Cysteinyl Leukotrienes Impair Hypoxic Pulmonary Vasoconstriction in Endotoxemic Mice

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ABSTRACT

Background: Sepsis impairs hypoxic pulmonary vasoconstriction (HPV) in patients and animal models, contributing to systemic hypoxemia. Concentrations of cysteinyl leukotrienes are increased in the bronchoalveolar lavage fluid of patients with sepsis, but the contribution of cysteinyl leukotrienes to the impairment of HPV is unknown.

Methods: Wild-type mice, mice deficient in leukotriene C4 synthase, the enzyme responsible for cysteinyl leukotriene synthesis, and mice deficient in leukotriene receptor 1 were studied 18 h after challenge with either saline or endotoxin. HPV was measured by the increase in left pulmonary vascular resistance induced by left mainstem bronchus occlusion. Concentrations of cysteinyl leukotrienes were determined in the bronchoalveolar lavage fluid.

Results: In the bronchoalveolar lavage fluid of all three strains, cysteinyl leukotrienes were not detectable after saline challenge; whereas endotoxin challenge increased cysteinyl leukotriene concentrations in wild-type mice and mice deficient in cysteinyl leukotriene receptor 1, but not in mice deficient in leukotriene C4 synthase. HPV did not differ among the three mouse strains after saline challenge (120 ± 26, 114 ± 16, and 115 ± 24%, respectively; mean ± SD). Endotoxin challenge markedly impaired HPV in wild-type mice (41 ± 20%) but only marginally in mice deficient in leukotriene C4 synthase (96 ± 16%, P < 0.05 vs. wild-type mice), thereby preserving systemic oxygenation. Although endotoxin modestly decreased HPV in mice deficient in cysteinyl leukotriene receptor 1 (80 ± 29%, P < 0.05 vs. saline challenge), the magnitude of impairment was markedly less than in endotoxin-challenged wild-type mice.

Conclusion: Cysteinyl leukotrienes importantly contribute to endotoxin-induced impairment of HPV in part via a cysteinyl leukotriene receptor 1-dependent mechanism.

H YPOXIC pulmonary vasoconstriction (HPV) is an essential vasomotor response to alveolar hypoxia, diverting blood flow from poorly ventilated lung regions to better ventilated areas, thereby improving ventilation-perfusion matching and raising the partial pressure of oxygen in the systemic circulation.1,2 HPV is impaired in patients with acute lung injury and adult respiratory distress syndrome (ARDS).3 Among patients with acute lung injury/ARDS, sepsis-induced ARDS is associated with the highest mortality
rate. Experimental endotoxemia also has been shown to impair HPV. However, the precise mechanisms by which endotoxin impairs HPV are incompletely understood.

Among the inflammatory mediators implicated in the impairment of HPV are the leukotrienes. Leukotrienes are lipid mediators that are rapidly generated from arachidonic acid. Arachidonic acid is converted to the unstable intermediate leukotriene A₄ (LTA₄) by 5-lipoxygenase. LTA₄ can be converted by either LTA₄ hydrolase to leukotriene B₄ (LTB₄), or it can be conjugated with glutathione by LTC₄ synthase to form leukotriene C₄ (LTC₄). LTC₄ is converted by sequential hydrolysis to leukotriene D₄ (LTD₄) and leukotriene E₄ (LTE₄). LTC₄, LTD₄, and LTE₄ are collectively called the cysteinyl leukotrienes (cysLTs). The cysLTs bind to the cysteinyl leukotriene receptors 1, 2, 3 or the cysteinyl leukotriene receptor E₄, whereas LTB₄ mediates its effects by binding to LTB₄ receptors 1 or 2.

LTB₄ and the cysLTs display different functions during inflammation. LTB₄ is a potent chemokinetic and chemotactic agent for polymorphonuclear neutrophils, whereas the cysLTs increase vascular permeability and stimulate bronchoconstriction and mucus secretion. Noncardiovascular effects of LTs include vasodilation, bronchodilation, and inhibition of gastric acid. Arachidonic acid is converted to the unstable intermediate leukotriene A₄ (LTA₄) by 5-lipoxygenase. LTA₄ can be converted by either LTA₄ hydrolase to leukotriene B₄ (LTB₄), or it can be conjugated with glutathione by LTC₄ synthase to form leukotriene C₄ (LTC₄).

Measurement of Hypoxic Pulmonary Vasoconstriction

To assess HPV, the change of slope of the left lung pulmonary blood flow-pressure relationship in response to acute left lung alveolar hypoxia was measured in nine animals per group, as described previously. Briefly, 18 h after challenge with either saline or lipopolysaccharide, mice were anesthetized and mechanically ventilated with a respiratory rate of 100 breaths/min and a tidal volume of 10 ml/kg body weight at an inspired oxygen fraction of 1.0. The peak inspiratory pressure was approximately 10 cm H₂O and the positive end-expiratory pressure 2 cm H₂O. An arterial line was placed in the left carotid artery, and a left-sided throracotomy was performed. A custom-made polyethylene catheter was positioned in the main pulmonary artery, and a flow probe was placed around the left pulmonary artery. Heart rate, systemic arterial pressure, pulmonary arterial pressure, and left pulmonary arterial blood flow were continuously measured and recorded (DI 720; Dataq Instruments, Akron, OH). Left lung alveolar hypoxia and collapse were induced by occluding the left mainstem bronchus. To estimate left pulmonary vascular resistance, the inferior vena cava was transiently occluded to decrease left pulmonary arterial blood flow by approximately 50%. Left pulmonary vascular resistance was calculated from the slope of the left pulmonary arterial blood flow–pulmonary arterial pressure relationship. The increase in left pulmonary vascular resistance induced by occlusion of the left mainstem bronchus was expressed as the percentage increase from baseline left pulmonary vascular resistance to left pulmonary vascular resistance after 5 min of occlusion of the left mainstem bronchus. After all hemodynamic measurements were obtained, blood was sampled from the left carotid artery, anticoagulated with heparin, and arterial blood gas analyses were performed using a Rapid Lab 840 (Chiron Diagnostics, Medfield, MA).

The following exclusion criteria were used: a preparation time of more than 60 min, at baseline a mean blood pressure less than 60 mmHg and a heart rate less than 400 beats/min, and inadvertent displacement of the arterial line or the flow probe.

Circulating Leukocyte Count

In additional mice (in each group six or seven animals), blood was obtained via an arterial line 18 h after challenge with saline or lipopolysaccharide. Erythrocytes were hemolyzed using a Unopette® (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), and leukocytes were counted with a hemocytometer (Hausser Scientific, Horsham, PA).
Myeloperoxidase Assay
Infiltration of polymorphonuclear neutrophils into the lungs was estimated by measuring myeloperoxidase concentrations at 18 h after saline (n = 6 in each group) or lipopolysaccharide challenge (WT, n = 5; LTC₄S⁻/-, n = 7; and CysLT₁⁻/-, n = 7), as described previously.⁴²

Lung Wet/Dry Weight Ratio
In additional experiments, mice were euthanized with pentobarbital (0.1 mg/kg intraperitoneal) at 18 h after challenge with saline (WT, n = 5; LTC₄S⁻/-, n = 5; CysLT₁⁻/-, n = 4) or lipopolysaccharide (WT, n = 9; LTC₄S⁻/-, n = 10; CysLT₁⁻/-, n = 10). Both lungs were removed, blotted, and immediately weighed. The tissue was dried in a microwave oven for 60 min and reweighed. The lung wet/dry weight ratio was expressed as a percentage of dry to wet weight.

Bronchoalveolar Lavage Fluid
The lungs of mice challenged with either saline or lipopolysaccharide 18 h earlier were lavaged with 3 × 1 ml ice-cold phosphate buffered saline. The recovered bronchoalveolar lavage fluid was pooled and centrifuged at 1,500 rpm for 10 min at 4°C. The supernatant was snap frozen and stored at −80°C until the measurement of leukotriene concentration. Samples were taken after challenge with either saline (WT, n = 4; LTC₄S⁻/-, n = 5; CysLT₁⁻/-, n = 5) or lipopolysaccharide (n = 6).

Measurement of Leukotriene Concentration
The samples of bronchoalveolar lavage fluid were thawed and acidified to a pH of 3.5. LTB₄ and cysLTs were extracted with methyl formate and methanol, respectively. Leukotriene acidified to a pH of 3.5. LTB₄ and cysLTs were extracted using enzyme immunoassay kits following the manufacture’s instructions (Neogen Corporation, Lexington, KY).

Statistical Analysis
Data are expressed as mean ± SD. P values <0.05 were considered statistically significant. Statistical analyses were performed using Stat 3.0 (Systat Software Inc., Richmond, CA). For the comparison between saline and lipopolysaccharide or the genotypes of WT, LTC₄S⁻/-, and CysLT₁⁻/-, data were analyzed using a two-way ANOVA with post hoc Bonferroni tests (two-tailed) for normally distributed data or using a Kruskal-Wallis test (two-tailed) with a post hoc Bonferroni test for non-normally distributed data. Hemodynamic changes between before and during occlusion of the left mainstem bronchus were compared with a paired t test (two-tailed).

Results

Hemodynamic Measurements before and during Unilateral Hypoxia
At 18 h, all saline-challenged mice survived, whereas approximately 50% of the lipopolysaccharide-challenged mice had died. Before left lung hypoxia was induced by occlusion of the left mainstem bronchus, the values of heart rate, systemic arterial pressure, pulmonary arterial pressure, and left pulmonary arterial blood flow did not differ between the mouse genotypes at 18 h after challenge with either saline or LPS (table 1). During occlusion of the left mainstem bronchus, the heart rate, systemic arterial pressure, and pulmonary arterial pressure were not different between saline- and lipopolysaccharide-challenged mice. A comparison between before and during occlusion of the left mainstem bronchus showed that the pulmonary arterial pressure was significantly lower in the lipopolysaccharide-challenged mice. Statistical differences (WT, LTC₄S⁻/-, and CysLT₁⁻/-) were assessed using a two-way ANOVA with post hoc Bonferroni tests (two-tailed).

Table 1. Hemodynamic Measurements

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>LTC₄S⁻/-</th>
<th>CysLT₁⁻/-</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sal</td>
<td>LPS</td>
<td>Sal</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>515 ± 37</td>
<td>497 ± 38</td>
<td>487 ± 41</td>
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<tr>
<td>LMBO</td>
<td>508 ± 39</td>
<td>500 ± 27</td>
<td>473 ± 38</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>79 ± 11</td>
<td>85 ± 9</td>
<td>81 ± 7</td>
</tr>
<tr>
<td>LMBO</td>
<td>81 ± 12</td>
<td>82 ± 11</td>
<td>80 ± 8</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>15 ± 0</td>
<td>15 ± 2</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>LMBO</td>
<td>18 ± 1†</td>
<td>17 ± 2†</td>
<td>17 ± 1†</td>
</tr>
<tr>
<td>QLPA (ml/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.4 ± 0.3</td>
<td>2.3 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>LMBO</td>
<td>1.5 ± 0.3</td>
<td>2.0 ± 0.2†</td>
<td>1.7 ± 0.2†</td>
</tr>
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</table>

Hemodynamic measurements before (baseline) and during occlusion of the left mainstem bronchus in WT, LTC₄S⁻/-, and CysLT₁⁻/- mice at 18 h after challenge with either saline or lipopolysaccharide (n = 9 per group). All values at baseline or during occlusion of the left mainstem bronchus were compared for challenge. All data mean ± SD. * P < 0.05 vs. saline-challenged mice of respective genotype by two-way ANOVA. † P < 0.05 vs. baseline by paired t test. ‡ P < 0.05 vs. lipopolysaccharide-challenged WT mice by two-way ANOVA.

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saccharide-challenged WT mice, and CysLT1
blood; WT mice of the respective genotype, #
(hypoxic pulmonary vasoconstriction
in the arterial blood during occlusion of the left mainstem bronchus). Saline-
concentration between WT, LTC4S
mice congenitally deficient in leukotri-
concentration of oxygen in the arterial
right lung was more than threefold greater at 18 h after
challenge with lipopolysaccharide. However, after intravenous challenge with
part of pulmonary vascular resistance in response to the occlusion of the left mainstem bronchus occlusion
in lipopolysaccharide-challenged CysLT1
mice than in lipopolysaccharide-challenged WT mice (P < 0.05, fig. 1A).

Preserved HPV is Associated with a Higher Systemic Arterial Oxygen Tension during Occlusion of the Left Mainstem Bronchus

To estimate the impact of HPV on systemic arterial oxygenation, arterial blood gas tensions were measured during occlusion of the left mainstem bronchus at the end of each HPV experiment (fig. 1B and table 2). The systemic arterial partial pressure of oxygen (PaO2) during occlusion of the left mainstem bronchus did not differ between the genotypes after saline challenge. However, after occlusion of the left mainstem bronchus, the PaO2 was markedly less in endotoxin-challenged WT mice than in saline-challenged WT mice (P < 0.05). In contrast, the PaO2 after occlusion of the left mainstem bronchus in lipopolysaccharide-challenged LTC4S−/− mice was similar to that in saline-challenged LTC4S−/− mice and greater than in both lipopolysaccharide-challenged WT mice and lipopolysaccharide-challenged CysLT1−/− mice (P < 0.05 for both). In lipopolysaccharide-challenged CysLT1−/− mice, the PaO2 during occlusion of the left mainstem bronchus tended to be higher than in lipopolysaccharide-challenged WT mice (P > 0.05).

There were no differences in the values of the arterial partial pressure of carbon dioxide between the genotypes after challenge with saline or lipopolysaccharide. The changes in pH, and the base excess were smaller in each of the three genotypes after saline challenge than after lipopolysaccharide challenge. Hemoglobin concentrations were similar in all mice.

Endotoxin Promotes Pulmonary Infiltration of Polymorphonuclear Neutrophils and Increases cysLT concentrations in the Bronchoalveolar Lavage Fluid

Challenge with lipopolysaccharide markedly decreased the concentration of circulating leukocytes in all three mouse strains (fig. 2A). There was no difference in the circulating leukocyte concentration between WT, LTC4S−/−, and CysLT1−/− mice after intravenous challenge with lipopolysaccharide.

In all three genotypes, the myeloperoxidase activity of the right lung was more than threefold greater at 18 h after lipopolysaccharide challenge than after saline challenge (fig.
Hb (g/dl) 13.4
pH a 7.35
PaCO2 (mmHg) 30.5

Arterial blood gas analyses at the end of the hemodynamic studies during occlusion of the left mainstem bronchus in WT, LTC4S−/−, and CysLT1−/− mice after challenge with either saline or lipopolysaccharide. All data mean ± SD.

** P < 0.05 vs. saline-challenged mice of respective genotype. † P < 0.05 vs. lipopolysaccharide-challenged WT mice (n = 9 in each group). ‡ P < 0.05 vs. LPS-challenged CysLT1−/− mice.

**BE** = base excess; **CysLT1−/−** = mice congenitally deficient in the cysteinyl leukotriene receptor 1; **g/dl** = gram per deciliter; **Hb** = hemoglobin; **LPS** = lipopolysaccharide; **LTC4S−/−** = mice congenitally deficient in leukotriene C4 synthase; **mmHg** = millimeters of mercury; **mmol/l** = millimoles per liter; **PaO2** = level of oxygen in the arterial blood; **PaO2** = level of carbon dioxide in the arterial blood; **pH a** = arterial pH; **Sal** = saline; **WT** = wild-type.

2B). Lung myeloperoxidase activity levels in lipopolysaccharide-challenged CysLT1−/− mice were greater than the levels measured in lipopolysaccharide-challenged WT mice (P < 0.05).

The lung wet-to-dry weight ratio did not differ among WT, LTC4S−/−, and CysLT1−/− mice after challenge with saline (4.4 ± 0.2, n = 5; 4.4 ± 0.2, n = 5; 4.5 ± 0.3, n = 4, respectively). Challenge with lipopolysaccharide did not alter the wet-to-dry weight ratio compared with saline challenge in WT, LTC4S−/−, and CysLT1−/− mice (4.5 ± 0.3, n = 9; 4.4 ± 0.3, n = 10; 4.4 ± 0.2, n = 10).

In all three genotypes, there were no differences in the bronchoalveolar lavage fluid LTB4 concentrations after challenge with saline or lipopolysaccharide (fig. 3A). In contrast, cysLT concentrations in the bronchoalveolar lavage fluid of the same mice were much higher in WT and CysLT1−/− mice after endotoxin challenge than after saline challenge. No cysLTs were detectable in bronchoalveolar lavage fluid obtained from LTC4S−/− mice (fig. 3B).

Discussion

Our data show that cysLTs play an important role in endotoxin-induced impairment of HPV. A congenital deficiency of cysteinyl leukotriene synthesis largely protects septic mice from lipopolysaccharide-induced impairment of HPV and preserves systemic arterial oxygenation. Activation of the CysLT1 receptor contributes significantly to the impairment of HPV after endotoxin challenge because mice lacking the CysLT1 receptor are to a great extent protected from the lipopolysaccharide-induced attenuation of HPV.

After a saline challenge, both LTC4S−/− and CysLT1−/− mice demonstrated the same marked increase of left pulmonary vascular resistance that was observed in WT mice. In a previous study, we reported that HPV was similarly preserved in mice deficient in either 5-lipoxygenase or LTA4 hydrolase under normal (nonseptic) conditions. Taken together, these results confirm that neither LTB4 nor cysLTs are required for the pulmonary vasoconstrictor response to hypoxia in healthy lung.

As reported previously, we observed that endotoxin challenge markedly impaired HPV in WT mice. In the current study, we found that a congenital deficiency of cysLT synthesis largely preserves HPV after endotoxin challenge. Because cysLTs can bind to cysteinyl leukotriene receptors 1, 2, 3, or E4, we sought to clarify the role of the CysLT1 receptor in endotoxin-induced impairment of HPV by using CysLT1 receptor-deficient mice. We found that CysLT1 deficiency significantly attenuates the endotoxin-induced impairment of HPV compared with lipopolysaccharide-challenged WT mice, albeit to a lesser extent than did a complete deficiency of cysLT synthesis. It is possible that activation of cysteinyl leukotriene receptor 2 and/or cysteinyl leukotriene receptor 3 by cysteinyl leukotrienes may have contributed to the impairment of HPV in CysLT1−/− mice. Taken together, our results show that cysLTs impair HPV after endotoxin challenge and that they exert their effects in major part via CysLT1.

We reported previously that 5-lipoxygenase deficiency prevented the impairment of HPV by endotoxin challenge associated with a reduction in the endotoxin-induced increase in pulmonary myeloperoxidase concentrations. To learn if cysLTs impair HPV by inducing pulmonary polymorphonuclear leukocyte accumulation, the peripheral leukocyte concentration and pulmonary myeloperoxidase concentrations were measured in WT, LTC4S−/−, and CysLT1−/− mice at 18 h after endotoxin challenge. In all three genotypes, the leukocyte concentrations were markedly decreased, and pulmonary myeloperoxidase concentrations were increased. On the other hand, HPV was impaired in WT mice but not in LTC4S−/− mice. Taken together, these results suggest that the recruitment of leukocytes to the lung after endotoxin challenge is mediated by LTB4 but not by cysLTs and that the accumulation of leukocytes in the lung per se does not contribute to the impairment of HPV.
La¨rfars et al. showed that leukotrienes can cause nitric oxide release from polymorphonuclear leukocytes.45 Nitric oxide is a potent vasodilator that acts primarily via stimulation of soluble guanylate cyclase. In a previous study, we reported that mice deficient in inducible nitric oxide synthase had preserved HPV after endotoxin challenge.9 In addition, pharmacologic inhibition of soluble guanylate cyclase attenuated the endotoxin-induced impairment of HPV in an isolated, perfused, and ventilated mouse lung.12 It is possible that cysLTs contribute to the endotoxin-induced impairment of HPV by causing vasodilation via the nitric oxide pathway.

Concentrations of cysLTs are increased in the bronchoalveolar lavage fluid of patients with ARDS,35 and cysLTs are known to increase vascular permeability.31,32 We sought to
determine whether the impairment of HPV by endotoxin was associated with increased concentrations of cysLTs in bronchoalveolar lavage fluid and with increased pulmonary microvascular permeability. Eighteen hours after challenge with lipopolysaccharide, cysLT concentrations were markedly increased in bronchoalveolar lavage fluid obtained from WT and CysLT1<sup>-/-</sup> mice, whereas in the bronchoalveolar lavage fluid of LTC4S<sup>-/-</sup> mice, as expected, no cysLTs were detectable. In all three genotypes, endotoxin challenge did not increase lung wet-to-dry weight ratios. These observations suggest that cysLTs did not impair HPV by increasing permeability in the current study.

The molecular mechanisms underlying HPV remain elusive. However, the current theories of how oxygen tension is sensed by the pulmonary arteries center around the biosynthesis of radical oxygen species and the cellular redox state. In a previous study from our laboratory, we showed that oxygen radical scavengers attenuated the impairment of HPV after lipopolysaccharide challenge. In animal models of either indomethacin-induced gastric ulcers or skin flap ischemia reperfusion injury, the cysLT receptor antagonist montelukast exerted antioxidant effects. Taken together, it is possible that the deficiency of cysLT synthesis prevented endotoxin-induced impairment of HPV by reducing oxidative stress.

The current study demonstrates that cysLTs contribute to the endotoxin-induced impairment of HPV in a rodent model. However, our study has limitations. The administration of lipopolysaccharide is widely used as an animal model for sepsis, but the lipopolysaccharide component of the bacterial cell wall does not cause all of the complex inflammatory processes seen in clinical sepsis. Our results are also limited because of the small number of animals used and the relatively large standard deviations in some experiments.

In summary, we have identified a key role for cysLTs in endotoxin-induced impairment of HPV using two strains of genetically modified mice. We found that a congenital deficiency of LTC4S almost completely protected mice from endotoxin-induced impairment of HPV, whereas deficiency of the CysLT<sub>1</sub> receptor significantly attenuated the endotoxin-induced impairment of HPV. Endotoxin-induced activation of cysLT pathway compromised HPV, thereby reducing systemic arterial oxygenation. The protective effects of cysLT deficiency were independent of changes in both pulmonary polymorphonuclear leukocyte accumulation and the presence of pulmonary edema. The current results suggest that cysLTs may be additional therapeutic targets in the treatment or prevention of the sepsis-induced impairment of HPV.

References


