Lymph of patients with a systemic inflammatory response syndrome inhibits lipopolysaccharide-induced cytokine production


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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 (tumor necrosis factor α, in ereluting [IL]-6) by whole blood and by a high level of endotoxin in the circulation. In healthy humans, endotoxin is cleared by the reticuloendothelial system, and even in septic patients, the endotoxin level is lower than in non-septic patients. The mechanisms by which LPS tolerance is induced are unknown. Several blood factors are involved, including lipopolysaccharide-binding protein (LPSBP), which binds LPS and prevents its uptake by macrophages. LPSBP is synthesized by the liver, and its level is increased in septic patients. The role of LPSBP in LPS tolerance is unclear, but it may be involved in the clearance of LPS from the circulation.

**Patients and Methods**

**Plasma and thoracic duct lymph collection.** Peripheral blood plasma and thoracic duct lymph were collected from patients with SIRS (6 men, 2 women, age range 62 ± 5 years) and from 7 patients without SIRS (4 men, 3 women, age range 64 ± 2 years). Plasma and thoracic duct lymph were obtained by catheterization of the thoracic duct. The lymph was collected in sterile plastic tubes (Sarsted, L.C.Lemaire@AMC.UVA.NL). Lymph was collected in pyrogen-free plastic tubes (Sarsted, L.C.Lemaire@AMC.UVA.NL).

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The studies were approved by the institutional review board. Written informed consent was obtained from all patients.

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**References**


Results

Incubation without LPS. Incubaion of normal PBMC with hou PBS only resul ed in de ec able levels of TNF- and IL- in he presence of lymph or plasma of pa ien s wi h SIRS. In hese samples, TNF- levels were 39 ± 15 pg/mL (incubaion wi h lymph) and 10 ± 7 pg/mL (incubaion wi h plasma); IL- concen ra ions were 1774 ± 88 and 502 ± 43 pg/mL, respesively. Therefore, he ex vivo produc ion of TNF- and IL- was calcula ed as he difference be ween cy okine concen ra ions found af er incuba ion wi h LPS and hose found af er incuba ion wi h LPS.

Effect of lymph. Lymph of pa ien s wi h SIRS and con rols (pa ien s wi h SIRS) reduced TNF- and IL- produc ion af er si mal ion wi h 1 ng/mL LPS (P < .01 vs. LPS only; figure 1). A 10 ng/mL LPS, IL- produc ion was significan ly inhibi ed only by SIRS lymph (P < .01 vs. LPS only; figure 1). SIRS lymph was more po en han lymph of con rols in inhibi ing TNF- produc ion elici ed by 1 ng/mL LPS (P < .05) and in inhibi ing bo h TNF- and IL- produc ion elici ed by 10 ng/mL LPS (P < .05; figure 1).

Comparison of lymph and plasma. Plasma obained af er heal by volun eers inhibi ed TNF- and IL- produc ion (P < .05 vs. LPS only, except for IL- release a 10 ng/mL LPS; figure 1), confrming a previous repor [2]. The ex of inhibi ion by heal plasma was less (P < .05) compared wi h he inhibi ion by SIRS plasma and non-SIRS plasma (P < .01 vs. LPS only; figure 1). TNF- and IL- release induced by 10 ng/mL LPS was inhibi ed more by SIRS plasma han 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 wi h SIRS conained higher concen ra ions of LBP and IL-10 han lymph of pa ien s wi h SIRS (P < .03), while BPI levels were below he de ec in lymph in lymph of bo h SIRS and non-SIRS pa ien s (able 1). Plasma of pa ien s wi h SIRS conained higher concen ra ions of LBP and BPI han plasma of pa ien s wi h non-SIRS lymph (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 demonds ra e ha lymph of pa ien s wi h SIRS can par ially reproduce LPS olerance, possibly indica ing ha his phenomenon also occurs in he es vascular compar men.

In pa ien s wi h SIRS, he body may comba LPS oxici y by reduing he capaci y of mononuclear cells o produc proinflammary cy okines upon res si mal 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 par ially reproduced LPS- oleran e and plasma of he pa ien s wi h SIRS o influence LPS-induced cy okine produc ion, since horacic duc lymph has been shown o represen he in ervol fluid compar men [5].

Pa ien s wi h SIRS, undergoing a rans horacic resec ion of he esophagus for a carcinoma of he esophagus, were used as con rols. I is possible ha hese pa ien s have charac eris cs ha differ from heal hy 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 approximate the condition of healthy volunteers, the ideal control group.

Indeed, lymph of patients with SIRS inhibited LPS-induced TNF-α and IL-6 production by normal PBMC. This was also found for plasma of patients with SIRS, as described previously [3]. Lymph and plasma of patients with SIRS, undergoing major surgery, also inhibited the LPS-induced proinflammatory 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-sensitization [14].

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

Table 1. Concentrations (mean ± SE) of lipopolysaccharide-binding protein (LBP), bactericidal/permeability-increasing protein (BPI), and interleukin-10 (IL-10) in lymph and plasma of patients with and without SIRS.

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<th>Lymph</th>
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<tr>
<td><strong>LBP (µg/mL)</strong></td>
<td>35 ± 10*</td>
<td>48 ± 9†</td>
<td>11 ± 6</td>
<td>8 ± 2</td>
</tr>
<tr>
<td><strong>BPI (pg/mL)</strong></td>
<td>&lt;200</td>
<td>924 ± 198†</td>
<td>&lt;200</td>
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</tr>
<tr>
<td><strong>IL-10 (pg/mL)</strong></td>
<td>885 ± 175*‡</td>
<td>328 ± 87</td>
<td>371 ± 109</td>
<td>261 ± 110</td>
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</table>

* P < .03 vs. lymph of patients without SIRS.
† P < .02 vs. plasma of patients without SIRS.
‡ P < .02 vs. lymph of patients with SIRS.
lymph and SIRS plasma inhibit ed cytokine release more strongly than lymph and plasma of pa ient s withou SIRS, respect ively.

Many other substances may bind LPS (e.g., antibodies, complements, albumin) or deac tivate mononuclear cells. For example, lipoprotein ions are known to bind and neu ralize LPS [12, 15]. Concent ra ions of apolipoprotein ions A-1 and B were significan tly lower in lymph and plasma of pa ient s with SIRS compared with concen ra ions in lymph and plasma of pa ient s withou SIRS (da a not shown). Lipoprotein ion concen ra ions can herefore no explain he more po en inhibi ion of proinflammatory cytokine produc ion by SIRS lymph or SIRS plasma compared with non-SIRS lymph or non-SIRS plasma, respect ively. The presen inves iga ion does no elucidate e which o her media ors migh be involved. It is seem ha al era ions in LBP concen ra ions do no con ribu e significan t ly to he development of LPS olerance.

LPS olerance is associa ed wi ha reduced capaci y of mononuclear cells to produce cytokines upon stimula ion wi ha LPS. Here we show ha lymph ab ained from pa ient s with SIRS can reproduce an LPS-olerant s a e when added o cul ures of normal PBMC. These da a sugge ha soluble media ors pres- en in lymph (and plasma) are responsible, a leas in par , for he phenomenon of LPS olerance.

References

5. Gas aldeii A, Schwarz JM, Caveggion E, e al. Glucose kinase in in ers i-plemen, albumin) or deac tivate mononuclear cells. For exam- ple, lipoprotein ions are known to bind and neu ralize LPS [12, 15].
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