Cysteinyl Leukotrienes Impair Hypoxic Pulmonary Vasoconstriction in Endotoxemic Mice

Bodil Petersen, M.D.,* K. Frank Austen, M.D.,† Kenneth D. Bloch, M.D.,‡ Yukako Hotta, M.D.,§ Fumito Ichinose, M.D.,‖ Yoshihide Kanaoka, M.D., Ph.D.,# Warren M. Zapol, M.D.**

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 C_4 synthase, the enzyme responsible for cysteinyl leukotriene synthesis, and mice deficient in cysteinyl 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 C_4 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 C_4 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.

What We Already Know about This Topic

- Hypoxic pulmonary vasoconstriction (HPV) is impaired in patients with sepsis and acute lung injury. Experimental data suggest that endotoxemia may play a role for impairment of HPV.

What This Article Tells Us That Is New

- This experimental study in genetically-modified mice identifies a key role for cysteinyl leukotrienes (cysLTs) in endotoxin-induced impairment of HPV which was prevented/attenuated in cysLT deficient animals.

HYPOXIC 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

* Postdoctoral Fellow, Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; Department of Anesthesia and Critical Care Medicine, University Hospital Leipzig, Leipzig, Saxony, Germany (current address). † Astra Zeneca Professor of Respiratory and Inflammatory Diseases, # Assistant Professor of Medicine, Department of Medicine and Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Harvard Medical School. ‡ William Thomas Green Morton Professor of Anesthesia, § Postdoctoral Fellow, ‖ Associate Professor of Anesthesia, ** Reginald Jenney Professor of Anesthesia, Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School.

Received from the Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; and the Department of Medicine and Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, Harvard Medical School. Submitted for publication December 14, 2010. Accepted for publication July 1, 2011. Supported by the grants P01HL36110 (to Dr. Austen), R01HL74352 (to Dr. Bloch), R01GM79360 (to Dr. Ichinose), R01HL090630 (to Dr. Kanaoka), and R01HL42397 (to Dr. Zapol) from the National Institutes of Health, Bethesda, Maryland.

Address correspondence to Dr. Zapol: Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, 55 Fruit Street, Boston, Massachusetts 02114. wzapol@partners.org. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

 Anesthesiology 2011; 115:804–11

rate.[4] Experimental endotoxemia also has been shown to impair HPV.[5–12] However, the precise mechanisms by which endotoxin impairs HPV are incompletely understood.[13–16]

Among the inflammatory mediators implicated in the impairment of HPV are the leukotrienes.[5–7,9–12,17] Leukotrienes are lipid mediators that are rapidly generated from arachidonic acid. Arachidonic acid is converted to the unstable intermediate leukotriene A_4 (LTA_4) by 5-lipoxygenase.[18,19] LTA_4 can be converted by either LTA_4 hydrolase to leukotriene B_4 (LTB_4),[20] or it can be conjugated with glutathione by LTC_4 synthase to form leukotriene C_4 (LTC_4).[21–23] LTC_4 is converted by sequential hydrolysis to leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4).[24] LTC_4, LTD_4, and LTE_4 are collectively called the cysteinyl leukotrienes (cysLTs). The cysLTs bind to the cysteinyl leukotriene receptors 1, 2, 3 or the cysteinyl leukotriene receptor E_4,[25–28] whereas LTB_4 mediates its effects by binding to LTB_4 receptors 1 or 2.[29,30]

LTB_4 and the cysLTs display different functions during inflammation. LTB_4 is a potent chemokinetic and chemoattractant agent for polymorphonuclear neutrophils, whereas the cysLTs increase vascular permeability and stimulate bronchoconstriction and mucus secretion.[31,32] Noncardiogenic pulmonary edema and the intrapulmonary accumulation of polymorphonuclear neutrophils are key features of acute lung injury/ARDS.[33,34] The generation of leukotrienes by leukocytes is enhanced during sepsis, and leukotriene concentrations are increased in the bronchoalveolar lavage fluid obtained from patients with ARDS.[35,36] These observations suggest the possibility that leukotrienes participate in the pathogenesis of acute lung injury and the impairment of HPV.

In a previous study, we showed that mice congenitally deficient in 5-lipoxygenase are protected from the impairment of HPV that follows endotoxin challenge.[10] Furthermore, congenital deficiency of either LTA_4 hydrolase or LTB_4 receptor 1 did not preserve HPV in endotoxemic mice, suggesting that LTB_4 does not contribute to the impairment of HPV in endotoxin-challenged mice. In contrast, the pharmacologic inhibition of the cysteinyl leukotriene receptor 1 ($CysLT_1$), using MK571, completely protected wild-type (WT) mice from endotoxin-induced impairment of HPV.[10] However, MK571 has multiple targets, such as the multidrug-resistant protein-1 and the purinergic receptors 1, 2, 4, and 6,[37,38] so we sought to clarify the role of cysLTs and $CysLT_1$ in the impairment of HPV by endotoxin using mice congenitally deficient in either LTC_4 synthase ($LTC_4S^{-/-}$) or the $CysLT_1$ receptor ($CysLT_1{}^{-/-}$). We hypothesized that cysLTs contribute to the impairment of HPV after endotoxin challenge and that they exert their effect *via* the $CysLT_1$ receptor-dependent mechanisms.

Materials and Methods

All animal experiments were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital, Boston, Massachusetts. $LTC_4S^{-/-}$ and $CysLT_1{}^{-/-}$ mice were generated as described previously.[39,40] $LTC_4S^{-/-}$ and $CysLT_1{}^{-/-}$ mice were backcrossed onto a C57BL/6J background for nine generations. WT mice (C57BL6/J) were purchased from Jackson Laboratory (Bar Harbor, ME). The studies were conducted in male WT, $LTC_4S^{-/-}$, and $CysLT_1{}^{-/-}$ mice. Mice weighing between 21 and 27 g were matched for body weight and intravenously challenged with saline or lipopolysaccharide (*Escherichia coli* O111:B4, σ, Sigma Aldrich Corp., St. Louis, MO; 10 mg/kg, dissolved in saline 0.1 ml/10 g body weight).

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.[10,41] 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 H_2O and the positive end-expiratory pressure 2 cm H_2O. An arterial line was placed in the left carotid artery, and a left-sided thoracotomy 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 was 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; $LTC_4S^{-/-}$, n = 7; and $CysLT_1{}^{-/-}$, 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; $LTC_4S^{-/-}$, n = 5; $CysLT_1{}^{-/-}$, n = 4) or lipopolysaccharide (WT, n = 9; $LTC_4S^{-/-}$, n = 10; $CysLT_1{}^{-/-}$, 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; $LTC_4S^{-/-}$, n = 5; $CysLT_1{}^{-/-}$, 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. LTB_4 and cysLTs were extracted with methyl formate and methanol, respectively. Leukotriene concentrations were quantified in duplicate 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 σ Stat 3.0 (Systat Software Inc., Richmond, CA). For the comparison between saline and lipopolysaccharide or the genotypes of WT, $LTC_4S^{-/-}$, and $CysLT_1{}^{-/-}$, 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 pres-

Table 1. Hemodynamic Measurements

	WT		$LTC_4S^{-/-}$		$CysLT_1{}^{-/-}$	
	Sal	LPS	Sal	LPS	Sal	LPS
HR (beats/min)						
Baseline	515 ± 37	497 ± 38	487 ± 41	485 ± 26	489 ± 44	504 ± 50
LMBO	508 ± 39	500 ± 27	473 ± 38	484 ± 26	485 ± 47	493 ± 55
SAP (mmHg)						
Baseline	79 ± 11	85 ± 9	81 ± 7	85 ± 12	79 ± 6	89 ± 10*
LMBO	81 ± 12	82 ± 11	80 ± 8	84 ± 9	78 ± 9	86 ± 9
PAP (mmHg)						
Baseline	15 ± 2	15 ± 2	15 ± 1	16 ± 1*	16 ± 1	15 ± 1
LMBO	18 ± 1†	17 ± 2†	17 ± 1†	18 ± 2†	18 ± 2†	18 ± 2†
QLPA (ml/min)						
Baseline	2.4 ± 0.3	2.3 ± 0.2	2.4 ± 0.2	2.3 ± 0.3	2.4 ± 0.2	2.4 ± 0.1
LMBO	1.5 ± 0.3†	2.0 ± 0.2*†	1.7 ± 0.2†‡	1.7 ± 0.2†	1.6 ± 0.2†	1.8 ± 0.2†

Hemodynamic measurements before (baseline) and during occlusion of the left mainstem bronchus in WT, $LTC_4S^{-/-}$, and $CysLT_1{}^{-/-}$ 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.

beat/min = beats per minute; $CysLT_1{}^{-/-}$ = mice congenitally deficient in the cysteinyl leukotriene receptor 1; HR = heart rate; LMBO = occlusion of the left mainstem bronchus; LPS = lipopolysaccharide; $LTC_4S^{-/-}$ = mice congenitally deficient in leukotriene C4 synthase; ml/min = milliliter per minute; mmHg = millimeters of mercury; PAP = mean pulmonary arterial pressure; QLPA = flow rate in left pulmonary artery; Sal = saline; SAP = mean systemic arterial pressure; WT = wild-type mice.

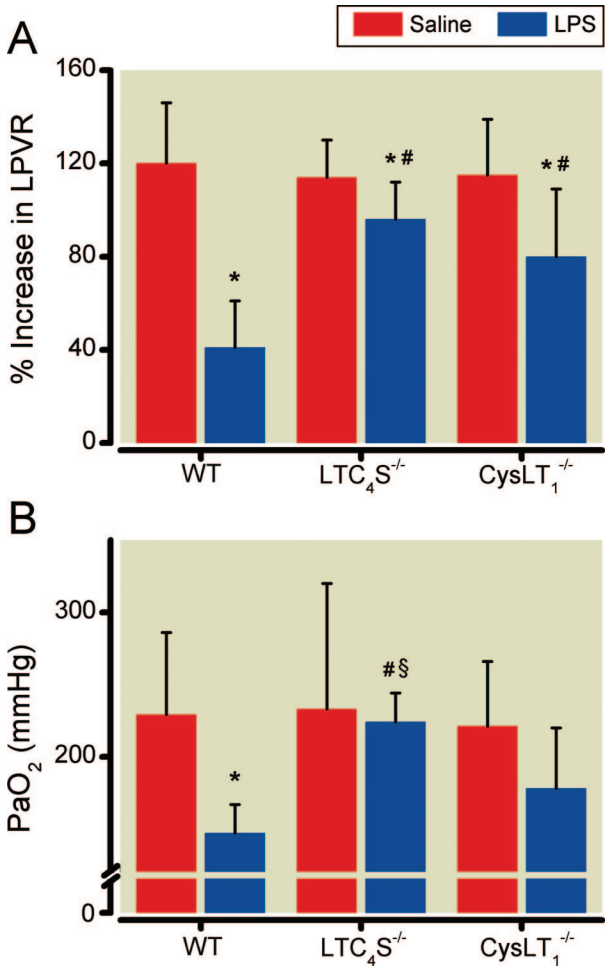

Fig. 1. Occlusion of the left mainstem bronchus-induced increase of left pulmonary vascular resistance in WT, $LTC_4S^{-/-}$, and $CysLT_1^{-/-}$ mice at 18 h after challenge with either saline or lipopolysaccharide (n = 9 in each group) (*A*). Values of oxygen in the arterial blood during occlusion of the left mainstem bronchus at the end of the hypoxic pulmonary vasoconstriction (HPV) measurements (*B*). * $P < 0.05$ *versus* saline-challenged mice of the respective genotype, # $P < 0.05$ *versus* lipopolysaccharide-challenged WT mice, § $P < 0.05$ *versus* lipopolysaccharide-challenged $CysLT_1^{-/-}$ mice. $CysLT_1^{-/-}$ = mice congenitally deficient in the cysteinyl leukotriene receptor 1; LPS = lipopolysaccharide; LPVR = left pulmonary vascular resistance; $LTC_4S^{-/-}$ = mice congenitally deficient in leukotriene C_4 synthase; Pa_{O_2} = concentration of oxygen in the arterial blood; WT = wild-type. All data mean ± SD.

sure increased and left pulmonary arterial blood flow decreased in all mice, whereas heart rate and systemic arterial pressure did not change, suggesting that the changes in pulmonary arterial pressure and left pulmonary arterial blood flow were not attributable to hemodynamic instability.

Hypoxic pulmonary vasoconstriction was assessed as the percentage change of left pulmonary vascular resistance in response to occlusion of the left mainstem bronchus (fig. 1A). Saline-challenged mice of all three genotypes (WT, $LTC_4S^{-/-}$, and $CysLT_1^{-/-}$) demonstrated a marked increase of left pulmonary vascular resistance in response to occlusion of the left mainstem bronchus. As expected, challenge with lipopolysaccharide markedly impaired the increase of left pulmonary vascular resistance during occlusion of the left mainstem bronchus in WT mice compared with saline-treated WT mice ($P < 0.05$). In contrast, in $LTC_4S^{-/-}$ mice, the increase in left pulmonary vascular resistance induced by left mainstem bronchus occlusion was largely preserved after challenge with lipopolysaccharide. In $CysLT_1^{-/-}$ mice, challenge with lipopolysaccharide modestly impaired the increase in left pulmonary vascular resistance in response to the occlusion of the left mainstem bronchus occlusion ($P < 0.05$ *vs.* saline-challenged $CysLT_1^{-/-}$ mice). However, the increase in left pulmonary vascular resistance was significantly greater in lipopolysaccharide-challenged $CysLT_1^{-/-}$ 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 (Pa_{O_2}) 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 Pa_{O_2} was markedly less in endotoxin-challenged WT mice than in saline-challenged WT mice ($P < 0.05$). In contrast, the Pa_{O_2} after occlusion of the left mainstem bronchus in lipopolysaccharide-challenged $LTC_4S^{-/-}$ mice was similar to that in saline-challenged $LTC_4S^{-/-}$ mice and greater than in both lipopolysaccharide-challenged WT mice and lipopolysaccharide-challenged $CysLT_1^{-/-}$ mice ($P < 0.05$ for both). In lipopolysaccharide-challenged $CysLT_1^{-/-}$ mice, the Pa_{O_2} 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_a 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, $LTC_4S^{-/-}$, and $CysLT_1^{-/-}$ 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.

Table 2. Arterial Blood Gas Analyses

—	WT		$LTC_4S^{-/-}$		$CysLT_1^{-/-}$	
—	Sal	LPS	Sal	LPS	Sal	LPS
Pa_{O_2} (mmHg)	229 ± 57	147 ± 20*	233 ± 87	224 ± 20†‡	221 ± 45	178 ± 42
Pa_{CO_2} (mmHg)	30.5 ± 5.2	31.0 ± 5.6	29.6 ± 7.3	31.0 ± 6.5	32.9 ± 8.7	29.3 ± 5.3
pH_a	7.35 ± 0.08	7.11 ± 0.08*	7.33 ± 0.06	7.07 ± 0.05*	7.34 ± 0.07	7.11 ± 0.07*
BE (mmol/l)	−7.5 ± 3.3	−21.1 ± 5.5*	−9.8 ± 2.9	−20.5 ± 2.8*	−7.7 ± 3.2	−19.4 ± 3.6*
Hb (g/dl)	13.4 ± 1.1	13.1 ± 0.8	13.7 ± 1.0	13.9 ± 0.7	13.5 ± 0.8	14.1 ± 0.6

Arterial blood gas analyses at the end of the hemodynamic studies during occlusion of the left mainstem bronchus in WT, $LTC_4S^{-/-}$, and $CysLT_1^{-/-}$ 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 $CysLT_1^{-/-}$ mice.

BE = base excess; $CysLT_1^{-/-}$ = mice congenitally deficient in the cysteinyl leukotriene receptor 1; g/dl = gram per deciliter; Hb = hemoglobin; LPS = lipopolysaccharide; $LTC_4S^{-/-}$ = mice congenitally deficient in leukotriene C4 synthase; mmHg = millimeters of mercury; mmol/l = millimoles per liter; Pa_{CO_2} = level of carbon dioxide in the arterial blood; Pa_{O_2} = level of oxygen in the arterial blood; pH_a = arterial pH; Sal = saline; WT = wild-type.

2B). Lung myeloperoxidase activity levels in lipopolysaccharide-challenged $CysLT_1^{-/-}$ 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, $LTC_4S^{-/-}$, and $CysLT_1^{-/-}$ 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, $LTC_4S^{-/-}$, and $CysLT_1^{-/-}$ 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 LTB_4 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 $CysLT_1^{-/-}$ mice after endotoxin challenge than after saline challenge. No cysLTs were detectable in bronchoalveolar lavage fluid obtained from $LTC_4S^{-/-}$ 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 $CysLT_1$ receptor contributes significantly to the impairment of HPV after endotoxin challenge because mice lacking the $CysLT_1$ receptor are to a great extent protected from the lipopolysaccharide-induced attenuation of HPV.

After a saline challenge, both $LTC_4S^{-/-}$ and $CysLT_1^{-/-}$ 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 LTA_4 hydrolase under normal (nonseptic) conditions.[10] Taken together, these results confirm that neither LTB_4 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.[10] 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 E_4,[25–28] we sought to clarify the role of the $CysLT_1$ receptor in endotoxin-induced impairment of HPV by using $CysLT_1$ receptor-deficient mice. We found that $CysLT_1$ 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 $CysLT_1^{-/-}$ mice.[43,44] Taken together, our results show that cysLTs impair HPV after endotoxin challenge and that they exert their effects in major part *via* $CysLT_1$.

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.[10] To learn if cysLTs impair HPV by inducing pulmonary polymorphonuclear leukocyte accumulation, the peripheral leukocyte concentration and pulmonary myeloperoxidase concentrations were measured in WT, $LTC_4S^{-/-}$, and $CysLT_1^{-/-}$ 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 $LTC_4S^{-/-}$ mice. Taken together, these results suggest that the recruitment of leukocytes to the lung after endotoxin challenge is mediated by LTB_4 but not by cysLTs and that the accumulation of leukocytes in the lung *per se* does not contribute to the impairment of HPV.

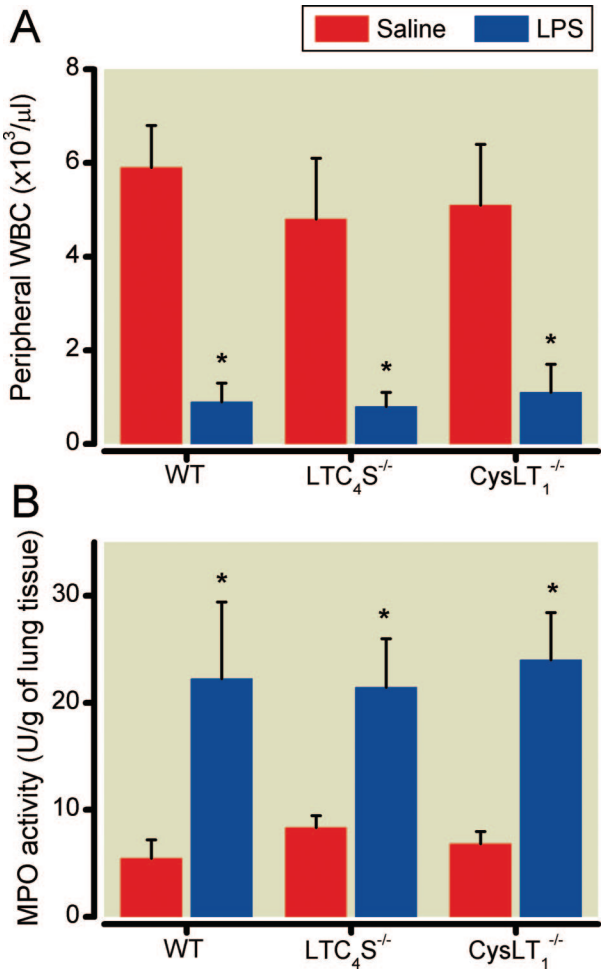

Fig. 2. In WT (n = 6), $LTC_4S^{-/-}$ (n = 6), and $CysLT_1^{-/-}$ mice (n = 7), the circulating leukocyte concentrations were markedly reduced after lipopolysaccharide challenge compared with WT (n = 6), $LTC_4S^{-/-}$ (n = 7), and $CysLT_1^{-/-}$ (n = 6) mice after saline challenge (*A*). Lung tissue myeloperoxidase activity was greater in lipopolysaccharide-treated WT (n = 5), $LTC_4S^{-/-}$ (n = 7), and $CysLT_1^{-/-}$ (n = 7) mice than in saline-treated WT (n = 6), $LTC_4S^{-/-}$ (n = 6), and $CysLT_1^{-/-}$ (n = 6) mice. Blood and tissue samples were taken 18 h after lipopolysaccharide challenge (*B*). * $P < 0.05$ *versus* saline-challenged mice of the respective genotype. $CysLT_1^{-/-}$ = mice congenitally deficient in the cysteinyl leukotriene receptor 1; LPS = lipopolysaccharide; $LTC_4S^{-/-}$ = mice congenitally deficient in leukotriene C_4 synthase; MPO = myeloperoxidase; WBC = leukocyte count; WT = wild-type. All data mean ± SD.

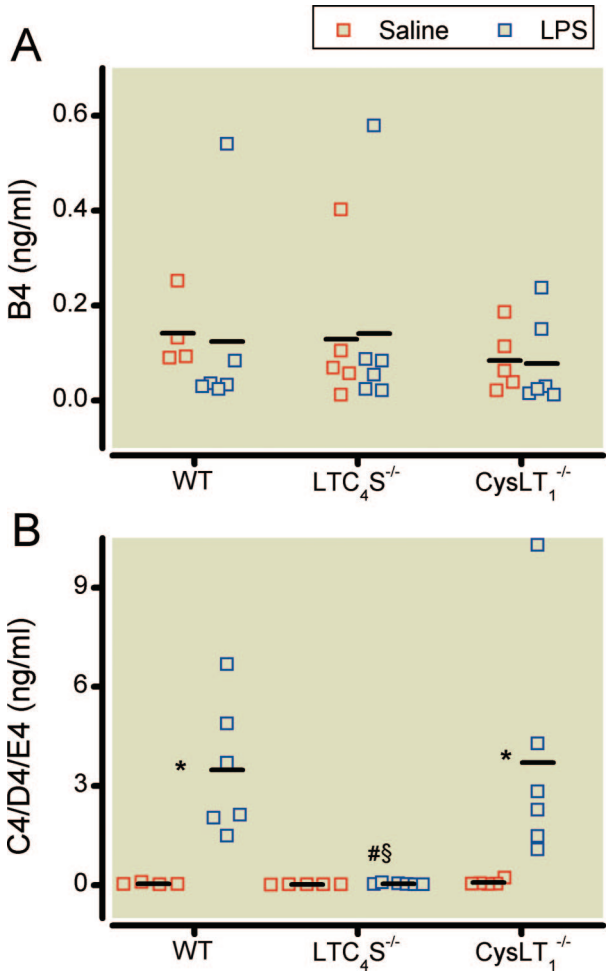

Fig. 3. The concentrations of LTB_4 in bronchoalveolar lavage fluid did not differ between the saline-challenged WT (n = 4), $LTC_4S^{-/-}$ (n = 5), and $CysLT_1^{-/-}$ (n = 5) mice and the lipopolysaccharide-challenged WT (n = 6), $LTC_4S^{-/-}$ (n = 6), and $CysLT_1^{-/-}$ (n = 6) mice 18 h after challenge (*A*). In the same mice, concentrations of cysLTs ($LTC_4/D_4/E_4$) in the bronchoalveolar lavage fluid were higher in WT and $CysLT_1^{-/-}$ mice after lipopolysaccharide challenge than in saline-challenged WT and $CysLT_1^{-/-}$ mice. As expected, no cysLTs were detectable in bronchoalveolar lavage fluid from the $LTC_4S^{-/-}$ mice after challenge with either saline or lipopolysaccharide (*B*). * $P < 0.05$ *versus* saline-challenged mice of the respective genotype, # $P < 0.05$ *versus* lipopolysaccharide-challenged WT mice, § $P < 0.05$ *versus* lipopolysaccharide-challenged $CysLT_1^{-/-}$ mice. B_4 = cysteinyl leukotriene B_4; $C_4/D_4/E_4$ = cysteinyl leukotriene $C_4/D_4/E_4$; $CysLT_1^{-/-}$ = mice congenitally deficient in the cysteinyl leukotriene receptor 1; LPS = lipopolysaccharide; LTB_4 = leukotriene B_4; $LTC_4S^{-/-}$ = mice congenitally deficient in leukotriene C_4 synthase; WT = wild-type. The concentrations of LTB_4 and cysLT are depicted as individual values with arithmetic means.

Lärfars *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 $CysLT_1^{-/-}$ mice, whereas in the bronchoalveolar lavage fluid of $LTC_4S^{-/-}$ 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.[13–16] 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.[11] In animal models of either indomethacin-induced gastric ulcers or skin flap ischemia reperfusion injury, the cysLT receptor antagonist montelukast exerted antioxidant effects.[46,47] 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 of sepsis, but the lipopolysaccharide component of the bacterial cell wall does not cause all of the complex inflammatory processes seen in clinical sepsis.[5–12] 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 LTC_4S almost completely protected mice from endotoxin-induced impairment of HPV, whereas deficiency of the $CysLT_1$ 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

1. Thomas HM 3rd, Garrett RC: Strength of hypoxic vasoconstriction determines shunt fraction in dogs with atelectasis. J Appl Physiol 1982; 53:44–51
2. Brimioulle S, LeJeune P, Naeije R: Effects of hypoxic pulmonary vasoconstriction on pulmonary gas exchange. J Appl Physiol 1996; 81:1535–43
3. Dantzker DR, Brook CJ, Dehart P, Lynch JP, Weg JG: Ventilation-perfusion distributions in the adult respiratory distress syndrome. Am Rev Respir Dis 1979; 120:1039–52
4. Sharma S, Kumar A: Septic shock, multiple organ failure, and acute respiratory distress syndrome. Curr Opin Pulm Med 2003; 9:199–209
5. Weir EK, Mlczoch J, Reeves JT, Grover RF: Endotoxin and prevention of hypoxic pulmonary vasoconstriction. J Lab Clin Med 1976; 88:975–83
6. Hales CA, Sonne L, Peterson M, Kong D, Miller M, Watkins WD: Role of thromboxane and prostacyclin in pulmonary vasomotor changes after endotoxin in dogs. J Clin Invest 1981; 68:497–505
7. Chang SW, Feddersen CO, Henson PM, Voelkel NF: Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin-treated rats. J Clin Invest 1987; 79:1498–509
8. Theissen JL, Loick HM, Curry BB, Traber LD, Herndon DN, Traber DL: Time course of hypoxic pulmonary vasoconstriction after endotoxin infusion in unanesthetized sheep. J Appl Physiol 1991; 70:2120–5
9. Ullrich R, Bloch KD, Ichinose F, Steudel W, Zapol WM: Hypoxic pulmonary blood flow redistribution and arterial oxygenation in endotoxin-challenged NOS2-deficient mice. J Clin Invest 1999; 104:1421–9
10. Ichinose F, Zapol WM, Sapirstein A, Ullrich R, Tager AM, Coggins K, Jones R, Bloch KD: Attenuation of hypoxic pulmonary vasoconstriction by endotoxemia requires 5-lipoxygenase in mice. Circ Res 2001; 88:832–8
11. Baboolal HA, Ichinose F, Ullrich R, Kawai N, Bloch KD, Zapol WM: Reactive oxygen species scavengers attenuate endotoxin-induced impairment of hypoxic pulmonary vasoconstriction in mice. ANESTHESIOLOGY 2002; 97:1227–33
12. Spohr F, Busch CJ, Teschendorf P, Weimann J: Selective inhibition of guanylate cyclase prevents impairment of hypoxic pulmonary vasoconstriction in endotoxemic mice. J Physiol Pharmacol 2009; 60:107–12
13. Paky A, Michael JR, Burke-Wolin TM, Wolin MS, Gurtner GH: Endogenous production of superoxide by rabbit lungs: Effects of hypoxia or metabolic inhibitors. J Appl Physiol 1993; 74:2868–74
14. Marshall C, Mamary AJ, Verhoeven AJ, Marshall BE: Pulmonary artery NADPH-oxidase is activated in hypoxic pulmonary vasoconstriction. Am J Respir Cell Mol Biol 1996; 15:633–44
15. Waypa GB, Chandel NS, Schumacker PT: Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. Circ Res 2001; 88:1259–66
16. Michelakis ED, Hampl V, Nsair A, Wu X, Harry G, Haromy A, Gurtu R, Archer SL: Diversity in mitochondrial function explains differences in vascular oxygen sensing. Circ Res 2002; 90:1307–15
17. Stevens T, Morris K, McMurtry IF, Zamora M, Tucker A: Pulmonary and systemic vascular responsiveness to TNF-alpha in conscious rats. J Appl Physiol 1993; 74:1905–10
18. Rouzer CA, Matsumoto T, Samuelsson B: Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A4 synthase activities. Proc Natl Acad Sci U S A 1986; 83:857–61
19. Dixon RA, Diehl RE, Opas E, Rands E, Vickers PJ, Evans JF, Gillard JW, Miller DK: Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. Nature 1990; 343:282–4
20. Evans JF, Dupuis P, Ford-Hutchinson AW: Purification and characterisation of leukotriene A4 hydrolase from rat neutrophils. Biochim Biophys Acta 1985; 840:43–50
21. Nicholson DW, Ali A, Vaillancourt JP, Calaycay JR, Mumford

RA, Zamboni RJ, Ford-Hutchinson AW: Purification to homogeneity and the N-terminal sequence of human leukotriene C4 synthase: A homodimeric glutathione *S*-transferase composed of 18-kDa subunits. Proc Natl Acad Sci U S A 1993; 90:2015–9

22. Lam BK, Penrose JF, Freeman GJ, Austen KF: Expression cloning of a cDNA for human leukotriene C4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A4. Proc Natl Acad Sci U S A 1994; 91:7663–7
23. Welsch DJ, Creely DP, Hauser SD, Mathis KJ, Krivi GG, Isakson PC: Molecular cloning and expression of human leukotriene-C4 synthase. Proc Natl Acad Sci U S A 1994; 91:9745–9
24. Carter BZ, Shi ZZ, Barrios R, Lieberman MW: Gamma-glutamyl leukotrienase, a gamma-glutamyl transpeptidase gene family member, is expressed primarily in spleen. J Biol Chem 1998; 273:28277–85
25. Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, Coulombe N, Abramovitz M, Figueroa DJ, Zeng Z, Connolly BM, Bai C, Austin CP, Chateauneuf A, Stocco R, Greig GM, Kargman S, Hooks SB, Hosfield E, Williams DL Jr, Ford-Hutchinson AW, Caskey CT, Evans JF: Characterization of the human cysteinyl leukotriene CysLT1 receptor. Nature 1999; 399:789–93
26. Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, Stocco R, Bellefeuille JN, Abramovitz M, Cheng R, Williams DL Jr, Zeng Z, Liu Q, Ma L, Clements MK, Coulombe N, Liu Y, Austin CP, George SR, O'Neill GP, Metters KM, Lynch KR, Evans JF: Characterization of the human cysteinyl leukotriene 2 receptor. J Biol Chem 2000; 275:30531–6
27. Maekawa A, Kanaoka Y, Xing W, Austen KF: Functional recognition of a distinct receptor preferential for leukotriene E4 in mice lacking the cysteinyl leukotriene 1 and 2 receptors. Proc Natl Acad Sci U S A 2008; 105:16695–700
28. Paruchuri S, Jiang Y, Feng C, Francis SA, Plutzky J, Boyce JA: Leukotriene E4 activates peroxisome proliferator-activated receptor gamma and induces prostaglandin D2 generation by human mast cells. J Biol Chem 2008; 283:16477–87
29. Yokomizo T, Izumi T, Chang K, Takuwa Y, Shimizu T: A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. Nature 1997; 387:620–4
30. Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T: A second leukotriene B(4) receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. J Exp Med 2000; 192:421–32
31. Lewis RA, Austen KF: The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions, and pharmacology. J Clin Invest 1984; 73:889–97
32. Lewis RA, Austen KF, Soberman RJ: Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. N Engl J Med 1990; 323:645–55
33. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE: Acute respiratory distress in adults. Lancet 1967; 2:319–23
34. Ware LB, Matthay MA: The acute respiratory distress syndrome. N Engl J Med 2000; 342:1334–49
35. Stephenson AH, Lonigro AJ, Hyers TM, Webster RO, Fowler AA: Increased concentrations of leukotrienes in bronchoalveolar lavage fluid of patients with ARDS or at risk for ARDS. Am Rev Respir Dis 1988; 138:714–9
36. Baenkler M, Leykauf M, John S: Functional analysis of eicosanoids from white blood cells in sepsis and SIRS. J Physiol Pharmacol 2006; 57(Suppl 12):25–33
37. Mamedova L, Capra V, Accomazzo MR, Gao ZG, Ferrario S, Fumagalli M, Abbracchio MP, Rovati GE, Jacobson KA: CysLT1 leukotriene receptor antagonists inhibit the effects of nucleotides acting at P2Y receptors. Biochem Pharmacol 2005; 71:115–25
38. Mitra P, Oskeritzian CA, Payne SG, Beaven MA, Milstien S, Spiegel S: Role of ABCC1 in export of sphingosine-1-phosphate from mast cells. Proc Natl Acad Sci U S A 2006; 103:16394–9
39. Kanaoka Y, Maekawa A, Penrose JF, Austen KF, Lam BK: Attenuated zymosan-induced peritoneal vascular permeability and IgE-dependent passive cutaneous anaphylaxis in mice lacking leukotriene C4 synthase. J Biol Chem 2001; 276:22608–13
40. Maekawa A, Austen KF, Kanaoka Y: Targeted gene disruption reveals the role of cysteinyl leukotriene 1 receptor in the enhanced vascular permeability of mice undergoing acute inflammatory responses. J Biol Chem 2002; 277: 20820–4
41. Ichinose F, Ullrich R, Sapirstein A, Jones RC, Bonventre JV, Serhan CN, Bloch KD, Zapol WM: Cytosolic phospholipase A(2) in hypoxic pulmonary vasoconstriction. J Clin Invest 2002; 109:1493–500
42. Hellman J, Roberts JD Jr, Tehan MM, Allaire JE, Warren HS: Bacterial peptidoglycan-associated lipoprotein is released into the bloodstream in gram-negative sepsis and causes inflammation and death in mice. J Biol Chem 2002; 277: 14274–80
43. Ortiz JL, Gorenne I, Cortijo J, Seller A, Labat C, Sarria B, Abram TS, Gardiner PJ, Morcillo E, Brink C: Leukotriene receptors on human pulmonary vascular endothelium. Br J Pharmacol 1995; 115:1382–6
44. Paruchuri S, Tashimo H, Feng C, Maekawa A, Xing W, Jiang Y, Kanaoka Y, Conley P, Boyce JA: Leukotriene E4-induced pulmonary inflammation is mediated by the P2Y12 receptor. J Exp Med 2009; 206:2543–55
45. Lärfars G, Lantoine F, Devynck MA, Palmblad J, Gyllenhammar H: Activation of nitric oxide release and oxidative metabolism by leukotrienes B_4, C_4, and D_4 in human polymorphonuclear leukocytes. Blood 1999; 93:1399–405
46. Dengiz GO, Odabasoglu F, Halici Z, Cadirci E, Suleyman H: Gastroprotective and antioxidant effects of montelukast on indomethacin-induced gastric ulcer in rats. J Pharmacol Sci 2007; 105:94–102
47. Gideroglu K, Yilmaz F, Aksoy F, Bugdayci G, Saglam I, Yimaz F: Montelukast protects axial pattern rat skin flaps against ischemia/reperfusion injury. J Surg Res 2009; 157:181–6