Lymph of patients with a systemic inflammatory response syndrome inhibits lipopolysaccharide-induced cytokine production


Published in:
The Journal of Infectious Diseases

DOI:
10.1086/515348

Citation for published version (APA):
Lymph of Patients with a Systemic Inflammatory Response Syndrome Inhibits Lipopolysaccharide-Induced Cytokine Production

Lucienne C. J. Lemaire, J. Jan B. van Lanschot, Tom van der Poll, Wim A. Buurman, Sander J. H. van Deventer, and Dirk J. Gouma

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, and interleukin-6, IL-6) by peripheral blood mononuclear cells (PBMC) that have been activated by LPS in vitro (1, 2). This phenomenon has been found in patients with SIRS (3–5) and in healthy humans (6). Allogeneic mechanisms of LPS tolerance are unclear, as the mechanisms of tolerance are unknown.

Severe circulatory failure is a common feature of SIRS. In patients with SIRS, the production of cytokines is often increased, and the ability of the immune system to respond to a second challenge with LPS is decreased. This phenomenon has been found in patients with SIRS (3–5) and in healthy humans (6). Allogeneic mechanisms of LPS tolerance are unclear, as the mechanisms of tolerance are unknown.

Patients and methods.

Plasma and thoracic duct lymph collection. Peripheral blood plasma and thoracic duct lymph were collected from 8 patients with SIRS (6 men, 2 women, age [mean ± SD] 62 ± 5 years) and from 7 healthy volunteers (4 men, 3 women, age 64 ± 2 years). Blood samples were collected from the thoracic duct for carcinoma of the esophagus or gas reoxygenation or resection of the esophagus. The collection of lymph from the thoracic duct was performed after the insertion of a thoracic duct catheter (14-gauge single-lumen) in the fifth intercostal space. Lymph was collected in pyrogen-free plastic tubes (Sarsted, Germany) containing pyrogen-free heparin (Tromboline; Organon, Oss, The Netherlands) and was assayed for the presence of LPS.

Received 20 January 1998; revised 10 April 1998.

The authors were approved by the ethics committee of the local hospital. Written informed consent was obtained from all patients.

Gran support: Royal Dutch Academy of Arts and Sciences (to T. v. d. F.)

Reprints or correspondence: Dr. Lucienne Lemaire, Department of Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.
Results

with LPS only resulted in detectable levels of TNF-α. In these samples, TNF-α incubation with LPS had differences from healthy individuals. However, we assume that this phenomenon also occurs in the extravascular compartment-specific ELISAs as described [9, 13]. The lower levels of detection were 7 pg/mL (TNF-α) were determined by ELISA according to the instructions of the SIRS can partially reproduce LPS tolerance, possibly indicating that patients with SIRS, and 10% plasma of 10 healthy volunteers of 10% plasma of patients with SIRS, 10% plasma of

PBMC isolation. Blood was obtained aseptically from 12 healthy male volunteers (age 33 ± 2 years). Blood from each volunteer was transferred immediately in pyrogen-free tubes in a room emergerate for 15 min at 600 g. Cells in the supernatant were collected, washed twice and hereafter brough to a conen rate ion of 1 × 10^6 PBMC/mL in HBSS containing 1% non-accelerated human serum (Centrifugation Ficoll Paque; Pharmacia, Woerden, The Netherlands) to a room temperature at 10 min.

Incubation of normal PBMC (final concentration 0.5 × 10^6/mL) of each healthy volunteer were incubated with 10% of the respective lymph-LPS suspensions for patients with SIRS (table 1). Plasma of patients with SIRS contained higher concentrations of LBP and IL-10 than lymph of the same lymph and plasma. Plasma obtained from healthy volunteers inhibited 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 inhibited more by SIRS plasma than by non-SIRS plasma (P < .05; figure 1).

Comparison of lymph and plasma. Plasma obtained from healthy volunteers inhibited by SIRS-α and IL-6 production (P < .05 vs. LPS only, except for IL-6 release at 10 ng/mL LPS; figure 1), confirming a previous report [2]. The extent of inhibition by heat inactivation was less (P < .05) compared with LPS he inhibitory effect on both 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 inhibited more by SIRS plasma than by non-SIRS plasma (P < .05; figure 1). SIRS plasma was more potent in reducing cytokine production than LPS levels in lymph (P < .03), as was non-SIRS plasma compared with non-SIRS lymph (P < .03; figure 1).

LBP, BPI, and IL-10. Lymph of patients with SIRS contained higher concentrations of LBP and IL-10 than lymph of healthy volunteers with SIRS (P < .03), while BPI levels were below the detection limit in lymph of both SIRS and non-SIRS patients (P < .02; table 1). Patients with SIRS demonstrated higher concentrations of LBP and BPI than plasma of healthy volunteers with SIRS (P < .02; table 1), while IL-10 concentration in patients with SIRS was lower than in healthy volunteers with SIRS lymph (P < .03). IL-10 concentration in patients with SIRS was higher in SIRS lymph than in SIRS plasma (P < .02).

Discussion

The presented findings demonstrate that ha lymph of patients with SIRS can partially reproduce LPS tolerance, possibly indicating that this phenomenon also occurs in the extravascular compartment.

In patients with SIRS, the body may combat LPS oxysym by reducing the capaciy of mononuclear cells to produce proinflammatory cytokines upon stimulation with LPS. I has been shown that soluble media ors are involved, since serum of sepic patients and endotoxemic volunteers partially reproduced LPS-oleran's effects in normal whole blood [2, 3]. I has not been previously shown that sepsis or IL-10 in septic patients has led to a change in the systemic response. Therefore, we aimed to investigate the effects of horacic duct lymph from patients with SIRS on the systemic response, possibly reflecting the processes of horacic duct lymph that has been shown to occur in septic patients in sepsis [5].

Paired and unpaired Wilcoxon tests were used as appropriate. P < .05 was considered significant.
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), bacterial/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-overload syndrome [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>385 ± 175*</td>
<td>328 ± 87</td>
<td>371 ± 109</td>
<td>261 ± 110</td>
</tr>
</tbody>
</table>

* P < .03 vs. lymph of patients without SIRS.
† P < .02 vs. plasma of patients without SIRS.
‡ P < .02 vs. plasma of patients with SIRS.

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 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-overload syndrome [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 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 decelerate mononuclear cells. For example, lipoprotein ions are known to bind and neutralize LPS [12, 15]. Concena ions of apolipoprotein A-I and B were significantly lower in lymph and plasma of patients with SIRS compared with concena ions in lymph and plasma of patients with SIRS (data not shown). Lipoprotein eon concena ions cannot therefore 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 IgA does not elucidate which other media ors might be involved. Thus, it seems that concena ions in LBP concentrations may play a role in LPS tolerance.

LPS tolerance is associated with 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 media ors present in lymph (and plasma) are responsible, at least in part, for the phenomenon of LPS tolerance.

References