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 (TNF-α), in interleukin (IL)-6) by white blood cells following an enhanced synthesis of IL-1 receptor or an agonist on extracellular matrix in the body. This phenomenon has been found in patients with SIRS and in healthy humans. The mechanism of tolerance is not clear, as it is caused by a lack in parabody to soluble media or in vivo. The present study, therefore, assessed the capacity of lymph to influence LPS-induced cytokine production, as lymph was present in vivo. Thoracic duct lymph from patients with SIRS inhibited LPS-induced cytokine 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.

Patients and Methods

Plasma and thoracic duct lymph collection. Peripheral blood plasma and thoracic duct lymph were obtained from 8 patients with SIRS (6 men, 2 women, age 62 ± 5 years) and from 7 patients without SIRS (4 men, 3 women, age 64 ± 2 years). From both patient groups, an enhanced synthesis of IL-1 receptor and agonist on LPS signaling was observed. Thoracic duct lymph from patients with SIRS reduced LPS-induced cytokine 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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The authors were approved by he ins i u tional scien ic and e hics committee. Written informed consent was obtained from patients or their relatives.

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an and plasma were aliquo ed and s ored a −80°C un il fur her processing.

PB C isolation. Blood was ob ained aseptically from 12 heal by male volun eers (age 33 ± 2 years). Blood from each volun eur was transser ined immedia e ly in o pyrogen-free ubes ha con ained pyrogen-free heparin. Blood was dilu ed 1:1 in Hanks’ Buffered Solu ion (HBSS; BioWhi aker, Verviers, Belgium) and subse quen ly by PBMC of each volun eur were isola ed by cen rifuga ion over a densi y gradien (Lymphopaque Ficoll Paque; Pharmacia, Woerden, The Ne herlands) a room empera ure for 15 min a 600 g. Cells in he in erphases were collec ed, washed wice and herea er brough a con cen ra ion of 1 × 10^7 PBMC/mL in HBSS con aining s eric nonac e human serum (Cen- tral Labora ory of The Ne herlands Red Cross Blood Transfusion Service (CLB), Ams erdam) [12].

Experimental design. Lymph of pa ien s wi h SIRS was po oed, as was lymph of he pa ien s wi hou SIRS; equal amoun s of lymph from each pa ien were used. The lymph pools were brough o final con cen ra i ons of 10% (vol%) in RPMI 1640 (BioWhi aker). Then, 0% lymph (RPMI 1640 only), 10% SIRS lymph, or 10% non-SIRS lymph was preincuba ed in he absence of LPS or in he presence of 1 or 10 ng/mL LPS (final con cen ra i ons) (Escherichia coli O111:B4; Sigma, S. Louis; 1 ng ≈ 12 endo oxin uni s) for 24 h in a CO2 incubu a 37°C. Therea er, PBMC (final con cen ra ion 0.5 × 10^6/mL) of each heal by volun ee er were incuba ed wi h he de heen lymph-LPS suspensions for 4 and 24 h in a CO2 incubu a 37°C for mea suremen of TNF-α and IL-6, respec ively. These dur a ions of icos were cho sen af er preliminary experimen s had es ablished ha he con cen ra ions of TNF-α and IL-6 peaked a hee ime poin s (da a no-shown).

Af er cen rifuga ion a 2000 g for 30 min a 4°C, superna an s were aliquo ed and s ored a −80°C un il assays were performed. An iden ical pro ocol was used o de ermine LPS-neu ra lizing ca pac i cs of 10% plasma of pa ien s wi h SIRS, 10% plasma of pa ien s wi hou SIRS, and 10% plasma of 10 heal by volun 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 accordan o he is u c ions of he manufac ural. LBP and BPI con cen ra ions were de ermined using his phenomenon also occurs in he ex ravaular compar isons found af er incuba ion wi h lymph or hose found af er incuba ion wi hou LPS.

Effect of lymph. Lymph of pa ien s wi h SIRS and con rols (pa ien s wi hou SIRS) reduced TNF-α and IL-6 produc ion af er s imula ion wi h 1 ng/mL LPS (P < .01 vs. LPS only; figure 1). A 10 ng/mL LPS, IL-6 produc ion was signi can 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 ng TNF-α produc ion elicied by 1 ng/mL LPS (P < .05) and in inhibi ing bo 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 heal by volun eers inhibi ed TNF-α and IL-6 produc ion (P < .05 vs. LPS only, excep for IL-6 release a 10 ng/mL LPS; figure 1), con firming a previous repor [2]. The ex en of inhibi ion by heal by 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-α and IL-6 release in deduced 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 con cen ra ions of LBP and IL-10 han lymph of pa ien s wi hou 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 wi h SIRS conained higher con cen ra ions of LBP and BPI han plasma of pa ien s wi hou SIRS (P < .02; able 1), while IL-10 con cen 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 con cen ra ions were higher in SIRS lymph han in SIRS plasma (P < .02).

Discussion

Discuss the presen findings demons ra e ha lymph of pa ien s wi h SIRS can par ially reproduc LPS olerance, possi bly indica ing ha his phenomenon also occurs in he ex ravaular compar ison. In pa ien s wi h SIRS, he body may comba LPS oxici y by reduc ing he ca pac iy of mononuclear cells o produc 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 par ially reproduc he LPS- oleran s a e in normal whole blood [2, 3]. I has no pre viously been s uided whe her, in he in er s i al fluid, an LPS- oleran s a e is presen, presumably relec ing processes a issue-level. Therefore, we aimed o invesiga e he ca pac iy of horacic duc lymph prom 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 er s i al fluid compar ison [5].

Pa ien s wi hou SIRS, undergoing a trans horacic resec ion of he esophagus for a carcinoma of he esophagus, were used as con rols. I is possible ha hee pa ien s have charac eris ics ha differ from heal by 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 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-oleran sample [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 concentration of IL-10 and increased plasma concentration of BPI in patients with SIRS may explain why SIRS

### Table 1. Concentrations (mean ± SE) of lipopolysaccharide-binding protein (LBP), bac tericidal/permeability-increasing protein (BPI), TNF-α, and IL-6 in lymph and plasma of patients with and without SIRS.

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<th>Lymph</th>
<th>Plasma</th>
<th>Lymph</th>
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<tr>
<td>LBP (μg/mL)</td>
<td>35 ± 10°</td>
<td>48 ± 9&quot;</td>
<td>11 ± 6</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>BPI (pg/mL)</td>
<td>&lt;200</td>
<td>924 ± 198*</td>
<td>&lt;200</td>
<td>&lt;200</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>885 ± 175±1</td>
<td>328 ± 87</td>
<td>371 ± 109</td>
<td>261 ± 110</td>
</tr>
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*P < .05 vs. lymph of patients without SIRS.
†P < .02 vs. plasma of patients with SIRS.
‡P < .02 vs. plasma of patients with SIRS.
lymph and SIRS plasma inhibited cy okine release more strongly than lymph and plasma of patients with SIRS, respectively.

Many other substances may bind LPS (e.g., antibodies, complements, albumin) or deacivate mononuclear cells. For example, lipoprotein ions are known to bind and neutralize LPS [12, 15]. Concentration of apolipoprotein ions A-1 and B were significantly lower in lymph and plasma of patients with SIRS compared with concentrations in lymph and plasma of patients without SIRS (data not shown). Lipoprotein ion concentrations can therefore not explain the more potent inhibition of proinflammatory cytokine production by SIRS lymph or SIRS plasma compared with non-SIRS lymph or non-SIRS plasma, respectively. The presence of IgG ion does not elucidate which other mediators might be involved. Thus, it seems that changes in LBP concentrations 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 response when added to cultures 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.

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