Modulation of the Triggering Receptor Expressed on the Myeloid Cell Type 1 Pathway in Murine Septic Shock

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The triggering receptor expressed on myeloid cell type 1 (TREM-1) is a cell surface molecule that has been identified on both human and murine polymorphonuclear neutrophils and mature monocytes. The activation of TREM-1 in the presence of microbial components amplifies the inflammatory response and may be responsible for the hyperresponsiveness observed during the initial stage of sepsis. To investigate the effect of the modulation of the TREM-1 pathway during experimental murine sepsis, we used analogue synthetic peptides derived from the extracellular moiety of TREM-1. The TREM-1 ligand was expressed on both peritoneal and peripheral neutrophils during experimental peritonitis in mice. The TREM-1 peptides inhibited the recognition by TREM-1 of its ligand and protected endotoxin mice from death. In septic rats, the TREM-1 peptides improved the hemodynamic status, attenuated the development of lactic acidosis, and improved survival. The protective effect of these peptides on arterial pressure could partly be explained by a decreased production of nitric oxide. These data suggest that in vivo modulation of TREM-1 might be a suitable therapeutic tool for the treatment of sepsis.

Sepsis constitutes a significant public health burden and remains an ever-present challenge in intensive care units. Its pathogenesis is now becoming better understood, and a greater comprehension of the complex network of immune, inflammatory, and hematological mediators involved in this disorder now allows for the development of rational and novel therapies.

Stimulatory immunoreceptors have a central role for the recognition of foreign antigens or pathogens by the immune system (9). These receptors are composed of ligand-binding sites and associated transmembrane adaptor proteins. The cytoplasmic domain of adaptor proteins contains immunoreceptor tyrosine-based activation motifs. Among such immunoreceptor tyrosine-based activation motif-containing adaptor proteins are CD3ζ, FcRγ, and DAP12 (DNA activating protein 12, also called KARAP) (9). Several immunoglobulin (Ig)-like activating receptors have been characterized including paired Ig receptors (18), NKP44 (5), and the SHPS-1 family (10). Recently, a new family of receptors expressed on myeloid cells (the triggering receptor expressed on myeloid cells [TREM]) has been identified on both human and murine polymorphonuclear cells and mature monocytes. Its expression by these effector cells is dramatically increased in skin, biological fluids, and tissues infected by gram-positive or gram-negative bacteria as well as by fungi (3, 7). By contrast, TREM-1 is not upregulated in samples from patients with noninfectious inflammatory disorders such as psoriasis, ulcerative colitis, or vasculitis caused by immune complexes (3). In mice, the engagement of TREM-1 with agonist monoclonal antibodies has been shown to stimulate the production of such proinflammatory cytokines and chemokines (1, 2) as interleukin-8 (IL-8), monocyte chemotaxtractant proteins 1 and 3, and macrophage inflammatory protein 1α, along with rapid neutrophil degranulation and oxidative burst (25). The activation of TREM-1 in the presence of Toll-like receptor 2 (TLR2) or TLR4 ligands amplifies the production of proinflammatory cytokines (tumor necrosis factor alpha [TNF-α], IL-1β, granulocyte-macrophage colony-stimulating factor), together with the inhibition of IL-10 release (1). In addition, the activation of these TLRs upregulates TREM-1 expression (2). Thus, TREM-1 and TLRs appear to cooperate in producing an inflammatory response. The role of TREM-1 as an amplifier of the inflammatory response has been confirmed in a mouse model of septic shock in which blocking signaling through TREM-1 partially protected animals from death (3, 14). Both in vitro and in vivo, synthetic peptides mimicking short highly interspecies-conserved domains of TREM-1 attenuated the cytokine production of human monocytes and protected septic animals from hyperresponsiveness and death (14). These peptides were efficient not only in preventing but also in down-modulating the deleterious effects of proinflammatory cytokines (14).

The aim of the present study was to investigate further the mechanisms of this protective effect in two experimental models of murine sepsis.
MATERIALS AND METHODS

Animals. Male BALB/c mice (20 to 23 g) and adult male Wistar rats (250 to 300 g) were used. The experiments were performed in adherence to the National Institutes of Health guidelines on the use of laboratory animals and approved by our Institutional Animal Care and Use Committee.

TREM-1 peptides. Based on the TREM-1 sequence in GenBank/EMBL/DDJB (accession numbers XM217336, AF287008, and AF242119), three highly conserved domains in rats, mice, and humans were found in the extracellular region of the protein. These three corresponding sequences (LVVTQRPRTFR, SEVHMGRKFTLKH, TTTRSLKPTAWSPG, and LVQTVDDGLYRCYVHNPP) called P1, P3, and LP17, respectively, were chemically synthesized (Pepscan Systems, Lelystad, The Netherlands, and Protein and Peptide Chemistry Facility, Institute of Biochemistry, Lausanne, Switzerland) as previously described (14). The correct peptides were obtained in >99% yield and were homogeneous after preparative purification, as confirmed by mass spectrometry and analytic reverse-phase high-performance liquid chromatography. These peptides were free from endotoxin. Scrambled peptides containing the same amino acids but in a totally different sequence order were similarly synthesized and served as control peptides.

Binding of mouse TREM-1-IgG1 on peritoneal exudate cells and mTREM-1-IgG1 binding competition with TREM-1-derived peptides. Peritoneal exudate cells (PEC) or peripheral blood cells from mice suffering from a cecal ligation and puncture (CLP)-induced peritonitis were subjected to flow cytometry analysis after incubation with a soluble mouse TREM-1-IgG1 chimera (mTREM-1-IgG1; kindly provided by Marco Colonna, Washington University) or with a control IgG1 (3). Binding was then revealed using a phycoerythrin (PE)-conjugated anti-human IgG1 (Jackson ImmunoResearch). Cells were double stained with a fluorescein isothiocyanate (FITC)-conjugated anti-GR1 or anti-Ly-6G (BD Bioscence). Competition with TREM-1 peptides was performed by preincubating cells with the indicated concentrations of peptides for 45 min on ice before adding mTREM-1-IgG1. To increase the sensitivity, some experiments were performed using tetramericized TREM-1-IgG1 generated as follows: for each staining sample, 8 μg of mTREM-1-IgG1 or control IgG1 was complexed with Alcya 488-conjugated protein A (Molecular Probes, Brussels, Belgium) at a 4:1 molar ratio for 30 min in a phosphate-buffered saline–bovine serum albumin 0.5% buffer, at room temperature in the dark. Additional human IgG1 (10 μg) was added to the solution to completely block the nonspecific binding sites of protein A antibody.

LPS-induced endotoxemia. Animals were randomly grouped (n = 10 to 20) and treated with 15 mg/kg Escherichia coli lipopolysaccharide (LPS) (O111:B4; Sigma-Aldrich, Lyon, France) intraperitoneally (i.p.) and with the TREM-1 or scrambled peptides (4 mg/kg for rats and at various doses for mice).

CLP polymicrobial sepsis model and hemodynamic measurements in rats. The procedure we used has been described in detail elsewhere (22). Briefly, 16 h after CLP, arterial blood pressure (systolic, diastolic, and mean), heart rate, abdominal aortic blood flow, and mesenteric blood flow were recorded using a procedure described elsewhere (22). Briefly, the left carotid artery and the left jugular vein were cannulated with PE-50 tubing. Arterial blood pressure was continuously monitored by a pressure transducer and an amplifier-recorder system (IOX EMKA Technologies, Paris, France). Perivascular probes (Transonic Systems, Ithaca, NY) wrapped up the upper abdominal aorta and mesenteric artery, were allowed to monitor their respective flows by means of a flowmeter (Transonic Systems). After the last measurement (4th hour during CLP experiments and 24th hour after CLP), animals were sacrificed by an intravenous overdose of sodium thiopental.

Biological measurements. Blood was sequentially withdrawn from the left carotid artery. All animals benefited from a corresponding fluid administration just after. Arterial lactate concentrations and blood gas analyses were performed on an automatic blood gas analyzer (ABL 735; Radiometer, Copenhagen, Denmark).

Concentrations of TNF-α and IL-1β in the plasma were determined by an enzyme-linked immunosorbent assay (Biosource, Nivelles, Belgium) according to the manufacturer’s recommendations. Plasma concentrations of nitrates/nitrites were measured using the Griess reaction (R&D Systems, Abingdon, United Kingdom).

RESULTS

TREM-1 ligand is expressed on granulocytes from septic mice. Flow cytometry analysis of mouse PEC drawn 5 h after the completion of a CLP revealed that about 80% of neutrophils are TREM-1 ligand positive (Fig. 1A). Conversely, neutrophils isolated from blood or peritoneal lavage of nonseptic animals did not bind to mTREM-1-IgG1 (data not shown). Analysis of the TREM-1 ligand expression at different time points showed that peritoneal neutrophils expressed the TREM-1 ligand as early as 3 h after the completion of the CLP (Fig. 1B). The maximum expression (approximately 90% of positive cells) was reached 6 h post-CLP. TREM-1 ligand expression on PEC appeared to be transient, being down-regulated 15 h post-CLP. The kinetics of TREM-1 ligand expression on the peripheral blood granulocytes appeared to be delayed with an ongoing increase in expression after the 15th hour post-CLP (Fig. 1B).

TREM-1 peptides inhibit the binding of mTREM-1-IgG1 to TREM-1 ligand-positive cells. To test the ability of the TREM-1 peptides to compete for the recognition of the TREM-1 ligand by the mTREM-1-IgG1, competition experiments were designed (Fig. 2). Both P1 and LP17 peptides inhibited the binding of mTREM-1-IgG1 with its ligand in a concentration-dependent manner. By contrast, P3 peptide demonstrated no effect.

TREM-1 peptides protect endotoxemic mice from death. To assess the ability of TREM-1 peptides to protect mice from LPS-induced lethality, we administered peptides P1, P3, and LP17 (300 μM) 1 h before a lethal dose of LPS (Fig. 3A). Lethality was monitored over time and compared to that of animals having received control injections of scrambled peptides. As we have previously described (14), LP17 injection conferred maximal protection, with 90% of the animals still alive 7 days after LPS injection, compared with 10% of control mice (P < 0.001). Sixty percent of the P1-treated mice survived endotoxemia compared with 10% of control mice (P < 0.01). All P3-treated animals died within 4 days after LPS injection. To investigate whether TREM-1 peptide treatment could be delayed until after the administration of LPS, we administered the peptides 4 h after LPS injection. Only in the case of P1 and LP17 did this delayed treatment confer significant protection against a lethal dose of LPS (Fig. 3B) (14). Eighty percent of the mice administered P1 4 h after LPS injection survived endotoxemia compared to 60% of mice treated 1 h before LPS and 10% of control animals (P < 0.001 and P < 0.01, respectively, compared to controls). Thus, both P1 and LP17 are effective even when injected after the outbreak of endotoxemia. No late death occurred over 1 week, indicating that these peptides did not merely delay the onset of LPS-induced lethality but provided lasting protection.

We have previously demonstrated a dose-dependent protection of LP17 (14). A similar finding was observed here for P1 (Fig. 3C). Indeed, P1 administration conferred maximal protection (80% survival) when administered at 600 μM (P < 0.01), and the level of protection dropped to 50% at 300 μM (P < 0.05) and
dropped further down to 30% at 150 µM compared to 20% of control mice, indicating a dose-dependent effect of P1 (Fig. 3C).

Considering that both P1 and LP17 were effective in protecting mice from LPS-induced lethality, we next analyzed their respective effects on hemodynamics, acid-base status, and cytokine release during sepsis in rats.

**Endotoxemia model.** Following LPS administration, arterial pressures, and aortic and mesenteric blood flows dropped rapidly in control animals (scrambled peptide-treated rats) while the heart rate remained unchanged (Table 1). The decrease of arterial pressures and aortic blood flow was delayed until the second hour in TREM-1 peptide-treated animals, with significantly higher values by that time than in control animals. There was no difference between the P1- and LP17-treated groups. By contrast, neither of these two peptides had any effect on the decrease of the mesenteric blood flow (Table 1).

Arterial pH remained constant over time until the fourth hour after LPS injection, where it severely dropped in the control group only (Table 1). The significant arterial lactate level elevation present in control animals after the third hour was abolished by the TREM-1 peptides (Table 1). There was no difference between P1 and LP17 with regard to pH, arterial bicarbonate, and lactate concentrations.

As expected, a peak of TNF-α plasma concentration was
induced by LPS between 30 min and 1 h after injection, followed by a progressive decline thereafter (Fig. 4A). P1 peptide injection had no effect on this production, while LP17 attenuated TNF-α production by ~30%.

P1 delayed the IL-1β peak until the third hour after LPS injection but without attenuation. By contrast, LP17 strongly reduced IL-1β release (Fig. 4B).

Nitrite/nitrate concentrations increased rapidly after LPS administration in control and P1-treated animals but remained stable upon LP17 treatment (Fig. 5).

**CLP polymicrobial sepsis model.** To investigate the role of the TREM-1 peptides in a more relevant model of septic shock, we performed CLP experiments.

As the severity of our model was at its highest 16 to 20 h after the completion of the CLP, we chose to investigate animals by the 16th hour. Importantly, there were no deaths before this time...
TABLE 1. Hemodynamic parameters during LPS-induced endotoxinemia

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Hour</th>
<th>Heart rate (bpm)</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Aortic blood flow (ml/min)</th>
<th>Mesenteric blood flow (ml/min)</th>
<th>pH</th>
<th>Lactate (mmol/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>486 ± 13</td>
<td>123 ± 21</td>
<td>45 ± 7</td>
<td>13.6 ± 3.4</td>
<td>7.31 ± 0.03</td>
<td>3.3 ± 0.8</td>
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<td></td>
<td>1</td>
<td>522 ± 16</td>
<td>103 ± 25</td>
<td>25 ± 8</td>
<td>9.6 ± 3.3</td>
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<tr>
<td></td>
<td>2</td>
<td>516 ± 13</td>
<td>98 ± 23</td>
<td>12 ± 5</td>
<td>8.0 ± 3.7</td>
<td>7.29 ± 0.03</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>490 ± 20</td>
<td>78 ± 8</td>
<td>8 ± 3</td>
<td>5.8 ± 1.1</td>
<td>7.26 ± 0.01</td>
<td>7.9 ± 1.8</td>
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<tr>
<td></td>
<td>4</td>
<td>510 ± 18</td>
<td>67 ± 9</td>
<td>6 ± 1</td>
<td>4.1 ± 0.8</td>
<td>7.03 ± 0.10</td>
<td>11.5 ± 0.7</td>
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<tr>
<td>P1</td>
<td>0</td>
<td>464 ± 25</td>
<td>116 ± 10</td>
<td>49 ± 11</td>
<td>12.0 ± 3.7</td>
<td>7.32 ± 0.04</td>
<td>2.7 ± 0.1</td>
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<tr>
<td></td>
<td>1</td>
<td>492 ± 26</td>
<td>119 ± 14</td>
<td>39 ± 12</td>
<td>10.5 ± 1.7</td>
<td>7.29 ± 0.04</td>
<td>4.9 ± 1.1</td>
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<tr>
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<td>492 ± 26</td>
<td>113 ± 21</td>
<td>26 ± 14</td>
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<td>7.30 ± 0.01</td>
<td>5.0 ± 0.9</td>
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<tr>
<td></td>
<td>3</td>
<td>480 ± 30</td>
<td>97 ± 29</td>
<td>22 ± 8</td>
<td>5.0 ± 1.0</td>
<td>7.26 ± 0.06</td>
<td>5.7 ± 0.7</td>
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<td></td>
<td>4</td>
<td>480 ± 20</td>
<td>92 ± 7</td>
<td>16 ± 6</td>
<td>4.8 ± 0.9</td>
<td>7.26 ± 0.08</td>
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<td>LP17</td>
<td>0</td>
<td>474 ± 49</td>
<td>115 ± 16</td>
<td>48 ± 9</td>
<td>12.8 ± 6.4</td>
<td>7.33 ± 0.04</td>
<td>3.4 ± 1.5</td>
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<td></td>
<td>1</td>
<td>498 ± 26</td>
<td>99 ± 22</td>
<td>32 ± 8</td>
<td>11.4 ± 2.7</td>
<td>7.28 ± 0.06</td>
<td>5.4 ± 1.4</td>
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<tr>
<td></td>
<td>2</td>
<td>510 ± 42</td>
<td>101 ± 18</td>
<td>23 ± 4</td>
<td>9.2 ± 1.9</td>
<td>7.32 ± 0.07</td>
<td>5.5 ± 1.6</td>
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<tr>
<td></td>
<td>3</td>
<td>517 ± 62</td>
<td>93 ± 21</td>
<td>20 ± 7</td>
<td>6.0 ± 0.8</td>
<td>7.29 ± 0.11</td>
<td>5.9 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>510 ± 26</td>
<td>89 ± 10</td>
<td>15 ± 6</td>
<td>5.0 ± 1.0</td>
<td>7.28 ± 0.12</td>
<td>7.4 ± 1.8</td>
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</table>

\[ a \ P < 0.05 \text{, P1 versus controls.} \]

\[ b \ P < 0.05 \text{, LP17 versus controls.} \]

There was a dramatic decline in arterial pressure in the control animals over time, and by hour 24, systolic, diastolic, and mean arterial pressures were 58 ± 7 mmHg, 25 ± 4 mmHg, and 38 ± 2 mmHg, respectively. This decrease was almost totally abolished with P1 or LP17 treatments, with no significant difference between hour 16 and hour 24 (Fig. 6). There was no difference between P1- and LP17-treated rats.

TREM-1 peptides also prevented the aortic and mesenteric blood flow decreases observed in control animals (Table 2). The protective effect on mesenteric blood flow alterations was even higher under LP17 treatment. The relative preservation of blood flows was not related to an increased heart rate, since the latter was rather slower than in control animals (Table 2).

The progressive metabolic acidosis that developed in control rats was attenuated by the P1 peptide and almost abrogated by LP17. The same protective trend was observed for arterial lactate elevation with a more pronounced effect for LP17 (Table 2).

Both P1 and LP17 induced a decrease in TNF-α production, again with a stronger effect for LP17. By hour 20, TNF-α in plasma was almost undetectable under LP17 treatment, whereas it remained elevated in the other groups of animals (Fig. 7).

Nitrite/nitrate concentrations were increased in control animals and mean arterial pressures were 58 ± 7 mmHg, 25 ± 4 mmHg, and 38 ± 2 mmHg, respectively. This decrease was almost totally abolished with P1 or LP17 treatments, with no significant difference between hour 16 and hour 24 (Fig. 6). There was no difference between P1- and LP17-treated rats.
but remained at a low level in both TREM-1 peptide-treated groups (Fig. 8).

As observed in mice, both LP17 and P1 peptides demonstrated a significant protective effect against CLP-induced lethality (Fig. 9).

**DISCUSSION**

This study confirmed the protective effect of LP17 and demonstrated that another TREM-1-related peptide, P1, also shows protective effects in a mouse model of endotoxemia. To further appreciate the mechanisms of this protection, two rat models were developed, allowing us to monitor more easily than in mice the physiological parameters altered in the course of sepsis. This allowed us to show that these two TREM-1 peptides (i) efficiently protected the animals from sepsis-related hemodynamic deterioration, (ii) attenuated the production of such proinflammatory cytokines as TNF-α and IL-1β, and (iv) decreased the generation of nitric oxide.

The TREM-1 consists of an extracellular region of 194 amino acid residues, a membrane-spanning region of 29 aa, and a short cytoplasmic tail of 5 aa. The extracellular Ig-like domain contains the motif DXGXYXC, which corresponds to a V-type Ig domain. The Ig domain is connected to the transmembrane region by a 60-aa portion containing three N-glycosylation sites. The spanning region contains a Lys residue which forms a salt bridge with an Asp residue of the transmembrane domain of DAP12, allowing the association between TREM-1 and its adaptor protein (1, 2). Engagement of TREMs triggers a signaling pathway involving ZAP70 (ζ-chain-associated protein 70) and SYK (spleen tyrosine kinase) and an ensuing recruitment and tyrosine phosphorylation of such adaptor molecules as GRB2 (growth factor receptor binding protein 2), the activation of phosphatidylinositol 3-kinase, phospholipase C-γ, extracellular signal-regulated kinase 1 and 2, and p38 mitogen-activated protein kinase (6, 23). The activation of these pathways ultimately leads to a mobilization of intracellular calcium, a rearrangement of the actin cytoskeleton, and an activation of transcriptional factors. The activation of TREM-1 in the presence of TLR2 or TLR4 ligands amplifies the production of proinflammatory cytokines (TNF-α, IL-1β, granulocyte-macrophage colony-stimulating factor). Note, although crystallographic analyses (17, 24) can predict TREM-1 ligand recognition by using antibody-equivalent complementary determining region (CDR) loops (such as T-cell receptors, CD8, and cytotoxic-T-lymphocyte-associated antigen 4), its natural ligand has yet to be determined.

We therefore synthesized several TREM-1 peptides matching the following criteria: (i) highest homology between human and

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**TABLE 2. Hemodynamic and selected biochemical parameters during CLP polymicrobial sepsis**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Hour</th>
<th>Heart rate (bpm)</th>
<th>Aortic blood flow (ml/min)</th>
<th>Mesenteric blood flow (ml/min)</th>
<th>pH</th>
<th>Bicarbonate (mmol/liter)</th>
<th>Lactate (mmol/liter)</th>
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<td>Control</td>
<td>16</td>
<td>516 ± 440&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.6 ± 3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.31 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.9 ± 2.7</td>
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<td>41 ± 12</td>
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<tr>
<td>LP1</td>
<td>16</td>
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<td>41 ± 14</td>
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<td>28 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>17.1 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.9 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> P < 0.05, P1 versus controls.

<sup>b</sup> P < 0.05, LP17 versus controls.

<sup>c</sup> P < 0.05, LP17 versus P1.

FIG. 6. Mean arterial pressure evolution during cecal ligation and puncture-induced peritonitis in rats. * P < 0.05 versus control animals.
mouse and rat TREM-1 and lowest homology with TREM-2 and (ii) spanning the CDRs of TREM-1. One peptide (P1) was designed in the CDR2 region, LP17 was designed in the CDR3 region, and P3 was designed in the neck region. Both peptides mapping in a CDR region (P1 and LP17), but not P3 mapping the neck region, competed with TREM-1 recognition of its endogenous ligand(s), suggesting a direct interaction of TREM-1 CDRs with TREM-1 ligand(s). The effect of these two peptides could therefore be explained by their ability to act as decoy receptors and block TREM-1 interactions with its ligand. An additional effect of LP17 on the TREM-1 pathway could stem from its ability to act as a putative homodimerization, and LP17 could thus impair the TREM-1 dimerization necessary for its engagement. The hypothesis of these peptides acting in impairing the TREM-1 signaling upon binding to its ligand led us to investigate whether the TREM-1 ligand could be expressed on the myeloid cells’ surface. We indeed found a high level of TREM-1 ligand expression on the neutrophils infiltrating the peritoneum 5 h after the completion of peritonitis in mice. By contrast, neutrophils isolated from sham-operated animals did not express the TREM-1 ligand. These findings clearly indicate a selective expression of the TREM-1 ligand during infection. The pattern expression of the TREM-1 ligand appeared to be delayed on peripheral blood granulocytes. This might reflect both a recirculation of peritoneal neutrophils as well as systemic spreading of bacteria.

We have recently shown in a mouse model of endotoxemia (14) that animals treated with a single dose of LP17 60 min before administration of a 100% lethal dose of LPS were protected from death in a dose-dependent manner. Delayed treatment with LP17 still conferred significant protection against an 100% lethal dose of LPS. Compared to controls, LP17 reduced cytokines levels by 30%. Similar results were obtained in a model of polymicrobial sepsis induced by CLP (14). The data reported here suggest a comparable effect on survival for the P1 peptide.

In the current report, we further extend these findings in highlighting the protective action of both LP17 and P1 on hemodynamics and survival in septic rats. Both arterial pressure and blood flows were preserved, independently of heart rate. Moreover, modulation of TREM-1 signaling reduced, although not completely, cytokine production and protected septic animals from hyperresponsiveness. The fact that cytokine production was not totally inhibited is a crucial point. Indeed, although inflammatory cytokines such as TNF-α are considered deleterious, they also display beneficial effects in sepsis, as underlined by the fatal issue of peritonitis models in animals with impaired TNF-α responses (11–13).

The activation of inducible nitric oxide synthase observed during septic shock leads to the production of a large amount of NO that partly explains some of the peripheral vascular disorders (notably, vasodilatation and hypotension). On the myocardium itself, most of the action of NO is mediated by an activation of the soluble guanylate-cyclase responsible for the production of cyclic GMP which impairs the effect of cytosolic calcium on contraction (19). Cyclic GMP is also able to stimulate the activity of some phosphodiesterases. The subsequent decrease of intracellular cAMP levels could explain the ability of NO to attenuate the effects of beta adrenergic stimulation (26). The preservation of arterial pressure could therefore be partly explained by a lessened production of NO, as reflected by the lower concentrations of plasma nitrite/nitrate in TREM-1 peptide-treated animals.

The decrease in inflammatory cytokine production could partly explain the effect noted on blood flows. Indeed, although the list
of potential cytokine mediators of myocardial depression is long, TNF-α and IL-1β have been shown to be good candidates (4, 20). Both these latter cytokines depressed myocardial contractility in vitro or ex vivo (16, 28). Moreover, the neutralization or removal of TNF-α or IL-1β from human septic serum partly abrogates the myocardial depressant effect in vitro and in vivo (20, 27). Although P1 and LP17 had an identical action on blood flows and arterial pressure during endotoxemia, their action on cytokine production differed with only a slight effect of P1 on TNF-α and IL-1β concentrations in plasma. The protective role of the TREM-1 peptides could therefore be only partly related to their action on cytokine release, or involve redundant pathways. The observed differences between the two peptides could simply be related to a variable bioavailability. Indeed, while LP17 is strictly conserved between mice and rats, P1 (also designed in mice) only shares 70% identity with its corresponding region in rats. Another hypothesis, although unproven, is that LP17, due to its overlapping with the “F” β-strand, could also act in inhibiting the putative TREM-1 homodimerization.

Another mechanism by which the TREM-1 peptides could exert their action has recently been suggested by Hamerman et al. (15). These authors demonstrated that DAP12-deficient macrophages produced higher levels of inflammatory cytokines in response to diverse microbial stimuli. Moreover, DAP12-deficient mice were more susceptible to endotoxemia and had enhanced resistance to infection by Listeria monocytogenes. These data suggest the existence of DAP12-pairing receptor(s) that negatively regulate the TLR-mediated signaling. One of these could be a specific receptor for soluble TREM-1 (and then recognize the TREM-1 peptides), since many DAP12-paired receptors have a related inhibitory receptor (21). The variable effects of the TREM-1 peptides on cytokine production could therefore be explained by a different affinity for this putative receptor.

The modulation of the TREM-1 pathway by the use of small synthetic peptides seemed to have salutary effects on hemodynamics during experimental septic shock in rats, along with an attenuation of inflammatory cytokine production. The mechanism of action of such TREM-1 peptides remains to be fully elucidated.

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