Downregulation of Caveolin-1 in a Murine Model of Acute Allergic Airway Disease

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Key Words
acute allergic airway disease; airway remodeling; caveolin; collagen; mouse model

Background: Airway remodeling refers to the structural changes in the airways of asthma. Caveolin-1 reduces cell growth and negatively regulates smooth muscle cell proliferation. The aim was to investigate lung caveolin-1 status in a murine model of acute allergic airway disease.

Methods: Six- to eight-week-old female BALB/c mice were sensitized by intraperitoneal injections of phosphate-buffered saline or ovalbumin (OVA) and aluminium hydroxide on Days 0 and 14, challenged with aerosolized saline or OVA (1%) on Days 21–25, 28–32, and 35. The mice were killed 1 day after the last OVA/saline challenge. Serum OVA-specific immunoglobulin E (IgE) was measured by enzyme-linked immunosorbent assay. Peribronchial inflammation was quantified by morphometric analysis. Lung caveolin-1 and Type I collagen mRNA expression was determined by real-time reverse-transcription polymerase chain reaction. Total lung collagen was measured using Sircol Assay Kit.

Results: Serum OVA-specific IgE levels were significantly elevated in OVA-challenged mice when compared with saline-challenged mice. Percentage of inflammatory cells in the bronchoalveolar lavage was significantly higher in the OVA-challenged animals. The animals’ lungs that were sensitized and challenged with OVA contained large numbers of inflammatory cells concentrated near the airways and in the perivascular areas. The thickness of the bronchial epithelial layer and smooth muscle layer and the numbers of total inflammatory cells and eosinophils significantly increased in OVA-challenged mice. Caveolin-1 mRNA expression...
1. Introduction

Asthma affects approximately 6% of the children.\(^1\) It is characterized by the presence of increased numbers of lymphocytes, eosinophils, and activated mast cells in the airway.\(^2\) In addition to the presence of inflammatory cells in the airway, the airways demonstrate varying levels of structural changes termed airway remodeling.\(^3,4\) Structural changes include thickening of basement membrane, subepithelial fibrosis, increased smooth muscle mass, and increased airway vascularity.\(^5,6\) Airway remodeling is associated with poorer clinical outcome in pediatric patients with asthma.\(^7\) Early diagnosis and prevention of airway remodeling has the potential to lessen disease severity and to prevent disease expression. Currently, no effective therapy is clinically available to prevent these structural changes. Therefore, it is important to explore the mechanisms and seek new strategies for the prevention and treatment of airway remodeling.

Caveolae are 50- to 100-nm omega-shaped invaginations of the plasma membrane in epithelial cells, endothelial cells, adipocytes, fibroblasts, smooth muscle cells, and striated muscle cells.\(^8,9\) Caveolae's functions range from endocytosis and transcytosis to signal transduction.\(^10–12\) Caveolin-1 is the principal structural component of caveolae. Caveolin-1 can reduce cell growth and increase apoptosis by inhibiting the activation of growth factor receptors and their downstream signaling pathways.\(^9\) In addition, caveolin-1 negatively regulates smooth muscle cell proliferation.\(^13\) The aims of this study were to investigate the lung caveolin-1 status and the relationship of lung caveolin and collagen and structural changes in a murine model of acute allergic airway disease.

2. Materials and Methods

2.1. Animal models

Six- to eight-week-old female BALB/c mice received standard animal care under the supervision of Institutional Animal Care and Use Committee at Taipei Medical University. The mice (n = 7) were sensitized by intraperitoneal injection of 50-µg Grade V chicken egg ovalbumin (OVA; Sigma Chemical Co., St. Louis, MO, USA) and 4 mg aluminium hydroxide (Imject Alum; Pierce, Rockford, IL, USA) in 0.2 mL saline on Days 0 and 14. The mice then were challenged with aerosolized OVA (1%) for 30 minutes on Days 21–25, 28–32, and 35. Aerosol challenge was performed in a whole-body inhalation exposure system attached to an ultrasonic nebulizer (PARI GmbH, Starnberg, Germany). Control mice (n = 8) were sensitized with 4 mg of aluminium hydroxide in 0.2 mL phosphate-buffered saline and challenged with aerosolized saline solution. The mice were killed 1 day after the last OVA/saline challenge. Serum was obtained through cardiac puncture of anaesthetized mice and was stored at −20 °C for OVA-specific IgE measurement.

2.2. Serum OVA-specific IgE

The OVA-specific IgE levels in serum were measured by a modified enzyme-linked immunosorbent assay.\(^14\)

2.3. Bronchoalveolar lavage

Immediately after cardiac puncture, the lung was removed, with the tracheostomy tube in place. The lungs were instilled with 1 mL of ice-cold saline, which was washed in and out of the lungs three times and then recovered. This washing procedure was repeated two more times before recording the total volume of the three washes. Differential cell counts of bronchoalveolar lavage fluid (BALF) were performed on cytocentrifuge preparations (Cytospin 3; Shandon Scientific, Cheshire, UK) stained with Liu’s stain (Tonyar, Diagnostic Inc., Taipei, Taiwan) using standard morphological criteria.

2.4. Morphometric analysis

Seven-µm lung sections were embedded in paraffin and stained with hematoxylin and eosin. Three bronchi measuring 150–350 µm in luminal diameter per mouse were analyzed for the thicknesses of the epithelium and the smooth muscle layers. Peribronchial inflammation was quantified by counting the number of inflammatory cells and eosinophils surrounding the airways and normalizing for airway size by dividing the square of the perimeter of the basement membrane.\(^15\)

2.5. Real-time reverse-transcription polymerase chain reaction

Lung caveolin-1 and Type I collagen mRNA expression was determined by reverse transcription, followed by real-time polymerase chain reaction (PCR) using appropriate primers (Table 1). Total RNA was extracted using TRIzol Reagent (Invitrogen Life technologies, Carlsbad, CA, USA) and treated with RNAase-free DNAase (Sigma-Aldrich, St. Louis, MO, USA). PCR reactions under conditions of 95 °C for 10 minutes (1 cycle), 94 °C for 15 seconds, and 60 °C for 1 minute (40 cycles) were performed on an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster...
City, CA, USA). Data were quantified using the comparative threshold cycle method with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference housekeeping gene and were expressed relative to the saline group. Triplicate experiments were carried out for each sample.

2.6. Lung collagen determination

Total soluble collagen in lung tissue was measured using the Sircol Collagen Assay Kit (Biocolor, Belfast, UK). The collagen content in each specimen was obtained by averaging three readings.

2.7. Statistical analysis

Results are presented as the means ± standard deviations. Comparisons between control and sensitized–challenged groups were made by nonparametric Mann-Whitney U test. Differences were considered significant at \( p < 0.05 \).

3. Results

3.1. OVA-specific IgE

The OVA-specific IgE levels in serum were significantly elevated by approximately 13-fold in sensitized mice challenged with OVA when compared with saline-challenged mice (Figure 1).

3.2. Inflammatory cell infiltrate in BALF

Differential cell count of BALF revealed a significantly increased percentage of eosinophils, neutrophils, lymphocytes, and macrophages in the OVA-challenged animals (Table 2).

3.3. Lung histology and morphometric analysis

The animals’ lungs that were sensitized and challenged with OVA contained large numbers of inflammatory cells concentrated near the airways and in the perivascular areas (Figure 2A). The thicknesses of the bronchial epithelial layer and the smooth muscle layer, and the numbers of total inflammatory cells and eosinophils significantly increased in OVA-challenged mice (Figure 2B).

3.4. Caveolin-1 and type I collagen mRNA expression and total collagen content

Caveolin-1 mRNA expression significantly decreased and Type I collagen mRNA expression significantly increased in OVA-challenged mice (Figures 3A and 3B). In this study, we measured only Type I collagen expression because it constitutes greater than 65% of the total lung collagen in normal human lung. Total lung collagen content increased after OVA challenge, though the difference was not statistically significant (Figure 3C).

4. Discussion

Airway remodeling refers to structural changes in airways of asthma, which include subepithelial fibrosis, elevated numbers of inflammatory cells, increased amounts of airway smooth muscle, and increased vascularization of the airway wall. In this study, the structural changes of our in vivo model are consistent with alterations known to occur in airway remodeling. The main findings of this study are that airway remodeling is associated with downregulation of caveolin-1 expression and upregulation of Type I collagen mRNA expression.

Caveolae is rich in proteins and lipids, such as cholesterol and sphingolipids, and have several functions in signal

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Oligonucleotide sequences of the primers used</th>
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<tr>
<td>Primer</td>
<td>Sequence 5’ → 3’</td>
</tr>
<tr>
<td>Caveolin-1 Forward</td>
<td>306GAAGGGACACACAGTTTCGAC328</td>
</tr>
<tr>
<td>Collagen-1 Forward</td>
<td>164GCCAAGAAGAATCCCTGAAG187</td>
</tr>
<tr>
<td>GAPDH Forward</td>
<td>97GAATGGGAAGCTTGTCATCAACGG121</td>
</tr>
<tr>
<td>Reverse</td>
<td>406GGATGCCGAAGATGATGACAC187</td>
</tr>
<tr>
<td>Reverse</td>
<td>307TCATTGCATTCGACGTCATC283</td>
</tr>
<tr>
<td>Reverse</td>
<td>204GTAGACTCCACGACATCAGCA180</td>
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transduction, endocytosis, and oncogenesis.\textsuperscript{10–12} Formation and maintenance of caveolae is primarily because of caveolin. The caveolin gene family has three members in vertebrates: CAV1, CAV2, and CAV3, coding for the proteins caveolin-1, caveolin-2, and caveolin-3, respectively. The primary caveolae protein expressed in airway smooth muscle is caveolin-1.\textsuperscript{9} The physiological importance of caveolin-1 in the lungs has been emphasized in airway

### Table 2 Bronchoalveolar lavage fluid differential cell counts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Eosinophils (%)</th>
<th>Neutrophils (%)</th>
<th>Lymphocytes (%)</th>
<th>Macrophages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7</td>
<td>0.2 ± 0.3</td>
<td>0.3 ± 0.5</td>
<td>2.1 ± 2.8</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>OVA</td>
<td>8</td>
<td>1.1 ± 0.3*</td>
<td>38.9 ± 3.0\textsuperscript{y}</td>
<td>7.3 ± 2.3*</td>
<td>3.1 ± 0.9\textsuperscript{z}</td>
</tr>
</tbody>
</table>

Differential cell counts in bronchoalveolar lavage fluid were performed on cytocentrifuge preparations using standard morphological criteria. The results were expressed as the percentage of total cells. Values are means ± standard deviations.

\*p < 0.01, \textsuperscript{y}p < 0.001, and \textsuperscript{z}p < 0.05 vs. the saline group.

OVA = ovalbumin.

Figure 2  (A) Representative photomicrographs (×100) and (B) morphometric analysis of structural changes and inflammatory cell counts in the airway wall of saline-challenged (n = 7) and OVA-challenged (n = 8) mice. Three bronchi measuring 150–350 μm in luminal diameter per mouse were analyzed for the thicknesses of the epithelium and the smooth muscle layers, and the total inflammatory cells and eosinophils surrounding the airways. (A) The lungs of animals that were sensitized and challenged with OVA contained large numbers of inflammatory cells concentrated near the airways and in the perivascular areas. (B) The thickness of the bronchial epithelium and smooth muscle layer and the numbers of total inflammatory cells and eosinophils increased significantly in OVA-challenged mice (\*p < 0.01 vs. the saline group). OVA = ovalbumin.
remodeling of allergen-challenged mouse model.\textsuperscript{17} In that pathology, decreased expression of the gene encoding caveolin-1 was associated with increased lung collagen deposition. In this study, we found that increased lung collagen mRNA expression and smooth-muscle-layer thickness was associated with decreased caveolin-1 expression.

Fibrosis is one of the predominant characteristics of airway remodeling, which appears by the thickening of the bronchial basement membrane, leading to subepithelial fibrosis.\textsuperscript{18} Fibrogenesis is characterized by the recruitment of inflammatory cells, which release inflammatory mediators and growth factors, leading to the activation and proliferation of fibroblasts and accumulation of extracellular matrix.\textsuperscript{19,20} Collagen is the major extracellular matrix component of the lungs and is vital for maintaining normal lung architecture. In this study, sensitization and challenge of BALB/c mice with OVA produced a significant increase (approximately onefold) in Type I collagen mRNA expression compared with that in the saline group. Total collagen content showed a similar trend, but the difference was not statistically significant. We speculate that the dissociated pattern might be the result of the insufficient increase in mRNA expression in generating significant product or post-transcriptional regulation.

There are two limitations in this study. First, we primarily measured the mRNA levels. Although mRNA levels are important regulators of the protein levels, other regulatory elements downstream of mRNA levels might be operative. It is still conceivable that this may also have affected, to some extent, the protein synthesis in the lungs investigated in our study. Second, caveolin-1 status in a chronic model of allergic airway disease was not measured and will need to be addressed in future experiments.

In conclusion, this study found decreased caveolin-1 expression in an acute model of allergic airway disease. These results suggest that caveolin-1 seems to be involved in the pathogenesis of airway remodeling, and caveolin-1 supplementation might prevent or treat airway remodeling.

References


