

The involvement of matrix metalloproteinases in basement membrane injury in a murine model of acute allergic airway inflammation

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Summary

Background Airway remodelling in asthma such as subepithelial fibrosis is thought to be the repair process that follows the continuing injury as of chronic airway inflammation. However, how acute allergic inflammation causes tissue injury in the epithelial basement membrane in asthmatic airways remains unclear. Matrix metalloproteinases (MMPs) capable of degrading almost all of the extracellular matrix components have been demonstrated to be involved in cell migration through the basement membrane *in vivo* and *in vitro*.

Objective We investigated the alterations of matrix construction and the role of MMPs in matrix degradation in the subepithelium during acute allergic airway inflammation.

Methods Airway inflammation, the ultrastructure of the subepithelium and injury of types III and IV collagen in tracheal tissues from ovalbumin (OVA)-sensitized mice after OVA inhalation with or without the administration of tissue inhibitor of metalloproteinase-2 (TIMP-2) and dexamethasone were evaluated by cell counting in bronchoalveolar lavage (BAL) fluids, electron microscopy and immunohistochemistry, respectively.

Results The disruption of the lamina densa and matrix construction and the decrease of the immunoreactivity for type IV collagen in subepithelium were observed in association with the accumulation of inflammatory cells in airways 3 days after OVA inhalation. This disorganization of the matrix components in the subepithelium, as well the cellular accumulation, was abolished by the administration of TIMP-2 and dexamethasone. The immunoreactivity for type IV collagen in the subepithelium in OVA-inhaled mice returned to the level of that in saline-inhaled mice 10 days after inhalation in association with a decrease of the cell numbers in the BAL fluid. The immunoreactivity for type III collagen was changed neither 3 nor 10 days after OVA inhalation.

Conclusion These results suggest that epithelial basement membrane gets injured by, at least in part, MMPs as a consequence of cell transmigration through the membrane during acute allergic airway inflammation.

Keywords airway remodelling, subepithelial fibrosis, acute allergic airway inflammation, eosinophils, lamina densa, lamina reticularis, type IV collagen, matrix metalloproteinases, tissue inhibitor of metalloproteinase, corticosteroids

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Introduction

Chronic airway inflammation is a fundamental feature of bronchial asthma, which is characterized by the accumulation and activation of inflammatory cells, such as eosinophils, that are tightly regulated by Th2 cytokines and chemokines. Inflammatory mediators released from activated eosinophils, such as eosinophil cationic protein (ECP), major basic protein (MBP), arachidonic acid metabolites and reactive oxygen species, evoke bronchoconstriction, bronchial mucosal oedema, epithelial shedding and airway hyper-responsiveness, which are the

pathophysiological features observed in asthmatics [1–3]. In addition to airway inflammation, tissue remodelling is recognized to play a pathogenic role in bronchial asthma. Airway remodelling, including goblet cell hyperplasia, thickening of epithelial basement membrane, an increase of fibroblasts and myofibroblasts and hyperplasia/hypertrophy of airway smooth muscle cells and submucosal glands, results in the thickening of the airway walls, which contributes to the severity and chronicity of asthma by amplifying the airway narrowing [4]. Airway remodelling in asthma is thought to be the repair process ensuing from chronic repetitious injury as a consequence of the chronic airway inflammation [3, 5–7].

In the epithelial basement membrane, three component layers are defined by electron microscopy: the lamina lucida, the lamina densa, and the lamina reticularis. The lamina lucida is a less electron-dense zone between the epithelium and a more

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electron-dense lamina densa. The basal lamina refers to both the lamina lucida and the lamina densa. The lamina densa is made up of type IV collagen, laminin, entactin, and heparan sulphate proteoglycan. The lamina reticularis is made up of collagen fibrils including types I, III, V, VI and VII collagen, fibronectin, tenascin, and proteoglycan. This layer is localized next to the lamina densa forming the transition to the subepithelial connective tissues [8, 9]. The thickening of the basement membrane is ultrastructurally the excess deposition of types III and V collagen, fibronectin and tenascin to the lamina reticularis, which is the so-called subepithelial fibrosis. Type IV collagen and laminin are not present in enhanced quantities [10, 11]. Fibrogenic growth factors such as TGF- β , platelet-derived growth factor (PDGF), and epidermal growth factor (EGF)/EGF receptor, the overexpression of which is observed in bronchial biopsy tissues from asthmatics, are thought to participate in subepithelial fibrosis through stimulating epithelial cells, fibroblasts and myofibroblasts to proliferate and synthesize extracellular matrix (ECM) proteins [3, 12]. However, the manner by which acute allergic inflammation causes tissue injury in the epithelial basement membrane in asthmatic airways remains unclear.

Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases capable of proteolytically degrading many of the components of the ECM [13]. They are produced by not only structural cells such as fibroblasts, endothelial cells, and epithelial cells [14, 15], but also inflammatory cells such as macrophages [16], lymphocytes [17], neutrophils [18], and eosinophils [19]. MMPs are involved in physiologic processes such as development, angiogenesis and wound healing, and in pathologic conditions such as tumour invasion and inflammation [14, 20]. The enzymes are secreted as latent forms followed by proteolytic processing to active forms. The proteolytic activation of latent forms and enzymatic activities by active forms of MMPs are inhibited by endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs) that form a 1 : 1 complex with MMPs. Among the members of the MMP family, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) cleave type IV collagen. TIMP-1 and TIMP-2 regulate their activities by making complexes with the active forms of either MMP-2 or -9 and with the latent forms of MMP-9 and -2, respectively [13]. The balance between the levels of MMPs and TIMPs is thought to be a critical factor in regulating the breakdown of connective tissues by MMPs in such cases as pulmonary emphysema [21].

Since we and others demonstrated that MMP-9 was overexpressed mainly by eosinophils and macrophages in asthmatic airways [16, 19], the participation of MMPs and TIMPs in the inflammation and remodeling in asthmatic airways has been extensively investigated [22]. Okada et al. showed that MMP-9 released from eosinophils plays a pivotal role in their trans-matrix membrane migration through the degradation of matrix components as demonstrated *in vitro* by electron microscopic examination [23]. MMP-2 and -9 have also been reported to be important for T cell transmigration through the basal lamina [17]. Furthermore, we recently demonstrated by the administration of TIMP-2 that MMPs including MMP-2 and -9 were involved in the extravasation and migration to airway lumen of inflammatory cells such as eosinophils and lymphocytes in airway inflammation using a murine model of allergic asthma [24].

Together with these observations, we hypothesized that the injury of the epithelial basement membrane was caused by MMPs in the course of the trans-basement membrane migration of inflammatory cells during acute allergic airway inflammation. To evaluate our hypothesis, we investigated the alteration of the structure and matrix components of the epithelial basement membrane in trachea after antigen challenge using a murine model of asthma by electron microscopic and immunohistochemical examination.

Materials and Methods

Sensitization and antigen challenge protocol

Specific pathogen-free female BALB/c mice, 6–8 weeks old, purchased from our animal facility (Institute for Experimental Animals, Tohoku University School of Medicine, Sendai, Japan), were sensitized and challenged as previously described [24]. Briefly, mice were sensitized by intraperitoneal injections of ovalbumin (OVA) (Sigma Chemical Co., St Louis, MO, USA) (8 μ g/mouse) adsorbed with aluminium hydroxide (Wako Pure Chemical Industries, Ltd, Osaka, Japan) at days 0 and 5. Ten to 14 days after the last injection, mice were challenged with aerosolized OVA or saline as a control. Three or 10 days after the challenge, mice were killed and bronchoalveolar lavage (BAL) fluids and tracheal tissues were collected. Total cell numbers in the BAL fluids were counted with a haemocytometer. Smears of BAL cells prepared with a Cytospin II (Shandon, Runcorn, UK) were stained with Diff-Quick solution (International Reagents Corp., Kobe, Japan) for differential cell counting. Tracheal tissues were processed for immunohistochemistry and electron microscopical analysis. These studies had been approved by the Ethics Committee for Animal Experiments of the Tohoku University School of Medicine.

Administration of TIMP-2 and dexamethasone

Recombinant human TIMP-2 (rhTIMP-2) (kindly provided by Dr K. Iwata, Fuji Chemical Industries, Ltd, Toyama, Japan) (25 μ g in 25 μ L of saline/mouse) or saline (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) alone (25 μ L/mouse) as control, each of which contained less than 0.005 EU of endotoxin, was instilled intranasally six times at 12-h intervals, beginning at 1 h before OVA inhalation as previously described [24]. Dexamethasone (Sigma) (5 mg/kg body weight in 0.5 mL PBS (Takara Shuzo Co., Ltd, Otsu Japan)/mouse) or PBS alone (0.5 mL/mouse) was injected intraperitoneally three times at 24-h intervals, beginning at 2 h before OVA inhalation as previously described. Three days after the OVA inhalation, mice were killed and samples were collected as mentioned above.

Transmission electron microscopy

Tracheal tissues collected as mentioned above were excised and fixed with 2.5% glutaraldehyde (TAAB, Berkshire, UK) in 0.1 M cacodylate (TAAB) buffer (pH 7.4) for 4 h at 4 °C. Fixed tracheal tissues were transversely divided into thin slices less than 1.0 mm in thickness with razor blades. The slices were stored in the same buffer containing 8% sucrose (Wako) for 5–6 h at 4 °C. After brief washing with the same buffer, the slices were postfixed with 2% osmium tetroxide (TAAB) for 1 h at 4 °C, dehydrated through a graded ethanol series, and embedded in

Epon-812 resin (TAAB). Sections 1 µm in thickness were stained with 1% toluidine blue (Merck, Darmstadt, Germany) and examined under a light microscope. Ultrathin sections 70 nm in thickness were prepared with an ultramicrotome (2088-V, LKB, Bromma, Sweden), stained with 2% uranyl acetate (Fluka, Buchs, Switzerland) and 0.1% lead citrate (Wako), and then two sections from each mouse were carefully observed under a transmission electron microscope (JEM-1200EX, JOEL, Tokyo, Japan) at an acceleration voltage of 80 kV.

Immunohistochemistry

Tracheal tissues were fixed with periodate-lysine-paraformaldehyde (PLP) solution [24] and embedded in OCT compound (Miles Laboratories, Naperville, IL, USA) with liquid nitrogen. Five µm-thick sections were immunostained with rabbit antisera for mouse type III (LSL Co., Ltd, Tokyo, Japan) and IV (LSL) collagen or normal rabbit serum (Dako Corporation, Carpinteria, CA, USA) as a control using the peroxidase anti-peroxidase method as previously described [25]. Sections were developed for 3 min in collagen III and 4 min in collagen IV staining with diaminobenzidine (Dako) according to the manufacturer's protocol, but not counterstained because the counterstaining would disturb the following evaluation of the immunoreactivity. After immunostaining, pictures of sections were taken in black and white at a magnification of $\times 400$ using a light microscope (Carl Zeiss, Jena, Germany). Then, the intensity of the staining in a 5-µm-deep and 10-µm-wide zone beneath the epithelium was estimated with National Institutes of Health (NIH) image 1.61 in a blind fashion, and expressed as a grey scale from 0 (= white) to 255 (= black). The intensity was measured in eight zones for each section. Two sections from each mouse were subjected to the immunohistochemical analysis. A mean of 16 zones was identified for the intensity in each mouse. Sections from two sensitized, but not challenged, mice were always included in each run of immunohistochemistry. The intensity of the mice examined was expressed as a percentage of the mean intensity of the two sensitized mice. The mean intraobserver coefficient of variance of the intensity was less than 10%.

Data analysis

Data are presented as mean $\pm$ SD. Significant differences between groups were determined using unpaired Student's *t*-test. These analyses were performed using Stat View 4.11 (Abacus Concepts, Berkeley, CA, USA) for Macintosh. A *P*-value of less than 0.05 was taken as significant.

Results

The effects of TIMP-2 and dexamethasone on cell counts in BAL fluids

As shown in Fig. 1, total cell numbers in BAL fluids were significantly increased, in association with significant increases of macrophages, eosinophils and lymphocytes, at 3 days after OVA inhalation compared with those after saline inhalation. The administration of either TIMP-2 (hatched columns) or dexamethasone (checked columns) significantly reduced the numbers of total cells ($3.4 \pm 1.0 \times 10^5/\text{mL}$ or $2.3 \pm 0.3 \times 10^5/\text{mL}$, respectively), macrophages ($2.3 \pm 0.5 \times 10^5/\text{mL}$ or $2.3 \pm 0.3 \times 10^5/\text{mL}$, respectively), eosinophils (0.6 ± 0.4

$\times 10^5/\text{mL}$ or $0.01 \pm 0.01 \times 10^5/\text{mL}$, respectively) and lymphocytes ($0.2 \pm 0.1 \times 10^5/\text{mL}$ or $0.01 \pm 0.01 \times 10^5/\text{mL}$, respectively) in the BAL fluid at 3 days after OVA inhalation ($n = 5$), compared with those after OVA inhalation with control treatment (closed columns) ($n = 5$); total cells ($8.5 \pm 1.9 \times 10^5/\text{mL}$), macrophages ($4.4 \pm 0.9 \times 10^5/\text{mL}$), eosinophils ($3.2 \pm 1.1 \times 10^5/\text{mL}$) and lymphocytes ($0.6 \pm 0.1 \times 10^5/\text{mL}$). On day 10, there was no significant difference in the numbers of macrophages and eosinophils in the BAL fluid between OVA-inhaled mice ($n = 5$) and saline-inhaled mice ($n = 5$) ($3.2 \pm 0.8 \times 10^5/\text{mL}$ vs. $2.4 \pm 0.7 \times 10^5/\text{mL}$ and $0.8 \pm 0.8 \times 10^5/\text{mL}$ vs. $0.01 \pm 0.01 \times 10^5/\text{mL}$, respectively), while the numbers of total cells and lymphocytes in OVA-inhaled mice were still significantly higher than those in saline-inhaled mice ($4.5 \pm 0.7 \times 10^5/\text{mL}$ vs. $2.7 \pm 0.6 \times 10^5/\text{mL}$ and $0.4 \pm 0.3 \times 10^5/\text{mL}$ vs. $0.04 \pm 0.01 \times 10^5/\text{mL}$, respectively) (Fig. 2).

Ultrastructural changes in subepithelium of tracheal tissues after OVA inhalation

In tracheal tissues from saline-inhaled mice, the continuity in the electron dense lamina densa was observed. Collagen fibres beneath the lamina densa were well organized and tightly attached to the lamina densa (Figs 3a and b). In contrast,

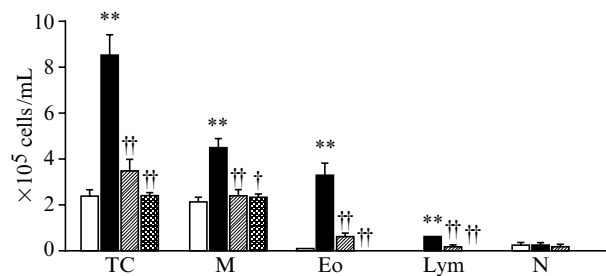


Fig. 1. The effects of TIMP-2 and dexamethasone on cell counts in BAL fluids from sensitized and challenged mice. The numbers of total cells (TC), macrophages (M), eosinophils (Eo), lymphocytes (Lym), and neutrophils (N) in BAL fluids from saline-inhaled mice with the administration of saline (open columns), OVA-inhaled mice with saline (closed columns) and OVA-inhaled mice with TIMP-2 (hatched columns) or dexamethasone (checked columns) were counted ($n = 5$) on day 3. ** $P < 0.01$, compared with saline inhalation group (open columns), and †† $P < 0.01$, compared with OVA inhalation group (closed columns).

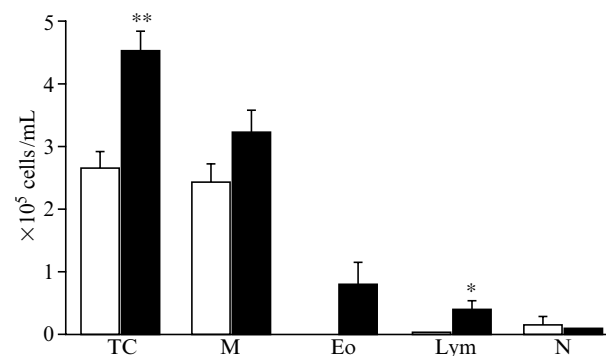


Fig. 2. Cell counts in BAL fluids from sensitized and challenged mice. The numbers of total cells (TC), macrophages (M), eosinophils (Eo), lymphocytes (Lym) and neutrophils (N) in BAL fluids from saline-inhaled mice (open columns) and OVA-inhaled mice (closed columns) were counted ($n = 5$) on day 10. ** $P < 0.01$ and * $P < 0.05$, compared with saline inhalation group (open columns).

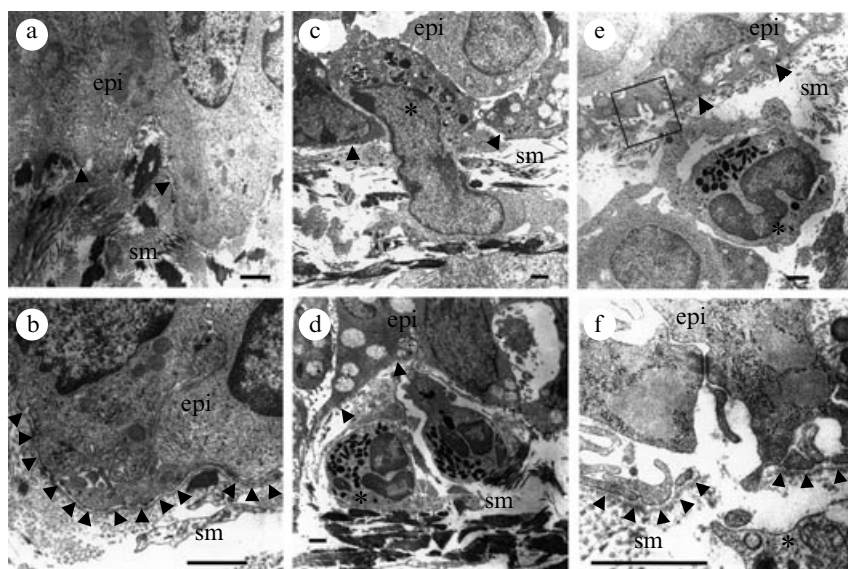


Fig. 3. Transmission electron micrographs of tracheal tissues from saline- and OVA-inhaled mice. Shown are representative pictures of tracheal tissues from mice 3 days after the inhalation of saline (a, b) or OVA (c–f). (f) is an enlarged picture of the square indicated in (e). The lamina densa is indicated by arrow heads. epi: epithelium; sm: submucosa; *: inflammatory cells. Bar = 1 µm.

discontinuity in the lamina densa was frequently observed in tracheal tissues from OVA-inhaled mice (Fig. 3f). Furthermore, the loss of the connection between the lamina densa and matrix bundles beneath the lamina was not rarely observed, and the integrity of the matrix components in the subepithelium was destroyed (Figs 3c and d). It was also observed that inflammatory cells, some of which appeared to be eosinophils, were present close to the disrupted portion of the lamina densa (Figs 3d and e), and that those were migrating through the basement membrane (Fig. 3c). In tracheal tissues from OVA-inhaled mice that were treated with either TIMP-2 or dexamethasone, discontinuity in the lamina densa was rarely observed. The matrix structure in the subepithelium and the connection of collagen fibres to the lamina densa were well conserved (Fig. 4), similar to those in saline-inhaled mice.

Immunohistochemical staining for types III and IV collagen in subepithelium

The immunoreactivity of type III collagen beneath the epithelium of tracheal tissues from mice 3 days after OVA inhalation (Fig. 5b) was similar to that from mice after saline inhalation (Fig. 5a). The immunoreactivity of type IV collagen beneath the epithelium of tracheal tissues from mice 3 days after OVA inhalation with the administration of saline (Fig. 6c) or PBS (Fig. 6e) appeared to be homogeneously less than that from mice after saline inhalation with saline (Fig. 6b) or PBS (not shown), respectively. In contrast, the reactivity of type IV collagen in OVA-inhaled mice with TIMP-2 (Fig. 6d) or dexamethasone (Fig. 6f) on day 3 was similar to that in saline-inhaled mice with saline (Fig. 6b) or PBS (not shown), respectively. On day 10, the immunoreactivity of type IV collagen as well as that of type III collagen (not shown) in OVA-inhaled mice (Fig. 7b) was almost the same as that in saline-inhaled mice (Fig. 7a).

Semiquantitative evaluation of the immunohistochemical staining

While there was no significant difference in the intensity for type III collagen in tracheal tissues between OVA-inhaled ($n = 5$)

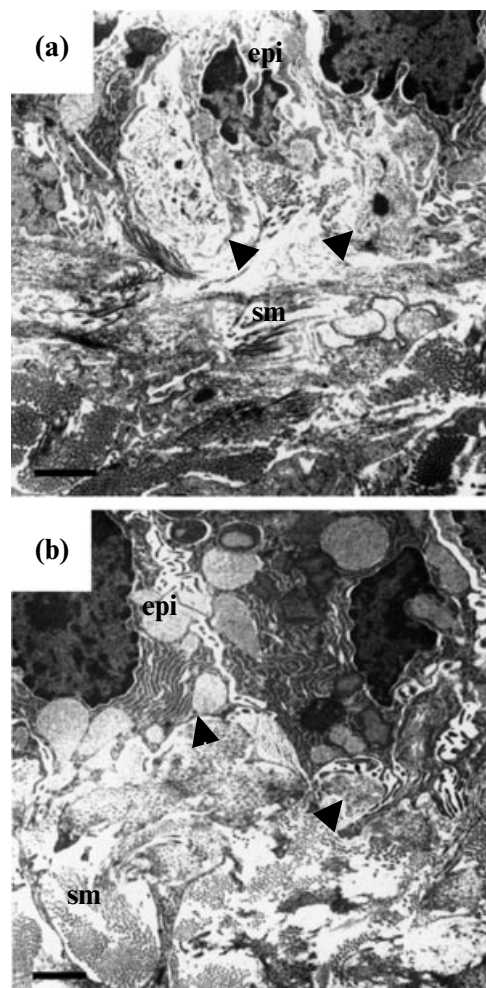


Fig. 4. Transmission electron micrographs of tracheal tissues from OVA-inhaled mice. Shown are representative pictures of tracheal tissues from OVA-inhaled mice treated with TIMP-2 (a) or dexamethasone (b). The interface between the epithelium and submucosa is indicated by arrow heads. epi: epithelium; sm: submucosa. Bar = 1 µm.

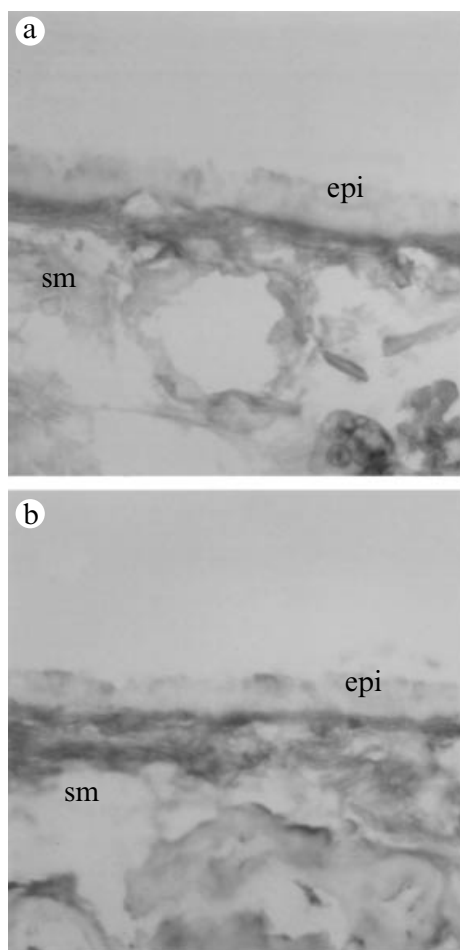


Fig. 5. Immunohistochemical staining for type III collagen in tracheal tissues. Shown are representative pictures of tracheal tissues, which were immunostained with antimouse collagen III antibody, from mice 3 days after the inhalation of saline (a) or OVA (b). The positive (brown) staining was not detected in any tissues with control antibody. epi: epithelium; sm: submucosa. ($\times 400$).

and saline-inhaled ($n = 5$) mice ($92.4 \pm 8.3\%$ vs. $100.7 \pm 7.3\%$) on day 3, the intensity of immunostaining for type IV collagen in OVA-inhaled mice was significantly lower than that in saline-inhaled mice. The significant decrease of the intensity for type IV collagen in OVA-inhaled mice treated saline ($n = 5$) or PBS ($n = 5$) compared to the intensity in saline-inhaled control mice ($n = 5$) ($69.7 \pm 14.0\%$ vs. $101.1 \pm 6.5\%$; $P < 0.01$, or $69.7 \pm 9.7\%$ vs. $103.5 \pm 3.5\%$; $P < 0.01$, respectively) on day 3 was abolished by the treatment with TIMP-2 ($106.1 \pm 3.1\%$, $n = 5$) or dexamethasone ($104.8 \pm 6.6\%$, $n = 5$), respectively (Fig. 8). On day 10, the intensity for type IV collagen in OVA-inhaled mice ($91.1 \pm 12.9\%$, $n = 5$) returned to the level of that in saline-inhaled mice ($93.9 \pm 3.4\%$, $n = 5$), and that for type III collagen in OVA-inhaled mice ($91.2 \pm 10.4\%$, $n = 5$) was still similar to that in saline-inhaled mice ($99.3 \pm 9.0\%$, $n = 5$).

Discussion

We demonstrated for the first time using a murine model of allergic asthma that the injury of the epithelial basement membrane during acute allergic airway inflammation following antigen challenge is one of the consequences of the transmigration of inflammatory cells through the membrane. The structural changes in the membrane included a disruption of the lamina densa and a disconnection between the lamina reticularis and the underlying connective tissues, which might be, at least in part, due to the degradation of matrix proteins including type IV collagen by MMPs such as MMP-2 and-9. It was also demonstrated that the integrity of the basement membrane was restored by 10 days after allergen exposure. The alterations in the matrix architecture, in terms of collagen deposition to the lamina reticularis and submucosa [10, 11, 26], and the matrix disorganization focusing on elastic fibres [27, 28] in asthmatic airways have been well investigated. However, the injury to matrix components in the epithelial basement membrane caused by acute allergic inflammation after antigen challenge is not well understood, although the cellular and biochemical changes in airway tissues and BAL fluids after antigen challenge

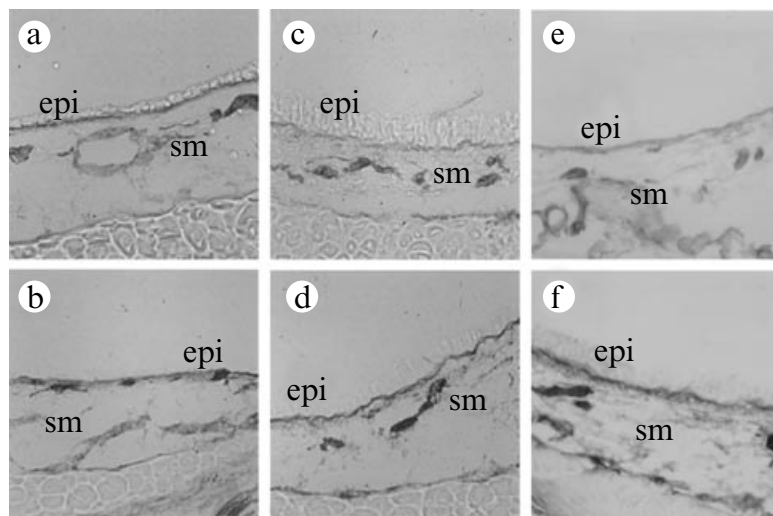


Fig. 6. Immunohistochemical staining for type IV collagen in tracheal tissues. Shown are representative pictures of tracheal tissues, which were immunostained with antimouse collagen IV antibody, from mice before inhalation (a), 3 days after the inhalation of saline with intranasal administration of saline (b), 3 days after the inhalation of OVA with intranasal administration of saline (c) or TIMP-2 (d), and 3 days after the inhalation of OVA with intraperitoneal administration of PBS (e) or dexamethasone (f). Pictures of tracheal tissues from mice 3 days after the inhalation of saline with intraperitoneal administration of PBS, which are similar to (b), are not shown. The positive (brown) staining was not detected in any tissues with control antibody. epi: epithelium; sm: submucosa. ($\times 200$).

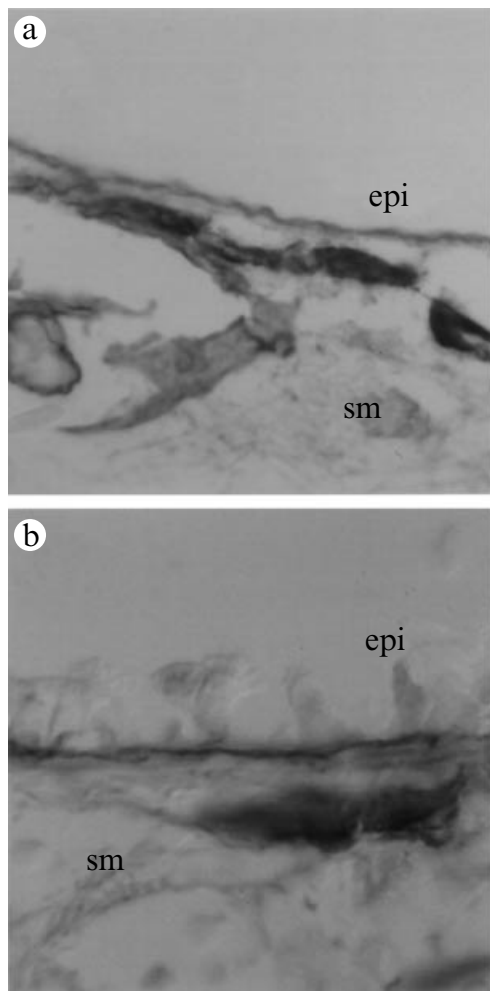


Fig. 7. Immunohistochemical staining for type IV collagen in tracheal tissues. Shown are representative pictures of tracheal tissues, which were immunostained with antimouse collagen IV antibody, from mice 10 days after the inhalation of saline (a) or OVA (b). The positive (brown) staining was not detected in any tissues with control antibody. epi: epithelium; sm: submucosa. ($\times 400$).

have been extensively studied in asthmatics [3]. There is only one report examining the structural alterations in the basement membrane in asthmatic airways after antigen challenge in which it was found that the reticular layer was partly missing due to dissolution of reticular fibres as demonstrated by electron microscopy, although the mechanisms and candidates of the alterations were not determined [29].

In this study, the discontinuity in the lamina densa and the disorganization of the lamina reticularis in the epithelial basement membrane were observed in association with the accumulation of inflammatory cells, predominantly eosinophils and lymphocytes, in the airway lumen. The basement membrane functions as a matrix barrier against the migration of cells [8]. Inflammatory cells would have to penetrate this barrier to reach the airway lumen. The basement membrane is constructed of various types of collagen, laminin, entactin, fibronectin, tenascin, and proteoglycan, which are substrates of endogenous matrix-degrading enzymes [9]. Among them, MMP-2 and -9 have been demonstrated to participate in the trans-membrane migration of eosinophils and lymphocytes through the

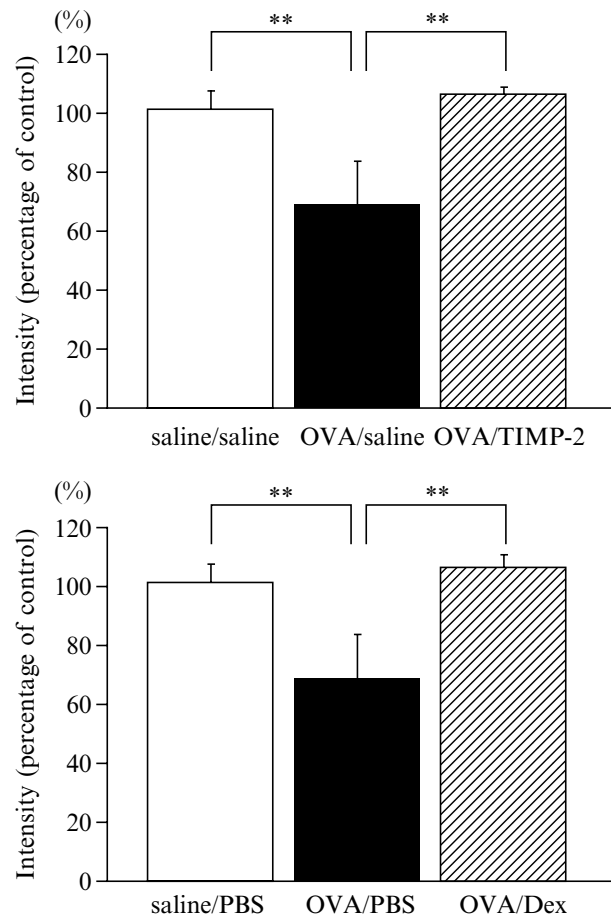


Fig. 8. The intensity of the immunostaining for type IV collagen in subepithelium of tracheal tissues. Tracheal tissues from mice 3 days after saline inhalation with the administration of saline (saline/saline) or PBS (saline/PBS), after OVA inhalation with saline (OVA/saline) or PBS (OVA/PBS) and after OVA inhalation with TIMP-2 (OVA/TIMP-2) or dexamethasone (OVA/Dex) were immunostained with antimouse collagen IV antibody. The intensity of the staining was determined as described in Materials and Methods. $**P < 0.01$.

degradation of matrix components *in vitro* [17, 23]. They are overexpressed, in association with cellular infiltration, in airway tissues and BAL fluids of asthmatics with acute exacerbations and in those of sensitized mice after antigen challenge [24, 30, 31]. Furthermore, we recently demonstrated using the same murine model as in the current study that the inhibition of MMP activities including MMP-2 and -9 by the administration of TIMP-2 reduced the migration of inflammatory cells to airway lumen after antigen challenge, and that the TIMP-2 indeed made a complex with MMP-2 [24]. Finally, as shown by electron microscopy and immunohistochemical staining for type IV collagen, the alterations in the basement membrane were not observed in antigen-challenged mice administered with TIMP-2. With these observations, it can be assumed that the basement membrane was injured, at least in part, by the proteolytic activity of MMPs including MMP-2 and -9 released from inflammatory cells during their transmigration through the membrane. MMPs could be also released from structural cells such as epithelial cells [15] during the inflammation. TIMP-2 might inhibit the matrix degradation by MMPs released from epithelial cells. Plasmin and elastase are capable of

degrading matrix components in the basement membrane [9]. Eosinophils have been shown to possess the membrane receptor for urokinase plasminogen activator which is involved in their transmigration through the matrix membrane [32], and to release elastase activity [33]. Accordingly, the contribution of these enzymes to the degradation of the basement membrane observed here can not be ruled out.

It is likely that type III collagen was degraded along with type IV collagen during the cellular migration. One would expect that the intensity of the immunostaining for type III collagen would be decreased as was type IV. However, it was not significantly decreased. While type IV collagen is localized in the lamina densa, type III collagen is distributed diffusely through the lamina reticularis to the submucosa [8, 9]. Due to the distribution of the collagen, the degraded type IV collagen would be localized in the lamina densa so that the decrease of the staining intensity might be significantly detectable by light microscopical examination. On the other hand, the degradation of type III collagen would be diffusely distributed so that the decrease might not be apparent. It was not determined in the current study whether the decreased immunoreactivity of type IV collagen was due to the destruction of antigen determinants and/or the decrease of the collagen content. There is a possibility that the degraded type IV collagen was disanchored from the lamina densa while the degraded type III collagen was still in the subepithelial area, which might result in a decrease of the reactivity to anti-type IV but not anti-type III collagen antibody in the area examined.

Our assumption that the injury of the basement membrane was a consequence of cell trans-membrane migration was supported by the results shown after the administration of corticosteroids. Dexamethasone is well known to inhibit the migration of inflammatory cells through suppressing the expression of chemoattractants and adhesion molecules [34], which would decrease the exposure of the membrane to migrating cells. Also, corticosteroids are able to reduce the expression of MMPs as reported in sputum, BAL fluids and bronchial biopsy tissues of asthmatics [16, 30, 35]. Tissue injury associated with eosinophil migration was also reported in cultured epithelial cells, in which the disruption of the epithelial cell integrity was demonstrated by immunohistochemical detection of decorin injury [36]. Invasion and metastasis of malignant cells is also well recognized as such a case of destruction of the basement membrane by MMPs released from migrating malignant cells [37].

It was also observed that the injury in the basement membrane, in terms of the immunoreactivity of type IV collagen, was repaired by 10 days after OVA inhalation. Decreased MMP expression in our model [24] and the low level of the MMP-9/TIMP ratio consequent to acute exacerbation [30] could reduce the injury of the membrane. The increased production of fibronectin [38] and the emergence of myofibroblasts [39] were demonstrated in asthmatic airways after antigen challenge. The overexpression of growth factors [12] and the presence of fibroblasts with enhanced potential for matrix synthesis [40] in asthmatic airways were also shown. Matrix fragments resulting from the degradation of basement membrane components have biological effects on the migration and activation of structural cells [8]. These cellular and molecular events could contribute orchestrally to the repair of the injury of the basement

membrane as observed here. Type III collagen, which is often observed to be increased in the lamina reticularis of asthmatic airways, was not changed immunohistochemically even 10 days after single antigen-exposure, while the collagen deposition to airway walls was observed in animal models after chronic antigen challenge [41, 42]. The repetition of injury and the repair processes must be important in the remodelling of the basement membrane.

In summary, the injury of epithelial basement membrane associated with acute allergic inflammation was demonstrated to be caused, at least in part, by MMPs during cell migration through the membrane. Further investigation of the mechanisms by which repair of the injured basement membrane occurs after a single episode of allergic airway inflammation would be helpful to understand the pathogenic role of chronic airway inflammation in tissue remodelling such as subepithelial fibrosis in asthmatic airways.

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