, Uden, Netherlands) according to the protocol of the manufacturer. Briefly, kidney and liver tissue samples from CLP (n = 6 (kidney) and 8 (liver)) CLP+BMSC (n = 7 (kidney) and 8 (liver)) and SHAM/BMSC (n = 2) were homogenized with a Kontes pellet pestle motor (Fisher Scientific, Pittsburgh, PA) in 600 µl of T-PER buffer (Pierce, Rockford, IL) containing protease inhibitor cocktail (Complete Mini, EDTA free; Roche, Indianapolis, IN). The protein concentrations of tissue homogenates were determined using the Micro BCA Protein Assay (Pierce), and 10 µg of the lysates were measured into the assay wells. All samples were assayed twice in duplicate. Data are presented as ng of MPO enzyme 100 μg^{-1} of tissue protein.

Histologic examination:

The 10% formalin-fixed, paraffin-embedded 10 µm thin kidney sections were stained with periodic acid-Schiff reagent (PAS) (Sigma Chemical Co.). Histologic changes in the cortex and in the outer stripe of the outer medulla (OSOM) were assessed by quantitative measurements of tissue damage as previously described[12]. Tubular damage was defined as tubular epithelial swelling, loss of brush border, vacuolar degeneration, necrotic tubules, cast formation, and desquamation. The degree of kidney damage was estimated at x400 magnification using five randomly selected fields for each animal by the following criteria: 0, normal; 1, areas of damage <25% of tubules; 2, damage involving 25% to 50% of tubules; 3, damage involving 50% to 75% of tubules; 4, damage involving 75% to 100% of tubules.

Immunohistochemical analysis of activated caspase-3 in spleen:

Immunohistochemical staining of 4 µm paraffin sections was performed as previously described with an anti-activated caspase-3 (a marker of apoptosis) antibody (Cell Signaling Technology, Beverly, MA, USA)[13], and was visualized using diamino-benzidine. The number of positively stained (brown) cells was examined in 5 randomly chosen x400 fields of white pulp and expressed as positive cells per high power field (HPF).

Cell labeling for histological cell tracking:

BMSCs were fluorescently labeled by incubation with 10 μM carboxy fluorescein diacetate (CFDA: Vybrant cell tracker kit, Molecular Probes, Eugene, OR, USA) in serum free tissue culture medium (SFM) for 30 min. After trypsinization, labeling was confirmed by fluorescence microscopy and cells were kept on ice until use. One million CFDA- labeled green fluorescent BMSCs were injected intravenously via the tail vein. For cell tracking studies mice were sacrificed at 1, 6 and 24 h after injection of labeled BMSCs and 8 μm snap-frozen lung, liver, kidney and spleen sections were cut and either examined for native green fluorescence or immunostained as described below.

Immunohistochemistry for histological cell tracking:

Eight micron thick sections of the various organs were used. The endogenous peroxidase activity was blocked using 3% H_2O_2 in absolute methanol for 20 minutes. The sections were then rehydrated, washed in PBS and incubated in a blocking solution (2.5% bovine serum albumin in PBS) for 1 hour at room temperature. Excess solution was discarded and the sections incubated with the 1:100 dilution of a primary antifluorescein/ Oregon green antibody (Molecular probe) overnight at 4 ºC. The staining was visualized using the ABC technique and the VectaStain Elite ABC kit (Vector Laboratories, Burlingame, CA). The slides were developed in 3,3-diaminobenzidine (Sigma FASTDAB tablet, Sigma Chemical, St. Louis, MO). The tissues were then counterstained with Mayer's hematoxylin, dehydrated and mounted.

Visualizing cell-cell interactions in the lung:

In a separate experiment to explore the detail of cell-cell interactions, labeling by nanocrystal Qtracker 655 (Invitrogen, Carlsbad, CA, USA) was used to pre-label the BMSCs before injection according to the manufacturer's instruction. To visualize the alveolar macrophages an antibody was used that recognizes a monocyte/macrophage/ microglia specific calcium binding protein (Iba-1, Wako Chemical Industries, Ltd.)[14]. Vascular endothelium was also immunostained using a rat monoclonal antibody (Abcam, ab7388) that recognizes CD31 or PECAM (Platelet Endothelial Cell Adhesion Molecule-1). The secondary antibodies were highly cross-absorbed anti-rabbit IgG-AlexaFluor 488 and anti rat IgG-Alexafluor-594 (Molecular Probes/Invitrogen). Immunostaining controls were done using no primary and/or no secondary antibodies.

Flow Cytometry Analysis

Flow cytometry was performed on FACSCalibur with CellQuest Pro software (BD Biosciences) using directly conjugated mAbs against the following markers: CD45-FITC, CD11b-PE, Gr1-FITC, F4/80-APC, CD3-PE, CD19-FITC, CD49b-APC, CD11c-APC, MHCII-FITC with corresponding isotype matched controls. To block unspecific antibody, binding cells were incubated with purified anti-mouse CD32/CD16 Mouse Fc Block prior to adding staining antibodies. For live/dead cell separation 7-AAD staining was used. All reagents were purchased from BD Biosciences. FACS buffer used for incubating cells with antibodies, and for washing, consisted of 1X PBS containing 0.5% BSA and 0.05% Azide. Preparation of the samples for FACS analysis was performed using standard FACS staining protocols.

For intracellular staining APC-IL-10 antibody (BD) was used. Isolated and Brefeldin A (eBioscience) treated cells were first surface stained, and then fixed and permeabilized with Cytofix/Cytoperm solution (BD). The cells were then resuspended in Perm/Wash buffer (BD), blocking step was performed with mouse IgG (BD) and the cells were incubated with APC-labeled anti-mouse IL-10. After washing, the cells were used for flow cytometric analysis.

COX activity assay and PGE2 ELISA measurements were performed according to the manufacturer's instructions. (Cayman Chemical).

Determination of nuclear NFkB

For determining the amount of nuclear NFκB (p65) first nuclear protein extracts were prepared using Nuclear Extract Kit (Active Motif), next following Micro-BCA standardization (Pierce) TransAM (Active Motif) assay was performed to determine the amount of translocated p65. Prior to performing COX activity assay, or nuclear NFκB detection BMSCs were isolated from the co-culture using CD11b magnetic beads (Miltenyi).

Western Blots

LPS stimulated wild type, TLR4 KO or neutralizing anti TNF-α pretreated BMSCs or macrophages were isolated from the co-culture (described above) using CD11b magnetic beads (Miltenyi). Isolated cells were lysed in M-PER lysis buffer (Pierce) containing protease inhibitors (Complete Mini EDTA, Roche). Total protein concentrations were

determined using the Micro BCA kit (Pierce). Fifteen micrograms of the total protein lysates were run on 4-12% Bis Tris gels and blotted to nitrocellulose membranes (Invitrogen). The membranes were blocked with 4% nonfat dry milk in TBS (1x TBS, 0.01% Tween 20) and incubated overnight at 4 °C with COX2 rabbit polyclonal antibody (Cayman) or iNOS rabbit polyclonal antibody (Biomol). As a secondary antibody, anti rabbit HRP (Jackson Immunoresearch) was used for 1 hour at RT. The blots were visualized with Western lightning enhanced chemiluminescence reagents (Perkin Elmer). The membranes were stripped and reprobed with a mouse monoclonal anti beta-actin antibody (Sigma) and an anti mouse HRP secondary (Jackson Immunoresearch.). Density measurements and quantification were performed using ImageJ Software (NIH, Bethesda).

Summary Table of all reagents used:

Name of Reagent/Kit	Concentration used	Source	Catalog #
PGE2 EIA kit		Cayman Chemicals	514010
COX activity assay		Cayman Chemicals	760151
AH 6809 EP2 antagonist	10 μM	Cayman Chemicals	14050
GW 627368X EP4 antagonist	10 μM	Cayman Chemicals	10009162
Indomethacin COX1/2 inhibitor	5 μM	Cayman Chemicals	70270
ODQ guanylyl cyclase inhibitor	50 μM	Cayman Chemicals	81410
SNAP nitrogen-oxide donor	10 μM	Biomol	CN210
SC-51089 EP1 antagonist	10 μM	Biomol	RA113-0005
NS-398 COX2 inhibitor	1 μM	Sigma	N194
SC-560 COX1 inhibitor	1 μM	Sigma	S2064
L-NAME iNOS inhibitor	1 mM	Sigma	N5751
1-Methyl-D-tryptophan IDO inhibitor	1 mM	Sigma	452483
Pertussis toxin EP3 inhibitor	0.5 μg ml^{-1}	Sigma	P7208
Lipopolysaccharides from Escherichia coli 0111:B4	1 μg ml^{-1}	Sigma	L4391
Evans Blue Dye		Sigma	E2129
Nuclear Extract Kit		Active Motif	40010
TransAM NFκB p65		Active Motif	40096
IL-10 neutrazlinig antibody clone 2A5	200-300 μg/mouse	Pierce Endogen	MM010
IL-10R neutralizing antibody clone 1B1.3a	5 μg ml^{-1} in vitro or 200-300 μg/mouse	BD Biosciences	550012
TGF-beta neutralizing antibody clone 1D11	10 μg ml^{-1}	R&D systems	MAB 1835
IFN-gamma neutralizing antibody clone XMG1.2	5 μg ml^{-1}	eBioscience	14-7311
TNF alpha neutralizing antibody clone MP6-XT22	5 μg ml^{-1}	R&D Systems	MAB4101
Anti-Asialo GM1 Antibody	50 μl diluted 1:10 in 500 total μl	Wako Chemicals	986-10001
CD45 FITC clone 30-F11	1 μg/million cells	BD Biosciences	553080
Cd11b PE clone M1/70	1 μg/million cells	BD Biosciences	553311
Gr-1 FITC clone 1A8	1 μg/million cells	BD Biosciences	551460
CD3 PE clone 17A2	1 μg/million cells	BD Biosciences	555275
CD19 FITC clone 1D3	1 μg/million cells	BD Biosciences	553785

CD49b APC clone HMa2	1 μg/million cells	BD Biosciences	558295
CD11c APC clone HL3	1 μg/million cells	BD Biosciences	550261
IL-10 APC clone JES5-16E3	1 μg/million cells	BD Biosciences	554468
MHCII FITC	1 μg/million cells	BD Biosciences	553569
CD16/CD32 blocking antibody clone 2.4G2	1 μg/million cells	BD Biosciences	553142
7-AAD staining	0.25 μg/million cells	BD Biosciences	559925
BD Cytofix/Cytoperm		BD Biosciences	554722
BD Perm/Wash		BD Biosciences	554723
F4/80 APC clone BM8	1 μg ml^{-1}	eBioscience	17-4801
Brefeldin-A Solution	3 μg ml^{-1}	eBioscience	00-4506-51
Vybrant® CFDA SE Cell Tracer Kit		Invitrogen	V12883
MPO ELISA kit		Hycult Biotechnology	H210
Collagenase, Type 1	300 U ml^{-1}	Worthington Biochemical	LS004196
Deoxyribonuclease I	50 U ml^{-1}	Worthington Biochemical	LS006328
CD11b microbeads		Miltenyi Biotec	130-049-601
Mouse IL-10 Quantikine ELISA Kit		R&D Systems	M1000
Mouse IL-6 Quantikine ELISA Kit		R&D Systems	M6000B
Mouse IFN-gamma Quantikine ELISA Kit		R&D Systems	MIF00
Mouse TNF-alpha/TNFSF1A Quantikine ELISA Kit		R&D Systems	MTA00
Recombinant Murine M-CSF		Peprotech	315-02
Liposomal clodronate	50 and 30 mg kg^{-1}	Encapsula NanoSciences	
Fetal Bovine Serum		BenchMark	100-106
MEM Alpha		Gibco	12571
GlutaMax		Gibco	35050
Pen Strep		Gibco	15140
Trypsin-EDTA		Gibco	25300
Micro BCA Protein Assay kit		Pierce	23235
M-PER mammalian Protein Extraction Reagent		Pierce	78501
Complete Mini, EDTA-free		Roche	11 836 170 001
	Dilution		
iNOS rabbit polyclonal	1/1000	Biomol	SA-200
COX2 rabbit polyclonal	1/200	Cayman	160106
anti rabbit IgG HRP	1/2000	Jackson	711-036-

		Immunoresearch	152
beta-actin mouse monoclonal	1/2000	Sigma	A 5441
anti mouse IgG HRP	1/2000	Jackson Immunoresearch	715-036-151

Nupage LDS Sample buffer (4x)	Invitrogen	NP0007
Nupage MES SDS Running buffer (20x)	Invitrogen	NP0002
Nupage Transfer buffer (20x)	Invitrogen	NP0006-1
Nitrocellulose membrane, 0.45 μm pore size	Invitrogen	LC2001
Nupage Antioxidant	Invitrogen	NP0005
Nupage 4-12% Bis-Tris Gel	Invitrogen	NP0321BOX
XCell SureLock Mini-Cell with XCell II Blot Module kit	Invitrogen	EI0002
Novex Sharp Pre-Stained Protein Standards	Invitrogen	LC5800
MagicMark XP Western Standards	Invitrogen	LC5602
OneMinute Western Blot Stripping Buffer	GM Biosciences	GM6001
Western Lightning Chemiluminescence Reagent Plus	Perkin Elmer	NEL104
10x TBS pH 7.4	Quality biological	351-086-101
Tween 20	Fluka	93773
Non fat dry milk	Safeway	
BSA (Albumin Bovine Fraction V)	MP Biomedicals	160069
Photometrics CoolSnap HQ2 CCD camera	Roper Scientific	

References

1. Naito, M., et al. Liposome-encapsulated dichloromethylene diphosphonate induces macrophage apoptosis in vivo and in vitro. J Leukoc Biol **60**, 337-344 (1996).
2. van Amerongen, M.J., Harmsen, M.C., van Rooijen, N., Petersen, A.H. & van Luyn, M.J. Macrophage depletion impairs wound healing and increases left ventricular remodeling after myocardial injury in mice. Am J Pathol **170**, 818-829 (2007).
3. van Rooijen, N., Sanders, A. & van den Berg, T.K. Apoptosis of macrophages induced by liposome-mediated intracellular delivery of clodronate and propamidine. J Immunol Methods **193**, 93-99 (1996).
4. Habu, S., et al. In vivo effects of anti-asialo GM1. I. Reduction of NK activity and enhancement of transplanted tumor growth in nude mice. J Immunol **127**, 34-38 (1981).
5. Kasai, M., et al. In vivo effect of anti-asialo GM1 antibody on natural killer activity. Nature **291**, 334-335 (1981).

6. Sherwood, E.R., et al. Beta 2 microglobulin knockout mice are resistant to lethal intraabdominal sepsis. Am J Respir Crit Care Med **167**, 1641-1649 (2003).
7. Fortier, A.H. & Falk, L.A. Isolation of murine macrophages. Current protocols in immunology / edited by John E. Coligan ... [et al **Chapter 14**, Unit 14 11 (2001).
8. Yuen, P.S., et al. A simplified method for HPLC determination of creatinine in mouse serum. Am J Physiol Renal Physiol **286**, F1116-1119 (2004).
9. Choi, W.I., et al. Systemic microvascular leak in an in vivo rat model of ventilator-induced lung injury. Am J Respir Crit Care Med **167**, 1627-1632 (2003).
10. Green, T.P., Johnson, D.E., Marchessault, R.P. & Gatto, C.W. Transvascular flux and tissue accrual of Evans blue: effects of endotoxin and histamine. J Lab Clin Med **111**, 173-183 (1988).
11. Yasuda, H., Yuen, P.S., Hu, X., Zhou, H. & Star, R.A. Simvastatin improves sepsis-induced mortality and acute kidney injury via renal vascular effects. Kidney Int **69**, 1535-1542 (2006).
12. Miyaji, T., et al. Ethyl pyruvate decreases sepsis-induced acute renal failure and multiple organ damage in aged mice. Kidney Int **64**, 1620-1631 (2003).
13. Dear, J.W., et al. Sepsis-induced organ failure is mediated by different pathways in the kidney and liver: acute renal failure is dependent on MyD88 but not renal cell apoptosis. Kidney Int **69**, 832-836 (2006).
14. Imai, Y., Ibata, I., Ito, D., Ohsawa, K. & Kohsaka, S. A novel gene iba1 in the major histocompatibility complex class III region encoding an EF hand protein expressed in a monocytic lineage. Biochemical and biophysical research communications **224**, 855-862 (1996).