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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-6 (IL-6) by whole blood of lipopolysaccharide-stimulated whole blood [1, 2]. This phenomenon has been found in patients with SIRS [3, 4]. Besides plasma, lymph may be another body compartment in LPS tolerance. Lymph is present in the thoracic duct lymph from patients with SIRS [4]. Moreover, thoracic duct lymph represents the extravascular, interstitial body compartment [5] and therefore may reflect processes at the tissue level. The effector lymph of lymph on LPS-induced cytokine production is unknown.

Several circulating factors are known to modulate the cytokine production of LPS in vivo. These include soluble mediators in the circulation, such as tumor necrosis factor-α, interleukin-6, and IL-10. The concentrations of these mediators in lymph and plasma were measured in this study to assess the potential role of LBP, BPI, and IL-10 in LPS tolerance.

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 [mean ± SD] 62 ± 5 years) and from 7 patients without SIRS (4 men, 3 women, age 64 ± 2 years). Plasma and thoracic duct lymph were obtained from patients undergoing thoracic duct lymph sampling for organ transplantation or carcinoma of the esophagus. From both patient groups, an arterial blood sample was drawn at 14-gauge indwelling catheter. Lymph was collected at 4.5-mL ube with an additional 0.048 mL of EDTA-K3 (Vacu ainer Sys ems; Becton-Dickinson, Rutherford, NJ). Lymph was collected in pyrogen-free plastic tubes (Sars ed, Nunnfrueh, Germany) containing pyrogen-free heparin (Tromboli-quine; Organon, Oss, The Netherlands) in the neck of the patient in sterile conditions. Written informed consent was obtained from the 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 asept ically from 12 heal hy male volun eers (age 33 ± 2 years). Blood from each volun eer was trans ferred immedi aly 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 subse quently by PBMC of each volun eer were iso la ed by cen rifuga ion over a densi y gradien (Lymphopaque Ficoll Paque; Pharmacia, Woe deren, The Ne herlands) a room empera ure for 15 min a 600 g. Cells in he in erphase were col lec ed, washed wice and hereaf er brough o a concen ra ion of 1 × 10^6 PBMC/mL in HBSS con aining 10% erile nonac e he manu fac urer. LBP and BPI concen ra ions were de ermined using ha his phenomenon also occurs in he ex rava sular compar menion with lymph) and 10% plasma of 10 heal hy volun eers (ege 33 ± 2 years). Blood from each figure 1). A 10 ng/mL LPS, IL-6 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-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 hy 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), confir ming a previous repor [2]. The ex en of inhibi ion by heal hy 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 redu cin 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 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 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 a ha lymph of pa ien s wi h SIRS can parially reproduce LPS ol erance, possibly indica ing ha his phenomenon also occurs in he ex rava sular compar men.

In pa ien s wi h SIRS, he body may comba LPS oxici y by reducin 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 solu ble media ors are involved, since serum of sep ic pa ien s and endo oxemic volun eers parially reproduced he LPS- ol eran s a e in normal whole blood [2, 3]. I has no previously been s uided whe her, in he in er s i al fluid, an LPS-ol eran s a e is presen, presumably re le ac ing processes a issue-level. Therefore, we aimed o inves ra e he 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 s i al 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 he pa ien s have charac eris ics ha differ from heal hy individuals. However, we assume ha

**Results**

**Incubation without LPS.** Incuba ion of normal PBMC wi hou LPS only resul ed in he e ca able levels of TNF-α and IL-6 in he presence of lymph or plasma of pa ien s wi h SIRS. In he e samples, TNF-α levels were 39 ± 15 pg/mL (incuba ion wi h lymph) and 10 ± 7 pg/mL (incuba ion wi h plasma); IL-6 concen ra ions were 1774 ± 88 and 502 ± 43 pg/mL, respec ively. Therefore, he ex vivo produc ion of TNF-α and IL-6 was calcula ed as he differ ence be ween cy okine concen ra ions found af er incuba ion wi h LPS and hose found af er incuba ion wi hou LPS.
**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 testing, 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 without 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-sensitized state [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

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**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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<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>
<td>&lt;200</td>
</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>
</tr>
</tbody>
</table>

* P < .03 vs. lymph of patients with SIRS.
† P < .02 vs. plasma of patients with SIRS.
‡ P < .02 vs. plasma of patients without SIRS.
lymph and SIRS plasma inhibited 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 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 plasma compared with non-SIRS lymph or non-SIRS plasma, respectively. The presence of IgG in the serum does not elucidate which other media or factors are involved. Thus, it seems that factors 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-sensitizing 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 development of LPS tolerance.

References