

Airway Remodeling in Asthma and Irreversible Airflow Limitation—ECM Deposition in Airway and Possible Therapy for Remodeling—

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ABSTRACT

Airway remodeling in asthma is characterized by goblet cell hyperplasia, subepithelial fibrosis, and hyperplasia and hypertrophy of airway smooth muscle cells. The airway wall thickness increases because of subepithelial fibrosis, and hyperplasia and hypertrophy of the airway smooth muscle cells and submucosal glands. Airway remodeling, therefore, can often cause irreversible airflow limitation and an increase of airway hyperresponsiveness. Recent studies have described the molecular and cellular mechanisms of collagen deposition in the airway wall such as subepithelial fibrosis. Fibroblasts or myofibroblasts play a critical role in the exaggerated deposition of collagen in asthmatic airways. Bone marrow derived fibroblasts may play a role in fibrotic remodeling in asthmatic airways. Airway remodeling is induced by cytokines and mediators produced in chronic allergic airway inflammation. Since, once formed, remodeling is resistant to asthma therapy, early intervention with inhaled corticosteroid should be considered to prevent the progress of airway remodeling.

KEY WORDS

bone marrow-derived cells, CTGF, myofibroblast, peripheral airway, TGF- β

INTRODUCTION

Bronchial asthma has been recognized as a respiratory disease that causes a long term decline of the lung function. As an obstructive lung disease, COPD is also widely recognized to cause a long term decline of the lung function including irreversible airflow limitation. As with COPD, reversible airflow limitation is a characteristic physiological feature of bronchial asthma. It had been thought that the impairment of pulmonary function in asthma could be reversed to a normal level with sufficient therapy. However, since inhaled corticosteroid therapy was introduced globally at the end of the last century, many clinicians noticed that the airflow limitation remained in some asthmatics after the administration of oral corticosteroid, high dose inhaled corticosteroid and bronchodilators.

Peat *et al.* and Lange *et al.* reported that FEV1 in asthmatics declined faster than in normal individuals

(Fig. 1).^{1,2} These airflow limitations are thought to result mainly from airway remodeling. In particular, fibrosis of the airway wall has been thought to play a major role in the irreversible airflow limitation in both asthma and COPD.

In this review, we describe the effects of airway remodeling on pulmonary function and the asthma severity, and the cellular mechanism of airway fibrosis in asthma.

AIRWAY REMODELING IN ASTHMATIC AIRWAY

Since long before, pathological analyses have revealed the dramatic structural changes in airways in autopsies of patients with severe asthma.^{3,4} Such changes are transformation of the epithelium to goblet cells, thickening of the epithelial basement membrane, deposition of extracellular matrix in the subepithelial layer, hyperplasia of submucosal glands, hypertrophy and hyperplasia of bronchial

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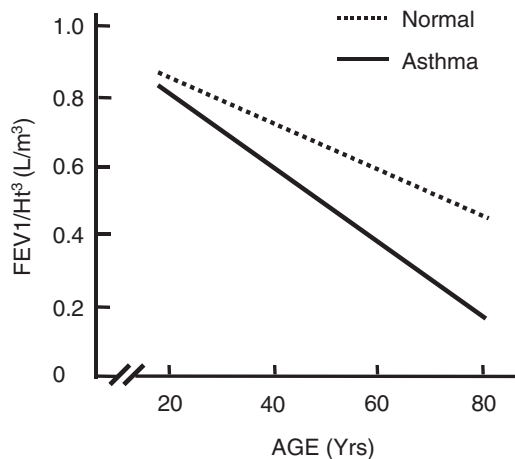


Fig. 1 The impairment of pulmonary function in asthma. Decrease of FEV1 in asthma was faster compared to that in normal individuals. (This figure was from Ref 1, and modified.)

smooth muscle, and an increase of submucosal vessels.⁵ These structural findings are characteristic for asthma and are referred to as airway remodeling.

From the view point of lung function, there have been reports since the 1970's describing patients with chronic asthma who exhibited persistent airflow limitation even during periods of complete remission or despite treatment with bronchodilators, including theophylline and β_2 stimulants, and oral corticosteroids.⁶ Persistent airflow limitation is not rare in patients with moderate/severe asthma during asymptomatic periods after the use of β_2 stimulants and there are many difficult asthmatics among these patients. Their airflow limitation is not fully improved by a high dose of corticosteroid, suggesting that the airflow limitation is not caused by transient airway inflammation.⁷ Airway remodeling results in thickening of the airway wall and was thought to cause irreversible airflow limitation.⁸ The relationship between the pathological changes and pulmonary function is important. There have been several reports which investigated the relationship between the pathological changes in specimens from transbronchial biopsies and pulmonary functions or asthma symptoms.^{10,11} However, the relationship is not fully understood because of limited numbers of asthmatics receiving transbronchial biopsy and the size of the transbronchial biopsy samples in which only the epithelium and restricted submucosal areas can be analyzed.

According to previous papers, airway remodeling was more prominent in the autopsy specimens than in transbronchial samples because the majority of patients with fatal asthma had had severe asthmatic attacks whereas patients with mild or moderate asthma tend to accept transbronchial biopsy.¹¹

EXAGGERATED EXTRACELLULAR MATRIX DEPOSITION IN ASTHMATIC AIRWAYS

The repair process after tissue injury often includes a fibrotic response. The chronic airway inflammation in asthma causes epithelial injury and the subsequent, repetitive repair process is thought to be responsible for the thickening of the epithelial basement membrane which has been observed as a characteristic feature in asthma and is reported to be found even in early asthma.¹² The thickening of the basement membrane observed by microscopy corresponds to the deposition of extracellular matrix (ECM) at the subepithelial space observed by electron microscopy and is called subepithelial fibrosis.^{13,14} In addition, Wilson reported the deposition of collagen not only in the subepithelial space but also in the deeper submucosal layer in asthmatics.¹⁵ The deposited ECM consisted of type III and V collagen, laminin, tenascin and fibronectin.¹⁵⁻¹⁸

One of the mechanisms of ECM deposition is thought to be an imbalance between synthesis and degradation. During transmigration through the basement membrane, inflammatory cells such as eosinophils produce and secrete matrix metalloproteinase-9 (MMP-9) capable of digesting type IV collagen, which is one of the components of the basement membrane.¹⁹ Over-production of tissue inhibitors of matrix metalloproteinases 1 (TIMP-1), an inhibitor of MMP-9, causes deposition of ECM and thickening of the basement membrane by inhibiting the degradation of ECM.

TGF- β is known to be a cytokine that increases the production of ECM. TGF- β is synthesized by a variety of cells such as macrophages, lymphocytes, fibroblasts, airway epithelial cells, eosinophils, mast cells, etc (Fig. 2).²⁰⁻²³ TGF- β also induces TIMP-1 expression and IL-13 augments TIMP-1 induction by TGF- β suggesting that ECM deposition proceeds in asthmatic airways in which Th2 cytokines are produced continuously.²⁴ In the airway, collagens have been thought to be produced mainly by fibroblasts, and the numbers of myofibroblasts in the submucosa are increased in the asthmatic airway. In addition, connective tissue growth factor (CTGF) has attracted attention as a new growth factor which is directly involved in the fibrotic response as a down-stream mediator of TGF- β .²⁵ Piao *et al.* demonstrated the up-regulation of CTGF gene expression after allergen exposure in an experimental asthma model with ovalbumin-sensitized mice and an association between the level of CTGF mRNA and collagen deposition in subepithelial tissue (Figs. 3A, B).²⁶ Recently, Kunzmann S *et al.* reported that histamine induced CTGF production in lung fibroblasts *via* H1 receptor suggesting allergic inflammation tends to induce the fibrotic response under the influence of histamine on fibroblasts in airways.²⁷

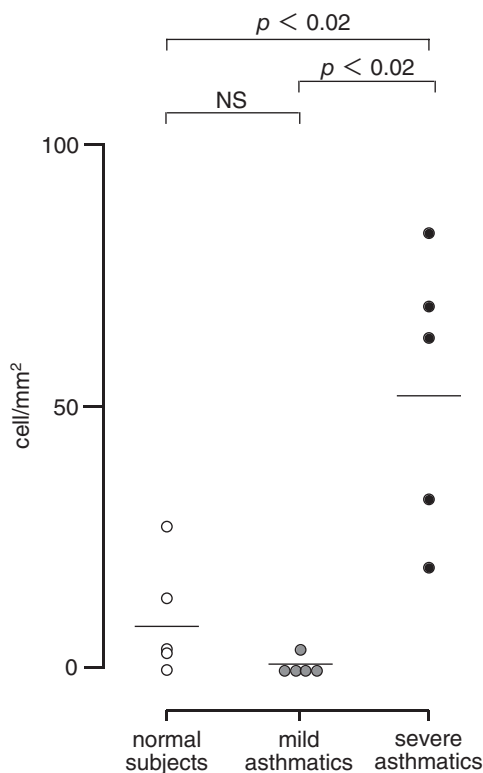


Fig. 2 Quantitative analysis of TGF β 1 gene expression in bronchial tissue. The number of cells expressing TGF β 1 mRNA in sections was scored. (This figure was from Ref 23, and modified.)

Since the thickening of the basement membrane in asthmatic airways is easily observed and can be analyzed quantitatively in the tissue samples from transbronchial biopsy, its relationship with pulmonary functions such as FEV1.0 or airway hyperresponsiveness and asthma severity has been investigated widely. Minshall *et al.* demonstrated a significant relationship between the thickness of the basement membrane and airflow limitation, and furthermore, a significant relationship between the thickness of the basement membrane and the strength of TGF- β mRNA expression in eosinophils.⁹ Smad7 is an inhibitory protein against the intracellular signal transduction of TGF- β , and is thought to be a modulator of TGF- β actions. Nakao *et al.* performed immunohistochemistry for Smad7 in the bronchial tissues obtained by transbronchial biopsy from asthmatics and normal individuals, and compared its expression in the airway of these two groups.²⁸ They demonstrated a significant reduction of Smad7 expression in asthmatic airways compared to normals. To date, several papers reported that the thickness of the basement membrane had a negative relationship with FEV1% (Fig. 4) and provocative dose of methacholine.^{29,30}

An increase of the collagen content in airways may alter the physical properties of the bronchial tree. In

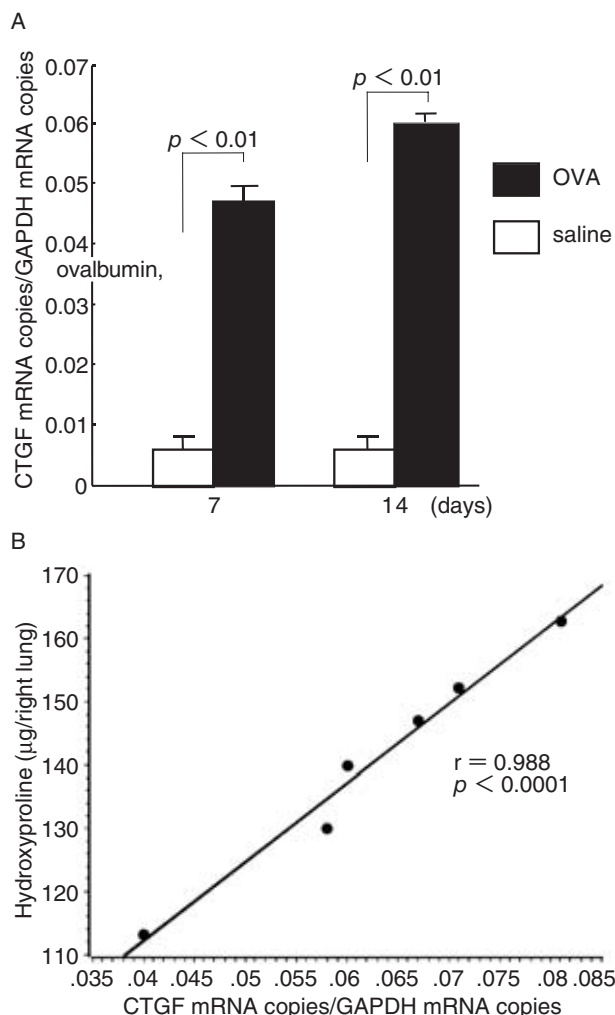


Fig. 3 A; CTGF mRNA expression in pulmonary tissues in an experimental murine asthma model. The level of CTGF mRNA in pulmonary tissues from the mice exposed to allergen increased markedly compared with those exposed to saline, **B;** Relationship between the level of CTGF mRNA and the content of hydroxyproline in the lung. (This figure was from Ref 26, and modified.)

fact, Ward *et al.* showed a negative relationship between the distensibility of the airway and the thickness of the basement membrane.³¹ Furthermore, Milanesi *et al.* measured thickness of epithelial basement membrane of bronchial tissues obtained by transbronchial biopsy from patients with mild perennial asthma, perennial allergic rhinitis, seasonal rhinitis and COPD. They revealed that the thickness of the basement membrane in the patients with mild perennial asthma and perennial allergic rhinitis was significantly greater than in the patients with seasonal rhinitis and COPD. In addition, the thickness of the basement membrane in the patients with asthma showed a positive relationship with the provocative dose of methacholine.³² These results suggested that

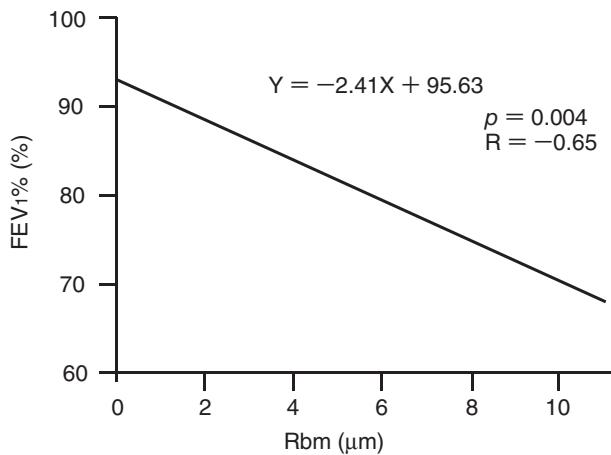


Fig. 4 Correlation Rbm thickness and FEV1%. FEV1% was negatively correlated with Rbm thickness. (This figure was from Ref 30 and modified.)

the reduction of elasticity in airway is associated with the thickening of the basement membrane, which resists the airway narrowing by smooth muscle contraction.

Deposited collagen makes the airway more rigid. The rigidity may contribute to a protective effect against airway smooth muscle (ASM) shortening or airway narrowing. In animal models, airway wall thickening,³³ or the deposition of ECM such as collagen or fibronectin in the airway wall after repeated allergen exposure,³⁴ was associated with attenuated ASM shortening. Furthermore, Niimi *et al.* measured the wall thickness of bronchi by helical computed tomography in patients with asthma and demonstrated that the airway reactivity negatively correlated with the airway wall thickness.³⁵ Bai *et al.* reported that older patients with fatal asthma had more muscle and collagen deposition in the airway wall than younger patients with fatal asthma (Fig. 5).³⁶ In this paper, histological analysis of the peripheral airway from autopsy samples of asthmatic patients revealed that the thickened airway wall consisted of smooth muscle, fibrotic tissue and inflammatory cell infiltration. The narrowing of the airway lumen suggested that the fibrosis of the airway wall attenuated the smooth muscle contraction by acting as a resistive force. The fibrosis of the airway wall might protect against the collapse of the airway lumen by an exaggerated contraction of the airway smooth muscle whose mass was extraordinarily increased by hypertrophy and hyperplasia.

PHENOTYPES OF FIBROBLASTS INVOLVED IN AIRWAY WALL FIBROSIS

Bone marrow derived cells have been recognized to play a critical role in tissue injury.^{37,38} Circulating fibrocytes can migrate to sites of wound-healing to serve as a source for fibroblasts and myofibroblasts

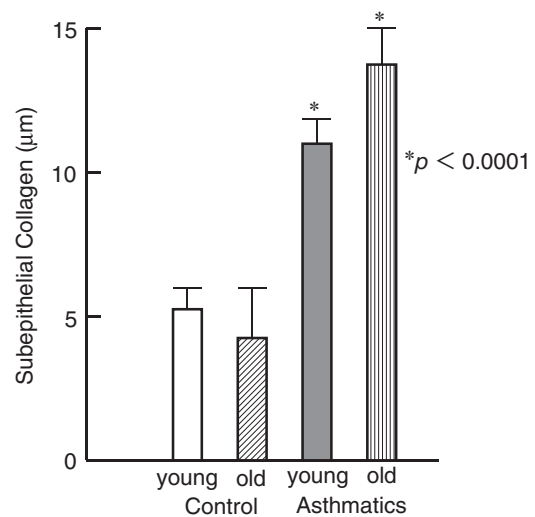


Fig. 5 Subepithelial collagen deposition in the peripheral airway of young and old asthmatics. (This figure was from Ref 36, and modified.)

that normally participate in the repair process.³⁹ Hashimoto *et al.* demonstrated that bone marrow-derived fibroblasts were involved in pulmonary fibrosis in rats treated with bleomycin. Their results suggested the possibility that bone marrow-derived precursor cells could serve as a source for fibroblasts in bleomycin-induced pulmonary fibrosis.⁴⁰ Furthermore LPS induced lung injury recruited bone marrow-derived progenitor cells which differentiated into endothelial cells and epithelial cells in alveolar tissue. These recruited bone marrow-derived progenitor cells may play a critical role in tissue repairing because suppression of the bone marrow-derived progenitor cells induced emphysematous changes.⁴¹

A recent study demonstrated the existence of circulating fibroblast-like cells in human beings.⁴² Schmidt *et al.* demonstrated that allergen exposure induces the accumulation of fibrocyte-like cells in the bronchial mucosa of patients with allergic asthma. These cells were CD34-positive and expressed collagen I and α -smooth muscle actin, and were localized to areas of collagen deposition below the epithelium (Fig. 6). They suggested that human circulating fibrocytes acquire the myofibroblast phenotype under *in vitro* stimulation with fibrogenic cytokines that are produced in exaggerated quantities in asthmatic airways. These results suggest that circulating fibrocytes may function as myofibroblast precursors and contribute to the pathogenesis of subepithelial fibrosis in asthma.⁴³ Nihlberg K *et al.* detected more fibrocytes that were positive for CD34, CD45RO, procollagen I, and α -smooth muscle actin in bronchial biopsies and BALF from mild asthmatic patients compared to controls. They also showed the basement membrane thickness could be correlated to the number of fibrocytes in tissue (Fig. 7).⁴⁴ These results

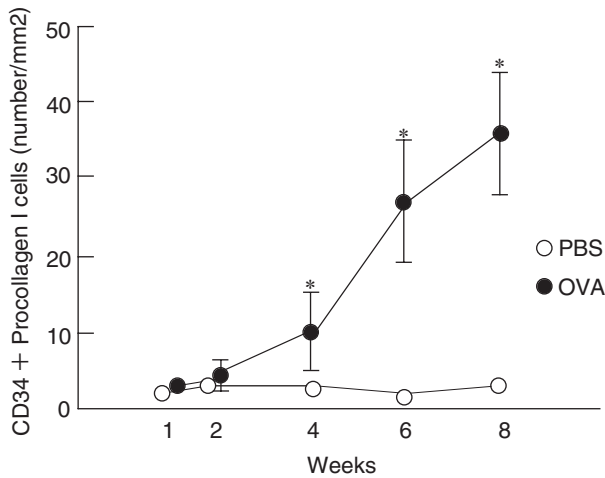


Fig. 6 Fibroblast-like cells in the airway wall of mice exposed to allergen. Count of cells double-stained with the anti-CD34 and anti-procollagen I. * $p < 0.05$ (This figure was from Ref 43, and modified.)

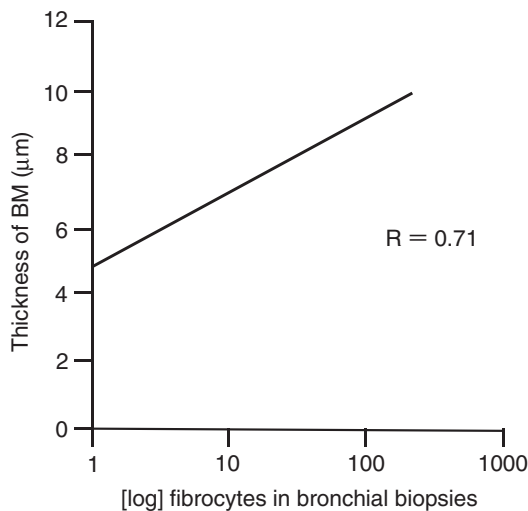


Fig. 7 Numbers of fibrocytes in bronchial tissues of asthmatics were counted and correlated ($r = 0.711$) to the thickness of basement membrane (μm). (This figure was from Ref 44, and modified.)

suggested that circulating fibrocytes could be one of the critical therapeutic targets of airway remodeling in asthma.

SITE OF REMODELING IN BRONCHIAL TREE

The cellular components and their biological responses appear different in the proximal and distal airways. Kotaru *et al.* suggested that at least two phenotypes of fibroblast exist in the lung and these phenotypic differences may partially explain the variable responses to injury and repair between proximal airways and distal lung/parenchyma in asthma and

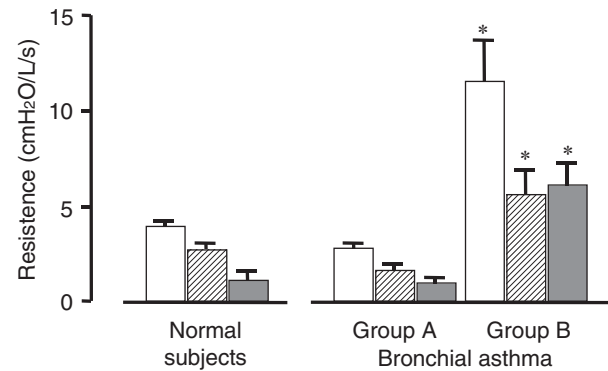


Fig. 8 Absolute values of pulmonary resistance, central airway resistance, and peripheral resistance in normals and asthmatics. Total pulmonary resistance: white column, central airway resistance: dashed column, peripheral resistance: gray column; Group A: asthmatics without airflow limitation; Group B: asthmatics with airflow limitation. * $p < 0.01$ (This figure was from Ref 47, and modified.)

other respiratory diseases.⁴⁵

Concerning pulmonary function, the site of obstruction in the bronchial tree in asthma was once thought to be in the central airway because the total area of the central airway is definitely smaller than that of the peripheral airway. However, measurement of the peripheral airway resistance with an antero-grade catheter revealed that the peripheral airway resistance in asthmatics was significantly higher than that in normal subjects, suggesting that a pathologic abnormality existed in the peripheral airway in asthma.^{46,47} Furthermore, Ohrui *et al.* measured the hyperresponsiveness to methacholine in the peripheral airway and demonstrated a relationship between the hyperresponsiveness in the peripheral airway and irreversible airflow limitation (Fig. 8).⁴⁸ Pathological studies on autopsy samples from patients who died of asthma have shown the histological changes from the central airway to the peripheral airway.⁴⁹ However, it is extremely difficult to obtain tissue of the peripheral airway by bronchoscopy. Hamid *et al.* obtained lung tissue from asthmatics who underwent an operation, and analyzed the inflammatory cells in the peripheral airway. They revealed that inflammatory changes including eosinophil infiltration in the peripheral airway (internal diameter <2 mm) were more severe compared with those in the central airway, suggesting that the major site of the pathological abnormalities in asthma is the peripheral airway.⁵⁰ Bai *et al.* also described smooth muscle hypertrophy and hyperplasia, tissue fibrosis and inflammatory cell infiltration in the peripheral airways of older patients who died from with asthma.³⁶

Peripheral airway is easily obstructed by smooth muscle contraction in the absence of cartilage and by mucous plugs. Previous reports revealed that asth-

matics with peripheral airway obstruction tended to have frequent asthma attacks.⁵¹ However, at present there is little reliable information about airway remodeling in the peripheral airway.

THERAPY FOR AIRWAY REMODELING IN ASTHMA

The development of airway remodeling has been reported to be associated with severe asthma.⁵² While some structural changes are reversible by therapy, others are thought to be irreversible.⁵³⁻⁵⁵ For example, goblet cell hyperplasia can be easily returned to normal epithelium by sufficient inhaled corticosteroid (ICS) therapy. In contrast, a thickened basement membrane and airway wall fibrosis are relatively resistant to ICS therapy. Irreversible airflow limitation lowers the QOL of severe asthmatics. Airway remodeling in asthma is thought to cause impairments of pulmonary function including irreversible airflow limitation. Since chronic airway inflammation induces airway remodeling in asthma, one of the effective treatments at present is to control airway allergic inflammation. However, once airway remodeling has been formed, it is hard to cure even if inhaled corticosteroid is used as described above in the case of subepithelial fibrosis. As we know, almost all patients with early asthma categorized as mild asthma, but some of these patients show increased severity over time. However, the factors that aggravate the severity of asthma have not been elucidated clearly.

Since, as described above, airway remodeling in the peripheral airway is critical for the pulmonary function and severity of asthma, one of the important targets of asthma therapy is the peripheral airway. To date, it has been thought that particles of inhaled corticosteroid hardly reached to the peripheral airway. Recently, new types of inhaled corticosteroids, such as HFA-beclomethasone or HFA-ciclesonid, showed high deposition on peripheral airway and lung parenchyma because the particles are ultra-fine. Hauber *et al.* reported that eosinophilic inflammation in the peripheral airway was suppressed by HFA-flunisolide in asthmatics.⁵⁶ Bergeron *et al.* showed a reduction of the smooth muscle area and an improvement of peripheral airflow limitation with HFA-flunisolide in patients with mild to moderate asthma, suggesting that treatment with ICS, the particle of which are smaller particle sized, would be effective to treat airway inflammation and remodeling in the peripheral airway.⁵⁷

Since some components of airway remodeling are resistant to ICS therapy, early intervention is thought to be an important strategy to prevent airway remodeling. Haahtela *et al.* described that a delay in starting ICS therapy induced irreversible airflow limitation which was thought to be caused by airway remodeling.⁵⁸ A large number of patients with severe asthma showing irreversible airflow limitation had a signifi-

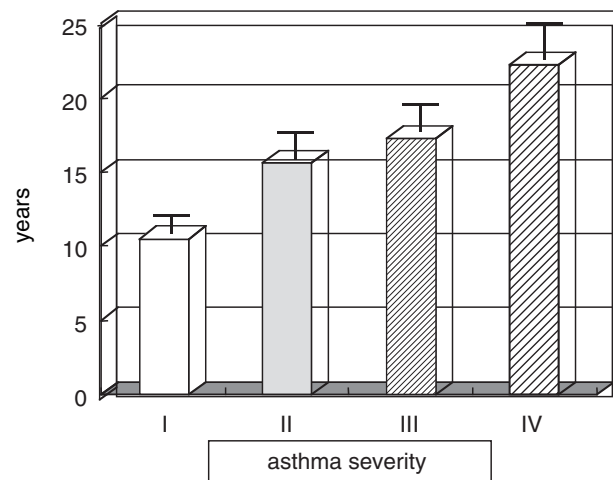


Fig. 9 Duration of asthma according to asthma severity in the asthma outpatient clinic of Iwate medical University School of Medicine in 2005. (This figure was from Ref 59, and modified.)

cantly longer duration of asthma compared to those with mild/moderate asthma. We can speculate that a considerable number of the patients with severe asthma had not received appropriate asthma therapy including ICS in the early stage of asthma. Since most clinicians started to adopt ICS therapy for asthma in Japan in the 1990's, we assume that many patients did not receive ICS therapy early enough in their treatment before the 1990's, which impaired their pulmonary function, at least in part by airway remodeling, resulting in a worsening of their asthma severity. As a matter of fact, FEV1% predicted is significantly lower in Japanese patients with severe asthma compared to those with mild/moderate asthma, and the asthma duration among Japanese patients with severe asthma was significantly longer (Fig. 9).⁵⁹ On the other hand, a significant reduction in the ratio of severe asthma was revealed in a 10-year study of a Japanese population of newly diagnosed as asthma, that received standard therapy for asthma including ICS (Figs. 10A, B).

Recently, Hendersen *et al.* reported that montelukast, a leukotriene receptor antagonist, suppressed subepithelial fibrosis and smooth muscle hypertrophy in a murine asthma model exposed to antigen. Furthermore, they also demonstrated that montelukast ameliorated the already formed airway remodeling in murine asthma model after one month exposure to allergen.^{60,61} Leukotriene C4 and D4 are thought to play an important role in fibroblast proliferation and ECS production. In this regard, the effects of montelukast on remodeling in human airway need to be evaluated.

In summary, at present early introduction of ICS to newly diagnosed asthmatics may be a useful and practical therapeutic strategy to prevent progress of

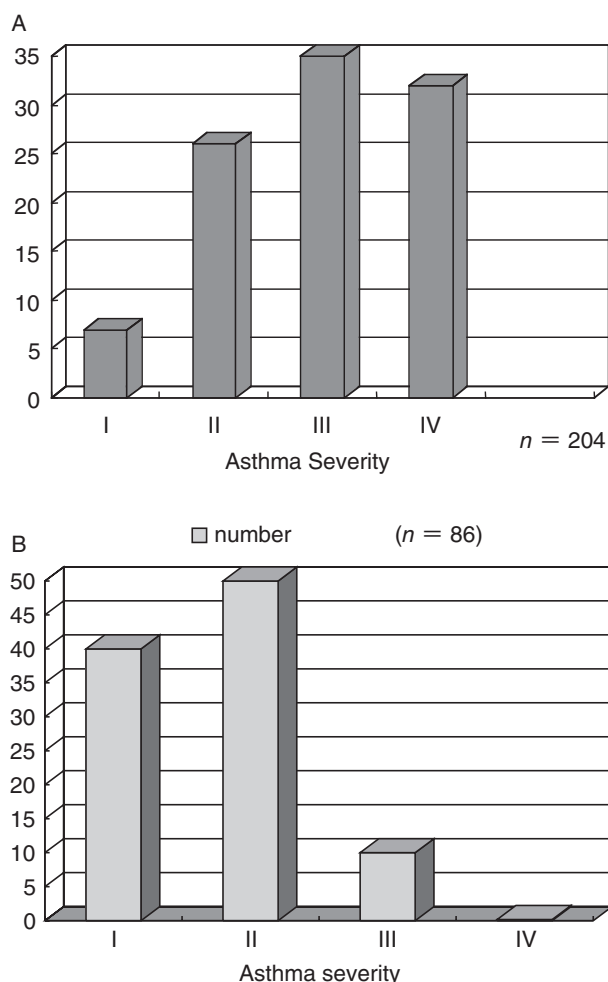


Fig. 10 **A**; Distribution of asthmatics according to asthma severity in the asthma outpatient clinic of Iwate medical University School of Medicine in 2005. The ratio of the patients with severe asthma exceeded more than 30% despite sufficient therapy with high dose ICS, **B**; Distribution of asthma severity of asthmatics who were newly diagnosed as asthmatic. No severe asthma was recorded in the newly diagnosed asthma from 1995 to 2005. (This figure was from Ref 59, and modified.)

airway remodeling.

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