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

Link to publication

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
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, in the leukemoid reaction) by peripheral blood mononuclear cells when challenged with LPS [1, 2]. This phenomenon has been found in patients with a systemic inflammatory response syndrome (SIRS) and in healthy humans, with the highest level of TNF-α production in low-dose LPS [1, 2]. Although the precise mechanisms of LPS tolerance are unclear, it is caused by a decrease in paracrine soluble media or in circulating immune complexes, since serum of patients with SIRS has a lower level of TNF-α [3, 4]. Besides plasma, lymph may be an important body compartment in LPS tolerance, as LPS is present in human lymph from patients with SIRS [4]. Moreover, horacic duct lymph represents a reservoir, in which LPS can be isolated, as it has been shown that LPS is present in paracrine soluble media or in circulating immune complexes, since serum of patients with SIRS has a lower level of TNF-α [3, 4].

Several circulating factors involved in LPS tolerance are unknown. These factors include TNF-α, IL-10, and IL-12, which are known to play a role in LPS tolerance. In patients with SIRS, tolerance to a second LPS challenge is well described [5]. Moreover, SIRS is characterized by an increase in circulating TNF-α, IL-10, and IL-12, which are known to play a role in LPS tolerance. In patients with SIRS, tolerance to a second LPS challenge is well described [5]. Moreover, SIRS is characterized by an increase in circulating TNF-α, IL-10, and IL-12, which are known to play a role in LPS tolerance.

In healthy humans, LPS tolerance is characterized by an increase in circulating TNF-α, IL-10, and IL-12, which are known to play a role in LPS tolerance. In patients with SIRS, tolerance to a second LPS challenge is well described [5]. Moreover, SIRS is characterized by an increase in circulating TNF-α, IL-10, and IL-12, which are known to play a role in LPS tolerance.

Plasma and thoracic duct lymph collection. Peripheral blood plasma and lymph were obtained from patients with SIRS (6 men, 2 women, age 62 ± 5 years) and from healthy volunteers (4 men, 3 women, age 62 ± 5 years). Lymph was collected from patients with SIRS (6 men, 2 women, age 62 ± 5 years) and from healthy volunteers (4 men, 3 women, age 62 ± 5 years).

Received 20 January 1998; revised 10 April 1998.

The Journal of Infectious Diseases 1998;178:883–6 © 1998 by the Infectious Diseases Society of America. All rights reserved.
Results

Incubation without LPS. Incubation of normal PMBC with hou LPS only resut led in de eable levels of TNF-α and IL-6 in he presence of lymph or plasma of pa ien s w i h SIRS. In hees samples, TNF-α levels were 39 ± 15 pg/mL (mula ion wih lymph) and 10 ± 7 pg/mL (mula ion wih plasma); IL-6 concen ra ions were 1774 ± 88 and 502 ± 43 pg/mL, repectively. Therefore, he e vivo produc ion of TNF-α and IL-6 was calcula ed as he difference be w een cy okine concen- ra ions found af er incuba ion wi h hou LPS and hose found af er incuba ion wi hou hou LPS.

Effect of lymph. Lymph of pa ien s w i h SIRS and con rol s (pa ien s w i hou SIRS) reduced TNF-α and IL-6 produc ion af er s imula ion wi h 1 ng/mL LPS (P < .01 vs. LPS only; 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 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 obained af er heal hy volun ers 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 e of inhibi ion by heal plasma was less (P < .05) compared wi h he inhibi ion by SIRS plasma and non-SIRS psama (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 psama han by non-SIRS psama (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 compar ed wi h non-SIRS lymph (P < .03; figure 1).

LB, BPI, and IL-10. Lymph of pa ien s w i h SIRS conained higher concen ra ions of LBP and IL-10 han lymph of pa ien s w i 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 w i h SIRS conained higher concen ra ions of LBP and BPI han plasma of pa ien s w i hou SIRS (P < .02; able 1), while IL-10 concen ra ions were no different be w een he e groups. LBP levels were higher in SIRS psama han in SIRS lymph (P < .03). IL-10 concen ra ions were higher in SIRS lymph han in SIRS psama (P < .02).

Discussion

The presen findings demons ra e ha lymph of pa ien s w i h SIRS can parially reproduc LPS olerance, possibly indica ing ha his phenomenon also occurs in he ex rascular compar men.

In pa ien s w i h SIRS, he body may comba LPS oxici y y reduci ng he capaci y of mononuclear cells o produc proin- flamma ory cy okines upon res imula ion ion wih LPS. I has been shown ha soluble media urs are involved, since serum of sep ic pa ien s and endo oxemic volun ers parially reproduc he LPS- oleran e a e in normal whole blood [2, 3]. I has no previously been s uided whe her, in he in urs ial fluid, an LPS- oleran e a e is presen, presumably reflec ing processes a issue-level. Therefore, we aimed o invesiga e he capaci y of horacic duc lymph from pa ien s w i h SIRS o influence LPS-induced cy okine produc ion, since horacic duc lymph has been shown o represen he in urs ial fluid compar men [5].

Pa ien s w i h SIRS, undergoing a rans horacic resec ion of he esophagus for a carcinoma of he esophagus, were used as con rol s. I is possible ha heese pa ien s have charac eris ics ha differ from heal hy individuals. However, we assume ha

PB C isolation. Blood was obained asepsically from 12 heal hy male volun ers (age 33 ± 2 years). Blood from each volun er was reserred immedia eiy in o pyrogen-free ubes ha conained pyrogen-free heparin. Blood was diu ed 1:1 in Hanks’ Buffered Sal Solu ion (HBSS; BioWhi aker, Verviers, Belgium) and subsequen ly by PBMC of each volun er were isola ed by cen- rifuga ion on 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 erphase were collec ed, washed wice and hereaf er brough a concen ra ion of 1 × 106 PBMC/mL in HBSS con aining a er ile nonacu e human serum (Cen- ral Labora ory of The Ne herlands Red Cross Blood Transfusion Service (CLB), Ams erdam) [12].

Experimental design. Lymph of pa ien s w i h SIRS was poed, as was lymph of he pa ien s w i 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/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 or 37°C. Thereaf er, PBMC (final concen ra ion 0.5 × 109/mL) of each heal hy volun er were incuba ed wi h he differen lymph-LPS suspensions for 4 and 24 h in a CO2 incuba or or 37°C for measuremen of TNF-α and IL-6, respce ively. These dura urs of incubation were cho- sen af er preliminary experimen es had es ablished ha he concen ra ions of TNF-α and IL-6 peaked a heese ime poin 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 cal pro ocol was used o de ermine LPS-neu ralizing ca- paci es of 10% plasma of pa ien s w i h SIRS, 10% plasma of pa ien s w i hou SIRS, and 10% plasma of 10 heal hy volun ers (10 women, age 29 ± 2 years). TNF-α (Medgenix, Fleurus, Bel- giurn), IL-6 (PharMingen, San Diego), and IL-10 (PharMingen) were de ermined by ELISA ac- cording o he ins uctions of he manufac urer. LBP and BPI concen ra ions were de ermined using specific ELISAs as described [9, 13]. The lower levels of de ec ion 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 ± SE. Da a were compared by paired and unpaired Wilcoxon es s as aproipa e. P < .05 was considered signiicant.
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.

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- tolerant state [14].

Table 1. Concentrations (mean ± SE) of lipopolysaccharide-binding protein (LBP), bac tericidal/permeability-increasing protein (BPI), and interleukin-10 (IL-10) in lymph and plasma of patients with SIRS and without SIRS.

<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 < .05 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 approximate the condition of healthy volunteers, the ideal control group.

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 SIRS and without SIRS.

<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 < .05 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 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-tolerant 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 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]. Concentrations of apolipoprotein A-I 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 concentration 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 LBP concentrations in lymph and plasma of patients without SIRS, on the other hand, may explain the more potent inhibition of proinflammatory cytokine production by SIRS lymph or plasma compared with non-SIRS lymph or plasma, respectively. The monocyte-based arginine assay identified interleukin-10 as a major functional deactivator of human monocytes. The phenomenon of 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 the LPS-tolerant phenotype when added to culture of normal PBMC. These data suggest a soluble mediator present in lymph (and plasma) that is responsible, at least in part, for the phenomenon of LPS tolerance.

References