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Cyclooxygenase 2 Plays a Pivotal Role in the Resolution of Acute Lung Injury[1]

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Acute lung injury (ALI) is a severe illness with excess mortality and no specific therapy. In its early exudative phase, neutrophil activation and accumulation in the lung lead to hypoxemia, widespread tissue damage, and respiratory failure. In clinical trials, inhibition of proinflammatory mediators has not proven effective. In this study, we pursued a new investigative strategy that emphasizes mediators promoting resolution from lung injury. A new spontaneously resolving experimental murine model of ALI from acid aspiration was developed to identify endogenous proresolving mechanisms. ALI increased cyclooxygenase 2 (COX-2) expression in murine lung. Selective pharmacologic inhibition or gene disruption of COX-2 blocked resolution of ALI. COX-2-derived products increased levels of the proresolving lipid mediators lipoxin A_4 (LXA_4) and, in the presence of aspirin, 15-epi-LXA_4. Both LXA_4 and 15-epi-LXA_4 interact with the LXA_4 receptor (ALX) to mediate anti-inflammatory actions. ALX expression was markedly induced by acid injury and transgenic mice with increased ALX expression displayed dramatic protection from ALI. Together, these findings indicate a protective role in ALI for COX-2-derived mediators, in part via enhanced lipoxin signaling, and carry potential therapeutic implications for this devastating clinical disorder. *The Journal of Immunology,* **2005, 174: 5033–5039.**

Aspiration of gastric contents is a common cause of acute lung injury (ALI)[3] and the acute respiratory distress syndrome (ARDS) (1). ALI is characterized by neutrophil (polymorphonuclear leukocyte (PMN))-rich inflammation and flooding of alveolar spaces with fluid and protein secondary to a loss of barrier integrity (2). The robust inflammatory response to airway injury often leads to unintended tissue damage to surrounding lung via the release of oxidants, proteases, and other potentially toxic agents from activated leukocytes (3–5). No specific therapy is currently available to modulate this inflammatory response and protect the lung.

Lipid mediators are important regulators of inflammation and arachidonic acid (C20:4)-derived products, such as PGs, thromboxane (TX), and leukotrienes (LTs), have been implicated as proinflammatory mediators of ALI (6). In addition, animals deficient in cytosolic phospholipase A_2, the key enzyme that releases C20:4 from cell membranes during inflammation for conversion to bioactive lipid mediators, display diminished early-phase pulmonary inflammation after ALI induced either by acid or LPS (7). Experimental strategies to block prophlogistic mediators have not proven successful (8–10) because of the multiple, redundant pathways that initiate inflammation.

Although resolution is well appreciated to be one of the four major outcomes for acute inflammation, it was held to be a passive process (11). Recently, resolution of acute inflammation was shown for the first time to be an active rather than a passive process (Ref. 12 and reviewed in Ref. 13). Specifically in ALI, resolution is characterized by clearance of PMN from the lung and restoration of epithelial barrier function and vascular permeability (2). Counterregulatory host responses to limit lung injury are predictive of clinical outcomes, since low airway concentrations of anti-inflammatory mediators correlates more closely than levels of proinflammatory mediators with ARDS severity (14). During resolution, multiple homeostatic mechanisms are enlisted to generate endogenous "braking signals." Lipoxins (LXs) are a unique class of C20:4-derived lipid mediators with a structure distinct from PGs and LTs (15). LXs display several features of proresolving mediators, including inhibition in vivo of PMN activation, cytokine release, and angiogenesis (reviewed in Ref. 16). Many of these properties are shared by the closely related 15-epimer-lipoxins (15-epi-LXs) that are generated during cell-cell interactions between leukocytes and tissue resident cells in the presence of aspirin (ASA)-acetylated cyclooxygenase (COX) 2 (16). LXs are generated in respiratory tissues and block airway inflammation and hyperresponsiveness in an experimental model of asthma (17).

In this study, we present evidence for a new, self-limited experimental model of ALI that has facilitated identification of COX-2 in a pivotal homeostatic pathway for resolution of acid-initiated airway injury, in part by promoting the endogenous formation of LXs.

Materials and Methods

Acid-initiated acute lung injury

Hydrochloric acid (0.1 N HCl, pH 1.5, 50 μl, endotoxin free; Sigma-Aldrich) was instilled selectively into the left lung of anesthetized mice

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[3] Abbreviations used in this paper: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; PMN, polymorphonuclear leukocyte; TX, thromboxane; LT, leukotriene; LX, lipoxin; ASA, aspirin; BAL, bronchoalveolar lavage; BALF, BAL fluid; ALX, lipoxin A_4 receptor; hALX, human lipoxin A_4 receptor; NHBE, normal human bronchial epithelial; COX, cyclooxygenase; MPO, myeloperoxidase; tg, transgenic.

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(FVB, male, 10–12 wk; Charles River Breeding Laboratories) via a 24-gauge angiocatheter inserted intratracheally. During the development of this model, Evans blue dye was instilled with acid for visual confirmation of selective targeting to the left lung (Fig. 1*A*). COX-2-deficient mice and LXA_4 receptor (ALX)-transgenic (tg) mice were generated as previously described (18, 19). All mice were maintained under specific pathogen-free conditions. All studies were reviewed and approved by the Harvard Medical Area standing committee on animals.

Acid was instilled only into the left lung because injury to both lungs was associated with a high mortality rate in preliminary experiments. In addition, a milder degree of injury was chosen so that the animals would not require mechanical ventilation for survival. At timed intervals (0–72 h), bronchoalveolar lavage (BAL) was performed and lung tissue obtained. Bilateral BAL was performed (2 aliquots of 1 ml of PBS plus 0.6 mM EDTA). After dissection, the lungs were inflated to 25 cm H_2O, fixed in 10% PBS-buffered Formalin (Fisher Scientific) or IHC zinc fixative buffer (BD Pharmingen), and paraffin embedded before immunohistochemistry. Differential leukocyte counts were determined in BAL fluid (BALF) as previously described (17). Protein concentration in BALF was measured with a DC protein assay kit (Bio-Rad). To select animals, ASA (0.125 g/kg; Aldrich), the selective COX-2 inhibitor NS-398 (5 mg/kg; Cayman Chemical) or vehicle was administered i.p. 30 min before acid instillation. In some animals, NS-398 and ASA were administered sequentially with NS-398 30 min before and ASA 2 h after acid. To select mice, the PGE analogue, misoprostol (200 μg/kg by gavage; Cayman Chemical) was given 30 min after i.p. injection of NS-398 and again 12 h after acid instillation. Analysis of COX-1 and COX-2 protein in lung homogenates was performed by Western blot analysis as described elsewhere (20). Membranes were immunoblotted using specific primary Abs (1/1000 dilution; Cayman Chemical) and rabbit anti-goat IgG secondary Abs conjugated to HRP (1/20,000 dilution; Amersham).

Myeloperoxidase (MPO) assay

Lungs were individually homogenized with a manual Dounce in potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. Materials were prepared as described previously (17). Briefly, after centrifugation (14,000 $\times$ *g*, 10 min, 4°C), 50 μl of supernatant was added to 950 μl of potassium phosphate buffer (pH 6.0) containing 0.2 mg/ml *o*-dianisidine dihydrochloride (Sigma-Aldrich) and 0.00002% hydrogen peroxide (Sigma-Aldrich). Changes in absorbance at 460 nm were monitored in a water-jacketed cassette (37°C) at 5-s intervals for 90 s. A calibration curve generated with known amounts of murine PMN was utilized for conversion of MPO activities to PMN number as described elsewhere (19).

Lipid mediator measurement

Specific lipid mediators were determined in BALF cell-free supernatants (200 $\times$ *g*, 10 min, 4°C) by sensitive and specific ELISAs for PGE_2, LTB_4, and TXB_2 (Cayman Chemical) and LXA_4 and 15-epi-LXA_4 (Neogen).

Airway epithelial cell culture and injury

Primary normal human bronchial epithelial (NHBE) cells (Cambrex) were cultured in an air-liquid interface system in 1:1 mixture of bronchial epithelial cell basal medium (Cambrex): DMEM with low glucose (Invitrogen Life Technologies) containing 0.5 mg/ml hydrocortisone, 0.5 μg/ml human recombinant epithelial growth factor, 0.5 mg/ml epinephrine, 10 mg/ml transferrin, 5 mg/ml insulin, 0.1 μg/ml retinoic acid, 6.5 μg/ml triiodothyronine, gentamicin, amphotericin B, and bovine pituitary extract (Cambrex). This medium was further supplemented with BSA (1.5 μg/ml) and retinoic acid (50 nM) as described previously (21). The cells were maintained in culture for 14 days until a differentiated cell population with mucus secretion and cilia was present. The human airway serous cell line Calu-3 (provided by S. Colgan, Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital, Boston, MA) was grown (5% CO_2, 37°C) in Eagle's MEM (American Type Culture Collection) supplemented with 10% FBS (Sigma-Aldrich), 100 IU/ml penicillin, and 100 μg/ml streptomycin (Invitrogen Life Technologies). MLE15 cells (provided by J. Whitsett, Neonatology, Perinatal and Pulmonary Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH) were grown (5% CO_2, 37°C) in DMEM supplemented with 5% FBS, 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells were incubated in the presence or absence of 0.1 N HCl (pH 1.5) for 5 min, then acid was removed. After rinsing twice with PBS (until pH $\geq$ 7), medium was added and the cells were cultured for 12 h after acid injury.

Lipid mediator-related gene expression

Total RNA was extracted from snap-frozen left lung using TRIzol reagent (Invitrogen Life Technologies). One nanogram of total RNA was reverse transcribed using a Titan One Tube RT-PCR System (Roche), and PCR was performed with specific primers for murine COX-2, COX-1, and GAPDH (internal control). Total cellular RNA from epithelial cells was obtained using RNeasy (Qiagen). cDNA synthesis was performed using Ready-To-Go RTPCR beads (Qiagen), and semiquantitative human ALX (hALX) gene expression was determined using specific primers for hALX (sense primer, 5′-TT GCT CTA GTC CTT ACC TTG C-3′, and antisense primer, 5′-GC AAG TAC AAA ATC ATT GAC ATC-3′) and β-actin (internal control). After electrophoresis, densitometry was performed using Scion Image software.

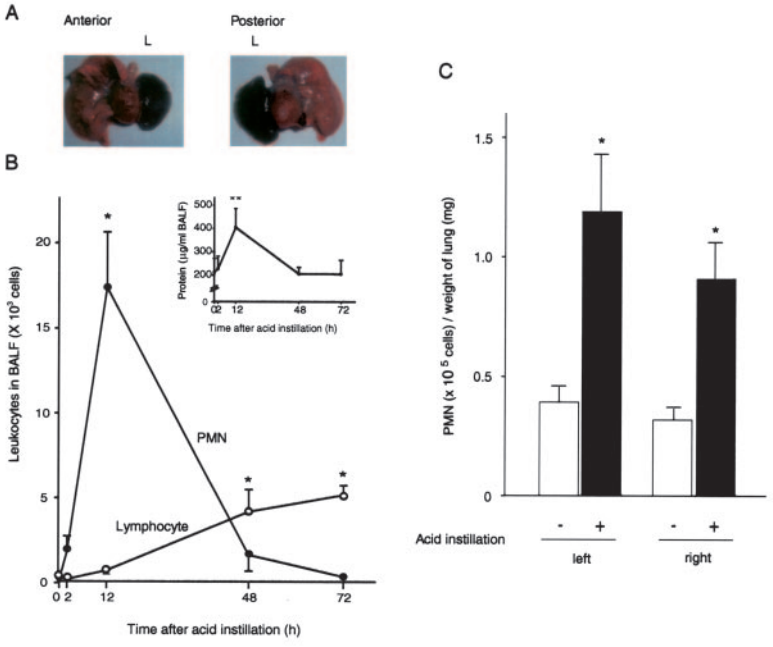

FIGURE 1. The kinetics of inflammation and resolution after acid-initiated acute lung injury. Acid was selectively instilled into the left lung, highlighted by the concomitant addition of Evans blue dye (*A*) and inflammatory cell trafficking into the lung (*B*) was determined in BALF by enumeration of PMN (●) and lymphocytes (○). *Inset,* Total protein in BALF was measured at the indicated time intervals. *C,* Twelve hours after acid injury, PMN accumulation in the left and right lungs was also determined by measurement of MPO activity. Values represent the mean ± SEM for n = 3–5 at each time point. *, $p < 0.01$; **, $p < 0.05$ vs t_0 (*B*) or control (*C*) by one-way ANOVA.

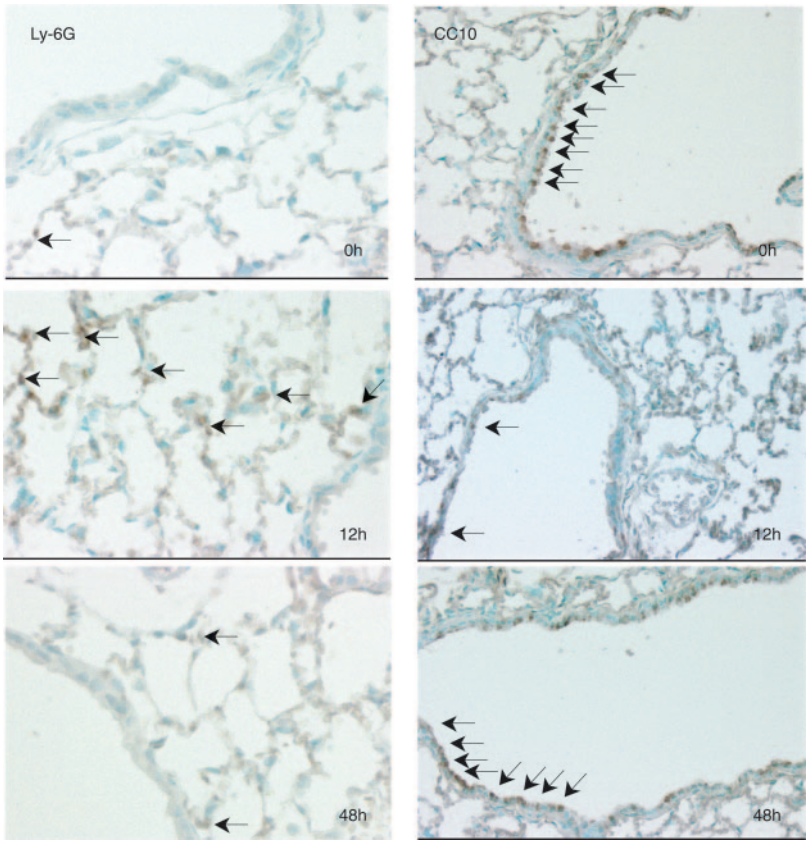

FIGURE 2. Histopathology of acid-injured murine lungs. PMN and airway lining cells were identified in paraffin-embedded tissue by expression of Ly-6G (*left panel*; original magnification, ×200) and CC10 (*right panel*; original magnification, ×100), respectively (examples highlighted by arrows).

Immunohistochemistry

Tissue blocks were obtained from midsagittal slices of lungs embedded in paraffin and immunostained with LY-6G (1/50 dilution), CD3 (1/25 dilution; BD Pharmingen), or CC10 (1/2000 dilution) as described previously (22).

Results

ALI and resolution after acid-initiated local airway trauma

To determine endogenous factors directing resolution of lung injury, we first developed a non-lethal experimental model of ALI

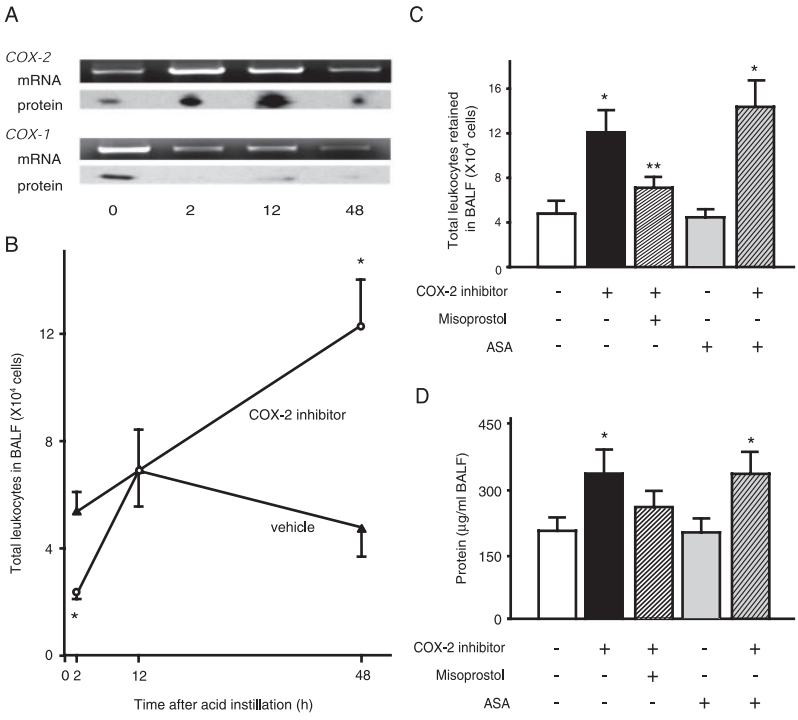

FIGURE 3. COX-2-derived mediators promote resolution of acute airway inflammation. *A,* COX-2 and COX-1 expression in the lungs of acid-injured mice was determined by RT-PCR and Western blot. *B,* The time course for leukocyte infiltration was monitored in the absence (▲) or presence (○) of a COX-2-selective inhibitor. Total leukocytes (*C*) and (*D*) protein were determined in BALF 48 h after acid instillation in mice exposed to a COX-2-selective inhibitor (filled bar), misoprostol (hatched bar), or ASA (shaded bar) (see *Materials and Methods*). Values represent the mean ± SEM for $n \geq 3$. *, $p < 0.05$ as compared with vehicle and **, $p < 0.05$ as compared with COX-2 inhibitor without misoprostol by one-way ANOVA.

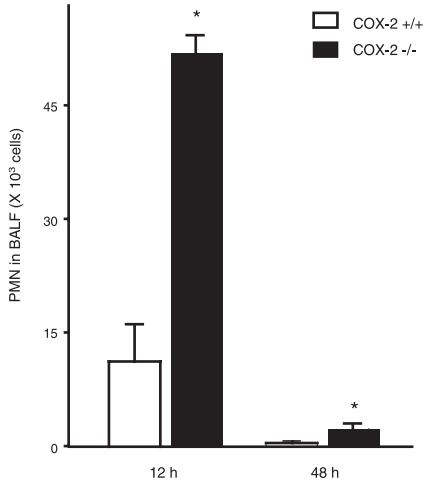

FIGURE 4. Increased inflammation after acid injury in COX-2-deficient mice. PMN recruitment was determined in BALF 12 and 48 h after acid injury in COX-2-deficient (COX-$2^{-/-}$) mice (■) or littermate control animals (COX-$2^{+/+}$) (□). Values represent the mean ± SEM for $n \geq 3$. *, $p < 0.02$ by one-way ANOVA.

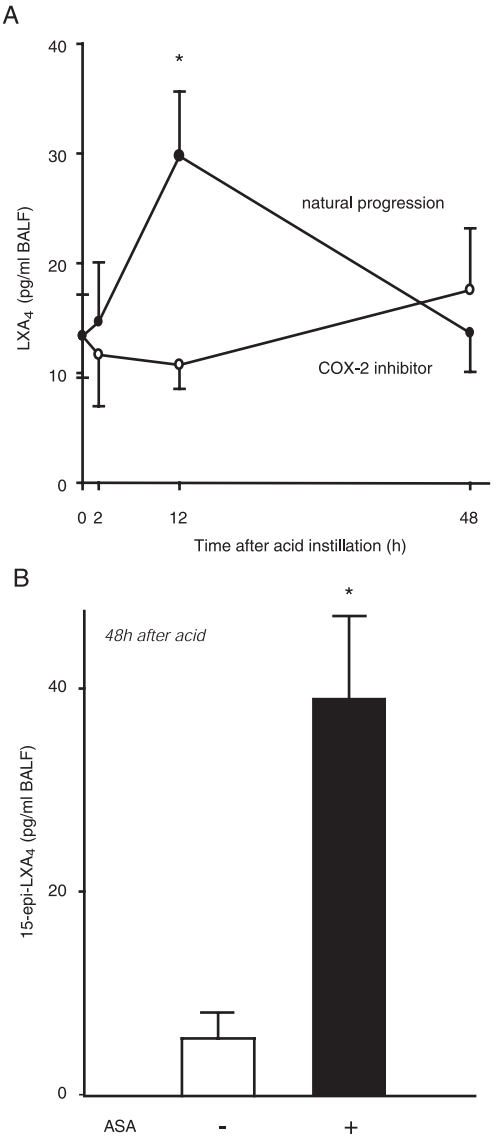

FIGURE 5. Impact of COX inhibition on LX formation after acid injury. The time course for LXA_4 in BALF (*A*) was determined in the absence (●) or presence (○) of a selective COX-2 inhibitor. *B,* The impact of ASA on 15-epi-LXA_4 levels 48 h after acid instillation was determined by specific ELISA. Values are expressed as mean ± SEM for $n \geq 4$. *, $p < 0.05$ by Student's *t* test.

that did not require mechanical ventilation for survival. A 24-gauge angiocatheter was inserted intratracheally into the left mainstem bronchus for unilateral instillation of a small aliquot of HCl (0.1 N, pH 1.5, 50 μl; Fig. 1*A*). In response to this acid injury, PMN were recruited to the lung with maximal numbers present in BALF within 12 h ($17.4 \pm 3.2 \times 10^3$ cells) (Fig. 1*B*). Maximal apparent lymphocyte trafficking occurred later than PMN with increased numbers present in BALF 72 h after acid ($5.2 \pm 0.6 \times 10^3$ cells). Although acid was selectively instilled into the left lung (Fig. 1*A*), leukocyte trafficking was increased bilaterally (Fig. 1*C*), indicative of a systemic inflammatory response to the lung injury. Intratracheal HCl instillation also significantly disrupted epithelial barrier integrity with increased protein in BALF (maximal at 12 h, 405.0 ± 81.2 μg/ml) (*inset*, Fig. 1*B*). The kinetics for leukocyte accumulation and epithelial injury and restitution after acid-initiated injury were determined in the lung parenchyma by immunohistochemistry (Fig. 2). After 12 h, acid had markedly decreased the number of airway lining cells and increased PMN trafficking to the lung (Fig. 2). Both airway epithelia expressing CC10 and PMN number approximated baseline levels within 48 h. Reflective of the leukocyte kinetics in BALF, CD3-positive T lymphocytes in the lung parenchyma also increased at later time points (48–72 h) (data not shown). Together, these findings in the lung and BALF are consistent with a mild form of acid-initiated ALI that might result clinically from gastric acid aspiration.

Identification of COX as a pivotal regulator of ALI

Because COX-catalyzed conversion of C20:4 to PGs and TXs can promote airway inflammation in ALI (6, 23), we next determined the time-dependent expression of COX after ALI. Constitutive expression of both COX-1 and COX-2 mRNA and protein were present in murine lung (Fig. 3*A*). After acid injury, levels of COX-2 markedly increased within 2 h, gradually returning to baseline within 48 h (Fig. 3*A*). This pattern markedly differed from COX-1 that decreased after acid injury, so that COX-2 predominated after injury. To determine the impact of COX-2 on inflammatory responses to ALI, a selective COX-2 inhibitor was administered 30 min before acid injury. COX-2 inhibition led to significant decrements in total leukocytes 2 h after acid injury (Fig. 3*B*). In sharp contrast, leukocyte recruitment was dramatically increased 48 h after acid injury ($12.3 \pm 1.8 \times 10^4$ total leukocytes vs $4.8 \pm 1.1 \times 10^4$ total leukocytes in BALF for the COX-2 inhibitor and vehicle control, respectively, $n = 4$–5, $p < 0.01$) (Fig. 3*B*). In addition to blocking leukocyte clearance from the injured lung, COX-2 inhibition also slowed restitution of epithelial barrier function as BALF protein levels were also increased at 48 h (Fig. 3, *C* and *D*). To explore the mechanism for COX-2-dependent resolution of ALI, we determined the amounts of C20:4-derived lipid mediators in BALF. PGE_2 was significantly increased 2 h (602.7 ± 59.9 pg/ml, $n = 4$, $p < 0.05$) after acid injury and maximal at 12 h (1299.3 ± 288.0 pg/ml, $n = 4$, $p < 0.05$). TXB_2 levels in BALF were also maximal at 12 h (46.1 ± 15.7 pg/ml). Administration of a COX-2-selective inhibitor blocked biosynthesis of both PGE_2 (460.1 ± 131.1 pg PGE_2/ml and 487.9 ± 200.3 pg PGE_2/ml BALF at 2 and 12 h, respectively) and TXB_2 (10.4 ± 2.3 pg/ml BALF at 12 h) after ALI. These findings indicate that significant, compensatory increases in COX-1 activity were not present at the time points examined. Because COX-2 inhibitors

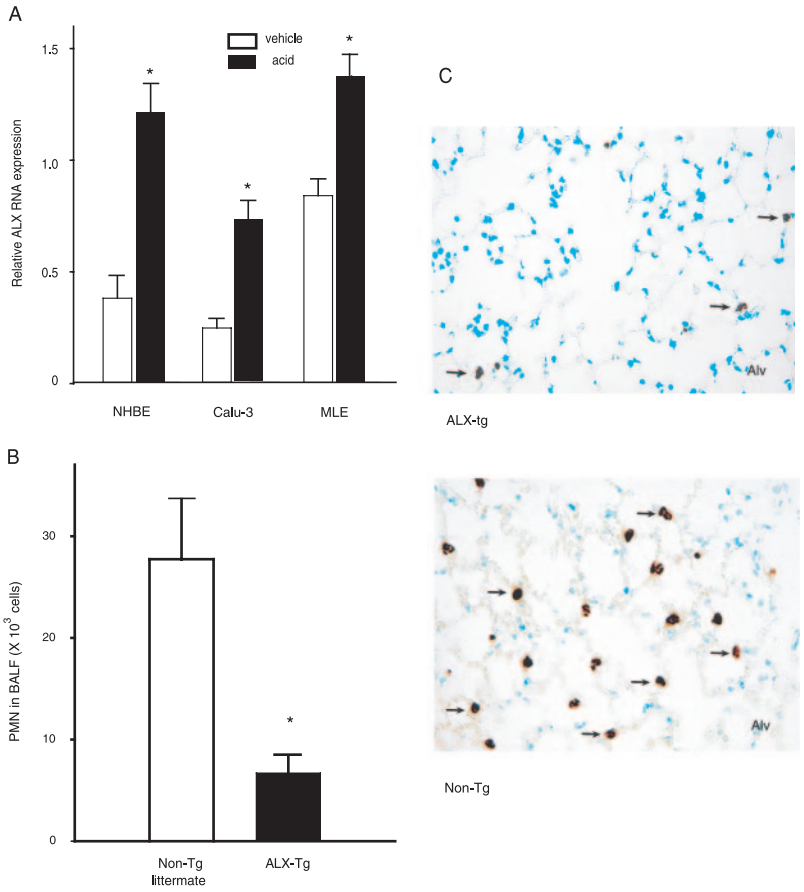

FIGURE 6. ALX overexpression blocks PMN recruitment after acid injury. *A,* Semiquantitative RT-PCR for ALX expression in NHBE, Calu-3, and MLE15 cells 12 h after transient exposure to acid (0.1 N HCl, pH 1.5, 5 min) (■) or vehicle (□). *B,* PMN were enumerated in BALF obtained from hALX tg mice (ALX-tg) (■) and littermate control animals (□) 12 h after acid instillation. Values represent the mean ± SEM for $n \geq 3$. *, $p < 0.05$ by Student's t test. *C,* Immunostaining for PMN (Ly-6G; original magnification, ×200) in murine lung tissue from ALX-tg (*upper panel*) or littermate non-tg control mice (*lower panel*) 12 h after acid administration (PMN highlighted by arrows). Representative of $n = 4$. Alv, Alveolar space.

may have PG-independent effects (24), we determined whether addition of the PGE stable analogue, misoprostol, would protect animals from the COX-2 inhibitor's adverse impact on resolution. Concomitant administration of misoprostol dampened the increases in both total leukocyte accumulation and protein in BALF observed with the COX-2 inhibitor alone (Fig. 3, *C* and *D*). Of interest, ASA, the lead nonsteroidal anti-inflammatory drug, inhibits both COX-1 and COX-2 and did not share the COX-2-selective inhibitor's dramatic impact on resolution of airway injury (Fig. 3, *C* and *D*). Moreover, animals that received the COX-2 inhibitor followed by ASA had similar airway responses as those receiving the COX-2 inhibitor alone, indicating a dominant COX-2 effect. Thus, COX-2 inhibition with a selective agent, but not ASA, delayed resolution of ALI.

We sought further evidence for a potentially protective role for COX-2 in the lung by determining the responses of mice deficient in this enzyme (COX-$2^{-/-}$) to lung injury. COX-$2^{-/-}$ injured mice also displayed markedly increased inflammatory responses to acid compared with their littermate controls with increased total leukocytes in BALF 48 h after injury ($13.1 \pm 1.8 \times 10^4$ vs $7.2 \pm 1.3 \times 10^4$ cells, $p < 0.03$). PMN number in BALF was also significantly increased at both 12 and 48 h after acid injury (52.0 ± 8.0 vs $11.3 \pm 4.9 \times 10^3$ cells at 12 h, 2.3 ± 0.7 vs $0.5 \pm 0.2 \times 10^4$ cells at 48 h, respectively, $n \geq 3$, $p < 0.02$) (Fig. 4). Together with the results in Fig. 3, these findings indicate that COX-2-derived products promote the timely resolution of acid-initiated airway injury.

Lipoxin formation after acid instillation

PGE_2 is a pivotal signal for switching on LX biosynthesis in acute exudative inflammation (12); therefore, we next determined whether this lipoxygenase-derived eicosanoid was present in BALF from injured animals. Significantly increased levels of LXA_4 were not identified until 12 h after acid instillation in BALF (Fig. 5*A*). LX biosynthesis in vivo generally followed the same time course after ALI as PGE_2, but unlike PGE_2, significant increases in LXA_4 were not observed at 2 h. Significant increases in LTB_4 were not evident in BALF at any of the time points measured. Of note, LXA_4 production was blocked by administration of the COX-2 inhibitor, likely as an indirect consequence of lower levels of COX-2-derived PGE_2.

Because nonselective COX inhibition with ASA did not prolong recovery from ALI, we next determined whether ASA initiated the formation of 15-epi-LXA_4. The levels of this ASA triggered lipid mediator 48 h after ALI in BALF increased ~7-fold in the presence of ASA (37.8 ± 7.8 pg 15-epi-LXA_4/ml with ASA vs 5.5 ± 2.4 pg 15-epi-LXA_4/ml without ASA) (Fig. 5*B*). Together these results indicate that COX-2 products enhance the in vivo formation of LXA_4 and, in the presence of ASA, 15-epi-LXA_4, potent proresolving lipid mediators for PMN-mediated inflammatory responses.

Anti-inflammatory signaling at LXA_4 receptors

LXA_4 and 15-epi-LXA_4 interact with anti-inflammatory LXA_4 receptors (ALX) to mediate protective actions (16). Semiquantitative RT-PCR revealed a 2.5-fold increase in murine lung ALX expression 48 h after acid-initiated ALI. ALX expression was not further altered by the COX-2 inhibitor, but was increased (36%) by misoprostol administration. To determine whether acid injury directly alters airway epithelial ALX expression, NHBE grown at an air-liquid interface, Calu-3 cells, or MLE15 cells were transiently exposed in vitro to HCl (5 min) (Fig. 6*A*). All of these upper airway

(i.e., NHBE, Calu-3) and alveolar (i.e., MLE15) epithelial cells expressed ALX at baseline, with the highest level of expression in the MLE15 cells. Twelve hours after acid injury, ALX mRNA significantly increased in both proximal and distal airway epithelial cell cultures (Fig. 6*A*). Of note, COX-2 mRNA also increased in NHBE 2 and 12 h after exposure to acid (data not shown). To determine whether ALX signaling regulates responses to ALI in vivo, we next utilized tg mice with leukocytes expressing hALX via targeting with a component of the CD11b promoter (ALX-tg) (17, 19). Total leukocytes in BALF 12 h after acid injury of ALX-tg mice was significantly decreased, compared with non-tg littermates ($7.4 \pm 0.8 \times 10^4$ vs $14.5 \pm 1.2 \times 10^4$ cells, $p < 0.001$). Notably, acid-initiated recruitment of PMN to the lung was significantly reduced in the ALX-tg mice compared with non-tg littermates ($6.7 \pm 1.7 \times 10^3$ vs $27.8 \pm 5.9 \times 10^3$ cells at 12 h, $p < 0.01$) (Fig. 6, *B* and *C*). In addition, leakage of plasma proteins into the air spaces was also decreased by 48% at this time point in ALX-tg animals. These results indicate that endogenous ligands, such as LXA_4, that are generated in response to airway injury and acute inflammation can interact with ALX to hasten the resolution of acid-initiated experimental ALI.

Discussion

Neutrophil recruitment and increased vascular permeability are hallmarks of ALI and ARDS. Our experimental model of ALI from acid aspiration recapitulated a mild form of this pathobiology with selective injury of the left lung limiting the severity of the injury and subsequent inflammation. This nonlethal injury enabled the animals to survive without mechanical ventilation, which can lead to additional ventilator-induced lung injury (25). Spontaneous resolution of ALI in this model facilitated identification of natural proresolving homeostatic mechanisms in the lung. Although lipid mediators derived from C20:4 are well appreciated to play pivotal roles in promoting the early inflammatory changes of ALI (7), evidence presented here indicates for the first time that select eicosanoids also promote recovery from lung injury.

Lung COX-2 expression and activity were significantly induced in our experimental model of ALI. Leukocyte COX-2 expression increases after acid aspiration (26) and during acute inflammation (27) and, in this study, acid injury also directly increased COX-2 expression in differentiated NHBE, suggesting contributions from multiple cell types to the increased COX-2 after ALI. Inhibition of COX-2 activity by pharmacologic or gene targeting decreased early PMN trafficking to the lung, but paradoxically led to dramatic increases in inflammation at later time points. A late, anti-inflammatory effect of COX-2, instead of the more widely appreciated early, proinflammatory action, was crucial to the timely recovery from ALI. Similarly, COX-2-catalyzed conversion of C20:4 to prostanoids has been identified as central to cardioprotection during ischemic preconditioning (28) and resolution of pleural inflammation (29, 30). COX-1-derived products may also have protective functions because its expression decreased after ALI, and deficiencies in COX-1 can exacerbate pulmonary inflammation in response to allergic and infectious stimuli (20, 31). Together these results indicate that the biosynthesis of COX products is temporally regulated in acute inflammation with early proinflammatory and, at least as important, late anti-inflammatory effects. These insights may help explain the failures of clinical trials that disrupted COX-derived product formation (9, 10).

In acute inflammation, COX-2-derived PGE_2 induces leukocyte 15-lipoxygenase expression to establish biosynthetic circuits for anti-inflammatory lipid mediators, such as the LXs (12). After acid injury, COX-2 inhibition blocked the increased LXA_4 production and resulted in an exacerbation of ALI with longer recovery times relative to animals with intact COX-2 function. ASA-acetylated COX-2 is still capable of enzymatic activity converting C20:4 to 15R-hydroxyeicosatetraenoic acid that can serve as a substrate for leukocyte 5-lipoxygenase-catalyzed conversion to 15-epi-LXs (16). In the presence of ASA, formation of 15-epi-LXA_4 increased in the injured lung and, unlike selective COX-2 inhibition, ASA did not adversely impact recovery from ALI. Of note, COX-2-selective inhibitors can block the COX-2 active site serine from acetylation by ASA to inhibit the ASA-triggered 15R-hydroxyeicosatetraenoic generation (32), and concomitant administration of these two agents here resulted in an increase in the severity of ALI that was similar to that seen when animals received the COX-2-selective inhibitor alone. In addition to 15-epi-LXs, ASA-acetylated COX-2 can also catalyze the formation of resolvins, structurally distinct lipid mediators derived from docosahexaenoic and eicosapentaenoic acids that also display tissue-protective properties during acute inflammation (33, 34). Thus, the inhibition of prostanoid formation by ASA is countered by increased generation of ASA-triggered lipid mediators that can promote ALI resolution.

Both LXA_4 and 15-epi-LXA_4 interact with ALX to initiate an array of proresolving actions for leukocyte-epithelial cell interactions during inflammation. LXs regulate airway epithelial cell proliferation and block IL-8 release and PMN-epithelial cell transmigration (reviewed in Ref. 16). Experimental ALI and direct airway epithelial cell injury with acid induced ALX expression. Coupled with the dramatic protection from ALI afforded ALX-tg mice, these findings suggest ALX agonists as potential novel therapeutic agents for ALI.

In summary, our findings have uncovered a new role for COX-2-derived lipid mediators in promoting resolution of acid-initiated experimental ALI. Although multiple anti-inflammatory circuits likely exist, endogenous LX formation and ALX signaling appear capable of potent protection from acid-initiated ALI. Selective COX-2 inhibitors are widely prescribed as analgesics and anti-inflammatory agents. Similar to recent concerns regarding cardiotoxity (35), selective COX-2 inhibition may have additional detrimental consequences for recovery from lung injury. Our findings support a new approach to ALI and ARDS that emphasizes enhancing natural resolution signaling pathways.

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Disclosures

The authors have no financial conflict of interest.

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