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 interleukin-6) but with an enhanced synthesis of IL-1 receptor or an agonist upon exposure to LPS. This phenomenon has been found in patients with systemic inflammatory response syndrome (SIRS) and in healthy humans in vivo and in vitro. Reduced LPS-induced cytokine production by normal peripheral blood mononuclear cells was assessed. 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 and thoracic duct lymph were obtained from 8 patients with SIRS (6 men, 2 women, age $\pm 62 \pm 5$ years) and from 7 patients without SIRS (4 men, 3 women, age $64 \pm 2$ years). Patients with SIRS, admitted to the intensive care unit of the Academic Medical Center, Amsterdam, fulfilled the SIRS criteria [11] and had organ failure of at least two organs. In these patients, the thoracic duct was cannulaed with a 14-gauge double-lumen catheter, and immediately, blood was collected in pyrogen-free plastic tubes (Sarstedt, Nümbrecht, Germany) containing EDTA-K$_2$ (Vacu ainter Sys ems; Bec on, NJ). Lymph was collected in prepyrogen-free plastic tubes (Sarstedt ed , Nümbrecht , Germany) containing pyrogen-free heparin (Tromboliquine; Organon, Oss, The Netherlands). The studies were approved by the institutional ethical and scientific committees. Written informed consent was obtained from all patients or their relatives.

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The s udes were approved by the ins i umonal scien ific and e hics commi - ees. Wrie in informed consent was obained from pa ien s or heir rela ives. Gran suppor : Royal Du ch Academy of Ar s and Sciences ( o T.v.d.P.). Reprin s or correspondence: Dr. Lucienne Lemaire, Dep . of Surgery, Aca- de mical Medical Cen er, Room 641-115, 1105 AZ Ams edam, The Ne herlands (L.C.Lemaire@AMC.UVA.NL). The Journal of Infectious Diseases 1998;178:883–6 © 1998 by the Infec ious Diseases Socie y of America. All righ s reserved. 0022–1899/98/7803–0040$02.00
an and plasma were aliquo ed and s ored a −80°C un il fur her processing.

**PB C isolation.** Blood was ob ained aseptic ally from 12 heal by male volun eers (age 33 ± 2 years). Blood from each volun eer was transferred immedia ely in o pyrogen-free ubes ha con ained pyrogen-free heparin. Blood was dilu ed 1:1 in Hanks’ Buffered Sal Solu ion (HBSS; BioWhi aker, Verviers, Belgium) and subsequently by PBMC of each volun eer 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 erphase were collec ed, washed wice and here af er brough o a concen ra ion of 1 × 10⁶ PBMC/mL in HBSS con aining 1% erle nonacu e human serum (Cen- nal 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 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 × 10⁶/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 at 37°C for measuremen of TNF-α and IL-6, respec ively. These dura ions of lymph-LPS incuba ion were cho- en af er preliminary experimen s had es ablished ha he concen ra ions o f TNF-α and IL-6 peaked a a heime po en 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 ralizing ca- paci es of 10% plasma o f pa ien s wi h SIRS, 10% plasma o f pa ien s wi hou SIRS, and 10% plasma o f 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 according o he ins ruc ions of he SIRS can par ially reproduce LPS olerance, possibly indica ing thi phenomenon also occurs in he ex ravascular compar men. LBP and BPI concen ra ions were de ermined using ha his phenomenon also occurs in he ex ravascular compar men. The lower levels of de ec ion men were 7 pg/mL (TNF-α). IL-6 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 by 10 ng/mL LPS; figure 1), con firma ing a previous repor [2]. The ex en of inhibi tion by heal by plasma was less (P < .05) compared wi he lower levels of de ec ion men wa N 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 o f 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 o f bo h SIRS and non-SIRS pa ien s (able 1). Plasma of pa ien s wi h SIRS con ai ned higher concen ra ions o f LBP and BPI han plasma o f 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 lymph han 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 o f pa ien s wi h SIRS can par iALLY produce LPS olerance, possibly indica ing ha his phenomenon also occurs in he ex ravascular compar men.

In pa ien s wi h SIRS, he body may comba LPS oxici y by reduci ng he capaci y o f mononuclear cells o produce proin- flamma ory cy okines upons res imula ion ion wi h LPS. I has been shown ha soluble media ors are involved, since serum o f sep ic pa ien s and endo oxemic volun eers par iALLY produced he LPS- eran s a e in normal whole blood [2, 3]. I has no previously been s udied whe her, in he in er s i al fluid, an LPS-eran s a e is presen, presumably reflec ing processes a issue-level. Therefore, we aim ed o inves iga e capaci y o f horacic duc lymph from pa ien s wi h SIRS o influence LPS-produced cy okine produc ion, since horacic duc lymph has been shown o represen he in er s i al fluid compar men [5].

Pa ien s wi h SIRS, undergoing a rans horacic resec ion o f he esophagus for a carcinoma o f he esophagus, were used as con rols. I is possible ha hepa 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.

Table 1. Concentrations (mean ± SE) of lipopolysaccharide-binding protein (LBP), bactericidal/permeability-increasing protein (BPI), TNF-α, and IL-6 production by normal PBMC. Indeed, lymph of patients with SIRS inhibited 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-resistant state [14].

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<th>Lymph Plasma</th>
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<tr>
<td>LBP (μg/mL)</td>
<td>35 ± 10∗</td>
<td>48 ± 9†</td>
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<td></td>
<td>11 ± 6</td>
<td>8 ± 2</td>
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<tr>
<td>BPI (pg/mL)</td>
<td>&lt;200</td>
<td>924 ± 198†</td>
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<td></td>
<td>&lt;200</td>
<td>&lt;200</td>
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<tr>
<td>IL-10 (pg/mL)</td>
<td>885 ± 175‡</td>
<td>328 ± 87</td>
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<td>371 ± 109</td>
<td>261 ± 110</td>
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∗P < .03 vs. lymph of patients without SIRS.
†P < .02 vs. plasma of patients without SIRS.
‡P < .02 vs. plasma of lymph of patients with SIRS.
lymph and SIRS plasma inhibit cyokine release more strongly than lymph and plasma of patients with 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]. Concentration of apolipoprotein ions A-I and B were significantly lower in lymph and plasma of patients with SIRS compared with conscious rats in lymph and plasma of patients with SIRS (data not shown). Lipoprotein ions can herefore 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 significant cytokine release does not explain the reduction in lymphocyte-mediated cytokine release.

LPS tolerance is associated with decreased production of mononuclear cells that produce cytokines upon stimulation with LPS. Here, we show that lymph obtained from patients with SIRS can reproduce an LPS-resistant effect 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
8. Brandzaeg P, Osnes L, Ovsen R, Joerg GB, Wessvik AB, Kierulf P. Necrocyte production by SIRS lymph or SIRS plasma compared with non-SIRS lymph or non-SIRS plasma, respectively. The presence of cytokines in lymph and plasma of patients with SIRS is responsible, at least in part, for the phenomenon of LPS tolerance.