Inhibitory effects of lysozyme on endothelial protein C receptor shedding in vitro and in vivo

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Supplementary Materials and Methods

*Enzyme-linked immunosorbent assay (ELISA) for membrane EPCR expression*

Modified whole-cell ELISA was performed as previously described to determine the expression levels of EPCR on HUVECs (1). Briefly, confluent monolayers of HUVECs were treated with or without lysozyme for 6 h, followed by treatment with PMA, TNF-α, or IL-1β for 1 h.

*Immunohistochemistry*

To analyze the expression pattern of EPCR, the descending thoracic aortic vessels from CLP-induced septic (Day 4) and sham-operated mice were removed and the EPCR was stained as described previously (10).

*ELISA for lysozyme, total and phospho p-38MAPK, ERK1/2, and JNK*

The plasma concentrations of lysozyme in mouse plasma were quantified using ELISA kits (LSBio, Seattle, WA). HUVECs were cultured in 96-well microplates for the quantitative determination of p38 MAPK, ERK1/2, and JNK phosphorylation. On the day of the experiments, culture medium was replaced with serum-free growth medium. Cells were then treated with or without lysozyme for 6 h, followed by treatment with PMA (1 μM) for 1 h. Activation of p38 MAPK, ERK 1/2, and JNK was quantified in nuclear lysates using ELISA kits for total/phosphorylated p38 MAPK (Invitrogen, Carlsbad, CA, for total p38 MAPK or Cell Signaling Technology, Danvers, MA, for phosphorylated-p38 MAPK), total/phospho ERK1/2 and JNK (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.
References

Supplementary Fig 1

EPCR → sEPCR

TACE

Lysozyme