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The IL-1 type 1 receptor is required for the development of LPS-induced airways disease

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Abstract

Background—The contribution of IL-1 β signaling through the IL-1 type 1 receptor (IL-1R1) to the development of persistent LPS-induced airway disease has not been investigated.

Objective—To determine the importance of signaling through the IL-1 type 1 receptor in the development of LPS-induced airway disease.

Methods—We exposed IL-1R1-deficient (C57BL/6^{IL-1RI^{-/-}}) mice to an aerosol of LPS or filtered air for 1 day, 1 week, or 4 weeks.

Results—After 4 weeks of LPS inhalation, C57BL/6^{IL-1RI^{-/-}} mice failed to develop significant submucosal thickening, whereas C57BL/6 mice had significantly thickened submucosa in small, medium, and large airways compared with those of unexposed control mice. Cell proliferation in the airways of both the 1-week and 4-week LPS-exposed C57BL/6^{IL-1RI^{-/-}} mice was significantly reduced compared with LPS-exposed C57BL/6 mice. mRNA for type III α -3 procollagen was significantly elevated over baseline in C57BL/6 yet remained unchanged compared with baseline in C57BL/6^{IL-1RI^{-/-}} mice after 1 week or 4 weeks of LPS inhalation. mRNA for tissue inhibitor of metalloprotease 1 in C57BL/6 mice in the 1-week and 4-week groups was significantly elevated over both control mice and C57BL/6^{IL-1RI^{-/-}} mice.

Conclusion—These data support the hypothesis that signaling through the IL-1 receptor modulates extracellular matrix homeostasis in response to inhaled LPS.

Clinical implications—Attenuating IL-1R1-mediated signaling might be an effective therapy against the development of airway remodeling in chronic inflammatory diseases.

Keywords

LPS; endotoxin; airway disease; IL-1; IL-1 receptor; airway remodeling

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Subchronic inhalation of endotoxin lipopolysaccharides (LPSs) in mice causes persistent airway disease with many classic features of asthma including reversible airflow obstruction and inflammation, airway remodeling, and persistent airway hyperreactivity^{1,2} and is used to model human grain dust-induced airway disease. We have shown that neutrophilic inflammation is critical to this process and that IL-1 β is produced abundantly in the lung during subchronic LPS inhalation challenge.^{1,2} However, the mechanistic contribution of IL-1 β to this process remains to be elucidated.

The ability to respond to LPS in mice and in human beings is imparted by the Toll-like receptor 4 (TLR4).^{3,4} TLR4 and the IL-1 type 1 receptor (IL-1R1) are both members of an expanding family of receptors defined by the Toll/IL-1 receptor domain that occurs in the cytoplasmic domain of these receptors.⁵ Both TLR4 and IL-1R1 activate downstream kinases and transcription factors resulting in nuclear factor- κ B-mediated gene transcription. Both receptors require MyD88, IL-1 receptor-associated kinase, TNF receptor-associated factor 6, and NF- κ B-inducing kinase (NIK) to activate NF- κ B.^{6,7} Mice rendered deficient in TLR4 either through a naturally occurring point mutation (C3H/HeJ)⁸ or through a targeted gene deletion (TLR4^{-/-}) are unresponsive to inhaled LPS.⁹ Mice with a targeted deletion of the IL-1R1 have a normal response to acute LPS inhalation,¹⁰ yet IL-1 β is typically associated with a proinflammatory phenotype, and LPS exposure in mice increases expression of IL-1 β mRNA and protein in the lung.¹¹ In addition, it has been shown that intratracheal administration of an adenoviral vector containing the cDNA for human IL-1 β induces TGF- β protein expression and consequent airway fibrosis in rats,¹² suggesting an important role for IL-1 β in the fibroproliferative process. We therefore asked whether C57BL/6^{IL-1R1-/-} mice would develop persistent LPS-induced airway disease. To address this question, we exposed C57BL/6 and C57BL/6^{IL-1R1-/-} mice to an aerosol of endotoxin for 5 days or 4 weeks.

METHODS

Experimental animals

Twelve male C57BL/6 mice and 12 male C57BL/6^{IL-1R1-/-} mice at 8 weeks of age were exposed to aerosolized LPS for 4 hours per day for either 1 week (1-week group) or 4 weeks followed by a 3-day recovery period (4-week group). Six C57BL/6 mice and six C57BL/6^{IL-1R1-/-} mice were sacrificed at each time point (1 week and 3 days after 4 weeks of exposure). Twelve male C57BL/6 mice and 12 male C57BL/6^{IL-1R1-/-} mice were used as controls and were exposed to filtered air alone.

LPS preparation and aerosol exposures

LPS was purchased as purified lyophilized powder (25 mg; 30,000,000 EU/mg; lot 110K4060), prepared by phenol extraction from *Escherichia coli* serotype 0111:B4 from Sigma (St Louis, Mo). LPS was reconstituted with 10 mL sterile HBSS, and stock aliquots (2.5 mg/mL) were stored at -20°C. Immediately before use, 160 μ L LPS stock (4.7 mg; 7,000,000 EU) was diluted in 75 mL HBSS for nebulization.

Mice were placed in stainless steel wire cage exposure racks in a 20-L chamber. Animals were exposed for 4 hours. LPS solution was aerosolized with a Collison 6-Jet Nebulizer (model CN-25; BGI Inc, Waltham, Mass) with all output directed to the exposure chamber. Filtered and dehumidified air was supplied to the nebulizer at 20-psi gauge pressure. The exposure chamber was vented at a flow rate of 28.0 L/min.

LPS assay

The airborne concentration of LPS was assessed by sampling 0.30 to 0.40 m³ of air drawn from the exposure chamber through 25-mm binder-free glass fiber filters (Gelman Sciences, Ann Arbor, Mich) held within a 25-mm polypropylene inline air-sampling filter holder (Gelman Sciences). Filters were placed in pyrogen-free petri dishes with 2 mL sterile PBS containing 0.05% Tween-20 (Sigma) and then placed on a rotating shaker at room temperature for 1 hour. Aliquots of the wash solution were serially diluted in pyrogen-free water and tested for endotoxin by using a chromogenic Limulus amebocyte lysate assay (QCL-1000; BioWhittaker, Walkersville, Md) according to the manufacturer's instructions.

Whole lung lavage

Mice were euthanized by CO₂ inhalation, and bilateral thoracotomy was performed. Lungs were lavaged with 6.0 mL sterile saline, 1 mL at a time, at a pressure of 20 cm H₂O. Processing of the lavage fluid has been described previously.¹³ Briefly, the lavage fluid was centrifuged for 5 minutes at 200g. The supernatant was decanted and stored at -70°C for further use. The cell pellet was resuspended with HBSS (without calcium or magnesium), and a small aliquot of this suspension was used to count total lavaged cells per animal by using a hemocytometer. One hundred microliters of the cell suspension was spun onto a slide using a cytocentrifuge (Shandon, Southern Sewickley, Pa). Cells were stained with Hema-3 (Biochemical Sciences Inc, Swedesboro, NJ) stain for differential, air-dried, and covered with a coverslip with Cytoseal (Stephens Scientific, Kalamazoo, Mich).

Tissue preparation

After whole lung lavage, the lungs were perfused with saline through the pulmonary artery, and the whole right lung was removed and snap-frozen in liquid nitrogen and stored at -70°C. Freshly prepared ice-cold 4% paraformaldehyde (Fisher Scientific, Pittsburgh, Pa) in 1×PBS (pH 7.4) was instilled through the tracheal cannula into the right lung at a constant pressure of 20 cm H₂O. The trachea was clamped, and the lung was fixed overnight at 48°C in 4% paraformaldehyde. This tissue was embedded in paraffin; sections 5 to 6 µm thick were cut and placed on positively charged slides (Super Frost Plus; Fisher Scientific, Pittsburgh, Pa).

Bronchoalveolar lavage leukocyte superoxide anion assay

Superoxide anion (O₂⁻) generation by BAL leukocytes was quantified as previously described.¹⁴ Briefly, animals were lavaged 6 hours after the beginning of LPS inhalation, and BAL leukocytes were enumerated. For each animal, 3.3 × 10⁵ BAL leukocytes were assayed for O₂⁻ generation induced by buffer, phorbol myristate acetate (PMA), and formyl-Met-Leu-Phe (fMLP).

Morphometry (quantitative analysis)

Morphometry was performed applying standard methods previously described.¹ Briefly, the area of the submucosa was calculated by using measurements of the external airway and basement membrane perimeter. Airways profiles were divided into 3 relatively equal groups determined by airway short diameter: small airways (0-90 μm), medium airways (>90-129 μm), and large airways (>129 μm). For every airway measured, submucosal area was normalized to the length of the adjacent basement membrane. To minimize the error that might arise from tangential sectioning, any airway profiles showing a length-to-width ratio greater than 2.5 were not used for analysis. The mean value of submucosal area standardized to length of basement membrane was calculated for each airway size for each study animal. These values were used to calculate means $\pm$ SEMs for each airway size for each study group.

Immunohistochemistry

Bromo-deoxy-uridine (BrdU) immunohistochemistry was performed as described previously² using a rat monoclonal anti-BrdU antibody at a dilution of 1:100 (Accurate Chemical and Scientific, Westbury, NY).

Quantitative analysis of cell proliferation

One BrdU-stained histological section from each animal was prepared as described. Digital images were acquired and categorized with respect to size as described in morphometry of all airways in each BrdU-stained histological section from each animal. All airway submucosal cells in each airway image were counted. Separate counts of BrdU-positive submucosal cells were also kept. The mean percent positive cells per airway was calculated for each airway size for each study animal. These values were used to calculate means $\pm$ SEMs for each airway size for each study group.

ELISA

ELISA kits for TNF- α , IL-1 β , and IL-6 were purchased from R&D Systems (Minneapolis, Minn). The ELISA kit for TGF- β was purchased from Promega (Madison, Wis). Standard curves were run with each ELISA. The lower limit of detection for each protein was as follows: 5.1 pg/mL for TNF- α , 3.0 pg/mL for IL-1 β , 10 pg/mL for IL-6, and 15.6 pg/mL for TGF- β .

Quantitative PCR

Total RNA (50 ng/reaction) was reversed-transcribed into cDNA and amplified in a buffer containing SYBR Green (Applied Biosystems, Foster City, Calif). PCR primers were specific for mouse pro- α 3 type III collagen (forward: CCCTGGTCCACAAGGATTACA; reverse: CTCCAGGTGCACCAGAATCA) and tissue inhibitor of metalloprotease 1 (TIMP-1; forward: ATAGCTTCCAGTAAGGC CTGTAGCT; reverse: GTACCGGATATCTGCGGCATT). TFIID (forward: ACGGACAACCTGCGTTGATTTT; reverse: ACTTAG CTGGGAAGCCCAAC) primers were used as an internal control. Samples were run in triplicate, and the mean value was normalized to the internal control.

Statistical analyses

All data are expressed as means $\pm$ SEMs. Primary comparisons were between the physiological and biological responses in C57BL/6^{IL-1R1^{-/-}} mice and their background control strains (C57BL/6). Total and differential cell counts from whole lung lavage, the airway responsiveness to inhaled methacholine challenge, and cytokine levels were compared between C57BL/6^{IL-1R1^{-/-}} mice and C57BL/6 mice. mRNA analyses compared pro- α 3 type III collagen and TIMP-1 from LPS-exposed mice with that from air-exposed mice. The differences between variables in each comparison were analyzed by ANOVA or Mann-Whitney *U* test where appropriate. Probability values of $P < .05$ were considered statistically significant.

RESULTS

Inflammation of the lower respiratory tract

Air-exposed C57BL/6^{IL-1R1^{-/-}} mice and C57BL/6 mice have the same number of resident cells in the lung, the majority of which are macrophages (Fig 1). Inflammation of the lower respiratory tract was not different between C57BL/6^{IL-1R1^{-/-}} mice and C57BL/6 mice at any time during the course of the LPS exposure. However, 3 days after the end of the last LPS inhalation exposure, C57BL/6^{IL-1R1^{-/-}} mice had a significantly reduced concentration of inflammatory cells in the lower respiratory tract compared with C57BL/6 mice (Fig 1, A). There was no difference in the differential counts of these inflammatory cells in terms of either percent neutrophils (Fig 1, B) or percent macrophages (Fig 1, C) at any time.

Cytokine concentration in whole lung lavage fluid

Although the concentration of IL-1 β was similar in C57BL/6^{IL-1R1^{-/-}} mice and C57BL/6 mice throughout the LPS inhalation exposure, C57BL/6^{IL-1R1^{-/-}} mice had significantly lower concentrations of TNF- α at 1 day and 1 week of exposure and of IL-6 at 1 week and 4 weeks in the lavage fluid than C57BL/6 mice (Fig 2). At baseline and in both strains of mice exposed to LPS for 4 weeks and then recovered for 3 days, there was no detectable TNF- α , IL-1 β , or IL-6 in whole lung lavage fluid.

Airspace leukocyte activation

Because we have previously shown that neutrophils are an essential component of LPS-induced airways remodeling in mice,¹ and because there is no difference between C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice in inflammatory cells in whole lung lavage during the course of the LPS exposure, we asked whether BAL cells from both C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice could be similarly activated. To address this question, we exposed both C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice to a single inhaled LPS challenge and evaluated the ability of the BAL leukocytes to produce oxygen radicals (Fig 3). Under these conditions, greater than 95% of total BAL leukocytes retrieved are neutrophils. At baseline (BAL leukocytes exposed to buffer only), lavage cells from C57BL/6^{IL-1R1^{-/-}} mice produce more superoxide than C57BL/6 lavage cells. In response to the protein kinase C activator PMA, neutrophils from both C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice produced equal amounts of superoxide, showing that there is not a primary defect in the ability to respond to stimulus or to produce

superoxide. However, in response to fMLP as an indicator of a response to microbial products, C57BL/6^{IL-1R1^{-/-}} mice had a significantly elevated superoxide response compared with C57BL/6 mice (Fig 3).

Morphometry

Only C57BL/6 mice that had been exposed to LPS for 4 weeks developed significant submucosal thickening in small, medium, and large airways (Fig 4). The submucosal area in the airways of the C57BL/6^{IL-1R1^{-/-}} mice was not different from airways from C57BL/6 and C57BL/6^{IL-1R1^{-/-}} air-exposed groups (Fig 4).

Airway submucosal cell proliferation

There was no difference in submucosal cell proliferation between air-exposed C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice (Fig 5). After 1 week of LPS exposure, C57BL/6 mice had a significantly greater percentage of cells proliferating in the submucosa than C57BL/6^{IL-1R1^{-/-}} mice, although cell proliferation in the LPS-exposed C57BL/6^{IL-1R1^{-/-}} mice was also significantly elevated over baseline (Fig 5). Although submucosal cell proliferation in C57BL/6 mice exposed to LPS for 4 weeks was significantly elevated compared with air-exposed controls, C57BL/6^{IL-1R1^{-/-}} mice had a similar percentage of cells proliferating in the submucosa compared with air-exposed controls (Fig 5).

Concentration of TGF-β1 in the lavage fluid

At baseline in both C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice, TGF-β1 was below the limits of detection in whole lung lavage fluid (data not shown). After a single acute LPS inhalation, there was no difference in the total TGF-β present in whole lung lavage fluid between C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice, and there was no detectable active TGF-β in either group (Table I). After 1 week of LPS inhalation, there was no difference in the concentration of whole lung lavage total TGF-β protein between C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice (Table I); however, there was more active TGF-β in C57BL/6^{IL-1R1^{-/-}} mice. Immediately after the end of 4 weeks of exposure to LPS, there was no difference between the groups in either total or active TGF-β. Three days after the end of the last exposure, however, C57BL/6^{IL-1R1^{-/-}} mice had significantly less total TGF-β1 in whole lung lavage fluid than C57BL/6 mice (although active TGF-β1 was not different between these 2 groups; Table I).

Matrix protein gene expression

After either 1 or 4 weeks of inhalation of LPS, type III α-3 procollagen mRNA in the whole lung tissue mRNA was significantly elevated in C57BL/6 mice compared with air-exposed controls (Fig 6, A). In contrast, type III α-3 procollagen mRNA expression remained unchanged in C57BL/6^{IL-1R1^{-/-}} mice from both the 1-week and 4-week LPS-exposed groups. In both C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice in the 1-week group, TIMP-1 mRNA expression was significantly elevated (Fig 6); however, TIMP-1 mRNA expression was significantly higher in the C57BL/6 mice. After 4 weeks of LPS inhalation, TIMP-1 mRNA expression remained significantly elevated only in the LPS-exposed C57BL/6 mice.

DISCUSSION

The key finding of this study is that although the IL-1R1 is not essential to the acute response to inhaled LPS, it is required for the development of airway remodeling in chronic LPS-induced airways disease. We find that, underlying this, cell proliferation is significantly reduced in chronically LPS-exposed C57BL/6^{IL-1R1-/-} mice compared with exposed C57BL/6 mice, and that collagen III and TIMP-1 are differentially expressed when comparing LPS-exposed C57BL/6^{IL-1R1-/-} mice and C57BL/6 mice. We have previously reported that neutrophils are critical to LPS-induced airways remodeling.¹ We show here, however, that C57BL/6^{IL-1R1-/-} mice are protected from airway remodeling despite airspace neutrophils that are equivalent in number and enhanced in oxidant-generating function. Thus, a second important finding of this study is that IL-1R1 appears to modulate chronic airway remodeling through a neutrophil-independent mechanism.

In the current study, we show that, although cellular inflammation of the lower respiratory tract in the C57BL/6^{IL-1R1-/-} mice returns to baseline more quickly than in C57BL/6 mice, C57BL/6 and C57BL/6^{IL-1R1-/-} mice have an equivalent number of lavageable neutrophils in the lung (Fig 1, *A and B*) throughout the course of exposure. Whole lung mRNA analysis (not shown) of neutrophil specific genes including neutrophil elastase and myeloperoxidase was also equivalent between C57BL/6 and C57BL/6^{IL-1R1-/-} mice, suggesting an equivalent number of neutrophils in the interstitium of the lung. Our findings of enhanced oxidant capacity of airspace neutrophils in C57BL/6^{IL-1R1-/-} mice are consistent with a recent study showing that TLR4-deficient mice have an increased oxidant burden in the lung and decreased antioxidant capacity¹⁵ and further suggest that IL-1R1-mediated signaling may also modulate oxidant/antioxidant balance in the lung, and may regulate the inflammatory phenotype triggered by inhaled LPS.

We show here that there is less lavageable TNF- α in C57BL/6^{IL-1R1-/-} mice at 1 day and 1 week and less IL-6 at 1 week and 4 weeks, suggesting that the IL-1R1 is important in sustained expression of these proinflammatory proteins. Taken together, our results suggest that signaling initiated through the IL-1R1 is not completely functionally redundant with signaling initiated by LPS through TLR4 despite significant sequence homology in the cytoplasmic domain of these 2 proteins, and further suggest that IL-1R1 deficiency alters the character of the inflammatory response to inhaled LPS. The minimal differences between C57BL/6 and C57BL/6^{IL-1R1-/-} mice in lavageable active or total TGF- β (Table I) suggest that IL-1R1 does not directly regulate production or expression of this critical growth factor. However, it may be that IL-1R1 provides a permissive signal for fibroproliferation in response to TGF- β . Whether this is a direct result of signal transduction events initiated by the IL-1R1 or this is downstream and dependent on further cytokine (or other gene) expression resulting from IL-1R1 initiated signaling remains to be determined.

Recent studies have demonstrated that IL-13 induces airway fibrosis,¹⁶ that it is a susceptibility locus for human asthma,¹⁷ and that it can induce TGF- β -independent fibrosis.¹⁸ Because the differences we observed in TGF- β between C57BL/6 and C57BL/6^{IL-1R1-/-} mice are minimal, we investigated whether IL-13 might be expressed in the lungs of mice exposed to chronic LPS inhalation challenge. Whole lung mRNA analysis during

the course of chronic LPS-induced airway remodeling reveals no differential expression of IL-13 or the IL-13 receptors (Brass et al, unpublished data, February 2007). In addition, during chronic LPS exposure, there was no detectable IL-13 protein in whole lung lavage in either C57BL/6 or C57BL/6^{IL-1R1^{-/-}} mice (data not shown). Our data show there is differential expression of both collagen III and TIMP-1 in whole lung mRNA between C57BL/6 and C57BL/6^{IL-1R1^{-/-}} mice. It has also been demonstrated recently that IL-1 increases fibronectin production in cultured rat cardiac fibroblasts,¹⁹ and another study suggests that IL-1 β may play a role in regulating collagenase and elastase activities.²⁰ In addition, IL-1R1 has been shown to regulate expression of the urokinase plasminogen activator receptor (uPAR),^{20,21} suggesting that IL-1 may indirectly regulate the conversion of plasminogen to plasmin, which has been shown to be important in regulation of extracellular matrix homeostasis. uPAR expression is significantly increased in fibroblasts from fibrotic lung tissue compared with those from normal tissue,²² and urokinase plasminogen activator binding to uPAR is mitogenic for fibroblasts,²² suggesting a plausible mechanistic connection between IL-1R1-mediated signaling and airway remodeling. In support of this, plasminogen activator inhibitor (PAI)-1-deficient mice are protected from the fibroproliferative effects of bleomycin, and transgenic mice overexpressing PAI-1 are more susceptible to these effects.²³ However, these studies have not evaluated IL-1 protein expression or IL-1R1-mediated signal transduction. Furthermore, we have shown that PAI-1-deficient mice are protected from the subchronic effects of LPS inhalation.²⁴ These findings and our data taken together support the hypothesis that IL-1R1 may regulate TGF- β -independent events such as extravascular coagulation. This in turn could explain why there is measurably elevated cell proliferation in the submucosa of C57BL/6^{IL-1R1^{-/-}} mice but no significant increase in submucosal thickening.

In summary, C57BL/6^{IL-1R1^{-/-}} mice are protected from submucosal thickening associated with chronic LPS inhalation challenge. Our results suggest this protection is a result in part of a failure to increase collagen expression and reduced expression of TIMP-1 as well as reduced cell proliferation in the submucosal region. Thus, the absence of significant submucosal thickening in C57BL/6^{IL-1R1^{-/-}} mice suggests that blocking the effects of IL-1 in chronic inflammatory airway diseases may be a viable therapy for the prevention of airway remodeling.

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Abbreviations used

BAL	Bronchoalveolar lavage
BrdU	Bromo-deoxy-uridine
fMLP	Formyl-Met-Leu-Phe
IL-1R1	IL-1 type I receptor

NF-κB	Nuclear factor-κB
PAI	Plasminogen activator inhibitor
PMA	Phorbol myristate acetate
TIMP-1	Tissue inhibitor of metalloprotease 1
TLR4	Toll-like receptor 4
uPAR	Urokinase plasminogen activator receptor

REFERENCES

1. Savov JD, Gavett SH, Brass DM, Costa DL, Schwartz DA. Neutrophils play a critical role in development of LPS-induced airway disease. *Am J Physiol Lung Cell Mol Physiol.* 2002; 283:L952–62. [PubMed: 12376348]
2. Brass DM, Savov JD, Gavett SH, Haykal-Coates N, Schwartz DA. Subchronic endotoxin inhalation causes persistent airway disease. *Am J Physiol Lung Cell Mol Physiol.* 2003; 285:L755–61. [PubMed: 12794002]
3. Poltorak A, He X, Smirnova I, Liu MY, Huffel CV, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science.* 1998; 282:2085–8. [PubMed: 9851930]
4. Poltorak A, Smirnova I, He X, Liu MY, Van Huffel C, McNally O, et al. Genetic and physical mapping of the Lps locus: identification of the toll-4 receptor as a candidate gene in the critical region. *Blood Cells Mol Dis.* 1998; 24:340–55. [PubMed: 10087992]
5. Bowie A, O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products. *J Leukoc Biol.* 2000; 67:508–14. [PubMed: 10770283]
6. Muzio M, Ni J, Feng P, Dixit VM. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science.* 1997; 278:1612–5. [PubMed: 9374458]
7. Zhang FX, Kirschning CJ, Mancinelli R, Xu XP, Jin Y, Faure E, et al. Bacterial lipopolysaccharide activates nuclear factor-kappaB through interleukin-1 signaling mediators in cultured human dermal endothelial cells and mononuclear phagocytes. *J Biol Chem.* 1999; 274:7611–4. [PubMed: 10075645]
8. Lorenz E, Jones M, Wohlford-Lenane C, Meyer N, Frees KL, Arbour NC, et al. Genes other than TLR4 are involved in the response to inhaled LPS. *Am J Physiol Lung Cell Mol Physiol.* 2001; 281:L1106–14. [PubMed: 11597901]
9. Hollingsworth JW 2nd, Cook DN, Brass DM, Walker JK, Morgan DL, Foster WM, et al. The role of Toll-like receptor 4 in environmental airway injury in mice. *Am J Respir Crit Care Med.* 2004; 170:126–32. [PubMed: 15020293]
10. Moreland JG, Fuhrman RM, Wohlford-Lenane CL, Quinn TJ, Benda E, Pruessner JA, et al. TNF-α and IL-1β are not essential to the inflammatory response in LPS-induced airway disease. *Am J Physiol Lung Cell Mol Physiol.* 2001; 280:L173–80. [PubMed: 11133507]
11. Wohlford-Lenane CL, Deetz DC, Schwartz DA. Cytokine gene expression after inhalation of corn dust. *Am J Physiol.* 1999; 276:L736–43. [PubMed: 10330029]
12. Kolb M, Margetts PJ, Anthony DC, Pitossi F, Gauldie J. Transient expression of IL-1β induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J Clin Invest.* 2001; 107:1529–36. [PubMed: 11413160]
13. George CL, Jin H, Wohlford-Lenane CL, O'Neill ME, Phipps JC, O'Shaughnessy P, et al. Endotoxin responsiveness and subchronic grain dust-induced airway disease. *Am J Physiol Lung Cell Mol Physiol.* 2001; 280:L203–13. [PubMed: 11158998]

14. Fessler MB, Arndt PG, Frasch SC, Lieber JG, Johnson CA, Murphy RC, et al. Lipid rafts regulate lipopolysaccharide-induced activation of Cdc42 and inflammatory functions of the human neutrophil. *J Biol Chem*. 2004; 279:39989–98. [PubMed: 15262974]
15. Zhang X, Shan P, Jiang G, Cohn L, Lee PJ. Toll-like receptor 4 deficiency causes pulmonary emphysema. *J Clin Invest*. 2006; 116:3050–9. [PubMed: 17053835]
16. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science*. 1998; 282:2261–3. [PubMed: 9856950]
17. Wills-Karp M. The gene encoding interleukin-13: a susceptibility locus for asthma and related traits. *Respir Res*. 2000; 1:19–23. [PubMed: 11667960]
18. Kaviratne M, Hesse M, Leusink M, Cheever AW, Davies SJ, McKerrow JH, et al. IL-13 activates a mechanism of tissue fibrosis that is completely TGF-beta independent. *J Immunol*. 2004; 173:4020–9. [PubMed: 15356151]
19. Fernandez L, Mosquera JA. Interleukin-1 increases fibronectin production by cultured rat cardiac fibroblasts. *Pathobiology*. 2002; 70:191–6. [PubMed: 12679595]
20. Elnor SG, Elnor VM, Kindzelskii AL, Horino K, Davis HR, Todd RF 3rd, et al. Human RPE cell lysis of extracellular matrix: functional urokinase plasminogen activator receptor (uPAR), collagenase and elastase. *Exp Eye Res*. 2003; 76:585–95. [PubMed: 12697422]
21. Iwamoto J, Takahashi K, Mizokami Y, Otsubo T, Miura S, Narasaka T, et al. Expression of urokinase-type plasminogen activator and its receptor in gastric fibroblasts and effects of nonsteroidal antiinflammatory drugs and prostaglandin. *Dig Dis Sci*. 2003; 48:2247–56. [PubMed: 14714609]
22. Shetty S, Kumar A, Johnson AR, Pueblitz S, Holiday D, Raghu G, et al. Differential expression of the urokinase receptor in fibroblasts from normal and fibrotic human lungs. *Am J Respir Cell Mol Biol*. 1996; 15:78–87. [PubMed: 8679225]
23. Eitzman DT, McCoy RD, Zheng X, Fay WP, Shen T, Ginsburg D, et al. Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J Clin Invest*. 1996; 97:232–7. [PubMed: 8550840]
24. Savov JD, Brass DM, Berman KG, McElvania E, Schwartz DA. Fibrinolysis in LPS-induced chronic airway disease. *Am J Physiol Lung Cell Mol Physiol*. 2003; 285:L940–8. [PubMed: 12818888]

FIG 1. Time course of lung lavage fluid mean concentration of total cells (A), percent neutrophils (B), and percentage of macrophages (C). Error bars are the SE. * $P < .05$.

FIG 2.

Time course of lung lavage fluid mean concentration of TNF-α (A), IL-1β (B), and IL-6 (C).

Error bars are the SE. **P* < .05.

FIG 3. Superoxide production by lavaged neutrophils, at baseline or stimulated with PMA or fMLP, from C57BL/6 and C57BL/6 $^{IL-1R1-/-}$ mice after a single LPS inhalation challenge. *Error bars are the SE. * $P < .05$.*

FIG 4.

Stereologic measures of the submucosal area from small, medium, and large airways from C57BL/6 and C57BL/6 $\text{IL-1R1}^{-/-}$ mice exposed to LPS for 1 month and allowed to recover for 3 days. Data from age-matched air-exposed C57BL/6 and C57BL/6 $\text{IL-1R1}^{-/-}$ are also presented. *Error bars* are the SE. * $P < .05$.

FIG 5.

BrdU labeling in small, medium, and large airway submucosa in C57BL/6 and C57BL/6^{IL-1R1-/-} mice at baseline, after 1 week, and in mice exposed to LPS for 4 weeks and allowed to recover for 3 days. Error bars are the SE. #*P* < .05 compared with baseline; **P* < .05 C57BL/6 compared with C57BL/6^{IL-1R1-/-}.

FIG 6. Real-time quantitative PCR for expression of type III α -3 procollagen and TIMP1 mRNA expression in C57BL/6 and C57BL/6^{IL-1R1-/-} mouse lung after 1 week and 4 weeks. *Dotted lines* represent no change. *Error bars* are the SE. * $P < .05$ C57BL/6 compared with C57BL/6^{IL-1R1-/-}.

TABLE ITime course of TGF- β protein in whole lung lavage fluid[†]

		Baseline	1 Day	1 Week	4 Weeks	Post
C57BL/6	Total	ND	67.11 $\pm$ 20.21	250.89 $\pm$ 26.00	359.29 $\pm$ 18.35	387.48 $\pm$ 28.00
C57BL/6	Active	ND	ND	6.85 $\pm$ 3.28	22.32 $\pm$ 12.74	27.77 $\pm$ 4.26
IL-1R1 ^{-/-}	Total	ND	50.94 $\pm$ 1.93	307.00 $\pm$ 12.66	398.80 $\pm$ 45.34	271.91 $\pm$ 18.63 [*]
IL-1R1 ^{-/-}	Active	ND	ND	31.76 $\pm$ 8.25 [*]	31.48 $\pm$ 4.31	38.64 $\pm$ 8.42

ND, None detected.

^{*} $P < .05$ vs C57BL/6.[†] TGF- β (pg/mL) in whole lung lavage fluid measured by ELISA (R & D; Minneapolis, Minn).