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Extra domain-A fibronectin is necessary for the development of nasal remodeling in chronic allergen-induced rhinitis

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ABSTRACT

Background: Extra domain A-containing fibronectin (EDA-FN) is necessary for the development of allergen-induced lower airway fibrosis. The pathogenesis of fibrosis in allergic rhinitis has not been well studied.

Objectives: To determine whether EDA-fibronectin is necessary for the development of nasal remodeling in a murine model of chronic allergic rhinitis and in human allergic rhinitis.

Methods: EDA(-/-) and wild-type (WT) C57Bl/6 mice were sensitized intraperitoneally and then challenged with inhaled ovalbumin (OVA) or saline for 2 and 5 weeks. Clinical signs of rhinitis and histological analysis of nasal tissue were evaluated. Immunohistological staining for EDA-FN was performed in human tissue of inferior nasal conchae from patients with allergic rhinitis and controls.

Results: After 2 weeks of allergen exposure, only goblet cell hyperplasia and perivascular eosinophilia were observed. After 5 weeks, goblet cell number, thickening of the subepithelial layer, and extent and area of collagen deposition were increased in the nasal tissue of WT OVA (ovalbumin)-challenged mice as compared with saline controls ($P < .0001$, $P < .0001$, $P = .018$, and $P = .03$, respectively). Clinical signs of rhinitis were observed only in WT OVA-challenged mice. In the EDA(-/-) mice exposed to OVA, collagen deposition, collagen area, and subepithelial thickness showed no increase and were similar to saline control mice, whereas goblet cell hyperplasia was similar to WT OVA-challenged mice. EDA-FN expression was prominent in inferior conchae from patients with allergic rhinitis but was absent in control patients.

Conclusion: EDA-containing fibronectin is necessary for the development of nasal tissue fibrotic remodeling process in both murine and human allergic rhinitis.

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Introduction

Allergic rhinitis is the most common form of noninfectious rhinitis and is associated with allergen-specific immunoglobulin E-mediated immune response.¹ Allergic rhinitis is a major global health problem, causing illness and disability worldwide.²

Epidemiologic, clinical, and immunopathologic data demonstrate an important link between upper and lower airway diseases, especially the well-recognized association of allergic rhinitis and asthma.² The nasal and bronchial mucosa share many similarities, and most patients with asthma also have rhinitis, giving rise to the concept of “one airway, one disease.”³

The development of structural changes in the airway of patients with asthma, termed “airway remodeling,” is thought to contribute

to severity of disease and refractoriness to therapy. These changes include epithelial disruption, goblet cell hyperplasia, subepithelial fibrosis, myofibroblast accumulation, increased smooth muscle, and neovascularity.^{4–6} The presence of eosinophilic inflammation and goblet cell hyperplasia in the nasal mucosa are characteristic features of allergic rhinitis.⁷ In contrast, remodeling changes in the nasal mucosa remain poorly characterized.

The subepithelial fibrosis observed in chronic asthmatic airways is composed of excessive deposition of extracellular matrix proteins.^{8–10} Fibronectin is a 440-kd dimeric major extracellular matrix glycoprotein that exists in several isoforms derived from alternative splicing of the fibronectin gene. Fibronectin containing the “extra-domain-A” or “EDA” is important for fibroblast differentiation to an activated myofibroblast phenotype. Increased EDA-containing FN (EDA-FN) levels have been reported in fibroblasts isolated from bronchoalveolar lavage (BAL) fluid of patients with asthma¹¹ and in bronchoalveolar lavage fluid of ovalbumin (OVA)-treated mice.¹² Using EDA-/- mice, we have previously demonstrated

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that EDA-FN is essential for the development of airway fibrosis and airway hyperresponsiveness in a model of chronic experimental asthma. OVA-treated EDA-/- mice are protected from the development of airway fibrosis, presumably through direct action on lung fibroblasts.¹³

In the current study, we describe the clinical and histological features of rhinitis in C57BL/6 mice in response to short-term (2 weeks) and more prolonged (5 weeks) allergen exposure. We demonstrate that allergen-exposed EDA-/- mice develop less nasal fibrosis than OVA-exposed WT mice, although epithelial goblet cell hyperplasia is unchanged. We also observed that nasal tissue from patients with chronic allergic rhinitis show prominent immunostaining for EDA-FN. These findings suggest that nasal tissue fibrosis is a prominent feature of chronic allergic rhinitis and that EDA-FN is necessary for development of this process.

Methods

Murine Model

Female C57BL/6 WT mice aged 8 to 10 weeks were purchased from Harlan Laboratories Ltd (Jerusalem, Israel). The Hebrew University–Hadassah Medical School Animal Ethics Committee approved all experimental protocols. EDA-/- mice have been previously described.¹⁴

Allergen Challenge Protocol

Mice were housed under specific pathogen-free conditions and provided adequate food and water. Four groups of mice were used, 9 to 11 mice/group: wild-type (WT) treated with OVA or saline and EDA-/- mice treated with OVA or saline. Animals were sensitized with intraperitoneal OVA (10 µg OVA/1 mg Al [OH]₃ in 0.5 mL 0.9% saline) (Sigma, St. Louis, MO), or with saline on days 1 and 10. Mice were then challenged with inhaled saline or OVA (2% weight/volume diluted in 0.9% saline; 4 mL/inhalation) 3 times per week for 2 or 5 weeks, starting on day 15 (Fig 1). For OVA and saline challenges, mice were transferred into a Perspex 30 x 40 x 40-cm box and exposed to saline or OVA, which were administered by means of a micromist nebulizer (Hudson RCI, Temecula, California) feeding directly into the box, with a flow rate of 7 L/min for 20 minutes. The box contained additional holes for air circulation. Mice were killed 24 hours after the last challenge.

Preparation of Murine Nasal Tissue

The skulls of the mice were skinned, and the nasal portion was excised from the head along the line of both eye sockets. The tissues were fixed in 4% buffered formaldehyde for 48 hours at room temperature. Specimens were then decalcified for 48 hours in sodium citrate (50%) and formic acid (50%) solution. The decalcified nasal cavity was transversely sectioned twice at the level of the

inferior conchae and vomeronasal cavity, and paraffin blocks were prepared. The blocks were sliced into 5-µm-thick sections and stained with hematoxylin-eosin to evaluate the general morphology (BX50; Olympus, Tokyo, Japan).

Clinical signs of rhinitis. Sneezes and nasal itching motions (nasal rubbing) were counted over a 5-minute interval 1 hour after saline/OVA inhalation was performed on the day before sacrifice.^{15,16}

Histological analysis. Goblet cells were visualized on periodic acid-Schiff (PAS)-stained nasal mucosa. Specimens were also stained with Masson-trichrome to visualize collagen distribution. For the sake of uniformity, histological evaluation was performed on tissue sections of the same area of the nasal septum in all mice. The analysis was conducted by 2 pathologists, blinded to the treatment groups of the mice.

Goblet cell number was determined by light microscopy as the average number of goblet cells in 4 different high-power fields using PAS stain. Tissue eosinophilia was demonstrated by hematoxylin-eosin stain using light microscopy 40× magnification. Inflammatory cells were evaluated in a semiquantitative manner after 2 and 5 weeks' OVA exposure. Collagen fibers were visualized using light microscopy (low magnification ×4) on Masson's trichrome-stained sections. Collagen distribution along the nasal septum was semiquantified and considered to be either "partial" (above the vomeronasal organ only) and scored as "0" or considered to be "complete" (along the entire nasal septum, reaching the nasal roof) (scored as "1"). Quantitative evaluation of collagen deposition was performed using Image-Pro version 6.0 (Media Cybernetics, Warrendale, Pennsylvania) scientific image processing and analysis software. The area that stained positively for collagen (Masson's trichrome) in the subepithelial layer of the nasal septum was calculated as a percentage of the total subepithelial layer area for each animal. We defined the subepithelial layer as the area between the epithelium and the cartilage of the nasal septum. Thickness of the subepithelial layer was measured using hematoxylin-eosin-stained tissue sections using light microscopy (×100). Subepithelial thickness was measured from the basal lamina to the perichondrium. To standardize our measurements, 2 sites along the nasal septum were selected; just above the vomeronasal organ and at the middle point of the septum. Results are the average of these measurements.

Human nasal tissue. The use of human nasal tissue was approved by the hospital ethics committee Internal Review Board. Nasal tissue was obtained from surgical specimens resected for medical indications. The study group included 4 patients suffering from refractory chronic allergic rhinitis who underwent therapeutic submucosal resection of the nasal septum and inferior conchectomy. Allergic rhinitis diagnosis was made according to established clinical guidelines,² including chronic rhinitis along with skin prick test positive for at least 1 common inhaled allergens (dust mites, cockroach particles, pollen, or mold graded as 3+/4+) and high serum total immunoglobulin E titer (>100 IU/mL). None of the patients used for this study received oral or nasal corticosteroids in the month before surgery. Control specimens were taken from patients with no evidence of sinonasal disease in whom the inferior nasal concha were removed for other reasons such as endoscopic base of skull surgery or trauma. Immunohistochemical staining for EDA-FN was performed on 4- to 6-µm paraffinized sections of human inferior concha tissue. Slides were incubated with EDA-Fibronectin antibody (Clone FN-3E2 [Sigma Aldrich, St. Louis, Missouri] diluted to a 1:100 ratio), in CAS-Block (Zymed/Invitrogen Corporation, Jerusalem, Israel) for 2 hours at room temperature, washed with Optimax (Biogenex, Fremont, California), incubated with MACH 3 Mouse HRP Polymer (Biocare Medical, Concord,

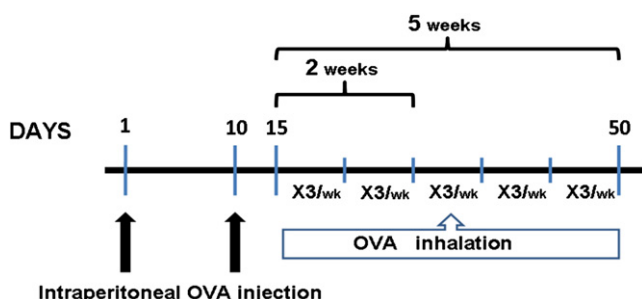


Figure 1. Schema to illustrate the timing of ovalbumin sensitization and inhalation challenges used in this study. OVA, ovalbumin.

California), and then developed with diamine aminobenzidine. The slides were counterstained with hematoxylin, dehydrated, and cleared. Positive findings of the primary antibody were marked by the presence of a brown precipitate.

Statistical Analysis

Means $\pm$ standard deviations are presented where appropriate. Two-way analysis of variance (ANOVA) was performed to analyze the possible differences of treatment (ovalbumin immunization) on allergic nasal signs, goblet cell number, and subepithelial layer thickness. Variables were type of mice (WT or EDA^{-/-}) and treatment group (study or control). Chi-square test was performed to compare collagen distribution along nasal septum between the treatment groups for each mice group (collagen stain along the nasal septum was considered as 1 for a complete and 0 for a partial distribution). Data analysis was performed using SAS 9.1e package (SAS Institute Inc, Cary, North Carolina). Statistical significance was accepted at a *P* value of .05 or below.

Results

Rhinitis after OVA Exposure in WT Mice

Clinical signs of rhinitis. Mice exposed to OVA for 5 weeks had significantly more sneezing and nasal itching motions measured over 5 minutes (29.5 ± 5.6) compared with saline-treated mice (5.3 ± 3.9) ($P = .01$) (Fig 2A).

Eosinophilic inflammation. Eosinophils were present only in OVA-exposed mice and only at the 2-week point but were not observed at 5 weeks. Eosinophils were largely restricted to the perivascular regions within nasal concha, with very few eosinophils in the subepithelial region (Fig 2B).

Goblet cell hyperplasia. Significant goblet cell hyperplasia was observed in OVA-exposed mice but not saline controls at both 2 and 5 weeks. Mean goblet cell count per high-power field (400 $\times$) at 2 weeks in OVA-treated mice was 26.25 ± 10.4 , compared with 1 ± 2 in the saline group ($P < .0001$). After 5 weeks' OVA inhalation, the mean goblet cell count was 12.7 ± 6.14 , compared with 1.5 ± 2 in the control group ($P < .0001$) (Fig 2C).

Fibrosis. After 2 weeks, no difference was observed in the amount of collagen or in the thickness of the subepithelial layer in OVA-exposed mice as compared with saline controls. In contrast, after 5 weeks' OVA inhalation, thickness of the subepithelial layer was significantly greater in the OVA 5 weeks study group ($244.3 \pm 45.5 \mu\text{m}$) compared with the control group ($132.4 \pm 46.8 \mu\text{m}$) ($P < .0001$) (Fig 2D). In 57% (4/7) of the sensitized mice, collagen deposition extended throughout the entire nasal septum, whereas in all control mice, there was only sparse, localized deposition ($P = .018$). The area of collagen stained by Masson's trichrome in the subepithelial layer was significantly greater ($P = .03$) in the OVA group ($37.8\% \pm 8.9\%$) compared with the saline control group ($16.8\% \pm 6.6\%$).

Rhinitis in EDA^{-/-} Mice

In a murine model of chronic "asthma," EDA^{-/-} mice exposed to OVA have reduced lower airway fibrosis and do not develop airway hyper reactivity but have no reduction in airway inflammation, goblet cell number, or airway smooth muscle.¹⁶ This suggests that airway hyper reactivity may be a consequence of remodeling rather than of inflammation. We determined whether developments of nasal remodeling changes are altered in EDA^{-/-} mice. At 5 weeks, goblet cell numbers were significantly higher in OVA-treated

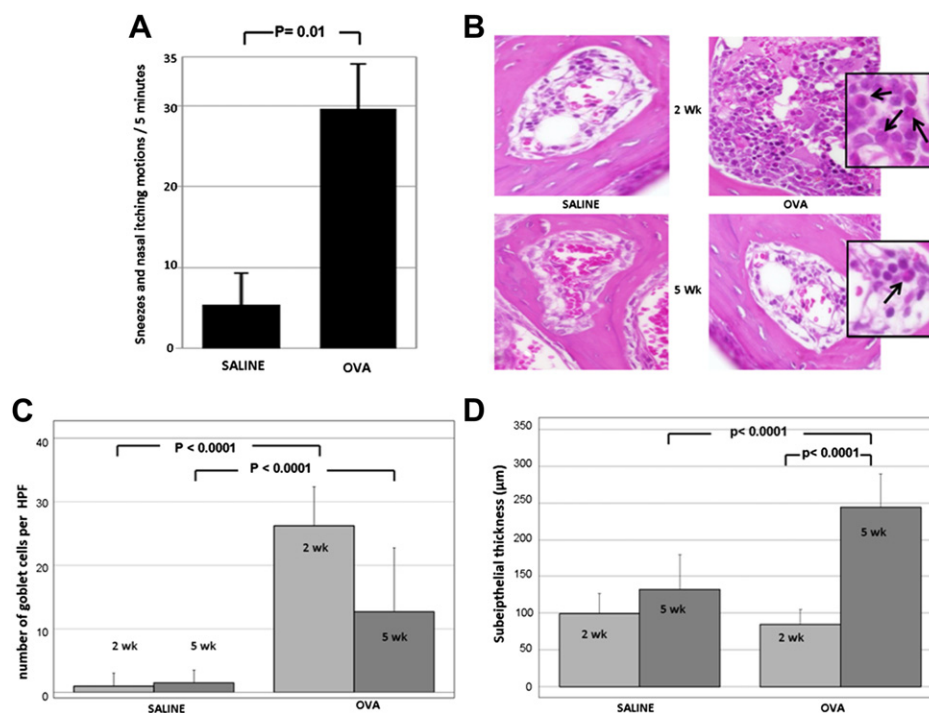


Figure 2. C57BL/6 mice were sensitized intraperitoneally to ovalbumin (IP) and then challenged 3 times/week by inhalation for 2 or 5 weeks. A, Average number of sneezes and nasal itching motions over 5 minutes after 5 weeks' exposure to OVA or saline in wild-type mice, $P = .01$. B, Presence of eosinophils in tissue sections stained with hematoxylin-eosin obtained after 2 or 5 weeks' saline and OVA exposure. Tissue eosinophilia was abundant only after short-term (2 weeks) OVA exposure, although occasional eosinophils are also seen at 5 weeks (arrows) ($\times 40$ magnification). C, Number of goblet cells was determined using periodic acid-Schiff staining (average in 4 different high-power fields, $\times 400$). Counts were increased at both 2 and 5 weeks in OVA-treated mice as compared with saline controls ($P < .0001$). D, Thickness of the subepithelial layer was measured using hematoxylin-eosin-stained tissue sections (magnification $\times 100$) and was increased after 5 weeks' OVA exposure as compared with saline control ($P < .0001$) and compared with short (2 weeks) OVA exposure ($P < .0001$). OVA, ovalbumin.

EDA^{-/-} mice (8.1 ± 4.3) compared with saline controls (1.7 ± 1.7) ($P = .024$). Although increased, the number of goblet cells was significantly less than that observed in OVA-treated WT mice (12.7 ± 6.14) ($P < .05$) (Fig 3A, B).

EDA^{-/-} mice exposed to OVA showed reduced nasal fibrosis compared with OVA-exposed WT mice. Subepithelial layer thickness was $125.8 \pm 41 \mu\text{m}$ in EDA^{-/-} exposed to OVA compared with $244.3 \pm 45.5 \mu\text{m}$ in OVA-exposed WT mice ($P < .0001$). This was similar to the findings in saline-treated EDA^{-/-} mice ($115 \pm 19.2 \mu\text{m}$, NS) (Fig 3C). Similarly, collagen deposition was attenuated in EDA^{-/-} OVA-treated mice as compared with OVA-treated WT mice ($P = .01$) and was similar to that observed in saline-treated mice ($P = .3$) (Fig 3D). Moreover, the area of collagen staining in the subepithelial layer was significantly reduced ($P < .03$) in the OVA-treated EDA^{-/-} group ($16.2\% \pm 8\%$) compared with the OVA-treated WT mice ($37.8 \pm 8.9\%$), similar ($P = .28$) to the saline EDA^{-/-} group ($14.8\% \pm 5.1\%$) (Fig 3E).

EDA-Fibronectin Expression in Human Allergic Rhinitis

To determine whether EDA-FN is relevant to human allergic rhinitis, immunohistochemical staining for EDA-FN was performed on histological sections of inferior concha from white patients with allergic rhinitis (all males, $n = 4$, mean age 36 ± 12.2 ; range, 25–51 years) and from controls (all males, $n = 4$, mean age 29 ± 18 ; range, 12–53 years). Strongly positive staining was observed in all allergic rhinitis patients and was absent in all control cases (Fig 4). Staining was observed in the subepithelial connective tissue.

Discussion

In this study, we initially established a model of “chronic” allergen-induced rhinitis in C57Bl/6 mice. After 2 weeks of exposure to allergen, tissue eosinophilia and goblet cell hyperplasia were present but there was no increase in nasal connective tissue deposition. After 5 weeks of exposure, clinical signs of rhinitis, goblet cell number, and collagen accumulation were significantly increased. The increase in nasal connective tissue deposition in response to OVA was attenuated in EDA knockout mice. We also observed increased expression of EDA-FN in nasal tissue of patients with chronic allergic rhinitis.

Asthma and rhinitis often coexist, share common risk factors, and might be manifestations of the same disease process.¹⁷ Epidemiologic studies show a link between the upper and the lower airways, and mechanistic studies suggest a cause-and-effect relationship rather than a coexistence of these conditions.¹⁸ The coexistence of rhinosinusitis in patients with asthma is associated with a more severe clinical phenotype and poorer response to anti-asthma therapy. Treatment of the rhinitis results in improved asthma control.¹⁹

In our study, we observed the presence of eosinophilic inflammation in nasal tissue as well as goblet cell hyperplasia after 2 weeks of allergen exposure. These findings are compatible with previous studies of acute murine models allergic rhinitis.^{15,16,20–23} Cui et al²⁴ demonstrated a significant increase of ciliated and goblet cells number both in turbinate and in septal mucosa. In histopathologic examination of mouse turbinates, Kim et al¹⁶ observed significantly increased infiltration of eosinophils after

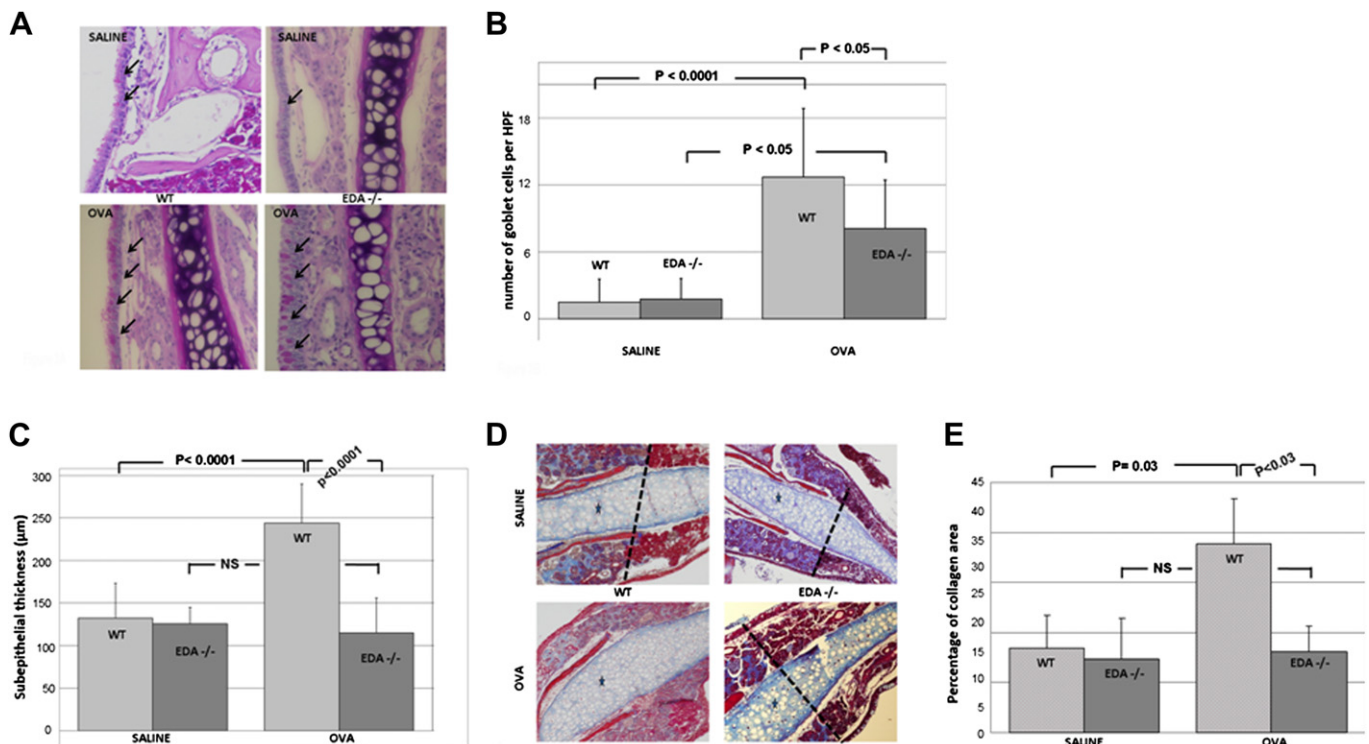


Figure 3. Remodeling changes in nasal tissue after 5 weeks' exposure to OVA or saline in wild-type (WT) and EDA^{-/-} mice. A, Representative tissue sections stained with PAS to demonstrate the presence of mucin in goblet cells in OVA- and saline-treated mice. B, An increase in goblet cell number (PAS-stained cells) was present in both WT and EDA^{-/-} groups exposed to OVA as compared with saline controls (arrows). C, Thickness of the subepithelial layer (hematoxylin-eosin staining) was increased in WT mice exposed to OVA vs saline controls but not in OVA-treated EDA^{-/-} mice. $P < .0001$ for OVA-treated WT vs OVA-treated EDA^{-/-} mice. D, Light microscopy ($\times 40$), using Masson trichrome stain, was used to demonstrate 2 distinct collagen distribution patterns along the nasal septum. Partial distribution (just above the vomeronasal organ) was present in the saline control mice. In contrast, OVA-exposed WT mice demonstrate collagen distribution along the entire nasal septum. Collagen distribution in OVA-exposed EDA^{-/-} mice is similar to that in saline controls. Asterisk marks nasal septum, dashed line indicate collagen distribution limit. OVA, ovalbumin; WT, wild type. E, Percentage of collagen area in the subepithelial layer (Masson trichrome staining) was increased in WT mice exposed to OVA vs saline controls but not in OVA-treated EDA^{-/-} mice. $P < .03$ for OVA-treated WT vs OVA-treated EDA^{-/-} mice.

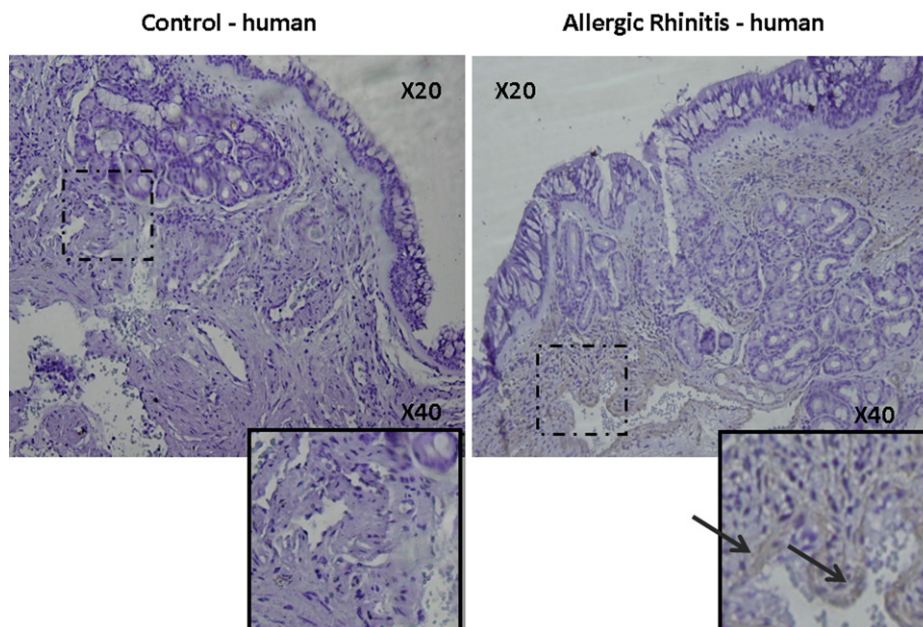


Figure 4. Representative sections of immunohistochemical staining for EDA-FN performed on paraffinized sections of human inferior conchae tissue from patients with allergic rhinitis and control patients ($n = 4$ per group). Significant staining (brown) indicative for abundant EDA-FN was present only in the allergic rhinitis group (arrows).

OVA exposure. Airway mucosal eosinophilia and goblet cell hyperplasia are characteristic features of both asthma and allergic rhinitis.⁷ In our model, nasal tissue eosinophilia was no longer present after 5 weeks' OVA exposure, even though there was persistent lower airway eosinophilia at this time (results not shown). This finding suggests that eosinophilic inflammation wanes over time in the nasal mucosa and may be less relevant to the chronic remodeling process in the nose.

The presence of structural changes in chronic allergic rhinitis is less prominent than that observed in asthma. Nevertheless, changes in the epithelial layer and connective tissue of the upper airways are present. A striking difference between the upper and lower airways relates to the presence or absence of submucosal smooth muscle, which is a prominent feature of remodeling in asthma but is absent in the nasal mucosa.²⁵ As regards extracellular matrix and collagen deposition in the upper airways, intense staining and altered distribution pattern of collagen and proteoglycans are present in the nasal mucosa of patients with mild and severe persistent allergic rhinitis as compared with healthy control subjects.²⁶ Sanai et al.²⁷ found an increase in the number of mucosal myofibroblasts within the upper airways in 13 perennial rhinitis patients compared with 13 nonallergic subjects. Myofibroblast activation would thus lead to increased airway collagen deposition. The presence of fibrotic changes after chronic allergen exposure has also been described in murine models of allergic rhinitis. Mice sensitized to ovalbumin for 3 months demonstrated a significant increase in subepithelial fibrosis and increased matrix metalloproteinase 9.^{28,29}

In our present study, we have demonstrated that the extracellular matrix protein EDA-fibronectin is necessary for the development of upper airway fibrosis in a murine model of chronic allergic rhinitis. The importance of EDA-FN in the development of fibrosis has been shown in the heart,^{30,31} kidneys,³² and in bleomycin-induced lung fibrosis.³³ We have also shown that EDA-FN is necessary for the development of lower airway fibrosis in a murine model of chronic allergen-induced "asthma."¹³

Because findings in an animal model are not always applicable to humans, we have also studied human nasal tissue to strengthen our findings. The attenuation of nasal remodeling in EDA-/- mice as well as the presence of pronounced immunostaining for EDA-FN in

nasal tissue from patients with allergic rhinitis demonstrated in the present study strongly suggest that EDA is necessary for allergic-rhinitis related upper airway remodeling.

As regards the lower airways, a correlation exists between severity of asthma and presence of remodeling.³⁴ The clinical and pathophysiological implications of upper airway remodeling are less established, and attenuating fibrosis via targeting EDA in the nose may possibly provide only limited clinical benefit. Nevertheless, we believe that such changes would be expected to have similar significance to those present in the lower airways. EDA-FN is an appealing target for attenuating fibrosis in general, because this splice variant is only expressed in response to injury and has a far narrower biological effect than other mediators of fibrosis such as transforming growth factor $\beta 1$.³⁵ Fibrosis in the nose as seen in chronic rhinitis and in nasal polyps is a particularly attractive target for development of future antifibrotic therapy in that it is easily accessible to administration of local rather than systemic treatment.

References

- [1] Greiner AN, Hellings PW, Rotiroli G, Scadding GK. Allergic rhinitis. *Lancet*. 2011;378:2112–2122.
- [2] Bousquet J, Khaltaev N, Cruz AA, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008. *Allergy*. 2008;63(suppl 86):8–160.
- [3] Bachert C, Vignola AM, Gevaert P, Leynaert B, Van Cauwenberge P, Bousquet J. Allergic rhinitis, rhinosinusitis, and asthma: one airway disease. *Immunol Allergy Clin North Am*. 2004;24:19–43.
- [4] Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. *J Clin Invest*. 1999;104:985–999.
- [5] Chiappara G, Gagliardo R, Siena A, et al. Airway remodelling in the pathogenesis of asthma. *Curr Opin Allergy Clin Immunol*. 2001;1:85–93.
- [6] Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med*. 2001;164(Suppl):S28–S38.
- [7] Braunstahl GJ, Fokkens WJ, Overbeek SE, Kleinjan A, Hoogsteden HC, Prins JB. Mucosal and systemic inflammatory changes in allergic rhinitis and asthma: a comparison between upper and lower airways. *Clin Exp Allergy*. 2003;33:579–587.
- [8] Laitinen A, Altraja A, Kampe M, Linden M, Virtanen I, Laitinen LA. Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. *Am J Respir Crit Care Med*. 1997;156:951–958.
- [9] Christie PE, Jonas M, Tsai CH, Chi EY, Henderson WR. Increase in laminin expression in allergic airway remodeling and decrease by dexamethasone. *Eur Respir J*. 2004;24:107–115.
- [10] Fernandes DJ, Bonacci JV, Stewart AG. Extracellular matrix, integrins and mesenchymal cell function in the airways. *Curr Drug Targets*. 2006;7:567–577.

- [11] Larsen K, Malmström J, Wildt M, et al. Functional and phenotypical comparison of myofibroblasts derived from biopsies and bronchoalveolar lavage in mild asthma and scleroderma. *Respir Res*. 2006;7:11.
- [12] Trifilieff A, El-Hashim A, Bertrand C. Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment. *Am J Physiol Lung Cell Mol Physiol*. 2000;279:L1120–L1128.
- [13] Kohan M, Muro AF, Bader R, Berkman N. The extra domain A of fibronectin is essential for allergen-induced airway fibrosis and hyperresponsiveness in mice. *J Allergy Clin Immunol*. 2011;127:439–446.
- [14] Muro AF, Chauhan AK, Gajovic S, et al. Regulated splicing of the fibronectin EDA exon is essential for proper skin wound healing and normal lifespan. *J Cell Biol*. 2003;162:149–160.
- [15] Mo JH, Kang EK, Quan SH, Rhee CS, Lee CH, Kim DY. Anti-tumor necrosis factor- α treatment reduces allergic responses in an allergic rhinitis mouse model. *Allergy*. 2011;66:279–286.
- [16] Kim YH, Yang TY, Park CS, et al. Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis. *Allergy*. 2012;67:183–190.
- [17] Grossman J. One airway, one disease. *Chest*. 1997;111(2 Suppl):11S–16S.
- [18] Corren J. Allergic rhinitis and asthma: How important is the link? *J Allergy Clin Immunol*. 1997;99:S781–S786.
- [19] Magnan A, Meunier JP, Saugnac C, Gasteau J, Neukirch F. Frequency and impact of allergic rhinitis in asthma patients in everyday general medical practice: a French observational cross-sectional study. *Allergy*. 2008;63:292–298.
- [20] Shin JH, Kang JM, Kim SW, Cho JH, Park YJ, Kim SW. Effect of oral tolerance in a mouse model of allergic rhinitis. *Otolaryngol Head Neck Surg*. 2010;142:370–375.
- [21] Akiyama K, Karaki M, Kobayashi R, Dobashi H, Ishida T, Mori N. IL-16 variability and modulation by antiallergic drugs in a murine experimental allergic rhinitis model. *Int Arch Allergy Immunol*. 2009;149:315–322.
- [22] Miyahara S, Miyahara N, Matsubara S, Takeda K, Koya T, Gelfand EW. IL-13 is essential to the late-phase response in allergic rhinitis. *J Allergy Clin Immunol*. 2006;118:1110–1116.
- [23] Hellings PW, Hessel EM, Van Den Oord JJ, Kasran A, Van Hecke P, Ceuppens JL. Eosinophilic rhinitis accompanies the development of lower airway inflammation and hyper-reactivity in sensitized mice exposed to aerosolized allergen. *Clin Exp Allergy*. 2001;31:782–790.
- [24] Cui YH, Wang YY, Liu Z. Transdifferentiation of Clara cell 10-kDa protein secreting cells in experimental allergic rhinitis. *Am J Rhinol Allergy*. 2011;25:145–151.
- [25] Bousquet J, Jacquot W, Vignola AM, Bachert C, Van Cauwenberge P. Allergic rhinitis: a disease remodeling the upper airways? *J Allergy Clin Immunol*. 2004;113:43–49.
- [26] Kim TH, Lee JY, Lee HM, et al. Remodelling of nasal mucosa in mild and severe persistent allergic rhinitis with special reference to the distribution of collagen, proteoglycans, and lymphatic vessels. *Clin Exp Allergy*. 2010;40:1742–1754.
- [27] Sanai A, Nagata H, Konno A. Extensive interstitial collagen deposition on the basement membrane zone in allergic nasal mucosa. *Acta Oto-Laryngol*. 1999;119:473–478.
- [28] Moon IJ, Kim DY, Rhee CS, Lee CH, Min YG. Role of angiogenic factors in airway remodeling in an allergic rhinitis murine model. *Allergy Asthma Immunol Res*. 2012;4:37–45.
- [29] Lim YS, Won TB, Shim WS, et al. Induction of airway remodeling of nasal mucosa by repetitive allergen challenge in a murine model of allergic rhinitis. *Ann Allergy Asthma Immunol*. 2007;98:22–31.
- [30] Booth AJ, Wood SC, Cornett AM, et al. Recipient-derived EDA fibronectin promotes cardiac allograft fibrosis. *J Pathol*. 2012;226:609–618.
- [31] Arslan F, Smeets MB, Riem Vis PW, et al. Lack of fibronectin-EDA promotes survival and prevents adverse remodeling and heart function deterioration after myocardial infarction. *Circ Res*. 2011;108:582–592.
- [32] Baelde HJ, Eikmans M, van Vliet AI, Bergijk EC, de Heer E, Bruijn JA. Alternatively spliced isoforms of fibronectin in immune-mediated glomerulosclerosis: the role of TGF β and IL-4. *J Pathol*. 2004;204:248–257.
- [33] Muro AF, Moretti FA, Moore BB, et al. An essential role for fibronectin extra type III domain A in pulmonary fibrosis. *Am J Respir Crit Care Med*. 2008;177:638–645.
- [34] Al-Muhsen S, Johnson JR, Hamid Q. Remodeling in asthma. *J Allergy Clin Immunol*. 2011;128:451–462.
- [35] Shull MM, Ormsby I, Kier AB, et al. Targeted disruption of the mouse transforming growth factor- β 1 gene results in multifocal inflammatory disease. *Nature*. 1992;359:693–699.