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Published in:
The Journal of Infectious Diseases

DOI:
10.1086/515348

Citation for published version (APA):

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Lymph of Patients with a Systemic Inflammatory Response Syndrome Inhibits Lipopolysaccharide-Induced Cytokine Production

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In patients with systemic inflammatory response syndrome (SIRS), tolerance of peripheral blood mononuclear cells to a second challenge with lipopolysaccharide (LPS) has been described. Thoracic duct lymph transports LPS and represents the extravascular, interstitial fluid compartment of the body. The aim of this study was to determine the capacity of lymph to influence LPS-induced cytokine production in vitro. Thoracic duct lymph was obtained from patients with SIRS and without SIRS (controls). The effect of lymph and simultaneously collected plasma on LPS-induced cytokine production by normal peripheral blood mononuclear cells was assessed. Both lymph and plasma of patients with SIRS reduced LPS-induced tumor necrosis factor-α and interleukin-6 production ($P < .01$); lymph of controls also inhibited cytokine production ($P < .01$), although to a lesser extent. This study suggests that LPS tolerance may occur both in the intra- and extravascular compartments.

Lipopolysaccharide (LPS) tolerance is characterized by down-regulation of the production of proinflammatory cytokines (e.g., tumor necrosis factor-α, in erelukin [IL]-6) by peripheral blood mononuclear cells when exposed to LPS [1, 2]. This phenomenon has been found in patients with systemic inflammatory response syndrome (SIRS) and in healthy humans injected with low-dose LPS [1, 2]. Although precise mechanisms of LPS tolerance are unclear, it is caused by a decrease in par, by soluble mediators in the extracellular space, and by the uptake of LPS by monocytes/macrophages in the extracellular space [5]. Besides plasma, lymph may be an important body compartment in LPS tolerance. In patients with SIRS, thoracic duct lymph represents the extravascular, interstitial fluid compartment of the body [5].

Several studies have shown that lymph is able to modulate the immune response to LPS [6, 7]. In patients with SIRS, thoracic duct lymph may represent a critical compartment in LPS tolerance. In this study, we have assessed the capacity of lymph to influence LPS-induced cytokine production in vitro. Thoracic duct lymph was obtained from patients with SIRS and healthy individuals (controls). The effect of lymph and simultaneously collected plasma on LPS-induced cytokine production by normal peripheral blood mononuclear cells was assessed. Both lymph and plasma of patients with SIRS reduced LPS-induced tumor necrosis factor-α and interleukin-6 production ($P < .01$); lymph of controls also inhibited cytokine production ($P < .01$), although to a lesser extent. This study suggests that LPS tolerance may occur both in the intra- and extravascular compartments.

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**Patients and Methods**

**Plasma and thoracic duct lymph collection.** Peripheral blood plasma and thoracic duct lymph were obtained from patients with systemic inflammatory response syndrome (SIRS) and healthy individuals. Peripheral plasma and thoracic duct lymph were collected from patients with SIRS (6 men, 2 women, average age 62 ± 5 years) and from healthy individuals (4 men, 3 women, average age 64 ± 2 years). The studies were approved by the institutional review committees. Written informed consent was obtained from all patients.

Received 20 January 1998; revised 10 April 1998.

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**The Journal of Infectious Diseases 1998;178:883–6**

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Results

Preclinical studies have shown that in the initial fluid of esophageal carcinoma of the esophagus, were used for incubation without LPS. In these samples, TNF-α, IL-6, and IL-10 were calculated as the difference between cytokine concentrations in the esophagus for a carcinoma of the esophagus.

Experimental design. Lymph of pa ien s w i h SIRS was pooled, as was lymph of pa ien s w i h SIRS; equal amount s of lymph from each pa ien were used. The lymph pools were brough o final concen ra ions of 10% (vol%) in RPMI 1640 (BioWhi aker). Then, 0% lymph (RPMI 1640 only), 10% SIRS lymph, or 10% non-SIRS lymph was preincubated in the absence of LPS or in the presence of 1 or 10 ng/mL LPS (final concen ra ions) (Escherichia coli O111:B4; Sigma, S. Louis; 1 ng 12 endo oxin uni s) for 24 h in a CO₂ incubator or at 37°C. Thereafter, PBMC (final concen ra ion 0.5 × 10⁶/mL) of each hea lvolun eer were incubated to reduce the lymph-LPS suspensions for 4 and 24 h in a CO₂ incubator or at 37°C for measurement of TNF-α and IL-6, respectively. These dura ions of incubation were chosen af er preliminary experimen s had es alished ha he concen ra ions of TNF-α and IL-6 peaked at a hea ltime point s (da a no shown).

Af er cen rifug a ion a 2000 g for 30 min a 4°C, superna an s were aliquo ed and s ored at −80°C un il assays were performed. An iden cal pro ocol was used o de ermine LPS-neu ralizing ca- lacities of 10% plasma of pa ien s w i h SIRS, 10% plasma of pa ien s w i h SIRS, and 10% plasma of 10 ha lvolun eers (10 women, age 29 ± 2 years). TNF-α (Medgenix, Fleurus, Bel- gi um), IL-6 (PharMingen, San Diego), and IL-10 (PharMingen) were de ermined by ELISA according o his ins ruc ions of he manu facile. LBP and BPI concen ra ions were de ermined using his phenomenon also occurs in the extravascular compar men tion wi h lymph) and 10% non-SIRS lymph (P < .05; figure 1). SIRS lymph was more po en han lymph of con rols in inhibing TNF-α produc ion elicied by 1 ng/mL LPS (P < .05) and in inhibing b h TNF-α and IL-6 produc ion elicied by 10 ng/mL LPS (P < .05; figure 1).

Comparison of lymph and plasma. Plasma ob ained from hea lvolun eers inhibi ed LPS-induced TNF-α and IL-6 produc ion (P < .05 vs. LPS only, except for IL-6 release by non-SIRS lymph; figure 1), confirming a previous repor [2]. The ex en of inhibi- tion by hea lplasma was less (P < .05) compared wi h he inhibi on by SIRS plasma and non-SIRS plasma (P < .01 vs. LPS only; figure 1). TNF-α and IL-6 release induced by 10 ng/mL LPS was inhibi ed more by SIRS plasma than by non-SIRS plasma (P < .05; figure 1). SIRS plasma was more po en in reducing cy okine produc ion han SIRS lymph (P < .03), as was non-SIRS plasma compared wi h non-SIRS lymph (P < .03; figure 1).

LBP, BPI, and IL-10. Lymph of pa ien s w i h SIRS conained higher concen ra ions of LBP and IL-10 han lymph of pa ien s w i h SIRS (P < .03), while BPI levels were below he de ec ion limi in lymph of bo h SIRS and non-SIRS pa ien s (able 1). Plasma of pa ien s w i h SIRS conained higher concen ra ions of LBP and BPI han plasma of pa ien s w i h SIRS (P < .02; able 1), while IL-10 concen ra ions were no differen be ween he 2 groups. LBP levels were higher in SIRS plasma han in SIRS lymph (P < .03). IL-10 concen ra ions were higher in SIRS lymph han in SIRS plasma (P < .02).

Discussion

The presen findings demons ra e ha lymph of pa ien s w i h SIRS can parially reproduce LPS ol erance, possibly indica ing ha his phenomenon also occurs in he ex rasascular compar men .

In pa ien s w i h SIRS, he body may comba LPS oxici y by reducing he capaci y of mononuclear cells o produce proin- flamma ory cy okines upon res imula ion wi h LPS. I has been shown ha soluble media ors are involved, since serum of sep ic pa ien s and endo oxemic volun eers parially reproduced he LPS-ol eran ce s a e in normal whole blood [2, 3]. I has no presen that has been s uided whe her, in he in ers i al fluid, an LPS-ol eran ce s a e is presen , presumably refl ecing processes a issue-level. Therefore, we aimed o inves iga e capaci y of horacic duc lymph from pa ien s w i h SIRS o influence LPS-induced cy okine produc ion, since horacic duc lymph has ben s hown o represen he in ers i al fluid compar men [5].

Pa ien s w i h SIRS, undergoing arans horacic resec ion of he esophagus for a carcinoma of he esophagus, were used as con rols. I is possible ha he presen pa ien s have charac eri cics ha differ from hea lly individuals. However, we assume ha
Figure 1. Mean (± SE) tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 concentrations: 10% lymph and plasma of patients with SIRS, 10% lymph and plasma of patients without SIRS, and 10% plasma of healthy volunteers were preincubated with lipopolysaccharide (LPS) for 24 h. Then peripheral blood mononuclear cells of 12 healthy volunteers were incubated with different lymph-LPS or plasma-LPS suspensions for 4 h (TNF-α measurements) and 24 h (IL-6 measurements). For results of statistical analysis, see text.

These patients closely approximated the condition of healthy volunteers, the ideal control group.

Indeed, lymph of patients with SIRS inhibited LPS-induced cytokine release, although to a lesser extent. These findings are in line with a recent report indicating that major surgery itself can induce an LPS-olean response [14].

To obtain insight into the possible roles of LBP, BPI, and IL-10, circulating factors known to modulate LPS toxicity, concentrations of these substances were measured in lymph and plasma of patients with and without SIRS. The increased lymph concentrations of IL-10 and the increased plasma concentration of BPI in patients with SIRS may explain why SIRS...
lymph and SIRS plasma inhibit ed cytokine release more strongly than lymph and plasma of patients with SIRS, respectively.

Many other substances may bind LPS (e.g., antibodies, complement, albumin) or deac e mononuclear cells. For example, lipoprotein ions are known to bind and neutralize LPS [12, 15]. Concentration of apolipoprotein ions A and B were significantly lower in lymph and plasma of patients with SIRS compared with concentrations in lymph and plasma of patients with SIRS (data not shown). Lipoprotein ions can therefore not explain the more potent inhibitory effect of proinflammatory cytokine production by SIRS lymph or SIRS plasma compared with non-SIRS lymph or non-SIRS plasma, respectively. These data suggest that soluble mediators present in lymph (and plasma) are responsible, at least in part, for the phenomenon of LPS tolerance.

LPS tolerance is associated with a reduced capacity of mononuclear cells to produce cytokines upon stimulation with LPS. Here we show that lymph obtained from patients with SIRS can reproduce an LPS-tolerant state when added to culures of normal PBMC. These data suggest that soluble mediators present in lymph (and plasma) are responsible, at least in part, for the phenomenon of LPS tolerance.

References