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Preprotachykinin-A Gene Products Are Key Mediators of Lung Injury in Polymicrobial Sepsis

Padman Puneet,* Akhil Hegde,* Siaw Wei Ng,* Hon Yen Lau,* Jia Lu,† Shabbir M. Moolchada,*‡ and Madhav Bhatia2,*

Preprotachykinin-A (PPT-A)3 gene products substance P (SP) and neurokinin-A have been shown to play an important role in neurogenic inflammation. To investigate the role of PPT-A gene products in lung injury in sepsis, polymicrobial sepsis was induced by cecal ligation and puncture in PPT-A gene-deficient mice (PPT-A−/−) and the wild-type control mice (PPT-A+/+). PPT-A gene deletion significantly protected against mortality, delayed the onset of lethality, and improved the long-term survival following cecal ligation and puncture-induced sepsis. PPT-A−/− mice also had significantly attenuated inflammation and damage in the lungs. The data suggest that deletion of the PPT-A gene may have contributed to the disruption in recruitment of inflammatory cells resulting in protection against tissue damage, as in these mice the sepsis-associated increase in chemokine levels is significantly attenuated. The Journal of Immunology, 2006, 176: 3813–3820.

Preprotachykinin-A (PPT-A)3 gene products substance P (SP) and neurokinin-A have been shown to play an important role in neurogenic inflammation. SP is an immunoregulatory neuropeptide produced at sites of inflammation. Sensory nerves can initiate inflammation or amplify the inflammatory responses initiated by noxious stimuli. SP and other neurokinins encoded by the PPT-A gene play a key role in this process, which is known as neurogenic inflammation (2). Neurokinins are thought to be stored primarily in unmyelinated nerve fibers (C-fibers) and released antidromically as part of the nociceptive response. The released molecules bind to a family of ubiquitous G protein-coupled receptors with at least three distinct receptors, of which the neurokinin-1 receptor has the highest affinity for SP. Activation of the neurokinin-1 receptor in smooth muscle cells, endothelial cells, and leukocytes decreases vascular tone, increases endothelial permeability, and promotes local flow of inflammatory cells, thereby enhancing the inflammation induced by the original stimulus (2). The presence of SP and other neurokinins in resident macrophages and circulating leukocytes is particularly intriguing because many of these cells also express neurokinin receptors (3, 4). Thus, it is possible that inflammatory cells use PPT-A gene-encoded neurokinins as a paracrine- or autocrine-signaling mechanism to propagate inflammation beyond the limited topographic spread of the C-fibers and intrinsic neurons (5). Exogenous administration or endogenous release of neurokinin-A and SP from C-fibers produces a variety of responses in mammalian airways including increased microvascular permeability, immune cell influx, and increased glandular secretion (6).

We have earlier shown that knockout mice deficient in PPT-A are protected against acute pancreatitis and associated lung injury (1). These results show that PPT-A gene products are critical inflammatory mediators in acute pancreatitis and the associated lung injury. Other investigators also reported the role of the PPT-A gene in airway inflammation (3), arthritis (7, 8), cystitis (9, 10), and inflammatory bowel disease (3). SP binds to neurokinin 1 receptors on the surface of effector cells and in addition to being a mediator of pain, acts as a proinflammatory mediator in many inflammatory states (4). Although increased SP immunoreactivity has been found in bronchoalveolar lavage samples from humans suffering from lung diseases as acute respiratory distress syndrome (11), to date, the role of PPT-A gene products SP and neurokinin-A have not been investigated in sepsis. Therefore, the present study addressed the impact of PPT-A gene products on the pathogenesis of lung injury in sepsis during experimental septic peritonitis in mice. Although the primary focus of this paper is lung injury, the hemodynamic instability in sepsis (12) may cause kidney damage and hepatocellular dysfunction (13). Hence, liver and kidney have been further investigated for tissue damage. This model of sepsis induced by cecal ligation and puncture (CLP) simulates an early hyperdynamic phase followed by a late, hypodynamic phase that is similar to clinical sepsis (14). We have also analyzed the mechanism in which PPT-A gene products play an important role in lung injury during sepsis.

Materials and Methods

Animal model of polymicrobial sepsis

Polymicrobial sepsis was induced by CLP as described previously (14). The cecum was ligated with 4/0 silk suture and punctured twice with a 22-gauge needle under sterile conditions. PPT-A−/− mice were a gift from Prof. A. Basbaum (University of California, San Francisco, CA) and bred as described previously (15). Male PPT-A−/− mice with BALB/c background and its wild-type PPT-A+/+ BALB/c male mice (20–23 g) were randomly selected to control, sham operated and CLP operated. In one set of experiments, comparative mice survival studies in between PPT-A−/− and PPT-A+/+ mice were conducted. Data was derived from 30 mice/group for each mice strain subjected to sham operation or CLP surgery for...
up to 5 days after these procedures. In another set of experiments to examine the course and transformation of the syndrome at different intervals, groups of eight mice subjected to sham or CLP operation in both PPT-A+/− and PPT-A+/− mice were sacrificed at 1, 5, 10, 16, and 20 h after the surgery. Sham-operated animals underwent the same surgical procedure except that the caecum was neither ligated nor punctured. The study was performed in accordance with the guidelines of the Medical Ethics Committee (Singapore) that conforms to the World Health Organization’s International Guiding Principles for Animal Research.

Survival studies

The time of onset of mortality and disease progression of PPT-A+/+ and PPT-A−/− male mice after sham and CLP operation were monitored every 12 h during the study. The survival of these mice was followed until day 5 (n = 30). Mice that survived beyond this time point were considered as permanent survivors.

Measurement of SP

Samples of lung and plasma were collected from the animals (n = 8). The lung fragments were homogenized in 2 ml of ice-cold assay buffer for 20 s. The homogenates were centrifuged (13,000 rpm, 20 min, 4°C) and the supernatants were collected. They were adsorbed on C18 cartridge columns (Bachem) as described (16). The adsorbed peptides were eluted with 1.5 ml of 75% v/v acetonitrile. The samples were freeze dried and reconstituted in sample buffer (16). SP content was then determined with an ELISA kit (Bachem; Peninsula Laboratories) according to the manufacturer’s instructions and expressed as picograms per milliliter for plasma and picograms per microgram of DNA for lung. DNA assay was performed fluorometrically by using Hoechst dye 33256 by the method of Labarca and Paigen (17) and salmon testes DNA as standard.

Myeloperoxidase (MPO) estimation

MPO activity as an assessment of neutrophil sequestration in lungs, liver, and kidney was quantified by measuring tissue myeloperoxidase activity as described previously (1) and the absorbance was then corrected for the DNA of the tissue sample. The results were expressed as fold increase over control (n = 8).

Measurement of pulmonary microvascular permeability

A separate group of animals was used for this assay and lavage was collected to assess protein concentration as an index of lung permeability (injury). PPT-A−/− and its wild-type PPT-A+/+ mice weighing 20–23 g were grouped into control, sham operated, and CLP operated (n = 8). Two hours before sacrifice, each animal received an i.v. bolus injection containing FITC-albumin (5 mg/kg; Sigma-Aldrich). The blood was collected by cardiac puncture and serum was separated. The trachea was exposed and the lungs were lavaged three times with 1 ml of saline. The lavage fluid was combined and FITC fluorescence was measured in lavage fluid and serum (excitation = 494 nm; emission = 520 nm). The ratio of fluorescence emission in lavage fluid to blood was calculated and used as a measure of pulmonary microvascular permeability.

**FIGURE 1.** Plasma and lung SP levels in wild-type mice subjected to CLP-induced polymicrobial sepsis (n = 8 mice/group at different time points). Mice were subjected to sham or CLP operation (with 22-gauge needle for two punctures) and sacrificed at different time points. SP in plasma (A) and lung (B) was assessed. Data are mean ± SEM (compared by one-way ANOVA and Tukey test for multiple comparisons, p < 0.05). *, CLP vs sham; **, 1 h CLP vs 5 h, 10 h, and 16 h CLP; ***, 1 h CLP vs 5 h and 10 h CLP. PPTA+/+ (PPTA+/+ control without any operation), PPTA+/+ S (PPTA+/+ sham-operated), and PPTA+/+ CLP (PPTA+/+ CLP-operated) mice.
Histopathological examination

A small portion of lung was excised and fixed with 10% buffered formaldehyde (Sigma-Aldrich), dehydrated, and embedded in paraffin for routine histology. Sections of 5-μm thickness were stained with H&E and evaluated by light microscopy and documented by photographs.

White blood corpuscles-differential count (WBC-DC)

Blood was collected by cardiac puncture and blood into EDTA-containing tubes on all the time points and samples were analyzed by CellDyne-3700 for WBC-DCs (n = 8).

Assessment of bacterial load in the peripheral blood

Blood smears from all the time points were spread and air dried (n = 5). Routine improved gram staining procedure was performed to identify the bacteria in each smear (18). Twenty different fields in each slide (smear) were examined and the results are expressed as total number of bacteria per smear (20 fields).

Chemokine ELISA analysis

Experiments were performed to evaluate the time-dependent production of chemokines in CLP-induced sepsis in both PPT-A−/− and its wild-type mice (n = 8). Plasma and organ-specific production of chemokine levels in plasma (picograms per milliliter) and tissue homogenates (picograms per microgram of DNA for tissue) were measured by ELISAs as specified by the manufacturer (R&D Systems) and documented. DNA assay was performed fluorometrically by using Hoechst dye 33256 by the method of Labarca and Paigen (17).

Immunohistochemical analysis

Immunostaining for MCP-1 was performed in paraffin-embedded sections of 5-μm thickness. Sections were deparaffinated and rehydrated. Ag sites were retrieved by heating the sections on the slides in 10 mM citrate buffer in a microwave oven and cooling for 10 min at room temperature and incubation in 3% H2O2 in methanol for 10 min to inactivate endogenous peroxidase. Sections were again washed in PBS for 10 min before blocking with normal goat serum (Vector Laboratories) for 30 min. Primary Ab specific for MCP-1, rabbit polyclonal to MCP-1 (anti-MCP-1, ab7202; Abcam) was then added at a dilution of 1/100 in 0.3% BSA for 60 min. The sections were then incubated with biotinylated goat anti-rabbit Ab (Vector Laboratories) for 30 min. Sections were again washed in PBS and incubated with AB reagent, avidin D-HRP (Vector Laboratories), in PBS for 30 min. After washing in PBS, staining was visualized using diaminobenzidine (DAB) (DakoCytomation) and counterstained by methyl green. The sections were dehydrated, permanently mounted with mounting medium, and observed by light microscopy. The DAB substrate chromogen yields a brown/reddish brown reaction end product at the site of the target Ag.

Statistical analysis

All values are expressed as the means ± SE of values obtained from multiple determinations in eight experiments. The significance of changes was evaluated by ANOVA when comparing three or more groups. The data were analyzed by using one-way ANOVA and Tukey’s method as a post hoc test for comparison among different groups. A p value of <0.05 was considered to indicate a significant difference. The overall survival analysis for lethal injury was described using Kaplan-Meier plots. The differences...
between the survival of PPT-A/+/+ and PPT-A−/− mice was tested by using the log rank test. Survival rates were expressed as percentages.

Results

Time-dependent increase in SP production after CLP

The SP level was observed significantly peaked at the 1-h point in both plasma (Fig. 1A) and lung (Fig. 1B) in the wild-type mice. SP level was observed toward the higher trend at 5 and 20 h in plasma and at the 16- and 20-h points in lung. As expected, no SP was detected in PPT-A−/− mice.

Effect of PPT-A gene deletion on neutrophil sequestration

The neutrophil sequestration was quantified by measuring tissue MPO activity. The rise in MPO activity indicates the presence of neutrophil sequestration in organs. The PPT-A−/− mice showed a significantly lower level of MPO activity in lung than the wild-type mice. The MPO activity in lung of PPT-A+/+ and PPT-A−/− mice showed a steady increase up to the 5-h time point. At 10 h, the PPT-A−/− mice started exhibiting a decreasing trend. At the 16- and 20-h points, the wild-type mice showed a significantly higher level of MPO activity than the PPT-A−/− mice (Fig. 2A). At the 16- and 20-h points, the wild-type mice showed a higher level of MPO activity in the liver than the PPT−/− mice (Fig. 3A). The PPT-A+/+ mice showed a significantly higher level of MPO activity in the kidney than the PPT-A−/− mice at the 5-, 10-, 16-, and 20-h time points (Fig. 3B).

PPT-A gene deletion significantly attenuated sepsis-associated lung injury

The clinical pathology of acute lung injury includes increased microvascular permeability and edema with a marked influx of polymorphonuclear leukocytes (PMNs). CLP-induced sepsis is associated with lung injury in both PPT-A−/− and the wild-type mice. Leakage of i.v. administered FITC-labeled albumin into the alveolar space, a measure of pulmonary microvascular permeability, is increased during sepsis in both PPT-A+/+ and PPT-A−/− mice compared with sham-operated and negative control mice. A significant reduction in pulmonary microvascular permeability in PPT-A−/− mice was observed compared with the wild-type mice in all time points (Fig. 2B).

Histological examination of lung revealed significantly higher alveolar congestion, edema, cellular infiltrates, and widening of alveolar septa in PPT-A+/+ mice than PPT-A−/− mice as per the disease progression. Normal lung histocharchitecture was observed in control and sham-operated mice (Fig. 2, C–J).

PPT-A gene deletion protects against CLP-induced mortality

PPT-A gene deletion delayed the onset of lethality and was significantly protected against mortality following CLP surgery. As shown in Fig. 4, the overall mortality in the PPT-A−/− mice was significantly more rapid when compared with the survival curves between both strains of mice. The onset of lethality in mice occurred at 24 h after CLP by which time PPTA+/+ mice had a significant 32% mortality than PPT-A−/− mice in which it was only 4% and delayed until 36 h. The survival at 36 h in PPT-A+/+ mice was 24% and in PPT-A−/− mice was 87% was observed, whereas at 48 h they were 0% vs 44% in PPT-A+/+ vs PPT-A−/− mice. Sham-operated mice were found normal and 100% survival was observed throughout the study.

Role of PPT-A gene deletion on neutrophil count and bacterial load

PPT-A+/+ mice exhibited an increased neutrophil count in circulation compared with the PPT-A−/− mice at various time points.

FIGURE 3. MPO activity in liver and kidney. Altered MPO activity in liver (A), and kidney (B) in between PPT-A+/− and PPTA+/+ mice subjected to CLP-induced sepsis (n = 8 mice/group for each strain) were assessed. Mice were subjected to sham or CLP operation with 22-gauge needle for two punctures and sacrificed at designated time points. Data are mean ± SEM (compared by one-way ANOVA and Tukey test for multiple comparisons; p < 0.05). *, CLP vs sham; @, 5 h PPTA+/− CLP vs 5 h PPT-A−/− CLP; #, 10 h PPT-A+/− CLP vs 10 h PPT-A−/− CLP; †, 16 h PPTA+/+ CLP vs 16 h PPT-A−/− CLP; & 20 h PPTA+/+ CLP vs 20 h PPT-A−/− CLP. PPTA+/− C (PPTA+/− control without any operation), PPTA+/− S (PPTA+/− sham operated), PPTA−/− C (PPTA−/− control without any operation), PPTA−/− S (PPTA−/− sham operated) and PPTA−/− CLP (PPTA−/− CLP operated). PPT-A+/− C (PPTA+/− control without any operation), PPT-A+/− S (PPTA+/− sham operated) and PPTA+/− CLP (PPTA+/− CLP operated) mice.

This difference was significantly higher at 10-, 16-, and 20-h points (Table I). Bacteria were observed in the blood at the 1-h point after the CLP operation. The quantitative bacterial count from the peripheral blood revealed lower bacterial load in PPT-A−/− mice compared with wild-type mice. At the 20-h point, the number of bacteria observed was significantly (p < 0.05) higher in PPT-A−/− mice (76 ± 2.16/smear) than in PPT-A+/− mice (58 ± 3.09/smear). Of these, the percentage of Gram-negative bacteria was significantly higher (p < 0.05) in PPT-A+/− mice (55.0 ± 1.96%) compared with PPT-A−/− mice (42.8 ± 2.35%).

PPT-A gene deletion impaired chemokine production in mice with sepsis

During sepsis, neutrophils and monocytes accumulate in the lungs. To address the role of chemokine production in mediating
the inflammatory cells, chemokine induction during polymicrobial sepsis was next investigated. Plasma- and organ-specific production of chemokine MCP-1 (CC chemokine), and MIP-2 (CXC chemokine) was quantified by ELISA as described in Materials and Methods. MIP-2 production was undetectable in plasma in normal mice and sham-operated mice and at the 1-h point. PPT-A/+/ mice showed a higher level of MIP-2 production in plasma at the 5-, 10-, and 20-h points than PPT-A−/− mice. This was observed significantly higher at the 5-h point in PPT-A+/+ mice than in PPT-A−/− mice (Fig. 5A). The MIP-2 level in lungs was significantly higher in the wild-type mice at 5 and 16 h than in the PPT-A−/− mice (Fig. 5B). Fig. 6A illustrates that the time-dependent MCP-1 production was significantly higher in the wild-type mice at 5, 10, and 20 h in plasma compared with PPT-A−/− mice. MCP-1 level in lungs was significantly higher in wild-type mice at the 5-, 10-, and 16-h points than in PPT-A−/− mice (Fig. 6B). Immunohistochemical analysis exhibited negative immunoreactivity in control (Fig. 6C) and weak immunoreactivity in sham mice (Fig. 6D). The immunostaining showed markedly enhanced reactivity at the 5-h point and stronger reactivity at the 10-h point (Fig. 6, E–H). However, at the 20-h point (Fig. 6, I and J), this was found to be less than at the 10-h point. The immunostaining was localized in the lung’s interstitium, alveolar epithelium, and endothelial cells at different time points. MCP-1 expression level in lungs was higher

**FIGURE 5.** Effect of PPT-A gene deletion on plasma (A) and lung (B) levels of MIP-2 following CLP-induced sepsis (n = 8 mice/group for each strain). Mice were subjected to sham or CLP operation with 22-gauge needle for two punctures and sacrificed at designated time points. Data are mean ± SEM (compared by one-way ANOVA and Tukey test for multiple comparisons; p < 0.05). MIP-2 was undetectable in plasma in normal mice, sham operated mice and at the 1 h point. *, CLP vs sham; †, 5 h PPT-A+/+ CLP vs 5 h PPT-A−/− CLP; ‡, 16 h PPT-A+/+ CLP vs 16 h PPT-A−/− CLP. PPT-A+/+ C (PPT-A+/+ control without any operation), PPT-A+/+ S (PPT-A+/+ sham operated), PPT-A+/+ CLP (PPT-A+/+ CLP operated), PPT-A−/− C (PPT-A−/− control without any operation), PPT-A−/− S (PPT-A−/− sham operated) and PPT-A−/− CLP (PPT-A−/− CLP operated) mice.

Table I. Neutrophil count (percent) in circulating blood

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<th>Time Point (h)</th>
<th>C+/+</th>
<th>S+/+</th>
<th>CLP+/+</th>
<th>C−/−</th>
<th>S−/−</th>
<th>CLP−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.46 ± 0.46</td>
<td>58.10 ± 2.08</td>
<td>70.60 ± 1.25</td>
<td>23.96 ± 0.39</td>
<td>39.50 ± 7.17</td>
<td>56.47 ± 3.81</td>
</tr>
<tr>
<td>5</td>
<td>23.46 ± 0.46</td>
<td>57.60 ± 5.36</td>
<td>63.81 ± 5.72</td>
<td>23.96 ± 0.39</td>
<td>57.98 ± 5.07</td>
<td>57.88 ± 2.80</td>
</tr>
<tr>
<td>10</td>
<td>23.46 ± 0.46</td>
<td>52.38 ± 10.38</td>
<td>68.23 ± 4.49</td>
<td>23.96 ± 0.39</td>
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<td>45.18 ± 8.59</td>
</tr>
<tr>
<td>16</td>
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<td>41.14 ± 1.07</td>
<td>52.51 ± 6.74</td>
<td>23.96 ± 0.39</td>
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<td>29.80 ± 3.65</td>
</tr>
<tr>
<td>20</td>
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<td>36.75 ± 2.44</td>
<td>63.12 ± 5.12</td>
<td>23.96 ± 0.39</td>
<td>37.35 ± 2.92</td>
<td>42.18 ± 4.73</td>
</tr>
</tbody>
</table>

* Effect of PPT-A gene deletion on neutrophil count (percent) in the circulating blood of CLP induced septic mice at different time points after sham or CLP operation. Data are mean ± SE (compared by one-way ANOVA and Tukey test for multiple comparisons; n = 8 mice per group at all time points; p < 0.05.

† CLP vs sham.

‡ Ten-hour PPT-A+/+ CLP vs 10-h PPT-A−/− CLP.

§ Sixteen-hour PPT-A+/+ CLP vs 16-h PPT-A−/− CLP.

¶ Twenty hour PPT-A+/+ CLP vs 20-h PPT-A−/− CLP.
in wild-type mice at the 5- and 10-h points in comparison to PPT-A−/− mice after the onset of sepsis.

Discussion

A great deal of preclinical data and some early clinical studies indicate that both tachykinins and their receptors are consistently expressed in the peripheral nervous system of several species, including humans (19). PPT-A gene expression in hemopoietic cells is essential for immune complex-mediated inflammation in the lungs (2). Resident macrophages have a clear topographic advantage to instigate inflammatory events because of their proximity to the injured epithelium and by extension to sensory nerve fibers that contain SP (20, 21). Our earlier work had shown that knockout mice deficient in neurokinin 1 receptors were protected against pancreatitis-associated lung injury suggesting an important proinflammatory role for SP (16). In a recent paper, we have shown the role of PPT-A gene products (SP and neurokinin-A) in the pathogenesis of acute pancreatitis and associated lung injury (1).

Our results in the present study demonstrate that PPT-A-gene encoded neurokinins play a key role in the pathogenesis of CLP-induced lung injury in sepsis. The data in the current report suggest that CLP-induced sepsis orchestrates a series of changes on different parameters in PPT-A−/− mice and wild-type mice. Results from these experiments indicate that PPT-A deletion significantly protected against mortality, delayed the onset of lethality, and improved the long-term survival following CLP-induced sepsis, raising the possibility of a therapeutic window for clinical therapy. At 48 h, 100% mortality was observed in PPT-A−/− mice, whereas 44% survival was observed in PPT-A−/− mice. The bacteria load, neutrophil count, MPO activity (indication of neutrophil sequestration) in lung, edema, cellular infiltrates, and tissue injury in lungs (leakage of i.v. administered FITC-labeled albumin into the alveolar space, a measure of pulmonary microvascular permeability) were all significantly more decreased in PPT-A−/− mice than in wild type. An earlier paper has reported plasma protein exudation responses to repeated i.v. injections of SP in rat trachea (22). The effect of SP on airway microvascular leak in guinea pigs (23, 24) and the amount of exudates in the tissue was quantified. In the present study, increased pulmonary microvascular permeability, an increased leakage of the i.v. administered FITC-labeled albumin into the bronchoalveolar lavage fluid, was observed in the wild-type mice. PPT-A−/− mice, however, had significantly less pulmonary microvascular permeability compared with the PPT-A+/+ mice.

Gene-targeted disruption of the tachykinin neurokinin 1 receptor protected the lungs of mice from immune complex injury. It has been also reported that immunoreactive SP was detectable in fluids lining the lung at time points before neutrophil influx and may thus be involved in an early step in the inflammatory response to immune complexes in the lung (25). In the present study, though a
time-dependent elevation of SP was noted, the highest SP level was observed in plasma and lung at the 1-h time point. The SP level was declined from 5-h point onwards and peaked again at 16 and 20 h in lungs and at the 20-h point in plasma. The same type of biphasic response was associated in the neutrophil count also. PPT-A⁻/⁻/ mice exhibited an increased neutrophil count at 1 h and was significantly higher at the 10-, 16-, and 20-h points than PPT-A⁻/⁻/ mice, resulting in protection against tissue damage in PPT-A⁻/⁻/ mice. The difference in the inflammatory initiator role between PPT-A⁻/⁻/ mice and wild-type mice starts at the 5-h point for various parameters. Production of a time-dependent, significantly elevated MPO activity, pulmonary microvascular permeability, and tissue injury shown by the wild-type mice than by the PPT-A⁻/⁻/ mice. In this study, the PPT-A⁻/⁻/ mice showed significantly lower MCP-1 levels in circulation, and lung homogenates than PPT-A⁻/⁻/ mice. The same trend was observed in MIP-2 levels in circulation and lung homogenates. These data provide evidence that the mechanism by which PPT-A gene products play a crucial role in CLP-related lung injury is by modulating leukocyte recruitment through chemokines, such as MCP-1 and MIP-2. Lung pathology and increased levels of MCP-1 and MIP-2 correlated with high viral loads in the lung parenchyma (26). Recent reports suggest that in response to specific inflammatory stimuli, PMN may also directly interact with CC chemokine receptors. Moreover, PMN from CLP, but not from sham rodents, bound MCP-1 and responded chemotactically in vitro to MCP-1. The ability of anti-MCP-1 to reduce MPO activity after CLP suggests a role for CCR2 in the build-up of inflammation in the lung (27). Ab blockade of CCR2 reduced the recruitment of macrophages and neutrophils to the infected peritoneal cavities during acute polymicrobial sepsis suggesting that CCR2 engagement contributes to the cellular response against polymicrobial septic peritonitis (32). The in vivo data in the mouse model of ischemia-reperfusion demonstrated that CCR1, CCR2, and CCR5 contribute to the recruitment of neutrophils during the early reperfusion phase through effects on intravascular adherence and subsequent transendothelial migration (33). Moreover, it was shown that chemokine receptors CCR2 and CCR5 are expressed on the surface of the native murine neutrophils (33, 34). Therefore, it seems plausible that CCR2 and CCR5 are dependent on firm adherence and transmigration of PMN by direct interactions between these chemokine receptors on PMN and their respective chemokine ligands. Holmes et al. (35) showed that LPS increased neutrophil-associated cytokine-induced neutrophil chemoattractant (CINC) and MIP-2 content in alveolar epithelial cells to intratracheal LPS stimulation. The CX3C chemokines CINC and MIP-2 are responsible for attracting neutrophils into the alveolar space (36, 37). Increased MIP-2 was associated with accumulation of inflammatory cells, especially PMN and acute lung injury. Intrapleural instillation of anti-MIP-2 antiseraum (38) and intrapulmonary instillation of anti-MIP-2 Ab (39, 40) decreased the influx of neutrophils in the lung and attenuated lung injury. Shibata et al. (41) reported that the CXCR2 is the receptor for CINC and MIP-2 on rodent neutrophils. Our results also show that lung MCP-1 and MIP-2 levels correlate well with neutrophil infiltration in the lung, measured by lung MPO activity. However, significantly lower levels of MPO activity and MCP-1 and MIP-2 were observed in PPT-A⁻/⁻/ mice compared with PPT-A⁻/+/+ mice providing important insights into the mechanism by which the PPT-A gene amplifies the inflammatory response.

Of the various neurokinins encoded by the PPT-A gene, SP exhibits the highest affinity for this receptor and therefore it is likely that its suppression contributed substantively to attenuation of the inflammatory response (2, 25). They also suggested that SP or, more likely, neurokinin A induces cytokine release via neurokinin receptors other than the neurokinin-1 receptor. In this study, the chemokine levels in PPT-A⁻/⁻/ mice showed a significantly lower level of MCP-1 in circulation and lung homogenates. Neurokinin-1 mediates proinflammatory and negative hemopoietic responses (42). Their study showed that there is a fine-tuned regulation between neurokinin-1 expression and cytokines to regulate a balance in immune and hemopoietic responses. The presence of SP and other neurokinins in resident macrophages and circulating leukocytes is particularly intriguing because many of these cells also express neurokinin receptors (3, 4) and act as a proinflammatory mediator in many inflammatory states (4). Thus, our findings raise the possibility that inflammatory cells use PPT-A gene-encoded neurokinins as a paracrine- or autocrine-signaling mechanism to amplify inflammation. SP was detectable in fluids lining the lung at time points before neutrophil influx and may thus be involved in an early step in the inflammatory response to immune complexes in the lung (25). It is reasonable to speculate that tachykinins produced by subepithelial sensory neurons may recruit inflammatory cells to amplify the inflammatory response. Also resident macrophages and circulating leukocytes expressing neurokinin receptors may trigger to enhance the response to injury. Thus, the present data illustrates that the deletion of the PPT-A gene disrupts the chemokine production followed by the inflammatory cell recruitment and ameliorates the sepsis-induced lung injury in septic mice.

In summary, the present study shows that PPT-A gene deletion delays the pathogenesis of sepsis raising the possibility of a therapeutic window for clinical therapy and offers insights into physiological and molecular mechanisms of PPT-A gene products in the modulation of the inflammatory cascade.

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Disclosures
The authors have no financial conflict of interest.

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