Leukocyte-Derived IL-10 Reduces Subepithelial Fibrosis Associated with Chronically Inhaled Endotoxin

Stavros Garantziotis, David M. Brass, Jordan Savov, John W. Hollingsworth, Erin McElvania-TeKippe, Katie Berman, Julia K. L. Walker, and David A. Schwartz

Division of Pulmonary, Allergy, and Critical Care Medicine, Duke University Medical Center, Durham, North Carolina; and National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Endotoxin (LPS), a Gram-negative cell wall component, has potent proinflammatory properties. Acute LPS exposure causes airway inflammation; chronic exposure causes airway hyperreactivity and remodeling. IL-10 is an important antiinflammatory cytokine, which is decreased in patients with airway disease, such as asthma and cystic fibrosis. To examine the physiologic and therapeutic role of IL-10 in acute and chronic LPS-induced airway disease. Mice were exposed to aerosolized LPS once or daily for 4 wk. Endpoints were airway inflammation, airway reactivity to methacholine, extracellular matrix protein expression, and histologic analysis. IL-10–deficient mice developed significantly enhanced airway cellularity and remodeling when compared with C57BL/6 mice after chronic LPS inhalation. However they demonstrated less airway hyperreactivity associated with higher inducible nitric oxide synthase (iNOS), endothelial NOS (eNOS), and lung lavage fluid nitrite levels. In a bone marrow transplantation model, the IL-10 antiinflammatory effect was dependent on the hematopoietic but not on the parenchymal IL-10 expression. Induced epithelial human IL-10 expression protected from the LPS effects and led to decreased collagen production. IL-10 attenuates chronic LPS-induced airway inflammation and remodeling. Physiologically, the antiinflammatory effect of IL-10 is mediated by hematopoietic cells. Therapeutically, adenovirus-driven expression of human IL-10 in airway epithelia is sufficient for its protective effect on inflammation and remodeling. The role of IL-10 on airway hyperreactivity is complex: IL-10 deficiency protects against LPS-induced hyperreactivity, and is associated with higher eNOS, iNOS, and airway nitrate levels.

Keywords: airway hyperreactivity; airway remodeling; endotoxin; IL-10

Endotoxin or LPS, a component of the wall of Gram-negative bacteria with significant proinflammatory properties, is present in high concentrations in organic dusts (1) and air pollution (2). Chronic occupational LPS exposure leads to airway inflammation and persistent obstruction (3), whereas LPS concentration in urban homes has been correlated with the clinical severity of allergic asthma (4). In animal models, acute LPS inhalation leads to neutrophilic airway inflammation, release of proinflammatory cytokines, and airway hyperreactivity (5, 6), whereas chronic LPS inhalation causes airway inflammation, persistent airway hyperreactivity, and airway remodeling with thickening of the subepithelial space (7). Regulation of the biological response to inhaled LPS is a complex process and requires the interaction of many cell types, including alveolar macrophages (8), airway epithelia (9), and smooth muscle (10). The specific cellular interactions that regulate this process and ultimately lead to airways remodeling remain to be elucidated.

CLINICAL RELEVANCE

This research elucidates the mechanism of chronic LPS-induced airway inflammation and remodeling and provides insights on the effect of IL-10. IL-10 is identified as a potential therapeutic agent for chronic LPS-induced airway inflammation and remodeling.

IL-10 is a potent antiinflammatory cytokine with direct antiinflammatory activity on macrophages and neutrophils (11, 12). IL-10 substantially reduces the inflammatory response to intravenous LPS by downregulating the release of cytokines, CC chemokines, free radicals, and coagulation factors (13–15). IL-10 may also play a role as an endogenous antiinflammatory agent in asthma (16, 17) and cystic fibrosis (18, 19). Overexpression of IL-10 in the airways inhibited airway inflammation in an ovalbumin model of allergic asthma (20), and attenuated acute lung injury to inhaled LPS (21) and grain dust exposure (22). However, the role of IL-10 expression in the context of chronic inflammation, a clinically more relevant situation, has not been examined.

Elucidating the role of IL-10 in chronic LPS-induced airway disease will lead to a better understanding of the nature and mechanisms of chronic inflammatory airway diseases, such as asthma and cystic fibrosis. Furthermore, because airway remodeling is thought to be a sequela of chronic inflammation and may be amenable to antiinflammatory treatment, IL-10 may represent a useful therapeutic option in these diseases. We hypothesized that IL-10 would minimize the effects of chronic LPS inhalation exposure in mice, and that IL-10 can be used therapeutically to decrease chronic LPS effects on the airways. To test this hypothesis, we compared the biological and physiologic response in IL-10–deficient (C57BL/6-IL10^{tm1Cgn}) and wild-type (WT; C57BL/6) mice after acute and chronic inhalation of LPS. We also induced expression of human IL-10 (hIL-10) in murine airways by using an adenoviral expression vector, and investigated the same endpoints. Finally, we used bone marrow transplant to determine whether hematopoietic cell-derived IL-10 or IL-10 derived from the lung parenchyma was involved in modulating the response to inhaled LPS.

MATERIALS AND METHODS

Experimental Animals

C57BL/6J and C57BL/6-IL10^{tm1Cgn} mice were purchased from Jackson Laboratories (Bar Harbor, ME). Experimental protocols were approved by the Duke Institutional Animal Care and Use Committee, and performed in accordance with the U.S. Animal Welfare Acts standards.

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Correspondence and requests for reprints should be addressed to Stavros Garantziotis, M.D., Division of Pulmonary, Allergy, and Critical Care Medicine, Duke University Medical Center, Box 3683, Durham, NC 27710. E-mail: garan001@mc.duke.edu

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Intratracheal Administration of Ad5RSVhIL-10

Ad5RSVhIL-10 (adenovirus carrying RSV promoter and hIL-10 gene) was purchased from the University of Iowa Vector Core Facility. A total of 5×10^8 plaque-forming units of the virus were given one time intratracheally in 50 μl of 3% Sucrose/PBS. Adenoviral vector-mediated expression after intratracheal infection usually abates after 2–3 wk, and would be insufficient for chronic LPS exposure, which lasts 4 wk. For chronic LPS exposures, mice were therefore treated intraperitoneally with 100 μg of anti-murine CD40L on days −3, 0, +3, and +6 of intratracheal instillation. This treatment prolongs vector-mediated expression to over 42 d (Ref. 23 and our observations). Control mice received adenovirus carrying the β-galactosidase gene (Ad5RSVLacZ). Acute LPS exposure was performed 7 d after adenoviral instillation (no CD40L used). Chronic LPS exposure was initiated 14 d after adenoviral instillation to avoid effects of anti-CD40L on the immune response. Immunohistochemical staining revealed the presence of hIL-10 in bronchial and alveolar epithelial cells, but not alveolar macrophages, at the end of both acute and chronic exposures.

Bone Marrow Transplantation

Chimeric animals were created by lethal irradiation (1,050 rad) of 6- to 8-wk recipient mice, which then received 4×10^6 donor bone marrow cells intravenously. The chimeric groups were: C57BL/6-IL10^{tm1Cgn} bone marrow into C57BL/6-IL10^{tm1Cgn} recipients, C57BL/6-IL10^{tm1Cgn} into C57BL/6, C57BL/6 into C57BL/6-IL10^{tm1Cgn}, and C57BL/6 into C57BL/6. CD45.1-positive C57BL/6 mice were used to differentiate from CD45.2-positive C57BL/6-IL10^{tm1Cgn}. Engraftment was verified 6 wk after transplantation. Peripheral engraftment of B-lymphocytes was > 99%, and that of T-lymphocytes was 83%, by flow cytometry. LPS exposure started 8 wk after transplantation.

LPS Exposure

Lyophilized, reconstituted LPS (*Escherichia coli* serotype 0111:B4; Sigma, St. Louis, MO) was used. LPS aerosol was generated as previously described (7). Briefly, we used a six-jet atomizer (Model 9306; TSI, Inc., Shoreview, MN) at a constant pressure of 35 psi. Mice were exposed for 2.5 h (acute exposure), or for 2.5 h/d, 5 d/wk, for 4 wk (chronic exposure). LPS concentrations were determined by sampling the total chamber outflow using the quantitative chromogenic Limulus amebocyte lysate assay (QCL-1000; Whittaker Bioproducts, Walkersville, MD). The concentrations of LPS aerosol (Limulus amebocyte lysate assay) in these experiments were 6–8 μg/m^3.

Airway pressure–time index (APTI) was measured as previously described (24). Mice were anesthetized, paralyzed, tracheally intubated, and received intravenous methacholine in doses of 25 μg/ml, 100 μg/ml, and 250 μg/ml.

Whole-Lung Lavage

Whole-lung lavage was performed, and lavage cells were spun and stained as previously described (7). In acute LPS exposure, mice were killed 4 h after the exposure. In chronic LPS exposures, mice were killed 3 d after the last exposure to avoid confounding acute LPS effects. Supernatants were stored at −80°C. Levels of murine TNF-α and transforming growth factor (TGF)-β in lavage fluid were measured by commercially available ELISA (R&D Systems, Minneapolis, MN).

Nitrite Level Determination

Nitrite in the whole-lung lavage fluid was measured as described previously (25) using a NOA 280 (Sievers, Boulder, CO).

Tissue Preparation

Lungs were perfused with 0.9% saline, the right lung removed, snap-frozen in liquid nitrogen, and stored at −80°C, and the left lung instilled and fixed with 4% paraformaldehyde in PBS. Tissue was embedded in paraffin, and 5 μm sections were stained as previously described (7).

Real-Time RT-PCR

Total lung RNA was isolated using the Trizol method. RT-PCR for hIL-10, murine IL-10, murine collagen I, III, and IV, fibronectin, eNOS, iNOS, neuronal NOS, and *S*-nitrosoglutathione reductase was performed using SYBR-Green assay and the 7900HT sequence detection system (Applied Biosystems, Foster City, CA). Fluorescence values for each gene were normalized to those of housekeeping genes, and expressed as fold-change over control groups.

Morphometric Analysis

Measurements of cross-sectional areas were performed on transverse bronchial sections (Masson-Trichrome stained) as previously described (7). Briefly, based on airway lumen diameter, all airway profiles were divided into small (≤ 90 μm), medium (> 90–129 μm), and large (> 129 μm). For every profile, the subepithelial area was normalized to the length of adjacent basal membrane. Measurements were averaged per animal. Data are reported as group mean ± SEM of these average values for each airway size.

Immunohistochemical Analysis

Staining for human and murine IL-10 was performed using respective goat antibodies (R&D Systems) and Vectastain ABC (Vector Laboratories, Burlingame, CA).

Statistical Analyses

All data were expressed as means ± SEM. Individual comparisons between groups were tested by the two-tailed Student's *t* test. Statistical calculations were performed using SPSS software (SPSS, Inc; Chicago, IL). Probability values of $P < 0.05$ (two-tailed) were considered statistically significant.

RESULTS

hIL-10 Attenuates Airway Inflammation after Acute LPS Exposure in Mice

First, we sought to confirm the efficacy of hIL-10 in ameliorating murine LPS–induced inflammation. After a single acute LPS inhalation challenge, IL-10–deficient mice had significantly increased cellularity and TNF-α protein in whole-lung lavage fluid compared with C57BL/6 control animals (Table 1). In contrast, expression of hIL-10 in the airways of C57BL/6 mice led to a decreased concentration of cells and TNF-α. Expression of hIL-10 in the airways of IL-10–deficient mice reconstituted the

TABLE 1. IL-10 REDUCES CELLULAR INFLAMMATION AND PROINFLAMMATORY CYTOKINE PRODUCTION IN WHOLE-LUNG LAVAGE FLUID AFTER ACUTE LPS EXPOSURE

	IL-10–Deficient	C57BL/6	IL-10–Deficient Expressing hIL-10	C57BL/6 Expressing hIL-10
Total cells /ml lavage fluid	1,134.0 ± 169.1	469.5 ± 45.8*	†594.4 ± 61.5	245.4 ± 43.5‡
Neutrophils/ml lavage fluid	1,082.6 ± 162.6	446.9 ± 46*	563 ± 59.8*	232.6 ± 43.5‡
TNF-α (pg/ml lavage fluid)	4,284.8 ± 545.9	2,864.6 ± 297.8†	1,404.3 ± 419.4*	564.9 ± 105.6‡

Definition of abbreviation: hIL = human IL.
Data are expressed as mean ± SEM.
* $P < 0.05$ compared to IL-10–deficient mice.
† $P < 0.01$ compared to IL-10–deficient mice.
‡ $P < 0.001$ compared to IL-10–deficient mice.

TABLE 2. IL-10 REDUCES CELLULAR INFLAMMATION AFTER CHRONIC LPS EXPOSURE

	IL-10–Deficient	C57BL/6	C57BL/6 Expressing hIL-10
Total cells/ml lavage fluid	813,234 ± 124,449*	479,285 ± 79,350†	263,100 ± 16,486
Macrophages/ml lavage fluid	692,222 ± 118,654	382,764 ± 72,194	230,876 ± 25,292
Percent macrophages	83.8 ± 3.2	78.8 ± 3.5	87.2 ± 6.3

For definition of abbreviation *see* Table 1.
Data are expressed as mean ± SEM.
* $P < 0.05$ compared to C57BL/6 mice and $P < 0.01$ compared to C57BL/6 hIL-10 mice.
† $P < 0.05$ compared to mice expressing hIL-10.

WT phenotype both in terms of cells and TNF-α (no statistical difference from C57BL/6 mice).

IL-10 Attenuates Airway Inflammation and Remodeling after Chronic LPS Exposure

IL-10 had a significant effect on cellular airway inflammation and remodeling after chronic LPS exposure (Table 2 and Figure 2). There was a statistically significant increase in inflammatory cells in the airways of IL-10–deficient mice, whereas the hIL-10–expressing mice had a significant decrease in cells. Additionally, real-time RT-PCR showed that murine IL-10 expression was increased by 350% in C57BL/6 mice after the end of chronic LPS exposure (Table 3). The subepithelial space in airways of IL-10–deficient mice was significantly thickened compared with that of LPS-exposed C57BL/6 mice (Figures 2 and 3A). In a separate experiment, hIL-10–expressing mice had significantly decreased thickness of the airway subepithelial area compared with mice treated with a control virus (Figures 2 and 3B). After a 4-wk recovery period, there was no difference in subepithelial thickness between IL-10–deficient and C57BL/6 mice (not shown). Expression of mRNA for collagen I, III, IV, and fibronectin was not significantly different between IL-10–deficient and control animals 3 d after chronic LPS exposure or 4 wk later (Table 3). Total levels of TGF-β1 in the lavage fluid were significantly higher in IL-10–deficient mice (616.3 ± 67.6 pg/ml versus 399 ± 43.9 pg/ml; $P = 0.02$); however, active TGF-β1 levels were similar (113.2 ± 11.6 pg/ml versus 94.6 ± 23.7 pg/ml). Mice that expressed hIL-10 in their airways had significantly decreased expression of collagen I and III in their lungs compared with control mice (Table 3).

We have previously demonstrated that chronic LPS exposure results in increased airway reactivity to methacholine even after a 4-wk recovery (7). Interestingly, APTI at that point demonstrated that LPS-treated IL-10–deficient mice had decreased airway hyperreactivity compared with C57BL/6 control animals (Figure 1). This was associated with statistically higher nitrite levels in the bronchoalveolar lavage fluid (154.4 ± 18.3 nM versus 79.4 ± 7.8 nM, $P < 0.01$), as well as increased mRNA expression of eNOS and iNOS (Table 3). Expression levels of neuronal NOS and *S*-nitrosoglutathione reductase did not differ between groups (data not shown).

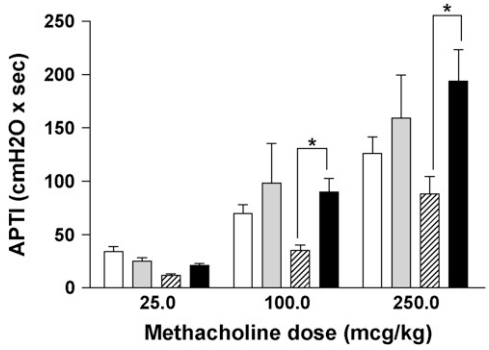

Figure 1. Airway hyperreactivity (measured as APTI) after chronic inhaled LPS exposure (*$P < 0.01$). *Open bars*, IL-10–deficient 3 d after chronic LPS; *shaded bars*, C57BL/6 3 d after chronic LPS; *striped bars*, IL-10–deficient 4 wk after chronic LPS; *solid bars*, C57BL/6 4 wk after chronic LPS.

Hematopoietic but Not Structural Cell-Derived IL-10 Is Important for the Attenuation of Airway Remodeling after LPS Exposure

To examine whether the origin of IL-10 is important for the effects on airway remodeling, we created bone marrow chimeric mice expressing IL-10 in their leukocytes but not in structural cells (WT > knockout [KO]) by engrafting bone marrow from C57BL/6 mice into IL-10–deficient mice (Figure 4F). Conversely, mice that expressed IL-10 in structural cells but not leukocytes (Figure 4C) were created by engrafting bone marrow from IL-10–deficient mice into C57BL/6 mice (KO > WT). Control groups were created by engrafting same-strain bone marrow to mice (WT > WT and KO > KO). We found that leukocyte-derived IL-10 was essential for the attenuation of airway remodeling after chronic LPS exposure. Mice with IL-10 expressing leukocytes had substantially less subepithelial thickening compared with mice with IL-10–deficient leukocytes, regardless of IL-10 expression in their pulmonary parenchyma (Figure 4). In morphometric analysis, mice expressing IL-10 in leukocytes (WT > KO and WT > WT) had significantly reduced subepithelial space thickness when compared with mice expressing IL-10 in structural cells only (Figure 3C).

DISCUSSION

Our findings indicate that IL-10 reduces LPS-induced inflammation and airway remodeling. In a bone marrow transplant model, leukocyte expression of IL-10 was sufficient to attenuate the chronic effects of inhaled LPS, whereas structural cell IL-10 expression alone was not. Furthermore, induced expression of hIL-10 in airway epithelial cells restores the protective effect of IL-10, suggesting that for therapeutic purposes, the source of airway IL-10 may be of secondary importance. Our results support the concept that IL-10 is effective in attenuating LPS-induced airway disease, suggesting that IL-10 may be therapeutic in chronic inflammatory diseases, such as asthma and chronic obstructive pulmonary disease (COPD).

This study and previous studies from this laboratory have demonstrated that repeated episodes of acute inflammation can lead to airway remodeling. We have also previously shown that reduced inflammation can attenuate chronic airway disease (7, 26, 27). In this set of studies, chronic LPS exposure increased expression of mRNA for endogenous IL-10 by 350%, whereas induced expression of IL-10 was protective from the effects of chronic LPS inhalation. Conversely, patients with chronic airway diseases, such as asthma, COPD, and cystic fibrosis, have reduced

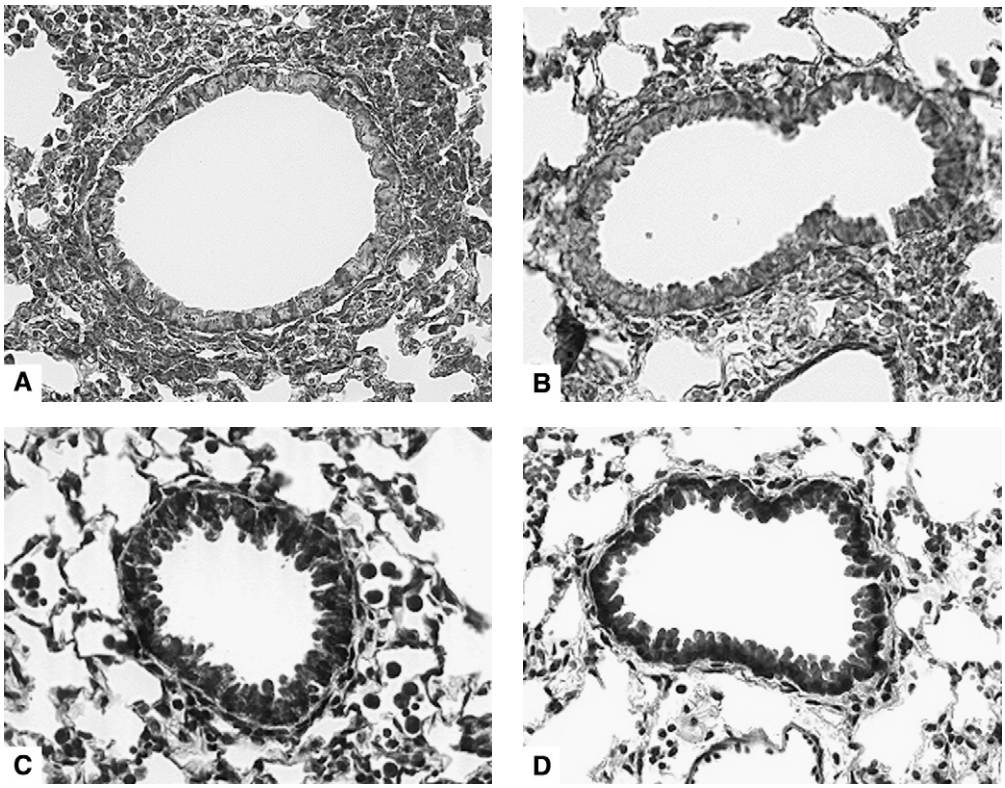

Figure 2. Airway changes after chronic LPS exposure. (*A*) IL-10–deficient mice. (*B*) C57BL/6 mice. (*C*) Mice expressing hIL-10. (*D*) C57BL/6 mice exposed to saline for same amount of time (control) (Masson-Trichrome).

endogenous IL-10 levels (17, 19), suggesting that IL-10 may be critical for maintaining lung architecture homeostasis in the face of environmental insult or injury. These patients are often colonized with gram-negative microorganisms, which are a steady source of LPS, and may be further susceptible to environmental sources of LPS (28). Such patients may be vulnerable to inhaled LPS in at least two ways: first, by having reduced baseline levels of IL-10; second, by failing to appropriately increase IL-10 expression after chronic exposure (16, 18). In our hands, induced expression of IL-10 in the airways significantly reduced the degree of inflammation after acute LPS exposure. More importantly, we demonstrated a significant decrease in airway remodeling, as well as pulmonary collagen deposition, after chronic (repeated) LPS exposure. Remodeling can account for the accelerated decline in pulmonary function observed in patients with asthma and those with COPD (29). Our results indicate that adequate control of acute inflammatory episodes will lead to decreased remodeling in the long term. Thus, exogenously administered IL-10 may prove to be useful as a therapeutic agent to decrease the persistent effects of chronic inflammatory airway diseases.

Hematopoietic cells are crucial to the response to inhaled LPS (7, 8). The present study demonstrates that hematopoietic cells can autoregulate their inflammatory response to LPS through IL-10 production. Teleologically this may be an adaptation to the continuous environmental exposure of the airways. Airway epithelial cells have decreased Toll-like receptor (TLR) 4 expression (30). Hematopoietic cells are plausibly the primary responder cells to inhaled LPS, and need to autoregulate their inflammatory response. In the ovalbumin model of allergic airway inflammation, adoptive transfer of regulatory T cells ameliorates inflammation in an IL-10–dependent manner (31). Our bone marrow transplant results indicate a similar hematopoietic cell–dependent effect of IL-10 on chronic LPS-induced remodeling. Additionally, we demonstrate that induced expression of IL-10 in the airway epithelial cells via an adenoviral vector decreases inflammation after LPS exposure. This implies that pharmacologic

TABLE 3. EVALUATION OF mRNA EXPRESSION BY REAL-TIME RT-PCR AFTER CHRONIC LPS EXPOSURE, EXPRESSED AS PERCENT OF CONTROL

	3 d after Chronic Exposure				4 wk after Chronic Exposure			
	mIL-10	Collagen I	Collagen III	Fibronectin	Collagen III	Collagen IV	eNOS	iNOS
Control	100 ± 4.5	100 ± 1.5	100 ± 3.7	100 ± 3	100 ± 0.8	100 ± 1.2	100 ± 3.7	100 ± 3.7
IL-10–deficient	ND	90.5 ± 8.7	421.6 ± 88.3*	1,576 ± 217‡	170.0 ± 38.8*	282.0 ± 22.5‡	133.9 ± 11.1*	153.1 ± 8.6*
C57BL/6	345.2 ± 94*	105.6 ± 10.7	329.1 ± 129.1*	1,375 ± 395‡	183.9 ± 57.9*	276.9 ± 46.3‡	92.9 ± 9.2	111.1 ± 11.2
C57BL/6 expressing hIL-10	NM	144.4 ± 26.7	265.0 ± 24.8*	686 ± 133‡	NA	NA	NA	NA
C57BL/6 expressing LacZ	NM	348.5 ± 72.5*	464.2 ± 113.8†	1,421 ± 410†	NA	NA	NA	NA

Definition of abbreviations: eNOS, endothelial nitric oxide synthase; hIL, human IL; iNOS, inducible nitric oxide synthase; mIL, murine IL; NA, not applicable; ND, not detected; NM, not measured.

Data expressed as mean ± SEM.

* $P < 0.05$ compared to control mice.

† $P < 0.05$ compared to hIL-10–expressing mice.

‡ $P < 0.01$ compared to control mice.

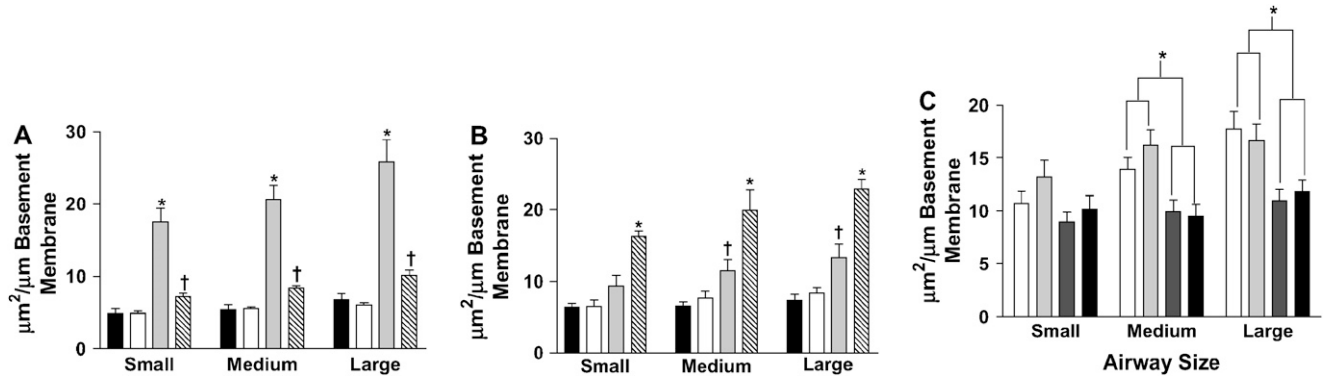

Figure 3. Stereometric measurement of subepithelial space thickness after chronic LPS exposure. (*A*) At 3 d after cessation of exposure, IL-10–deficient mice have dramatically increased subepithelial space thickness (*$P < 0.001$ compared with all other groups). C57BL/6 mice have moderate thickening of the subepithelial space (†$P < 0.001$ compared with control C57BL/6 mice; $P < 0.05$ compared with control IL-10–deficient mice). *Filled bars*, IL-10 KO PBS; *open bars*, C57BL/6 PBS; *shaded bars*, IL-10 KO PBS; *striped bars*, C57BL/6 LPS. (*B*) C57BL/6 mice expressing hIL-10 have decreased epithelial thickness compared with control mice expressing β-galactosidase (*$P < 0.001$ compared with all other groups; †$P < 0.05$ compared with control animals). *Filled bars*, AdhIL-10 PBS; *open bars*, AdLacZ PBS; *shaded bars*, AdhIL-10 LPS; *striped bars*, AdLacZ LPS. (*C*) At 3 d after cessation of exposure in the bone marrow transplantation model. Mice receiving IL-10–deficient bone marrow had significantly increased thickness of the subepithelial space of medium and large airways regardless of the recipient strain (*$P < 0.05$). *Open bars*, IL-10 KO bone marrow to IL-10 KO mice; *light gray bars*, IL-10 KO bone marrow to C57BL/6 mice; *dark gray bars*, C57BL/6 bone marrow to IL-10 KO mice; *solid bars*, C57BL/6 bone marrow to C57BL/6 mice.

delivery of IL-10 into the airways will attenuate LPS-induced inflammation regardless of the mode of delivery.

We demonstrate that IL-10 deficiency appears to protect from LPS-induced airway hyperreactivity. The protective effect of IL-10 deficiency despite increased inflammation is particularly interesting. Previous studies in the ovalbumin model of allergic sensitization resulted in the same observation (32, 33). A possible mechanism has been recently proposed by Ameredes and colleagues

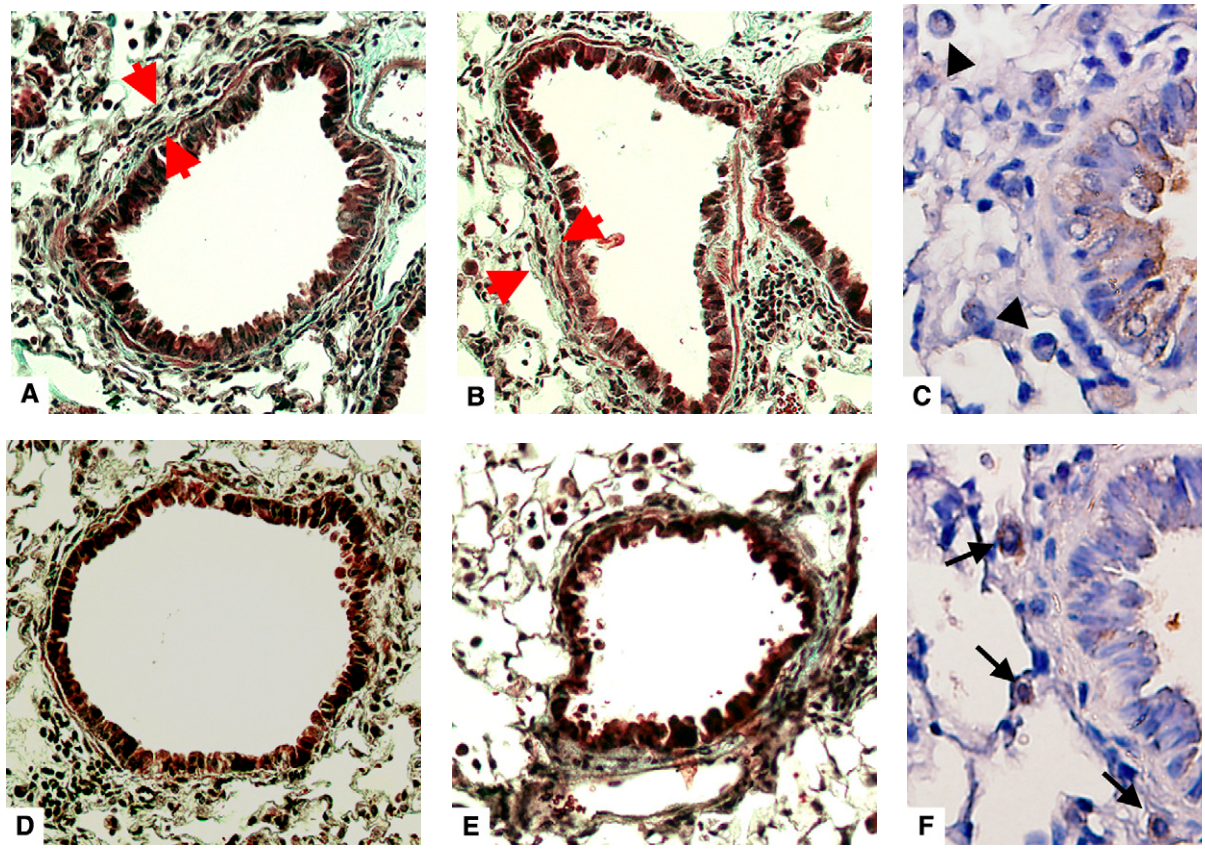

Figure 4. Airway remodeling after chronic LPS exposure, bone marrow transplantation model. (*A*) IL-10–deficient bone marrow engrafted into IL-10–deficient mice. (*B*) IL-10–deficient bone marrow engrafted into C57BL/6 mice. (*D*) C57BL/6 bone marrow engrafted into IL-10–deficient mice. (*E*) C57BL/6 bone marrow engrafted into C57BL/6 mice. Subepithelial space thickness is depicted between the arrows. (*C* and *F*) Representative immunohistochemistry for murine IL-10 in a C57BL/5 mouse receiving IL-10–deficient bone marrow (*C*) and an IL-10–deficient mouse receiving C57BL/6 bone marrow. Note absent staining from IL-10–deficient leukocytes (*arrowheads*, [*C*]) and IL-10–deficient bronchial epithelia (*F*). IL-10–positive leukocytes are also shown in (*F*) (*arrows*).

(34). These investigators showed that IL-10–deficient mouse airways had enhanced NO production and decreased baseline methacholine responsiveness. In this study of chronic LPS exposure, invasive measurement (APTI) of airway resistance demonstrated a similar decrease in airway hyperreactivity, and we detected increased levels of nitrite and expression of iNOS and eNOS in IL-10–deficient mice. Although this finding does not provide definite proof about the mechanism of hyporesponsiveness in IL-10 deficiency, it does support previous observations in the allergic model of airway inflammation (35).

In summary, we have demonstrated the important role of IL-10 in the response to chronic LPS exposure of the airways. *In vivo*, leukocyte-derived IL-10 attenuates airway remodeling after chronic inhaled LPS exposure. Induced IL-10 expression in the lung reduces LPS-induced inflammation, both in deficient mice and in WT mice. Pulmonary IL-10 expression could provide a novel therapeutic option in nonallergic asthma.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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