Supplemental Materials:

Methods:

Quantification of Tissue Histological score:

The liver, kidney, lung, and small intestine were removed immediately after sacrifice at 22 h after LPS or saline administration. These tissue specimens were fixed overnight in 4% buffered formaldehyde, processed by standard methods, and stained with hematoxylin and eosin (H and E). One observer, who was blinded to the treatment of animals, performed the tissue analysis. The severity of the organ injury was based on previous literature.\textsuperscript{1,2} Lung injury was judged by the appearances of alveolar edema, interstitial edema, hemorrhage, inflammatory infiltration, epithelial destruction, micro-atelectasis, and over-distension. The severity of the feature was scored as follows: 0, normal appearance; 1, slight effect; 2, middle effect; and 3, severe effects. The severity of small intestine injury was scored from 0 to 3 as follows: 0, normal, no damage; 1, mild; focal epithelial edema and necrosis; 2, moderate; diffuse swelling or necrosis of the villi; 3, severe; diffuse necrosis of the villi with evidence of neutrophil infiltration in the submucosa and/or hemorrhage. The severity of liver injury observed in the tissue sections was scored as follows: 0, minimal or no evidence of injury; 1, mild injury consisting of cytoplasmic vacuolation and focal nuclear pyknosis; 2, moderate to severe injury with extensive nuclear pyknosis, cytoplasmic hypereosinophilia, and loss of intercellular borders; and 3, severe necrosis with disintegration of the hepatic cords, hemorrhage, and neutrophil infiltration. The severity of renal tubular injury was scored by estimating the percentage of tubules in the cortex or the outer medulla that showed epithelial necrosis or had luminal necrotic debris, tubular dilation, and hemorrhage, as follows: 0, none; 1, <5%; 2, 5–25%; 3, 25–75% and 4, >75%. All evaluations were made on 5 fields per section and 5 sections per organ.

Measurements of myeloperoxidase activity (MPO) activity in tissues from endotoxemic WT and BK \( \beta \)-KO mice.
MPO activity was determined in lung, liver, kidney and small intestine as an index of neutrophil accumulation as described.³ Tissues were homogenized in a solution containing 0.5% hexa-decyl-trimethylammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7.0) and were centrifuged for 30 min at 20,000×g 4°C. An aliquot of the supernatant was allowed to react with a solution of tera-methyl-benzidine (1.6 mM) and 0.1 mM H₂O₂. The rate of change in absorbance was measured by spectrophotometry at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol hydrogen peroxide/min at 37°C and was expressed in units per 100 mg of tissue.

**Immunohistochemistry staining of PMN accumulation in lung, liver, and kidney:**

The paraffin-embedded tissue slices from liver, lung, and kidney were cut into 6 mM-thick slices, and stained for PMNs using a rabbit anti-PMN antibody (Abtu-neutrophil Clone 7/4, 1:2500).⁴ After incubation with the primary antibody, tissue sections were incubated with biotinylated goat anti-rabbit IgG, avidin-conjugated alkaline phosphatase, and Vector Red substrate to stain PMNs.

References:


Supplemental data:

Elevated MPO activity and PMN accumulation in organ tissues from endotoxemic BK β1-KO mice:

22h post LPS treatment, BK β1-KO mice exhibited mild elevation of MPO activity in the lung and liver (Fig. S1A, S1B, S1C), but not in kidney (Fig. S1D), compared to tissues from WT mice. MPO activity in BK β1-KO duodenum was lower than WT mice (Fig. S1B), perhaps because severe damage had occurred in response to PMN infiltration prior to the time at which sections were obtained 22 h post LPS administration (See Fig. 4I, 5B).

Higher levels of PMN infiltration in BK β1-KO lung and livers were confirmed in immunohistochemical studies, the typical images from 4 animals for each group are shown in Fig S2. Results were consistent with the MPO measurement (Fig S2), indicated a higher PMN infiltration in BK β1-KO lung and livers, but not in kidney. Unfortunately, the assessment of PMN in tissues of duodenum failed due to the pealing of mucosa from the slices during the staining process.

Figure Legends:

Fig S1. Comparison of tissue MPO activity in lung (A), duodenum (B), liver (C), and kidney (D) from 22 hours post saline or LPS treated WT and BK β1-KO mice. * Significantly different from saline treated WT mice. # Significantly different from LPS treated WT mice. (P<0.05)

Fig S2. Immunohistochemistry staining of PMN accumulation in organs from LPS-WT and BK β1-KO mice. More positive staining of cells was found in LPS-BK β1-lung, liver, but not kidneys.
Fig S1

A  Lung

- WT (n=7)
- BK β1-KO (n=8)

Saline  LPS-22h

MPO (U/100 mg tissue)

B  Duodenum

- WT (n=4)
- BK β1-KO (n=4)

Saline  LPS-22h

MPO (U/100 mg tissue)

C  Liver

- WT (n=7)
- BK β1-KO (n=8)

Saline  LPS-22h

MPO (U/100 mg tissue)

D  Kidney

- WT (n=7)
- BK β1-KO (n=8)

Saline  LPS-22h

MPO (U/100 mg tissue)
Fig S2

WT  BK β1-KO

LPS-22h-PMN

Lung  Liver  Kidney

100 μM

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