Effect of Human Umbilical Vein Endothelial Cells on Immune Responses of Lipopolysaccharide-Induced THP1 Macrophages

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To the Editor: Sepsis is one of the leading causes of death in Intensive Care Units. It is known as the syndrome of organ dysfunction caused by infection. Immune response has a critical role in sepsis. High concentrations of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 have been suggested to be harmful and one of the major causes of organ injury.1 Immune paralysis in sepsis causes a secondary infection.

In sepsis, macrophages phagocytize pathogenic bacteria and secrete large amounts of cytokines. They can be polarized to be M1 and M2 macrophages. M1 macrophages can be generated when stimulated by microbial products and produce large amounts of pro-inflammatory cytokines, with high human leukocyte antigen (HLA)-DR expression. On the contrary, M2 macrophages produce anti-inflammatory cytokines and express cluster of differentiation (CD)-163. M1 macrophage polarization can be of great importance in aggressive inflammatory response in sepsis, while M2 macrophages contribute to immune paralysis.2 Endothelial cell apoptosis and dysfunction are considered as one of the central events in sepsis.3 Vascular permeability increases in sepsis, leading to the movement of a large number of inflammatory cells into the tissue gap, with increased tissue edema and cell hypoxia.4 These events are critical for organ damage and failure in sepsis. However, little is known about the effects of endothelial cells on macrophages.

In the present study, THP-1 cells differentiated into macrophages on administering 200 nmol/L of phorbol-12-myristate-13-acetate for 72 h. Human umbilical vein endothelial cells (HUVECs) were cocultured with THP-1 macrophages for 8 h. Lipopolysaccharide (LPS) (100 µg/mL) was added to the culture. Cell-free supernatants were collected and kept in the refrigerator at −80°C. The enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-6, IL-10, and IL-12 were purchased from Thermo Scientific, San Jose, CA, USA. Nonspecific isotype-matched antibodies served as controls. The cells were analyzed using flow cytometry in a FACSCalibur (BD). Data were analyzed using GraphPad Prism 6.0 (Intuitive Software for Science, San Diego, CA, USA). Data were presented as mean ± standard deviation. Analysis of variance with Bonferroni post hoc test was used to determine significance. A P < 0.05 was considered statistically significant.

LPS is a component of the outer cell wall of Gram-negative bacteria, which can be recognized by immune cells such as macrophages. It induces cytokine production. The present study explored whether cytokine production induced by LPS was further affected by HUVECs. As shown in Figure 1a, HUVECs cocultured with LPS-induced THP1 macrophages showed enhanced IL-1β, TNF-α, IL-6, IL-10, and IL-12 production compared with LPS-induced THP1 macrophages alone (P < 0.01). HUVECs cocultured with THP1 macrophages without LPS also showed higher concentrations of these cytokines compared with THP1 macrophages alone. The expression of IL-1β, TNF-α, IL-6, IL-10, and IL-12 mRNA in THP1 macrophages was analyzed by real-time PCR. As shown in Figure 1b, the expression of IL-1β, TNF-α, IL-6, IL-10, and IL-12 was upregulated by LPS. Their expression was further upregulated by HUVECs. Besides, THP1 macrophages enhanced the mRNA expression of pro-inflammatory cytokines cocultured with HUVECs in the absence of LPS. HUVECs enhanced the expression of HLA-DR in THP1 macrophages in the absence of LPS.

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LPS and showed little effect on CD163 without LPS treatment. LPS increased the expression of HLA-DR and CD163 in THP1 macrophages. The expression of HLA-DR and CD163 in LPS-induced THP1 macrophages increased after coculture with HUVECs [Figure 1c], indicating promoting effects of HUVECs on the polarization of macrophages.

Pro-inflammatory cytokines secreted by macrophages affected endothelial cells and increased endothelial permeability. However, the effects of endothelial cells on macrophages were not well understood. In the present study, HUVECs enhanced the production of pro-inflammatory cytokines and the expression of HLA-DR in THP-1 macrophages. Pro-inflammatory cytokines IL-1β, TNF-α, IL-6, and IL-12 enhanced the innate and adaptive immune responses. HLA-DR is an important molecule in antigen presentation of immune cells such as macrophages and dendritic cells. Meanwhile, the expression of IL-10 and CD163 in THP1 macrophages increased after coculture with HUVECs. IL-10 inhibited immune responses of many types of immune cells. CD163 is an important marker for M2 macrophages. Thus, anti-inflammatory immune responses were upregulated. It is interesting that both pro-inflammatory and anti-inflammatory immune responses of macrophages were enhanced by endothelial cells.

Both pro-inflammatory and anti-inflammatory immune responses of macrophages are important in the occurrence and development of sepsis. Endothelial cells increased the pro-inflammatory immune responses, resulting in severe endothelial injury. Meanwhile, the enhanced anti-inflammatory immune responses resulted in immunosuppression. Thus, the amplification effects of endothelial cells on immune responses of macrophages suggested the critical role of endothelium. Inhibiting the amplification effects of endothelial cells might serve as a novel target for treating sepsis. Further studies should explore the mechanisms involved in the effect of endothelial cells on macrophages. In addition, drugs that block these effects should be developed. In conclusion, HUVECs enhanced both the pro-inflammatory and anti-inflammatory immune responses of LPS-induced THP1 macrophages, suggesting a potential target for sepsis therapy.

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**Conflicts of interest**

There are no conflicts of interest.
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