Endothelial barrier dysfunction caused by LPS correlates with phosphorylation of HSP27 in vivo

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Abstract

Lung edema during sepsis is triggered by formation of gaps between endothelial cells followed by macrophage infiltration. Endothelial gap formation has been proposed to involve changes in the structure of the actin filament cytoskeleton. Heat shock protein 27 (HSP27) is believed to modulate actin filament dynamics or structure, in a manner dependent on its phosphorylation status. We hypothesized that HSP27 may play a role in endothelial gap formation, by affecting actin dependent events in endothelial cells. As there has been no report concerning HSP27 in lung edema in vivo, we examined induction and phosphorylation of HSP27 in lung following LPS injection, as a model of sepsis. In lung, HSP27 mainly localized in capillary endothelial cells of the alveolus, and in smooth muscle cells of pulmonary arteries. HSP27 became significantly more phosphorylated at 3 h after LPS treatment, while the distribution of HSP27 remained unchanged. Pre-treatment with anti-TNF-α antibody, which has been shown to reduce lung injury, blocked increases in HSP27 phosphorylation at 3 h. HSP27 phosphorylation was also increased in cultured rat pulmonary arterial endothelial cells (RPAEC) by treatment with TNF-α, LPS, or H2O2. This phosphorylation was blocked by pre-treatment with SB203580, an inhibitor of the upstream kinase, p38 MAP kinase. Increased endothelial permeability caused by H2O2 in vitro was also blocked by SB203580. The amount of actin associated with HSP27 was reduced after treatment with LPS, or H2O2. In summary, HSP27 phosphorylation temporally correlated with LPS induced pathological endothelial cell gap formation in vivo and in a cell culture model system. This is the first report of increased HSP27 phosphorylation associated with pathological lung injury in an animal model of sepsis.

Abbreviations: ARDS, adult respiratory distress syndrome; BSA, bovine serum albumin; DMEM, Dulbecco minimal essential medium; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; H&E, hematoxylin and eosin; HRP, horseradish peroxidase; HSP27, heat shock protein 27; IEF, isoelectric focusing; IL, interleukin; IPB, immunoprecipitation buffer; LPS, lipopolysaccharide; MAP kinase, mitogen activated protein kinase; MHC, myosin heavy chain; MLCK, myosin light chain kinase; MTT, 3-(4,5-dimethyl-thiazoloyl-2-yl)2,5-diphenyltetrazolium bromide; PBS, phosphate buffered saline; PVDF, polyvinylidene fluoride; RPAEC, rat pulmonary arterial endothelial cells; SDS, sodium dodecyl sulfate; TNF-α, tumor necrosis factor α
Introduction

Adult respiratory distress syndrome (ARDS) occurs in 18–25% of sepsis syndrome patients and results in up to 90% mortality. ARDS associated pulmonary edema, poorly compliant lungs, and refractory hypoxemia are due to impairment of endothelial barrier function and advancement of neutrophils through the endothelium into the tissues (reviewed in Martin and Silverman, 1992).

The vascular endothelium is a semi-selective diffusion barrier between the blood plasma and interstitial tissue. Inter-endothelial cell gap formation with loss of barrier function leads to neutrophil infiltration and tissue edema. Trans-endothelial permeability is increased by endothelial cell shape change and disruption of tight junctions between cells. It has been shown that reorganization of the actin-containing cytoskeleton is essential for shape changes and loss of tight junction integrity associated with increased permeability in endothelial cells (Alexander et al., 1988; Phillips et al., 1989; Shasby et al., 1982).

HSP27 was identified as an inhibitor of actin polymerization when it was co-purified with vinculin from avian smooth muscle Miron et al., 1991). Several reports indicate that HSP27 plays an important role in regulating the actin-containing cytoskeletal structure. HSP27 can inhibit actin polymerization in vitro (Miron et al., 1991) and this ability appears to be dependent on its phosphorylation status (Benndorf et al., 1994). In cell lines transfected to over-express HSP27, cortical actin arrays were increased as was pinocytic activity (Lavoie et al., 1993a). These increases were not observed in cell lines transfected with a mutant form of HSP27 incapable of being phosphorylated (Lavoie et al., 1993a). Microfilaments in cells transfected to over-express HSP27 were more stable to heat-shock treatment or cytochalasin D treatment (Lavoie et al., 1993b, 1995). Thus, HSP27 can affect actin filament structure by a mechanism that depends on the phosphorylation state of HSP27.

Phosphorylation of HSP27 is induced by a number of agents or treatments that cause edema. TNFα treatment, which causes lung edema in sheep (Horvath et al., 1988), induced phosphorylation of HSP27 in endothelial cells (Arrigo, 1990; Robaye et al., 1989). Thrombin, another agent capable of disrupting the barrier function of endothelial cells (Phillips et al., 1989), induces HSP27 phosphorylation (Mendelsohn et al., 1991). Treatment of cultured endothelial cells with hydrogen peroxide (H₂O₂) caused increased endothelial cell monolayer permeability (Gilmont et al., 1996) and phosphorylation of HSP27 (Huot et al., 1997). Furthermore, H₂O₂-induced endothelial microfilament fragmentation was blocked by an inhibitor of p38 mitogen activated protein (MAP) kinase, which is an upstream kinase involved in HSP27 phosphorylation (Huot et al., 1997). These reports imply that HSP27 phosphorylation may be involved in loss of endothelial barrier function. Recently, p38 MAP kinase activation was reported to be a critical step in pertussis toxin-induced increased endothelial permeability, and phosphorylation of HSP27 was suggested as an effector of p38 MAP kinase (Garcia et al., 2002). We examined expression or phosphorylation of HSP27 in the in vivo and in vitro models of sepsis-induced pulmonary edema.

Materials and methods

Sepsis model

Adult, male Sprague-Dawley rats (approximately 200 g) were used in these experiments. The sepsis group (18 rats) were given intraperitoneal injections of LPS (E. coli. serotype 0127:B8, Sigma, St Louis, MO, USA) 2 mg/kg from a 2 mg/ml stock solution in phosphate buffered saline (PBS) and were euthanized in