Adhesion molecule and proinflammatory cytokine gene expression in hepatic sinusoidal endothelial cells following cecal ligation and puncture

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INTRODUCTION
Multiple organ dysfunction syndrome (MODS) is thought to be a frequent consequence of sepsis[1-3]. Despite substantial advances in our knowledge and understanding of the basic pathophysiologic mechanisms[4-7], in critically ill patients infections and sepsis are still associated with a high mortality[8,9]. There is evidence that the development of tissue damage in sepsis and shock is closely associated with the release of an ever increasing number of mediators and accumulation of neutrophils at the sites of infection or injury[10,11].

The endothelium is an intimal layer of simple squamous cells which provides a continuous, fluent surface for circulating blood. It is not the passive, metabolically inert barrier that it was once thought to be, and it is now known to be a metabolically and physiologically dynamic tissue with multiple functions. On the basis of recent discoveries in the field of endothelial cell biology (such as endothelium-derived mediators and the expression of adhesion molecules), endothelial cells are now thought to be not only target cells of injury, but also actively involved in inflammatory reactions and subsequent organ damage[12,13].

Polymicrobial sepsis induced by cecal ligation and puncture (CLP) is a model of sepsis which reproduces many of the inflammatory and pathological sequelae that are observed clinically. Following CLP, animals develop bacteremia, hypothermia, hypotension, and damage to multiple organ systems[14]. The present study was designed to observe the gene expression of adhesion molecules and proinflammatory cytokines in hepatic sinusoidal endothelial cells with a CLP model, in order to investigate the role of endothelial cells in tissue damage during sepsis.

MATERIALS AND METHODS
Animal model and CLP
NIH mice were obtained from the animal center of the General Hospital of PLA. The mice were randomly divided into 2 groups: CLP group and sham group. Sepsis was induced in the CLP group by CLP. The mice were anesthetized, and the cecum was ligated below the ileocecal junction: intestinal continuity was maintained. The cecum was punctured twice with a 20-gauge needle and a small amount of cecal contents was expressed through the punctures. The incision was closed and 1 mL of normal saline was administered subcutaneously. Sham-operated mice underwent the same surgical procedure, but without CLP. The mice were sacrificed at 3 or 12 h after the procedure.

Isolation and purification of hepatic sinusoidal endothelial cells
Hepatic sinusoidal endothelial cells were isolated by collagenase perfusion of the liver, isopyknic sedimentation in a two-step percoll gradient, and selective adherence[15]. The purified sinusoidal endothelial cells were identified by staining with anti-von willebrand factor (vWF, factor VII I-related antigen). Flow cytometric analysis showed a purity greater than 85% in hepatic sinusoidal endothelial cells.

Analysis of adhesion molecules and proinflammatory cytokines mRNA by reverse transcription-PCR
Total RNA was extracted from endothelial cells. We used a phenol-chloroform extraction method reported by Chomczynski. The RNA was then quantitated spectrophotometrically. Total RNA from experimental samples was used to synthesize cDNA using AMV reverse transcriptase. β-actin and β2-MG were used as internal control primers. The primers for the adhesion molecules and controls were as follows: β- actin (478bp), 5’ AGG GAA ATC GTG CGT GAC ATC AAA 3’, 5’ ACT CAT CGT ACT CCT GCT TGC TGA 3’; β2-MG (300 bp), 5’ GGC TCG CTC GGT GAC CCT AGT CTT T 3’.
5’TCT GCA GGC GTA TGT ATC AGT CTC A 3’; VCAM-1 (442bp), 5’CCT CAC TTG CAG CAC TAC GGG CT 3’, 5’TIT TCC AAT ATC CTC AAT GAC GGG 3’; ICAM-1 (326bp), 5’TGC GTTTTG GAG CTA GGC GAC CA 3’, 5’CGA GGA CCA TAC ACG TGC AG 3’; E-selectin (435bp), 5’CCT GAA CTG CTC CCA CCC GTT CG 3’, 5’GTG AAG TTA CAG GAT GAC TTA AAC GCA 3’; TNF-α (349 bp), 5’TTC GTG CCC TTT CAC TCA CTG G 3’, 5’TIT TGG GTT TGC TAC GAC GTG G 3’. E-selectin (435bp), 5’ATT ACG CAG CTC CAC TAC AGG CTC 3’, 5’AGA TTC CAT GGT GAA GTC AAT TAT 3’; IL-1β (441 bp), 5’TGG AAG TTA CAG GAT GAC TAA GGA CCA TAC 3’; ICAM-1 (326bp), 5’TGC GTT TTG GAG CTA 3’, 5’TCT GGC GTA TGT ATC AGT CTC A 3’; VCAM-1 (435bp), 5’CCT CAG TAA GGG 3’; ICAM-1 (326bp), 5’TGG AAG TTA CAG GAT GAC TAA GGA CCA TAC 3’; IL-6 (349bp), 5’TGC GTT TTG GAG CTA 3’. These primers were designed for experiments using the polymerase chain reaction (PCR) method. A normalization quotient (Q) was calculated based on the integrated optical density values (IOD) for the adhesion molecules and the β-actin or β2-MG bands (Q = IOD, adhesion molecules band/internal control band). The level of adhesion molecules mRNA was expressed as the quotient of the integrated optical density values for the adhesion molecules and the β-actin or β2-MG bands.

Statistical analysis
All data were reported as means ± SD. Data were analyzed by t test for comparisons between the two groups. A P value of less than 0.05 was deemed significant.

RESULTS

Adhesion molecules mRNA expression in hepatic sinusoidal endothelial cells
E-selectin mRNA levels markedly increased at 3 h after CLP in hepatic sinusoidal endothelial cells, and returned to baseline at 12 h after CLP. Increased ICAM-1 mRNA level was found at 3 h after CLP, and this level became higher at 12 h after CLP. VCAM-1 mRNA expression in hepatic sinusoidal endothelial cells increased significantly 3 h after CLP but declined at 12 h after CLP (Table 1).

Proinflammatory cytokines mRNA expression in hepatic sinusoidal endothelial cells
A significant increase in TNF-α and IL-6 gene expression was observed at 3 and 12 hours after CLP. The level of TNFα and IL-1β at 3 hours was higher than 12 hours, and the level of IL-6 gene expression at 12 hours was higher than 3 hours (Table 2).

DISCUSSION
The liver, with its rich supply of blood and sinusoid, is directly exposed to bacteria and endotoxins drained from the GI tract[16-19]. Previously, researchers in our institute have reported that the liver is the most susceptible and vulnerable organ during sepsis and multiple organ failure[20]. Vascular endothelial cells form an interface between tissues and inflammatory cells. This unique location allows localization of the inflammatory reaction to the site of injury while protecting adjacent healthy tissue. Endothelial cells mediate the local inflammatory response through modulation of vascular tone, vascular permeability and stimulation of leukocyte extravasation[21,22]. The role of neutrophil extravasation and accumulation has been emphasized in recent years[23,24]. This is mediated by the induced expression of multiple cell adhesion molecules on the surface of neutrophils and endothelial cells[25,26]. These include the selectins which are a group of surface glycoproteins essential to leukocyte margination and rolling along the vascular endothelium. Specifically, endothelial E- and P-selectin and L-selectin are expressed on neutrophils[27]. Another group of cell adhesion molecules involved in endothelial cell-leukocyte interaction is the immunoglobulin supergene family. This group comprises intercellular adhesion molecules-1 and 2 (ICAM-1, ICAM-2), vascular cell adhesion molecules-1 (VCAM-1) [28]. The most well studied of these molecules is ICAM-1, which play a critical role in events subsequent to initial leukocyte margination. In this study, we found that the up-regulation of the expressions of adhesion molecules in liver sinusoidal endothelial cells is a crucial step for the migration of leukocytes to and accumulation at the site of inflammation. Although neutrophils are important for killing microorganisms, activation of recruited neutrophils coupled with excessive release of oxygen metabolites and proinflammatory mediators may induce tissue injury which can lead to organ dysfunction[29,30].
Cytokines are polypeptides or glycoproteins of low molecular weight. Most cytokines are not stored as preformed molecules, hence their production requires new gene transcription and translation. Unlike mediators derived from the classical endocrine system, cytokines are produced. The production of cytokines at various tissue sites depends, in part, on the proximity of the site to the inflammatory stimulus. In this study we assessed the gene expression of proinflammatory cytokines in hepatic sinusoid endothelial cells. Recent discoveries in the field of endothelial cell biology have shown its capability of production cytokines, but the role of endothelial cell-derived cytokines in sepsis induced tissue injury was largely ignored. Proinflammatory cytokines TNF and IL-1 are known to play predominant roles in the normal inflammatory response. Exaggerated endogenous production is likely responsible for the complications associated with sepsis as tissue injury and ultimate organ failure. We observed a significant increase in TNF and IL-1 gene expression shortly after the induction of sepsis. This indicates that endothelium is an important source of cytokines during sepsis, and may play a role in sepsis induced organ dysfunction. The consensus of the concept of systemic inflammatory responses has brought about a likely promising approach in the treatment of SIRS/MODS, anti-inflammatory instead of anti-infection. Various approaches aimed at interrupting the cascade of host inflammatory responses have been tested. These include interventions targeted at the inflammation effector cells as monoclonal antibodies or receptor antagonist to pro-inflammatory cytokines. However, many of these seemingly effective measures in experimental study failed when moved from the laboratory bench to clinical ward.

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