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 he production of proinflammatory cytokines (e.g., tumor necrosis factor-α and interleukin-6) in response to a second challenge. Increased levels of these LPS-modulating substances have been described in plasma of patients with sepsis. However, the precise mechanisms of LPS tolerance are unclear. In this study, we sought to determine the capacity of lymph to influence LPS-induced cytokine production. We therefore analyzed the effects of lymph and simultaneously collected plasma on cytokine production by normal peripheral blood mononuclear cells (PBMC) stimulated with LPS. In patients with SIRS, lymph and plasma inhibited LPS-induced cytokine production (P < .01). 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). Paired plasma and thoracic duct lymph samples were obtained from these patients upon routine cannulation of the thoracic duct for respiratory purposes. Lymph was obtained from the first 14-gauge cannula inserted in the neck, and plasma was obtained from a peripheral venous site. The samples were centrifuged at 1600 g for 20 minutes to obtain lymph supernatants and plasma. The lymph supernatants were stored at −70°C until analysis. The plasma samples were stored at 4°C until analysis.

**Lymph and plasma analysis.** The effects of lymph and plasma on cytokine production were assessed by in vitro stimulation of normal peripheral blood mononuclear cells (PBMC) with LPS. The cytokine production was determined by enzyme-linked immunosorbent assay (ELISA) for tumor necrosis factor-α and interleukin-6.

**Results.** Lymph and plasma from patients with SIRS inhibited LPS-induced cytokine production (P < .01). Lymph from patients without SIRS 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.

**Discussion.** LPS tolerance is characterized by down-regulation of the production of proinflammatory cytokines. Increased levels of these LPS-modulating substances have been described in plasma of patients with sepsis. However, the precise mechanisms of LPS tolerance are unclear. In this study, we sought to determine the capacity of lymph to influence LPS-induced cytokine production. We therefore analyzed the effects of lymph and simultaneously collected plasma on cytokine production by normal peripheral blood mononuclear cells (PBMC) stimulated with LPS. In patients with SIRS, lymph and plasma inhibited LPS-induced cytokine production (P < .01). This study suggests that LPS tolerance may occur both in the intra- and extravascular compartments.

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an and plasma were aliquo ed and s ored a 
\( -80^\circ \text{C} \) 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 eor was transfused immedia ely in a pyrogen-free ubes ha con ained pyrogen-free heparin. Blood was dilu ed 1:1 in Hanks’ Buffered Sal Solu ion (HBSS; BioWhi aker, Verriers, Belgium) and su bseqen by PBMC of each volun eor were isola ed by cen- rigufa ion over a densy gradien (Lymphopaqe Ficoll Paque; Pharmacia, Woerden, The Ne herlands) a room empera ure for 15 min a 600 g. Cells in he in erphase were collec ed, washed wice and hereaf er brough o a concen ra ion of 1 × 10^7 PBMC/mL in HBSS con aining 0.05% erile nonac he uman serum (Cent- Lboral a 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 pooled, 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 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 preincuba ed in he absence of LPS or in he 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 incuba or at 37°C. Thereaf er, PBMC (final concen ra ion 0.5 × 10^6/mL) of each heal by volun eer were incuba ed wi h he differen lymph-LPS suspensions for 4 and 24 h in a CO₂ incuba or or a 37°C for measuremen of TNF-α and IL-6, respec ively. These dura ions of lymph were cho sen af er preliminary experimen s had es ablished ha he lymph of pa ien s wi h SIRS con- ra ions of TNF-α and IL-6 peake a he same ime poin s (da a no shown).

Af er cen rigufa ion a 2000 g for 30 min a 4°C, supra na an s were aliquo ed and s ored a 
\( -80^\circ \text{C} \) un il assays were performed. An iden ical pro ool wa used o de ermine LPS-neu ra lizing ca- picies of 10% plasma of pa ien s wi hou 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, Belgium), IL-6 (PharMingen, San Diego), and IL-10 (PharMingen) were de ermined by ELISA aco oring o he ins ution ions of he manuac urer. LBP and BPI concen ra ions were de ermined using his phenomenon also occurs in he ex ravascular compar -specific ELISAs as described [9, 13]. The lower levels of de ec ion men .were 7 pg/mL (TNF-α) and 15 pg/mL, IL-6 release induced by 10 ng/mL LPS was inhibi ed more by SIRS plasma han by non-SIRS plasma (P < .05; figure 1). SIRS lymph was more po en han lymph of con rol s in inhibi ing TNF-α produc ion elici ed by 1 ng/mL LPS (P < .05) and in inhibi ing bo h TNF-α and IL-6 produc ion elici ed 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, except for IL-6 release a 10 ng/mL LPS; figure 1), confirming 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 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 con-ained higher concen 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 hou SIRS con ained higher concen ra ions of LBP and BPI han plasma of pa ien s wi hou 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 wi h SIRS can par ailly reproduce LPS olerance, possibly indica ing ha his phenemon also occurs in he ex ravascular compar -men .

In pa ien s wi h SIRS, he body may comba LPS oxic y by reducin he capaci y 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 ailly reproduced he LPS- oleran s a e in normal whole blood [2, 3]. I has no previously been s uded whe her, in he in er ial fluid, an LPS- oleran s a e is presen , presumably refe ing processes a issue-level. Therefore, we aimed o inves ra e capaci y of horacic duc lymph from 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 ial fluid compar men [5].

Pa ien s wi hou SIRS, undergoing a rans horacic resec ion of he esophagus for a carcinoma of he esophagus, were used as con role. I is possible ha he lymph of 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-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.

Table 1. Concentrations (mean ± SE) of lipopolysaccharide-binding protein (LBP), bactericidal/permeability-increasing protein (BPI), 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-sensitive state [14].

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<tr>
<td>LBP (μg/mL)</td>
<td>35 ± 10*</td>
<td>48 ± 9†</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>
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<tr>
<td>IL-10 (pg/mL)</td>
<td>885 ± 175*</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 without SIRS.
‡ P < .02 vs. plasma of patients with SIRS.

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-sensitive state [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 concentrations of BPI in patients with SIRS may explain why SIRS...
lymph and SIRS plasma inhibited cytokine release more strongly than lymph and plasma of patients without SIRS, respectively.

Many other substances may bind LPS (e.g., antibodies, complement, albumin) or deacivate mononuclear cells. For example, lipoprotein ions are known to bind and neutralize LPS [12, 15]. Concentrated ions 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 plasma compared with non-SIRS lymph or non-SIRS plasma, respectively. The presence of high-levels of cytokines does not elucidate which other media or factors might be involved. Thus, it seems that changes in LBP concentrations correlate ions do not con ribute significantly to the development 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 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.

References