

Lung Parenchyma Remodeling in a Murine Model of Chronic Allergic Inflammation

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This study tested the hypotheses that chronic allergic inflammation induces not only bronchial but also lung parenchyma remodeling, and that these histologic changes are associated with concurrent changes in respiratory mechanics. For this purpose, airway and lung parenchyma remodeling were evaluated by quantitative analysis of collagen and elastin, immunohistochemistry (smooth-muscle actin expression, eosinophil, and dendritic cell densities), and electron microscopy. *In vivo* (airway resistance, viscoelastic pressure, and static elastance) and *in vitro* (tissue elastance, resistance, and hysteresivity) respiratory mechanics were also analyzed. BALB/c mice were sensitized with ovalbumin and exposed to repeated ovalbumin challenges. A marked eosinophilic infiltration was seen in lung parenchyma and in large and distal airways. Neutrophils, lymphocytes, and dendritic cells also infiltrated the lungs. There was subepithelial fibrosis, myocyte hypertrophy and hyperplasia, elastic fiber fragmentation, and increased numbers of myofibroblasts in airways and lung parenchyma. Collagen fiber content was increased in the alveolar walls. The volume proportion of smooth muscle-specific actin was augmented in distal airways and alveolar duct walls. Airway resistance, viscoelastic pressure, static elastance, and tissue elastance and resistance were significantly increased. In conclusion, prolonged allergen exposure induced remodeling not only of the airway wall but also of the lung parenchyma, leading to *in vivo* and *in vitro* mechanical changes.

Keywords: actin; collagen; remodeling; tissue mechanics

Asthma is described as an inflammatory disease that predominantly involves the large airways mainly because of scanty investigation of the small airways due to the difficulty of *in vivo* sampling. However, pathologic and physiologic evidence have emerged in the last few years suggesting that the inflammatory process extends beyond the central airways to the distal airways and the lung parenchyma (1, 2). Several studies have shown that the distal airways are responsible for airflow limitation and airway hyperresponsiveness (3–7).

Airway remodeling is applied to describe the dynamic processes that lead to structural changes in the airways in asthma.

These structural changes include: subepithelial fibrosis, mucous metaplasia, wall thickening, smooth muscle cell hypertrophy and hyperplasia, myofibroblast hyperplasia, vascular proliferation, and changes in the extracellular matrix, such as deposition of collagen fiber and elastic fiber fragmentation (8–11). The thickening around the airways together with excessive mucus secretion lead to airway narrowing and reduced lung function (12–15). The nature of airway wall remodeling has been well described in central airways. However, little is known about structural remodeling of the distal airways and lung parenchyma.

This study was undertaken to test the hypotheses that chronic allergic inflammation might induce not only bronchial but also lung parenchymal remodeling, and that this remodeling might be associated with changes in respiratory mechanics. For this purpose, lung histology, elastic and collagen fibers content in the airways and in the alveolar septa, the amount of smooth muscle-specific actin present in distal airways and alveolar duct walls, the density of dendritic cells, costimulatory molecules, and eosinophils in the airways and in the lung parenchyma were analyzed. In addition, *in vivo* and *in vitro* respiratory mechanics were measured in a murine model of chronic allergic inflammation.

Some of the results of this study have been previously reported in the form of an abstract (16, 17).

METHODS

Animal Preparation

Twenty-four BALB/c mice were randomly divided into two groups. In the ovalbumin group (OA), mice (15–20 g) were immunized using an adjuvant-free protocol by the intraperitoneal injection of ovalbumin (OVA, 10 µg) on each of 7 alternate days (18). Forty days after the beginning of sensitization, 20 µg of OVA in 20 µl sterile saline were intratracheally instilled. This procedure was performed 3 times with a 3-day interval between them. The control group (C) received saline using the same protocol.

Assessment of *In Vivo* and *In Vitro* Respiratory Mechanics

Twenty-four hours after the last challenge, animals were sedated (diazepam 1 mg intraperitoneally), anesthetized (pentobarbital sodium: 20 mg/kg intraperitoneally), and tracheotomized. Airflow, volume, and transpulmonary pressure were registered. Lung resistive and viscoelastic pressures, lung resistive plus viscoelastic pressures, static and dynamic elastances, and the difference between dynamic and static elastances were computed by the end-inflation occlusion method (19). Strips (2 × 2 × 10 mm) were cut from the periphery of the left lung of each animal in both groups and suspended vertically in a Krebs-Henseleith organ bath maintained at 37°C and continuously bubbled with 95% O₂–5% CO₂ (20–22). One end of the strip was attached to a force transducer and the other one was fastened to a vertical rod. This fiber-glass stick was connected to a woofer cone that was driven by a wave-form generator. A side arm of the rod was linked to a second force transducer by means of a silver spring of known Young's modulus, allowing the measurement of displacement. Strips were preconditioned by sinusoidally oscillating the tissue during 1 hour (21, 22). After stress adaptation, the final basal

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force was approximately 0.5×10^{-2} N and strips were oscillated at a frequency of 1 Hz. Tissue resistance, elastance, and hysteresivity (η) were calculated from the oscillatory recordings (23).

Airway Responsiveness

The airway responsiveness was measured on the basis of the response of airway resistance to increasing (10, 33, 100, and 330 $\mu\text{g/kg}$) intravenous doses of methacholine (24, 25).

Morphometric Analysis

Airway and parenchyma from the right lung and additional parenchymal strips from the left lung were used for morphometric analysis. The samples were fixed for light (26) and transmission electron microscopy. Slices (4 μm thick) were cut and underwent hematoxylin–eosin staining for morphometric analysis of lung architecture. Volume fraction of collapsed and normal pulmonary areas was determined by the point-counting technique (27, 28). Specific staining methods to quantify the collagen (Picrosirius-polarization method [29]) and elastic fibers (Weigert's resorcin fuchsin method modified with oxidation [30]) in the airways and alveolar septa were also used. The amount of smooth-muscle-specific actin (31), dendritic cells (DCs) (CD11c), and costimulatory molecule CD80 (32) was evaluated by immunohistochemistry, and the density of eosinophil was analyzed by histochemistry (33).

Statistical Analysis

SigmaStat 2.0 statistical software package (Jandel Corporation, San Raphael, CA) was used. Differences between the two groups were assessed by Student's *t* test or Mann-Whitney test for parametric and nonparametric conditions, respectively. Correlation between mechanical and histologic data was determined by the Spearman correlation test. A *p* value less than 0.05 was considered significant.

RESULTS

The survival rate was 100% in OA, but four mice from C died during the anesthesia procedure with pentobarbital sodium. During the experiment the animals did not lose weight (initial weight: 15–20 g; final weight: 20–25 g).

The fraction of area of alveolar collapse was higher in OA than in the C (Table 1). It can be seen that OA manifested smaller mean alveolar and central airway diameters (larger contraction index) than did C. Collagen fiber content in the alveolar septa ($C = 0.018 \pm 0.006 \mu\text{m}^2/\mu\text{m}$, $OA = 0.149 \pm 0.031 \mu\text{m}^2/\mu\text{m}$, $p < 0.001$) and airways ($C = 18.73 \pm 2.91 \mu\text{m}^2/\mu\text{m}$, $OA = 27.00 \pm 1.85 \mu\text{m}^2/\mu\text{m}$, $p = 0.02$) was greater in OA than in C. There was no statistically significant difference in the amount of elastic fibers in the alveolar septa ($C = 0.30 \pm 0.03 \mu\text{m}^2/\mu\text{m}$, $OA = 0.28 \pm 0.02 \mu\text{m}^2/\mu\text{m}$) and airways ($C = 3.97 \pm 0.94 \mu\text{m}^2/\mu\text{m}$, $OA = 4.68 \pm 0.73 \mu\text{m}^2/\mu\text{m}$) between the groups. However, there was fragmentation and disorganization of the elastic fibers in the airways in OA (Figure 1).

Electron microscopy of OA showed epithelial damage in the terminal bronchioles with hyperplasia of myofibroblasts, mucous cell hypertrophy, neovascularization, and fibrosis (Figure 2). In

addition, inflammatory cells including eosinophils, neutrophils, and lymphocytes, together with myofibroblasts were seen in the alveolar duct and alveolar wall (Figures 3 and 4). The alveolar interstitium was thickened because of increased amounts of extracellular matrix elements, such as collagen fibers. Myofibroblasts were frequently found together with types I and III collagen fibers. Elastic fiber content was normal in alveolar septa (Figure 1).

The volume proportion of smooth-muscle-specific actin was higher in OA in comparison to C in terminal bronchioles ($C = 26.00 \pm 1.29\%$ and $OA = 42.00 \pm 1.29\%$, $p < 0.001$) and alveolar ducts ($C = 9.00 \pm 0.81\%$ and $OA = 14.25 \pm 1.49\%$, $p = 0.015$) (Figure 5).

CD11c- and CD80-positive cells and eosinophils were significantly more abundant in the bronchial, perivascular, and alveolar wall in OA compared with C (Figure 6).

There was no statistically significant difference in flow ($C = 1.01 \pm 0.001 \text{ ml}^{-1}$ and $OA = 1.01 \pm 0.002 \text{ ml}^{-1}$) and volume ($C = 0.20 \pm 0.002 \text{ ml}$ and $OA = 0.20 \pm 0.001 \text{ ml}$) between the groups. Lung resistance and viscoelastic/inhomogeneous pressures, resistive plus viscoelastic pressures, static and dynamic elastances, the difference between dynamic and static elastances (Figure 7), tissue elastance, and resistance (Table 2) were higher in OA than in C.

Airway resistance in OA was higher than in C, even at baseline. Furthermore, only in OA was airway resistance augmented significantly with increasing doses of methacholine (Figure 8).

Considering C and OA together, viscoelastic pressure and static elastance were well correlated with the fraction of area of alveolar collapse and with the mean linear intercept between alveolar walls. Resistive pressure was correlated with the contraction index. Tissue elastance and resistance were significantly associated with the volume proportion of smooth-muscle-specific actin and with collagen fiber content in alveolar septa (Figure 9).

DISCUSSION

This study provides evidence that chronic allergic inflammation induces remodeling not only of central airways but also of distal airway units and lung parenchyma (Figures 1–4), leading to *in vivo* and *in vitro* respiratory mechanical changes (Figure 7, Table 2). A marked eosinophilic infiltration was seen in large and distal airways as well as in lung tissue. Neutrophils, lymphocytes, and DCs cells also infiltrated the lungs. Collagen fiber content increased significantly in the airway and alveolar walls. There was fragmentation and disorganization of elastic fibers in airways, and no alterations were found in lung parenchyma (Figure 1). The volume proportion of smooth-muscle-specific actin staining was increased (Figure 5). In addition, CD11c and CD80 densities (Figure 6) were increased in distal airways and alveolar duct walls. Ultrastructural analysis confirmed the histologic changes observed in light microscopy.

TABLE 1. MORPHOMETRIC PARAMETERS

Groups	Normal Area (%)	Alveolar Collapse (%)	Lm (μm)	Contraction Index
C	93.70 ± 0.77	6.30 ± 0.77	50.63 ± 4.57	1.82 ± 0.06
OA	$79.81 \pm 1.56^*$	$18.98 \pm 2.10^*$	$31.87 \pm 1.51^*$	$2.35 \pm 0.05^*$

Definition of abbreviations: C = control group; Lm = mean linear intercept between alveolar walls; OA = ovalbumin group.

Values are means $\pm$ SEM. In the OA group, mice were sensitized with ovalbumin (OVA) and exposed to repeated challenges with intratracheal instillation of OVA ($n = 12$). The C group received 0.1 ml saline using the same protocol ($n = 8$). Data were gathered from 10 random noncoincident fields per mouse.

*Values significantly different from C group ($p < 0.05$).

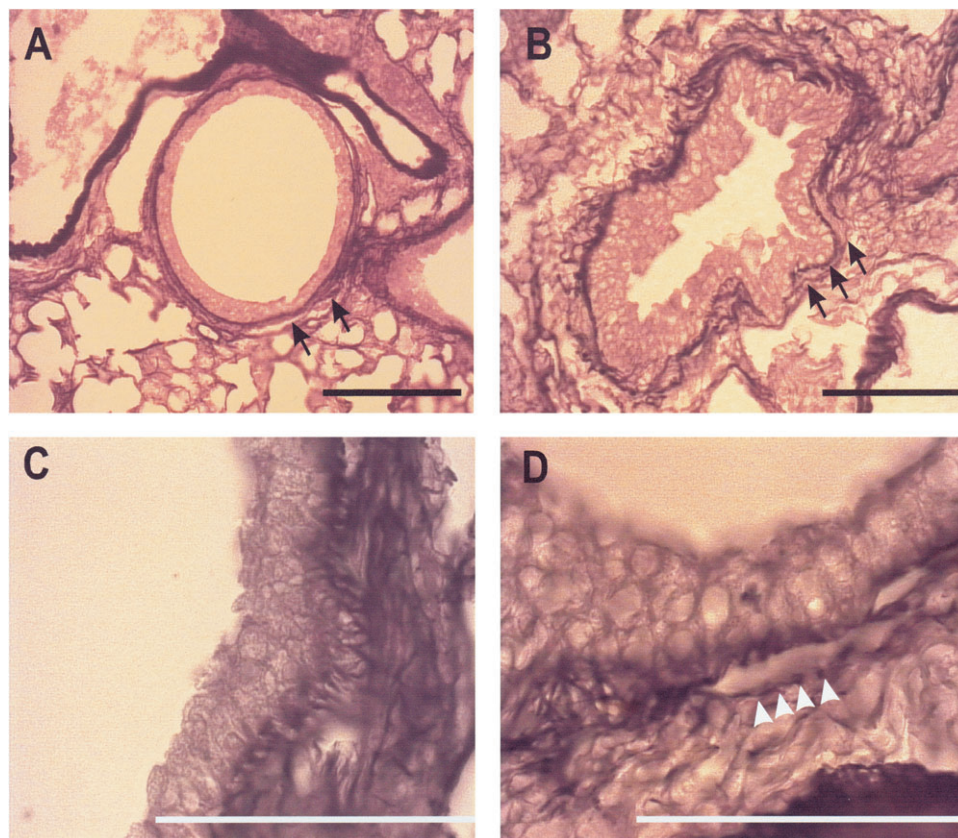


Figure 1. Representative photomicrographs of airways stained with Weigert's resorcin-fuchsin with oxidation in the control (C) group (A and C) and the ovalbumin (OA) group (B and D). In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA ($n = 12$). The C group received saline using the same protocol ($n = 8$). Elastic fibers are stained in black (arrows). In B and D note the fragmentation of elastic fibers in the OA group (arrowheads). Original magnification: $\times 400$ (A and B) and $\times 1,000$ (C and D). Bars = 100 μm .

Chronically challenged mice can develop antigen tolerance with loss of eosinophilic inflammation, avoiding the chronic inflammatory and epithelial changes observed in human asthma (15, 34, 35). The model of chronic allergic inflammation used in the present study was previously reported to reproduce many

characteristic features of the human disease, such as eosinophilic infiltration, macrophage activation, airway epithelium thickening, and goblet cell hyperplasia (18). Although this is a chronic preparation compared with the most commonly used protocols, we should be aware that the morphologic and functional changes

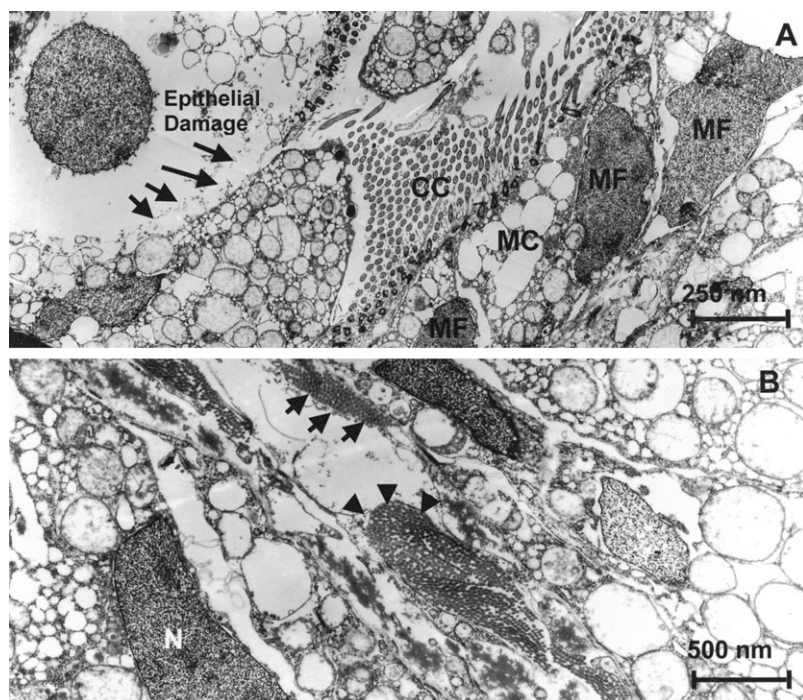


Figure 2. Electron microscopy of terminal bronchiole from mice with chronic allergic inflammation. Note the epithelial damage (arrows) (A) and the presence of myofilaments (short arrows and arrowheads) (B). In addition, in A, mucous cell (MC) metaplasia and myofibroblast (MF) hyperplasia are depicted. CC: ciliate cells; N: nucleus. The photo is representative of electron microscopic changes observed in 12 specimens. Bars = 250 nm (A) and 500 nm (B).

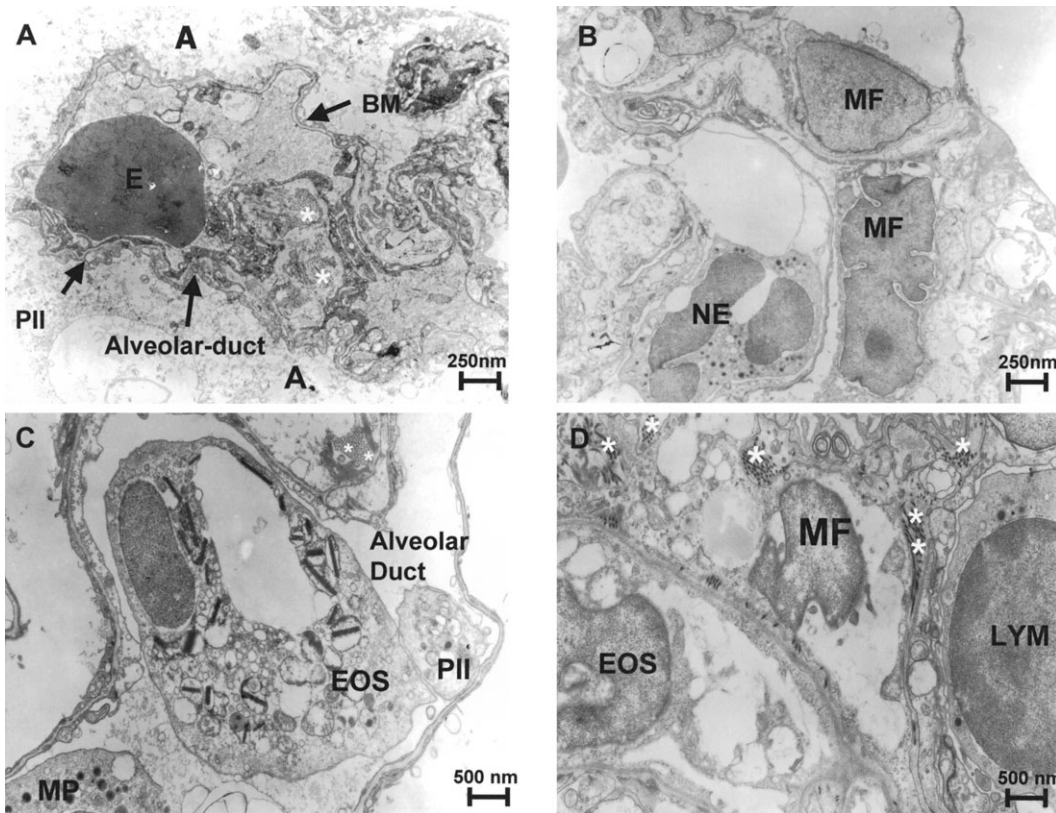


Figure 3. Electron microscopy of alveolar duct in the C group (A) and the OA group (B–D). In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA. The C group received saline using the same protocol. A: alveolar space, PII: pneumocyte type II, BM: basement membrane, E: erythrocyte, NE: neutrophil, EOS: eosinophil, LYM: lymphocyte. Numerous type III collagen fibers (asterisks) are observed in the lung interstitium of the OVA-sensitized/challenged mice. Eosinophils and lymphocytes are seen in close proximity to the collagen bundles in the OVA-treated mice. The photo is representative of electron microscopic changes observed in 12 specimens from the OA group and 8 specimens from the control group. Bars: (A and B) 250 nm; (C and D) 500 nm.

observed can potentially vary with even longer preparations as well as with different mouse strains. In the present study, mice were challenged with OVA intratracheally instead of intranasally to guarantee that each animal received the same amount of allergen.

Although the pathologic characteristics of asthma are well known, the association between these features and physiologic impairment is less clear. Airway resistance increased in the present model of chronic allergic inflammation (Figure 7). This increment was probably determined by the amount of bronchoconstriction (Table 1) as well as by airway remodeling (Figure 2). Airway responsiveness was measured by the methacholine dose–response curve, and there was a significant increase in airway reactivity (rate of increase in respiratory resistance for a given increase in methacholine dose) in OA compared with C (Figure 8). Airway remodeling is considered to have physiologic effects, including decreased distensibility of the airway (36), exaggerated narrowing of the airway lumen when smooth muscle shortens (13), and/or irreversible airflow obstruction (37). A previous study, using the forced oscillation technique, showed that mice sensitized to OVA developed increased respiratory resistance and decreased reactance (38). In our study, static elastance and viscoelastic/inhomogeneous pressure also increased in OA (Figure 7) and these data were correlated with the fraction of area of alveolar collapse and with the mean linear intercept between alveolar walls (Figure 9). The finding that peripheral airways are involved in functional changes of asthma is not new (39, 40). Studies using surgically resected lung tissue, postmortem lung specimens, and transbronchial biopsy specimens demonstrated that inflammatory and structural changes also occur in the small airways and in lung parenchyma of persons with asthma (39, 41–43). In addition, Wagers and coworkers (44), using a more acute protocol, showed increased distal lung closure without any increase in airway contractility. Thus, lung parenchyma

also plays a role in asthma. To clarify this issue, tissue mechanics were also analyzed in this murine model of chronic allergic inflammation.

Tissue resistance has been directly measured with alveolar capsules and increased with both endogenous and exogenous contractile stimulation (45, 46). The site of that response has been controversial and includes smooth muscle cells at the level of the alveolar duct, pericytes, filament-containing cells alongside capillaries, and contractile interstitial cells or myofibroblasts (47). In the present study, the lung parenchymal strip was used as a model for the study of the mechanical properties of the lung periphery. *In vitro* lung parenchyma preparation offers some potential advantages, as some of the mechanisms contributing to hysteresis *in vivo* should not be present in the *in vitro* preparation, i.e., surfactant and localized atelectasis. Thus, a direct analysis of the role of fiber–fiber networking within the connective tissue matrix on tissue mechanical properties is ensured (21–23, 48). The present study is the first analysis of tissue mechanical properties in a murine model of chronic allergic inflammation by oscillation of lung parenchymal strips. Elastance and resistance of lung parenchyma in OA were significantly increased in comparison with C, suggesting that parenchymal mechanical dysfunction plays an important role in the pathophysiology of this model (Table 2). The changes in tissue mechanics were accompanied by deposition of collagen fibers and increase in volume proportion of smooth-muscle-specific actin (Figure 9). In this context, Nagase and Ludwig demonstrated that resistance, elastance, and η increased during ovalbumin challenge in the bath in sensitized tissue strips (49). However, in the present study η did not increase in OA. The absence in η increment could be attributed to the nondisorganization in the extracellular matrix. Although there was a muscularization of small airways and an increment in collagen fiber content, they were not enough to distort lung parenchyma and increase η . Along this line, Rocco

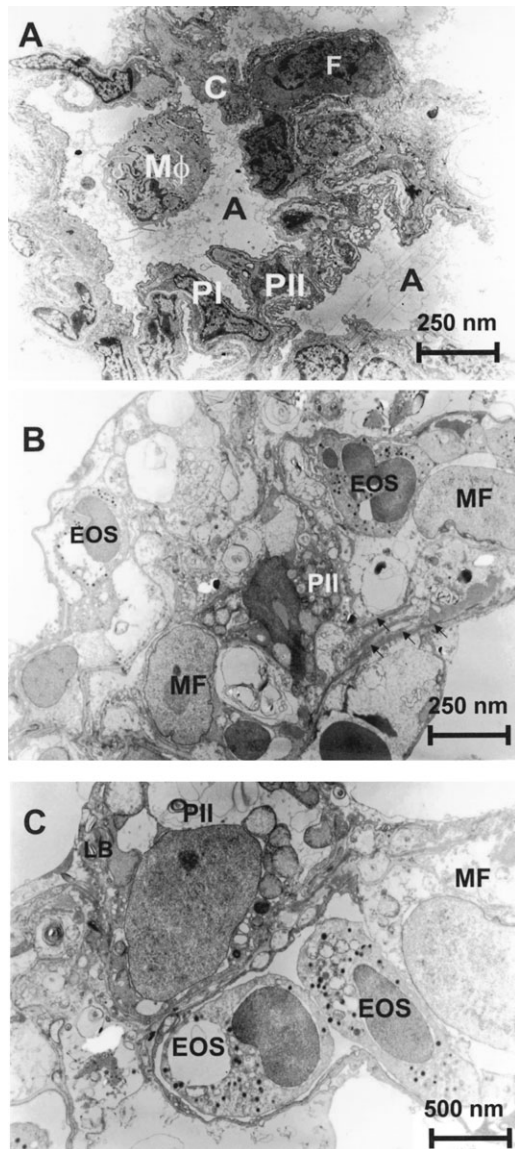


Figure 4. Electron microscopy of alveolar space in the C group (A) and OA groups (B and C). In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA. The C group received saline using the same protocol. C: capillary, A: alveolar space, F: fibroblast, M ϕ : macrophage, LB: lamellar bodies, PI: pneumocyte type I. Arrows indicate collagen fiber. The photo is representative of electron microscopic changes observed in 12 specimens from the OA group and 8 specimens from the C group. Bars: (A and B), 250 nm; (C), 500 nm.

and colleagues observed that η increases when there is an important structural modification of extracellular matrix (50).

Fibroblasts play a key role in the remodeling process of asthma (51). Although they are regarded as fixed cells of the extracellular matrix, the fibroblast number was increased in lung parenchyma of OA (Figure 4). In contrast to the fibroblast, the myofibroblast has bundles of filaments with electron-dense condensations indicative of a contractile phenotype. Myofibroblasts can potentially contribute to tissue remodeling by releasing extracellular matrix components (51). In the present study, ultrastructural analysis of lung parenchyma in OA showed an increased number of myofibroblasts in alveolar ducts and alveo-

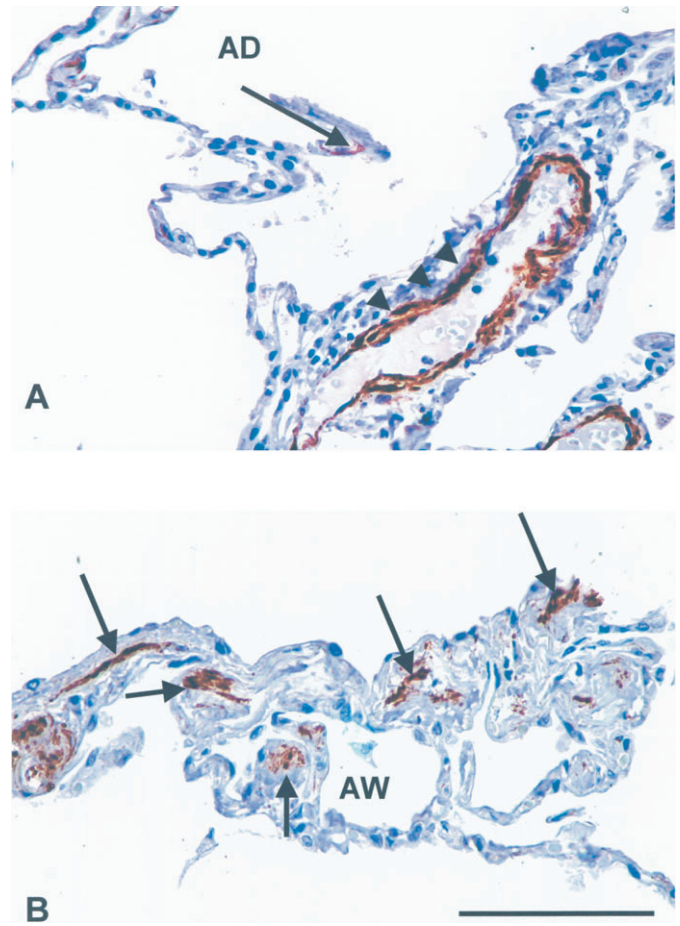


Figure 5. Immunohistochemical staining for smooth-muscle-specific actin in (A), the C group, and (B), the OA group. In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA. The C group received saline using the same protocol. Note positive staining for actin in the alveolar duct (AD) in A and in alveolar walls (AW) (arrows) in B. Arrowheads: intraacinar arteriole. Bar = 100 μ m. Analysis was performed in 12 specimens from the OA group and 8 specimens from the C group.

lar walls (Figures 3 and 4). Deposition of collagen types I, III, and V has been shown to account for basement membrane thickening in asthma (52). In our study, collagen fiber content increased significantly in OA not only in airways but also in lung parenchyma.

Elastic fibers provide support for the airway patency and lung elastic recoil. The amount of elastic fibers in airway and lung parenchyma was similar in both groups, but elastic fiber fragmentation and disorganization were present in the airway wall of animals sensitized with OA. The fragmentation of elastic fiber in the airways may be due to inappropriate repair or to mechanical distortion of damaged fibers induced by mucosal edema or elastolysis (53, 54). Delamination of elastic fibers within the subepithelial layer of the airways was reported in bronchoconstricted guinea pig airways (55), with similar configuration to that observed in the present study. These observations support the theory that elastic fibers may modulate bronchoconstriction. In addition, other studies (53, 54) showed both elastosis and elastic fiber fragmentation in airway walls of patients with fatal asthma. Changes in elastic fibers may also be influenced by the deposition of other matrix proteins like collagen, and should not be attrib-

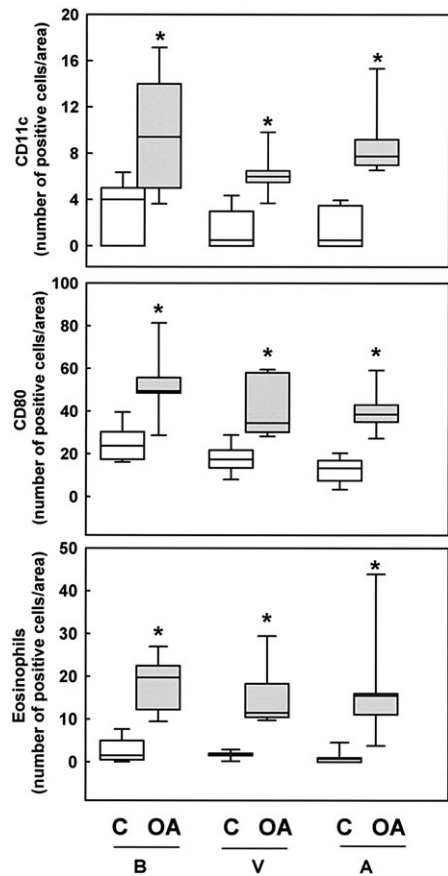


Figure 6. Box plot of eosinophil (histochemistry) and CD11c and CD80 (immunohistochemistry) in B: bronchial, V: perivascular, and A: alveolar wall. In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA (n = 12). The C group (n = 8) received saline using the same protocol. Boxes show interquartile range, whiskers show range, and horizontal lines represent median values. * Values significantly different from the C group (p < 0.05).

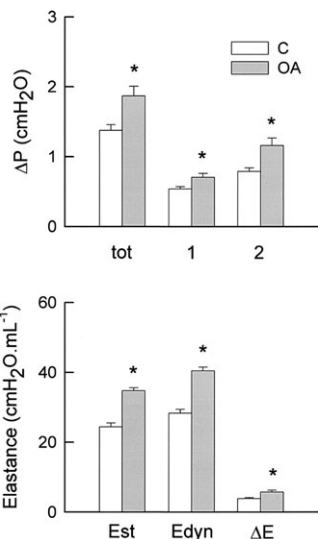


Figure 7. Bars are means ± SEM of 12 animals in the OA group and 8 animals in the C group (10 determinations per animal). In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA. The C group received saline using the same protocol. ΔP_{tot}, ΔP₁, and ΔP₂ = total, resistive, and viscoelastic/inhomogeneous pressures, respectively. Est = pulmonary static elastance; Edyn = pulmonary dynamic elastance; ΔE = difference between dynamic and static elastances. * Values significantly different from the C group (p < 0.05).

TABLE 2. TISSUE MECHANICAL PARAMETERS			
Groups	E (10 ⁴ N·m ⁻²)	R (10 ² N·s ⁻¹ ·m ⁻²)	η
C	1.12 ± 0.03	1.03 ± 0.04	0.063 ± 0.001
OA	1.35 ± 0.05*	1.25 ± 0.05*	0.060 ± 0.002

Definition of abbreviations: C = control group; E = tissue elastance; η = tissue hysteresivity; OA = ovalbumin group; R = tissue resistance. Values are means ± SEM. In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA (n = 12). The C group received 0.1ml saline using the same protocol (n = 8). *Values significantly different from C group (p < 0.05).

uted solely to increased degradation (56). The absence of elastosis in the present model of chronic allergic inflammation could be attributed to the fact that the present degree of lung inflammation was not enough to induce elastogenesis. In addition, we cannot rule out the possibility that elastogenesis could happen late in the course of lung disease (50).

The volume proportion of smooth-muscle-specific actin was prominent in terminal bronchioles and alveolar ducts (Figure 5) and was associated with chronic allergic inflammation and tissue elastance and resistance (Figure 9). These findings are contrary to those reported by Dolhnikoff and colleagues (31). The discrepancies between the two studies could be attributed to the extracellular matrix modification together with the increased amount of smooth-muscle-specific actin in the present study, which were not found in the study by Dolhnikoff and colleagues. Smooth muscle cells have been also implicated as modulators of inflammation in asthma. In addition to contractile responses and mitogenesis, airway smooth muscle cells have the potential to alter the composition of the extracellular matrix environment and orchestrate key events in the process of chronic airway remodeling (57).

Th2 cells play a key role in the initiation and maintenance of lung inflammatory responses in asthma (58, 59). Previous studies demonstrated that DCs are implicated in the capture and presentation of allergens to T cells (8, 60–62). In accordance with the literature (62), in the present study the number of DCs was higher in the airway and alveolar walls in OA. Costimulatory molecules expressed by immunocompetent cells are involved in Th2 responses and the maintenance or amplification of lung inflammatory responses in allergic asthma (63). Our data showed that costimulatory molecule (CD80) density was increased not only in the airways but also in the lung parenchyma (Figure 6). A previous study reported that DCs might play a pivotal role

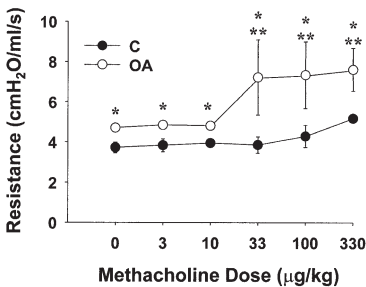


Figure 8. Airway responsiveness in BALB/c mice after chronic exposure to saline or OVA. Airway resistance was measured in response to increasing doses of methacholine. In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA. In the C group, mice received saline using the same protocol. Data are presented as means ± SEM of eight animals in each group. * Values significantly different from the C group (p < 0.05). ** Values significantly different from the lower doses of methacholine (< 33 μg/kg) (p < 0.05).

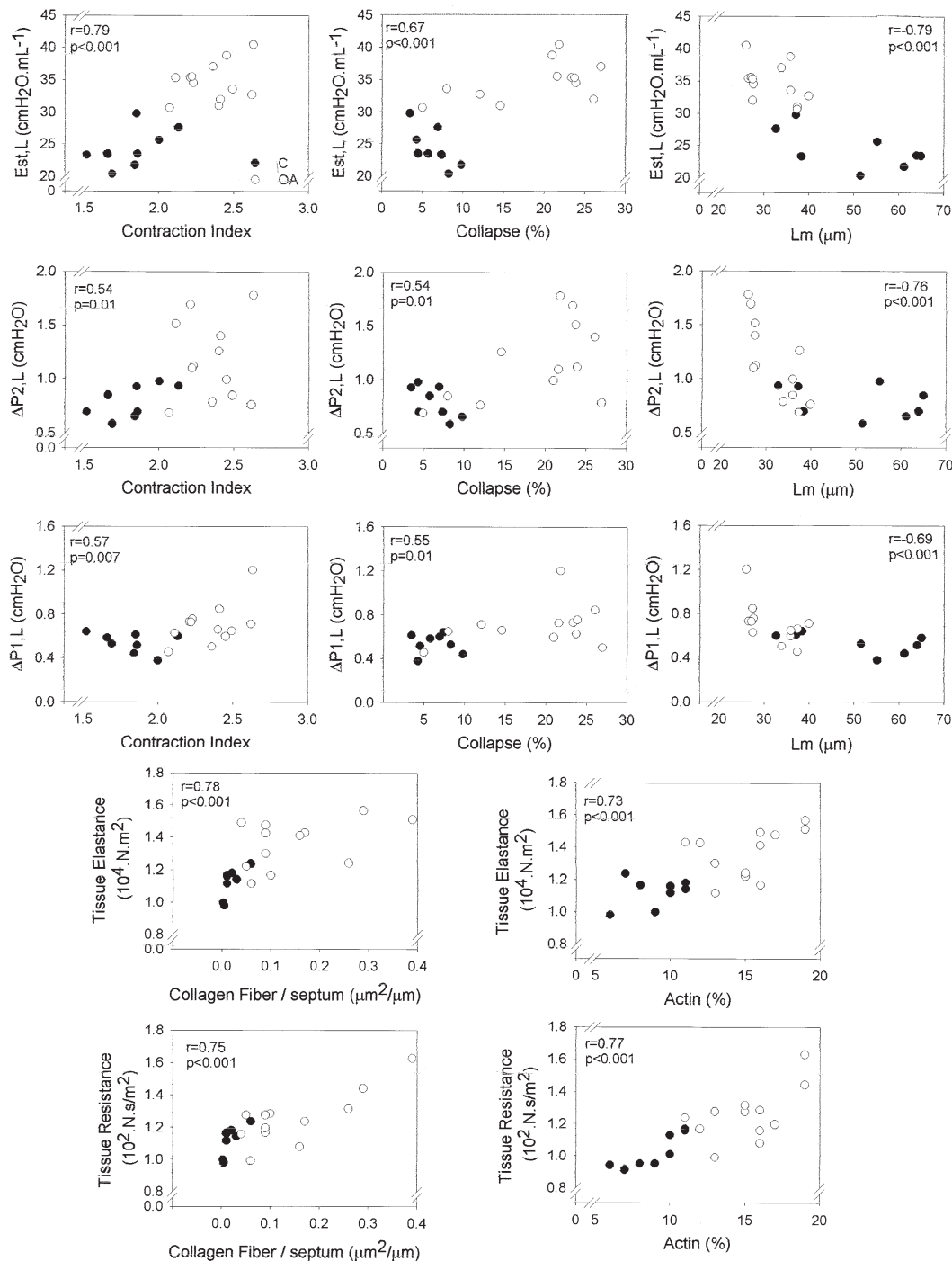


Figure 9. Correlations between mechanical and morphological data obtained through Spearman's correlation test in the C group (closed circles, $n = 8$), and the OA group (open circles, $n = 12$). In the OA group, mice were sensitized with OVA and exposed to repeated challenges with intratracheal instillation of OVA. The C group received saline using the same protocol. Static elastance (Est,L), resistive ($\Delta P1,L$), and viscoelastic ($\Delta P2,L$) pressures values correlated with the contraction index, the amount of alveolar collapse, and the mean linear intercept between alveolar walls (Lm). Tissue elastance and resistance values correlated with changes in collagen fiber content and volume proportion of smooth-muscle-specific actin (actin). Collagen fibers were expressed as the amount of each fiber per unit septum length ($\mu m^2/\mu m$).

in the maintenance and amplification of the allergic immune response via upregulation of CD80 (64). Burastero and colleagues found that allergic individuals have a significantly higher proportion of alveolar macrophage expressing CD80 than control subjects (65). We hypothesized that DCs in the lung parenchyma might play an important role in the maintenance of lung inflammation and remodeling via CD80.

Increased numbers of lymphocytes, eosinophils, and neutrophils were distributed along the distal airways compared with those in C mice (Figures 2–4). Eosinophilic infiltration was observed in lung parenchyma and in large and distal airways (Figure 6), as previously reported by many authors (18, 66–69). Tissue

eosinophilia and eosinophil degranulation have been associated with several fibrotic syndromes (70, 71), and eosinophils are the major source of tissue remodeling factors, such as transforming growth factor- α , transforming growth factor- β , and vascular endothelial growth factor (72, 73). Therefore, eosinophils stimulate myofibroblast differentiation through the production of transforming growth factor- β and contribute to airway remodeling (74–76). Lymphocytes may also play a role in pulmonary fibrosis through their cytotoxic effect on lung parenchymal cells (77). Although it is still unknown whether T lymphocytes can directly cause tissue damage and remodeling in asthma, these cells contribute to the pathogenesis of structural changes of the airways

and probably in lung parenchyma by orchestrating the recruitment and activation of inflammatory cells. In addition, neutrophils could also participate in the airway remodeling in asthma (78).

In conclusion, we have demonstrated that the present murine model of chronic allergic inflammation is characterized by remodeling not only of the airway wall but also of the lung parenchyma, leading to *in vivo* and *in vitro* mechanical changes. Although the results cannot be extrapolated directly to humans, they suggest that therapeutic strategies against remodeling also need to reach distal airways and lung parenchyma.

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