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 (e.g., tumor necrosis factor-α, or TNF-α, in erelukin [IL]-6) by peripheral blood mononuclear cells when challenged with LPS [1, 2]. This phenomenon has been found in pa ien s wi h SIRS [4]. Moreover, horacic duct lymph represses the ex vacavus, in ers i a l body compar men [3] and therefore may reflect processes at issue level. The effec of lymph on LPS-induced cytokine production is unknown.

Several circula ing fac ors known o modula e he oxici y of LPS migh be involved in LPS tolerance. These are sub-s ances ha ei her facili a e LPS-induced ac iva ion of cells, such as LPS-binding pro e (LBP) [6], or neu raiz LPS, such as bac ericial/permeabil y-increasing pro e (BPI) [7]. In ad di e, he horacic duct lymph is considered o an agonize LPS-induced oxici y by deac iva ion of monocy es [8]. Increased levels of these LPS-modulating sub-s ances have been de ed in plasma of pa ien s wi h SIRS [9, 10]. In his s udy, we sough o de ermine he capaci y of lymph o influence LPS-induced cytokine production. We herefore ob ain ed horacic duct lymph of pa ien s wi h SIRS and wi hou SIRS and assessed he effec of lymph and simul aneou e collect ed plasma on cy okine produc ion by normal peripheral blood mononuclear cells (PBMC) in simula ed wi h LPS. Fur her, o ob ain ed he horacic duct lymph in pa ien s wi h SIRS and wi hou SIRS (6 men, 2 women, age 62 ± 5 years) and from 7 pa ien s wi h SIRS (4 men, 3 women, age 64 ± 2 years). Pa ien s wi h SIRS, admi ed ed o he in e cir Ing fa ors known o modula e he oxici y of LPS migh be involved in LPS tolerance. These are sub-s ances ha ei her facili a e LPS-induced ac iva ion of cells, such as LPS-binding pro e (LBP) [6], or neu raiz LPS, such as bac ericial/permeabil y-increasing pro e (BPI) [7]. In ad di e, he horacic duct lymph is considered o an agonize LPS-induced oxici y by deac iva ion of monocy es [8]. Increased levels of these LPS-modulating sub-s ances have been de ed in plasma of pa ien s wi h SIRS [9, 10].

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Patients and Methods

Plasma and thoracic duct lymph collection. Peripheral blood plasma and horacic duct lymph were ob ain ed from 8 pa ien s wi h SIRS (6 men, 2 women, age 62 ± 5 years) and from 7 pa ien s wi h SIRS (4 men, 3 women, age 64 ± 2 years). Pa ien s wi h SIRS, admi ed ed o he in e cir Ing fa ors known o modula e he oxici y of LPS migh be involved in LPS tolerance. These are sub-s ances ha ei her facili a e LPS-induced ac iva ion of cells, such as LPS-binding pro e (LBP) [6], or neu raiz LPS, such as bac ericial/permeabil y-increasing pro e (BPI) [7]. In ad di e, he horacic duct lymph is considered o an agonize LPS-induced oxici y by deac iva ion of monocy es [8]. Increased levels of these LPS-modulating sub-s ances have been de ed in plasma of pa ien s wi h SIRS [9, 10].

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The s udies we approved by he ins u lonal scien ic and e hics commi - cees. Wri en informed consen was ob ain ed from pa ien s or heir rela ives. Gran suppor : Royal Du ch Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen er, Academ Cen...
Results

Preclinical studies of TNF-α and IL-6 from primary cells of the esophagus for esophageal carcinoma, were used in cultures without LPS. As seen in healthy individuals, we assume that IL-6 concentrations were 1774 ± 94 pg/mL (normal cytokine production) in healthy individuals. LBP and BPI concentrations were determined using enzyme-linked immunosorbent assays (ELISA) as described [9, 13]. The lower levels of detection were 7 pg/mL (TNF-α). The detection limit in lymph from patients with SIRS was higher than in lymph from non-SIRS patients (P < 0.05; figure 1). SIRS lymph was more potent in lymph from patients in inhibiting TNF-α production of lymphocytes by 1 ng/mL LPS (P < 0.05) and in inhibiting IL-6 by 10 ng/mL LPS (P < 0.05; figure 1).

Experimental design. Lymph from patients without SIRS was pooled, as was lymph from healthy volunteers; equal amounts of lymph from each patient were used. The lymph pools were treated with formaldehyde to inactivate virions in RPMI 1640 (BioWhiaker). Then, 0% lymph (RPMI 1640 only), 10% SIRS lymph, or 10% non-SIRS lymph was preincubated in the absence of LPS or in the presence of 1 or 10 ng/mL LPS (final concentrations) (Escherichia coli 0111:B4; Sigma, St. Louis; 1 ng/mL endotoxin unit/mL) for 4 hours in a CO2 incubator at 37°C. Then, PBMC (final concentration 0.5 × 10^6/mL) of each healthy volunteer were incubated with or without LPS in the absence of LPS or in the presence of lymph-LPS suspensions for 4 hours; cultures were performed in triplicate. The lower limits of detection were 7 pg/mL (TNF-α), 14 pg/mL (IL-6), 8 pg/mL (IL-10), 100 pg/mL (LBP), and 200 pg/mL (BPI).

Statistical analysis. All values are expressed as mean ± SD. Data were compared by paired and unpaired Wilcoxon tests as appropriate. P < 0.05 was considered significant.

Discussion

The presence of findings demonstrates the role of lymph from patients with SIRS can partially reproduce LPS tolerance, possibly indicating that the phenomenon also occurs in the extracellular compartment. As seen in healthy individuals, we assume that IL-6 concentrations were 1774 ± 94 pg/mL (normal cytokine production) in healthy individuals. LBP and BPI concentrations were determined using enzyme-linked immunosorbent assays (ELISA) as described [9, 13]. The lower levels of detection were 7 pg/mL (TNF-α). The detection limit in lymph from patients with SIRS was higher than in lymph from non-SIRS patients (P < 0.05; figure 1). SIRS lymph was more potent in lymph from patients in inhibiting TNF-α production of lymphocytes by 1 ng/mL LPS (P < 0.05) and in inhibiting IL-6 by 10 ng/mL LPS (P < 0.05; figure 1).

LBP, BPI, and IL-10. Lymph from patients with SIRS contains higher concentraions of LBP and IL-10 than lymph from patients without SIRS (P < 0.03), while BPI levels were below the detection limit in lymph from patients with SIRS and non-SIRS patients (P < 0.03; figure 1). Plasma from patients with SIRS contained higher concentrations of LBP and BPI than plasma from patients without SIRS (P < 0.02; figure 1), while IL-10 concentration in plasma from patients with SIRS was higher than in plasma from patients without SIRS (P < 0.03). IL-10 concentration in plasma from patients with SIRS was higher than in plasma from patients without SIRS (P < 0.02).
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), bacercidal/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 without SIRS, undergoing major surgery, also inhibited LPS-induced 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].

<table>
<thead>
<tr>
<th></th>
<th>Lymph (n = 8)</th>
<th>Plasma (n = 8)</th>
<th>Lymph (n = 7)</th>
<th>Plasma (n = 7)</th>
</tr>
</thead>
<tbody>
<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>
<td>&lt;200</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</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 with SIRS.

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 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].

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 the increased plasma concentration of BPI in patients with SIRS may explain why SIRS...
lymph and SIRS plasma inhibit ed cytokine release more strongly than lymph and plasma of pa ient s wi h SIRS, re spectively.

Many other substances may bind LPS (e.g., antibodies, com-

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lymph ab ained from pa ien s wi h SIRS can reproduce an LPS- oleran s a e when added o cul ures of normal PBMC. These da a sugges ha soluble media ors pres-

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