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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, or IL-6) by normal cells in response to a second challenge with LPS. In SIRS patients, lymph and simultaneously collected plasma reduced LPS-induced cytokine production by normal peripheral blood mononuclear cells. This study suggests that LPS tolerance may occur both in the intra- and extravascular compartments.
an and plasma were aliquo ed and s ored a −80°C un il fur her processing.

**PB C isolation.** Blood was ob ained aseptically from 12 heal hy male volun eers (age 33 ± 2 years). Blood from each volun eer was transferred immedia e in o pyrogen-free ubes ha con aigned pyrogen-free heparin. Blood was dilu ed 1:1 in Hanks’ Buffered Sal Solu ion (HBSS; BioWhi aker, Verviers, Belgium) and subseq uently by PBMC of each volun eer were iso la ed by cen- rifuga ion over a densi y gradien (Lympopaque Ficoll Paque; Pharmacia, Woerden, The Ne herlands) a room empera ure for 15 min a 600 g. Cells in he er phase were col leced, washed twice and hereaf er brough a con cen ra ion of 1 × 10⁶ PBMC/mL in HBSS con aining e s erile nonac u e human serum (Cen- tral Labora ory of The Ne herlands Red Cross Blood Tran fusion 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; equa al so, lymph of he 2 groups. LBP levels were higher in SIRS lymph han in SIRS plasma (\(P < .05\)) as was lymph of he pa ien s wi hou SIRS; equal amoun s lymph of each pa ien were used. The lymph pools were brough o final con cen 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 con cen ra ions) (Escherichia coli O111:B4; Sigma, S. Louis; 1 ng ≈ 12 endo oxin uni s) for 24 h in a CO₂ incubu a or a 37°C. Thereaf er, PBMC (final con cen ra ion 0.5 × 10⁶/mL) of each heal hy plasma was incuba ed wi h 10 ng/mL LPS-LPS suspensions for 4 and 24 h in a CO₂ incubu a or a 37°C for mesuremen of TNF-α and IL-6, respec ively. These dura ions of lymph were cho sen af er preliminary experimen s had es abolished ha he con cen ra ions of TNF-α and IL-6 peaked a he same ime point s (da a no shown).

Af er cen rifuga ion a 2000 g for 30 min a 4°C, superna an s were aliqueo ed and s ored a −80°C un il assays were performed. An iden ical pro ocol was used o de ermine TNF-α, IL-6, LBP, BPI, and IL-10 concen ra ions (Medgenix, Fleurus, Bel- guim), IL-6 (PharMingen, San Diego), and IL-10 (PharMingen) in SIRS lymph were de ermined by ELISA according o he ins ruc ions of he SIRS can par ially reproduce LPS olerance, possibly indica ing that he SIRS lymph was more po en han lymph of con rols in he absence of lymph or plasma of pa ien s wi h SIRS. LBP, BPI, and IL-10.

**Discussion**

The presen findings demons ra e ha lymph of pa ien s wi h SIRS can par ially reproduce 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 reduc ing he capaci y of mononuclear cells o produce proin- flammasome ory cy okines upon res imula ion wi h LPS. I has been shown ha soluble media ors are involved, since serum of sep ic pa ien s and endo oxemic volun eers par ially reproduced he SIRS olerance ha lymph of cons angos in he ex ravascular compar men.

In pa ien s wi h SIRS, lymph of pa ien s wi h SIRS can par ially reproduce LPS olerance, possibly indica ing ha his phenomenon also occurs in he ex ravascular compar men.

Pa ien s wi h SIRS, undergoing a rans horacic resec ion of he esophagus for a carcino ma of he esophagus, were used as con rols. I is possible ha he lymph of pa ien s have charac ers which differ from heal hy 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 ex.

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-oligan sence [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...

Table 1. Concentrations (mean ± SE) of lipopolysaccharide-binding protein (LBP), bacterial/permeability-increasing protein (BPI), TNF-α and IL-6 in lymph and plasma of patients with and without SIRS.

<table>
<thead>
<tr>
<th>Patients</th>
<th>LBP (μg/mL)</th>
<th>BPI (pg/mL)</th>
<th>IL-10 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph</td>
<td>Plasma</td>
<td>Lymph</td>
<td>Plasma</td>
</tr>
<tr>
<td>SIRS</td>
<td>(n = 8)</td>
<td>SIRS</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>Lymph</td>
<td>35 ± 10*</td>
<td>48 ± 9†</td>
<td>11 ± 6</td>
</tr>
<tr>
<td>Plasma</td>
<td>48 ± 9†</td>
<td>200</td>
<td>&lt;200</td>
</tr>
<tr>
<td>SIRS</td>
<td>35 ± 10*</td>
<td>48 ± 9†</td>
<td>11 ± 6</td>
</tr>
<tr>
<td>Plasma</td>
<td>48 ± 9†</td>
<td>200</td>
<td>&lt;200</td>
</tr>
</tbody>
</table>

* P < .05 vs. lymph of patients without SIRS.
† P < .05 vs. plasma of patients without SIRS.
‡ P < .02 vs. plasma of patients with SIRS.

hese patients closely approximate the condition of healthy volunteers, the ideal control group.
lymph and SIRS plasma inhibited cy okine release more strongly than lymph and plasma of pa ien s wi h SIRS, respec ively.

Many o her subs ances may bind LPS (e.g., an ibodies, com- plemen , albumin) or deac i e mononuclear cells. For example, lipopro eins are known o bind and neu ralize LPS [12, 15]. Concen ra ions of apolipopop eins A-1 and B were significan ly lower in lymph and plasma of pa ien s wi h SIRS compared wi h concen ra ions in lymph and plasma of pa ien s wi hou SIRS (da a no shown). Lipopro ein concen ra ions can here- fore no explain he more po en inhibi ion of proinflamma ory cy okine produc ion by SIRS lymph or SIRS plasma compared wi h non-SIRS lymph or non-SIRS plasma, respec ively. The presen inves iga ion does no elucida e which o her media ors migh be involved. I hus seems ha al era ions in LBP concen- ra ions do no con ribu e significan ly o he developmen of LPS olerance.

LPS olerance is associa ed wi ha reduced capaci y of mononuclear cells o produce cy okines upon s imula ion wi h LPS. Here we show ha lymph ob ained from pa ien s wi h SIRS can reproduce an LPS-o leran s a e when added o cul ures of normal PBMC. These da a sugges ha soluble media ors pres- en in lymph (and plasma) are responsible, a leas in par , for he phenomeon of LPS olerance.

References


5. Gas aldelli A, Schwarz JM, Caveggion E, e al. Glucose kine ics in in ers i-plemen , albumin) or deac iva e mononuclear cells. For exam- ple, lipopop eins are known o bind and neu ralize LPS [12, 15].


11. American College of Ches Physicians/Socie y of Cri ical Care Medicine Consensus Conference Commi ee. Defini ions for sepsis and organn failure and guidelines for he use of innova ive herapies in sepsis. Crien in lymph (and plasma) are responsible, a leas in par , for he phenomeon of LPS olerance.