

Airway Remodeling and Airway Smooth Muscle in Asthma

Mitsuru Munakata¹

ABSTRACT

Airway remodeling in asthma has been recognized as structural changes of airways such as smooth muscle hypertrophy (an increase in size of airway smooth muscle cells) and hyperplasia (an increase in the number of airway smooth muscle cells), thickening and fibrosis of sub-epithelial basement membrane, hypertrophy of bronchial glands, goblet cell hyperplasia, and thickening of airway epithelium. In these pathological changes, airway smooth muscle remodeling has been recognized as one of the most important factors related to *in vitro* and *in vivo* airway responsiveness and the severity of asthma. Both hypertrophy and hyperplasia have been shown in asthmatic airways by morphometrical analyses, although there is a wide variation in the contribution of each mechanism in each patient. Such changes could also be recognized as a phenotypic modulation of airway smooth muscle. On the background of airway smooth muscle remodeling, the existence of several contributing factors, such as inflammatory mediators, growth factors, cytokines, extra-cellular matrix proteins, and genetic factors have been suggested. On the other hand, recent studies revealed that airway smooth muscle could also be a source of inflammatory mediators promoting airway inflammation. In this article, the recent understanding in the mechanisms of airway smooth muscle remodeling in asthma, its relations to airway inflammation and airway physiology, and possible usefulness of early intervention with inhaled glucocorticoids have been discussed.

KEY WORDS

airway hyperresponsiveness, hyperplasia, hypertrophy, *in vitro*, *in vivo*

INTRODUCTION

Asthma is a syndrome characterized by the presence of two physiological characteristics, reversible airflow limitation and airway hyperresponsiveness. However, recent advance in asthma research revealed the importance of airway inflammation existed behind these physiological characteristics. According to such progress in the concept of asthma, the strategies of the treatment have changed dramatically, and the importance of anti-inflammatory therapy with inhaled glucocorticoids has been recognized. Such concept has been reflected to the guidelines for asthma diagnosis and management including Japanese Guideline for Asthma.^{1,2} The pronounced improvement in asthma control and quality of life of the patients, and a dramatic reduction in the number of emergency room visit and asthma death have been achieved. In these situations, concerns of the pulmonary physicians

have been focused more on the patients with severe intractable asthma. These patients have continuous symptoms, irreversible airflow limitation, and resistance to anti-inflammatory treatment with inhaled glucocorticoids. Airway remodeling, recognized as structural changes of airways produced by continuous airway inflammation, is speculated to be one of the most important causes for such severe intractable asthma. In this article, the recent understanding in the mechanisms of airway smooth muscle remodeling, and its relations to airway inflammation and physiology have been discussed.

LONG-TERM OUTCOME OF AIRWAY FUNCTION IN ASTHMA

In several studies, long-term outcomes of airway function in patients with asthma have been studied and the accelerated decline in forced expiratory volume in one second (FEV₁) has been demonstrated.³⁻⁵

¹Department of Pulmonary Medicine, School of Medicine, Fukushima Medical University, Fukushima, Japan.
Correspondence: Mitsuru Munakata, MD., Ph.D., Department of Pulmonary Medicine, School of Medicine, Fukushima Medical University, Hikarigaoka-1 Fukushima City, Fukushima 960-1295, Ja-

pan.
Email: munakata@fmu.ac.jp
Received 5 April 2006.
©2006 Japanese Society of Allergology

Large scale epidemiological study by Lange and associates revealed that the annual decline in FEV₁ in asthma and non-asthma were 38 ml/year and 22 ml/year, respectively, and asthmatics had additional 16 ml/year over-decline in FEV₁.⁵ Similar enhanced annual decline in FEV₁ was also observed in relatively large-scale surveys, such as Busselton Health Surveys reported by James and associates and the survey by Apostol and associates.^{3,4} Such accelerated decline is also reported for the subjects with airway hyperresponsiveness by Rijcken and associates.⁶ The subjects with airway hyperresponsiveness were revealed to have additional 12 ml/year over-decline in FEV₁ compared to those without airway hyperresponsiveness. Several studies including ours also demonstrated that, in asthmatic patients, there is a significant negative correlation between the duration of their disease and % predicted FEV₁ measured during stable phase or after inhalation of bronchodilators, but no significant correlation could be observed between % predicted FEV₁ and the age of the patients.⁷ These findings suggest that the events continuing in asthmatic airways may promote their airflow limitation irreversible, and become critical factors for severe and intractable asthma.

AIRWAY SMOOTH MUSCLE REMODELING IN ASTHMA

Pathologists have long recognized that there are significant pathological changes in asthmatic airways, such as airway smooth muscle hyperplasia and hypertrophy, thickening and fibrosis of sub-epithelial basement membrane, hypertrophy of bronchial glands, goblet cell hyperplasia, and thickening of airway epithelium. In these pathological changes, airway smooth muscle remodeling has been recognized as one of the most important factors related to *in vitro* airway responsiveness and the severity of asthma. These changes have been reported not only in severe asthmatics but also in mild and moderate asthmatics. In our previous studies for autopsied or operated lungs of the patients with asthma showed that the airway epithelial thickness was doubled and airway smooth muscle thickness was tripled in asthmatics when compared to controls.⁸ Such pathological alterations were observed even in young or mild asthmatic subjects. The recent study by Niimi and associates revealed that even in patients with cough variant asthma, possible early manifestation of bronchial asthma, airway remodeling recognized as a thickening of airway wall by high-resolution computer tomography could be observed.⁹ In addition, the appearance of airway remodeling has been confirmed even in children with bronchial asthma.¹⁰ These findings suggest that airway remodeling is a relatively common phenomenon observed widely in patients with asthma, and may have significant importance in clinical outcome of the patients.

The most prominent pathological change of airway smooth muscle in asthmatic is an increase in smooth muscle mass. Such change could be induced by several mechanisms. Hypertrophy (an increase in size of airway smooth muscle cells) and hyperplasia (an increase in the number of airway smooth muscle cells) are well known mechanisms. However, there is a wide variation in the contribution of each mechanism in each patient. Ebina and associates performed 3-D morphometry of airway muscle cells on serial sections of autopsied lungs from 10 asthmatic patients.¹¹ They are consisted with 5 cases of type I (thickened muscles is found only in the central bronchi) and 5 cases of type II (thickened muscles is found over the whole airway tree) asthmatic lungs as defined in their earlier study.¹² In type I asthmatics, smooth muscle cell hyperplasia was observed in the larger bronchi without hypertrophy. In contrast, in type II asthmatics, smooth muscle cell hypertrophy was observed over the whole airway and hyperplasia was mild and localized only in the bronchi. It has not been clarified whether these differences affect clinical features of the patients. In addition, there are still debates in whether there is an enhancement of smooth muscle proliferation in asthmatic airways. This question is of physiological importance since it might be related to phenotypic modulation of airway smooth muscle.¹³⁻¹⁵ The relation between increase in airway smooth muscle mass and the duration of asthma has also been recognized. Bai and associates compared the size of airway smooth muscle mass in autopsy specimen obtained from young (17–23 yr) and relatively aged (40–49 yr) subjects. Smooth muscle area in both asthma groups was greater than that in age-matched control subjects. But, in relatively old individuals it was 4-fold increase, and in young individuals it was 2-fold increase compared with age-matched controls.¹⁶ The increase in extra-cellular matrix proteins may also increase airway smooth muscle mass.^{17,18} The increase in connective tissue matrix could be observed both within muscle bundles and around individual cells. Additional interesting observation is the presence of mast cells in asthmatic airway smooth muscle bundles. Brightling and associates examined pathological differences of airways between asthma and eosinophilic bronchitis, and observed substantially higher number of tryptase-positive mast cells in the smooth muscle bundles from asthmatic airways compared to that from subjects with eosinophilic bronchitis.¹⁹ This finding suggests that the infiltration of airway smooth muscle by mast cells is associated with the disordered airway functions found in asthma. In consecutive study, they revealed that IL-4 and IL-13 may play an important role in this mast cell-airway smooth muscle interactions.²⁰

AIRWAY SMOOTH MUSCLE REMODELING AND AIRWAY PHYSIOLOGY

IN VIVO AND IN VITRO AIRWAY RESPONSIVENESS IN ASTHMA

Airway hyper-responsiveness is one of the major characteristics of asthma. Clinically, it can be demonstrated by measuring FEV₁ or airway resistance after inhalation of incremental dose of non-specific airway smooth muscle constrictor, such as acetylcholine, methacholine, and histamine. It seems not difficult to connect airway smooth muscle remodeling and airway hyperresponsiveness in asthma. The increased airway wall area including smooth muscle mass may directly cause excess airways narrowing of asthma. James and associates showed this mechanism elegantly using a computer model and real airway dimensional data obtained from asthmatic and control airways.¹⁰ The airway wall area in asthmatics is greater than non-asthmatic airways, and the airway smooth muscle shortening required to occlude the lumen was less in asthmatic than non-asthmatic airways. An increased mass of airway smooth muscle bundle, demonstrated as airway smooth muscle hypertrophy or hyperplasia, may increase the force generated during airway contraction. In smooth muscle hyperplasia, increased number of smooth muscle cells may cause parallel increase in contractile filaments. In hypertrophic smooth muscle, there might be an increase in intracellular contractile filaments. These could increase the smooth muscle force to contract the airway and to reduce airway caliber.

However, there is a fair amount of debate whether airway responses to inhaled smooth muscle constrictors *in vivo* is correlated with airway smooth muscle responsiveness *in vitro*. In subjects without lung diseases, no significant correlation is observed between *in vitro* airway responsiveness and *in vivo* airway smooth muscle responsiveness.²¹⁻²³ There are only few studies in which enhanced contractile responses of airway smooth muscle from asthmatics compared to non-asthmatic airway smooth muscle was confirmed. De Jongste and associates examined the responses of isolated human airways obtained from a single asthmatic patient and compared to the responses of airways from 10 non-asthmatic subjects *in vitro*. Asthmatic bronchiolar strips exhibited increased contractile responses to histamine, methacholine and leukotriene-C₄, and relaxed normally in response to *l*-isoprenaline and forskolin.²⁴ Bai and associates also examined *in vitro* responsiveness of tracheal smooth muscle obtained from 10 asthmatics and 34 non-asthmatic controls, and observed significant increase in maximum contraction induced by electrical field stimulation and contractile agonists, and also revealed disturbed relaxation responses to isoproterenol and theophylline.²⁵ However, other studies failed to show the difference in contractile re-

sponses of asthmatic airway smooth muscle. Cerrina and associates examined correlation between excised human airway smooth muscle responsiveness *in vitro* and *in vivo* airway responsiveness to histamine in 19 subjects including 5 patients with asthma and 7 patients with COPD. Although they could not observe a significant correlation between *in vivo* and *in vitro* responsiveness to histamine, there was a significant negative correlation between *in vivo* histamine responsiveness to *in vitro* airway smooth muscle responsiveness to isoproterenol.²⁶ Same trends, no correlation in contractile characteristics but negative correlation to relaxation responses, are also recognized in other studies.²⁷ To summarize the results of above studies, it seems reasonable to estimate that asthmatic airway smooth muscle has abnormalities in relaxation response rather than contractile response. The reduced relaxation responses to isoproterenol and theophylline seems to be important in relation to increased plasticity of airway smooth muscle in asthmatics.²⁸ It is speculated that the increased plasticity might be related to the lack of airway dilatation after deep inspiration, the most prominent characteristics of asthmatic airways.²⁹

However, it is still possible to explain the lack of *in vivo* and *in vitro* correlation in contractile responses in other ways. First example is for the measurement system itself. In above studies isometric tension measurements were applied. The claim is that isometric properties of airway smooth muscle relate to the stiffness of the airway wall and its role in regulating resistance is minor. In this aspect, it may not be able to show the difference in smooth muscle contractility without applying isotonic length measurements. Actually, Ma and associates demonstrated statistically significant increases in maximum shortening capacity and velocity of airway smooth muscle cells from asthmatics compared those from normal subjects.³⁰ Another possible explanation is an increase in elastic load of asthmatic airways. Airway wall fibrosis, including sub-epithelial fibrosis and an increase in matrix proteins in and around airway smooth muscle, may act as an elastic overlord for airway smooth muscle to reduce airway caliber.¹⁰ This phenomenon has been demonstrated to be true by Niimi and associates. They measured the airway wall thickness by high-resolution computer tomography and *in vivo* methacholine airway responsiveness in asthmatics. There are a significant negative correlation between airway wall thickness and methacholine airway responsiveness, suggesting that thickened fibrous airways are hard to be compressed.³¹

PHENOTYPIC MODULATION OF AIRWAY SMOOTH MUSCLE

When considering *in vivo* airway hyperresponsiveness, it may also involve reversible phenotypic modulation. Now, three smooth muscle phenotypes have

been recognized: contractile, synthetic and hypercontractile phenotypes. Contractile cells, characterized by a high density of contractile proteins including smooth muscle α -actin and myosin heavy chains (MHC) and a few cytoskeletal proteins including non-muscle-MHC and vimentin, are mitotically quiescent and retain their ability to contract in response to spasmogens. Synthetic cells, with a low density of contractile proteins and high fraction of cytoskeletal proteins, are mitotically active and may lose their ability to contract.³² These cells may have additional functions such as production and release of pro-inflammatory mediators and extra-cellular matrix element described in later section. Hyper-contractile cells are obtained by prolonged withdrawal of serum from primary cultures of confluent canine tracheal smooth muscle.³³ The content of smooth muscle myosin light chain kinase in these cells are increased 30 fold, and they shorten faster and to a greater extent.^{33,34} Whether such phenotypic modulation happens during remodeling of airway smooth muscle in asthma has not been clarified yet. However, our preliminary data suggest possible existence of such phenotypic modulation. Immuno-histochemical staining of airway smooth muscle with antibodies for different MHC isoforms revealed that the expression of non-muscle (embryonic) type of MHC isoform is significantly enhanced in asthmatic airway smooth muscle compared to control (unpublished data). Woodruff and associates reported that there is a proliferation of smooth muscle in asthmatic airways, but they could not observe the phenotypic modulation of smooth muscle to hyper-contractile phenotype.¹³ The existence of such phenotypic modulation may make things more complicated, since the co-appearance of synthetic as well as hyper-contractile phenotypes may result the overall effect of increasing, decreasing, or causing no change in airway smooth muscle contractility despite an increase in airway wall smooth muscle content.

MECHANISMS OF AIRWAY SMOOTH MUSCLE REMODELING

GENETIC BACKGROUND OF AIRWAY REMODELING

Some pulmonary physicians may have recognized that there is a large intra-individual variation of airway remodeling in asthma. Recent work by Roth and associates revealed that there might be an intrinsic abnormality in asthmatic airway smooth muscle. They demonstrated the enhanced proliferation of bronchial smooth-muscle cells derived from subjects with asthma and the failure of glucocorticoids to inhibit proliferation of these cells *in vitro*, possibly explained by a cell-type-specific absence of C/EBP α .³⁵ Recently, final results of several large scale positional cloning for asthma susceptibility genes have been reported, and now we have 4 possible asthma suscepti-

bility genes.³⁶⁻³⁹ Interestingly, some of these genes are not expressed in immune regulatory cells such as lymphocytes but are expressed mainly in airway structural cells such as airway smooth muscle, epithelium, and fibroblast. One of which is ADAM33, a family member of proteases known as the ADAM superfamily.³⁶ High-level expression of ADAM33 is observed in airway smooth muscles and fibroblasts and suggests possible roles in airway remodeling in patients with asthma. The ADAM33 levels in bronchoalveolar lavage fluid (BALF) were increased significantly in patients with asthma, and they correlate inversely with the %predicted FEV₁, suggesting their relation to the asthma severity.⁴⁰ Van Diemen and associates recently demonstrated in the general population that polymorphisms in ADAM33 gene are associated with accelerated decline in FEV₁.⁴¹ Another gene is a GPRA, which belongs to the G protein-coupled receptor family (GPCRs). GPRA is also expressed mainly in airway epithelium and smooth muscle, and is speculated to have some relation to airway remodeling.³⁹

AIRWAY INFLAMMATION AND AIRWAY SMOOTH MUSCLE REMODELING

Recently, tight interaction between airway inflammation and airway remodeling has been recognized. Many inflammatory mediators are increased in asthmatic lung and some of them are inducers of airway smooth muscle proliferation (Table 1). These mediators include growth factors, inflammatory mediators, contractile agonists, cytokines, and extra-cellular matrix proteins.⁴²

For the growth factors, there has been a controversy whether the expression of growth factors and their receptors are enhanced in asthmatic airways. In early studies, for platelet derived growth factor (PDGF) an insulin like growth factor (IGF), their over-expression or increased levels in BALF were not observed in asthmatics.⁴³⁻⁴⁵ In addition, although there was an increased expression of PDGF-mRNA in asthmatic airways, PDGF receptor protein expression was not observed by immunohistochemical staining.⁴⁴ Increased PDGF-mRNA expression seemed due to the effect of corticosteroid used in the asthmatic patients.⁴⁴ On the other hand, there is an enhanced expression of epidermal growth factor (EGF) in asthmatic airways obtained by autopsy and lung resection.⁸ At the same time, there is an over-expression of EGF receptor on the epithelial cells, bronchial gland cells, and smooth muscle cells in asthmatic airways.⁸ These findings were confirmed in the biopsy specimen obtained from asthmatic patients by bronchoscopy.⁴⁶ In addition to smooth muscle proliferative activities, EGF has also been demonstrated to have smooth muscle contractile activities.⁴⁷ Recently, Holgate and associates proposed the hypothesis that an abnormal interaction between airway

Table 1 Major Inflammatory Mediators with Potential Smooth Muscle Proliferative Activity

Growth Factors	Cytokines
epidermal growth factor (EGF)	IL-1 β
insulin-like growth factors (IGF)	IL-6
platelet-derived growth factor (PDGF)	tumor necrosis factor- α (TNF- α)
basic fibroblast growth factor (BFGF)	Plasma- or Inflammatory Cell-derived Mediators,
fibroblast growth factor-2 (FGF-2)	lysosomal hydrolases
Contractile Agonists	α -thrombin
histamine	tryptase
endothelin-1 (ET-1)	sphingosine 1-phosphate
substance P (SP)	Extracellular Matrix (ECM) Proteins
phenylephrine	collagen I, III, V
serotonin (5HT)	fibronectin
thromboxane A2 (TXA2)	tenascin
leukotriene D4 (LTD4)	hyaluronan
	versican

epithelium and mesenchymal cells, called epithelial-mesenchymal trophic unit (EMTU), mediated by EGFR system might be an important mechanism of airway remodeling observed in asthma.^{46,48} Transforming growth factor (TGF)- β , known as a growth factor inducing cell differentiation and fibrosis, is also increased in asthmatic airways.⁴⁹ In addition, eosinophils, a major player of allergic airway inflammation, have been shown to be a main source of TGF- β .⁵⁰ Goldsmith and associates suggested the relation of TGF- β to smooth muscle hypertrophy.⁵¹ TGF- β increased cell size and total protein synthesis, expression of alpha-smooth muscle actin and smooth muscle MHC, formation of actomyosin filaments, and cell shortening to acetylcholine.⁵¹ On the other hand, TGF- β itself or together with fibroblast growth factor 2 (FGF2) also reported to induced airway smooth muscle hyperplasia or proliferation.^{52,53}

As shown in Table 1, many inflammatory mediators increased in asthmatic airways induce airway smooth muscle proliferation *in vitro*. Classical mediators, such as histamine, thromboxane, and leukotriene are potent airway smooth muscle constrictors and are potential smooth muscle mitogens. Endothelin-1, a potent smooth muscle mitogen, are increased in BALF from steroid-naïve asthmatics and correlate well with % predicted FEV₁.⁵⁴ Pro-inflammatory cytokines, such as interleukin-1 β , IL-6, and TNF- α are also increased in asthmatic airways and have some evidence to enhance smooth muscle proliferation.⁵⁵⁻⁵⁷ Th₂ cytokine receptors, such as IL-4 and 13 receptors, are also expressed in airway smooth muscle cells, and IL-13 has been revealed to interfere the reduction in cell stiffness induced by isoproterenol.⁵⁸

Another important aspect of interaction between airway inflammation and airway smooth muscle is that airway smooth muscle itself could be a source of mediators of airway inflammation and remodeling.

Recent studies have revealed that airway smooth muscle cells release several mediators including cytokines (such as GM-CSF, IL-2, -5, -6, -11, -12, -13, IFN- γ), chemokines (such as eotaxin, RANTES, IL-8, MCP-1, -2, -3, TARC), growth factors (PDGF, IGF, SCF, VEGF) and inflammatory mediators in the airways (PGE₂, PLA₂).^{59,60} Therefore, airway smooth muscle cells might be a source of mediators of airway inflammation, and may modulate autocrine proliferative responses.

MECHANISMS OF SMOOTH MUSCLE PROLIFERATION INDUCED BY INFLAMMATORY MEDIATORS

The main intracellular pathways of smooth muscle proliferation by inflammatory mediators have been summarized well in the recent review articles.^{32,42,61} Figure 1 shows the brief outline of such mechanisms in airway smooth muscle. Mediators classified into growth factors, such as PDGF, EGF and IGF, act via growth factor receptors with intrinsic receptor tyrosine kinase (RTK) activity. Activation of RTK induces p21ras activation and stimulates two parallel signaling pathways, namely, the extracellular signal-regulated kinase (ERK) or the phosphatidylinositol 3-kinase (PI3K) pathways. ERK phosphorylates nuclear protein such as cyclin D1 to induce DNA synthesis and cell proliferation. PI3K activation also induced phosphorylation of cyclin D1 through activation of the S6 ribosomal kinase (p70^{S6K}), Rac1, and PKC ζ . Inflammatory mediators such as histamine, thromboxane-A₂ and leukotriene-D₄ stimulate G protein-coupled receptors (GPCRs). Stimulation of GPCRs induces degradation of PIP₃ into diacylglycerol (DAG) and inositol triphosphate (IP₃). DAG activates protein kinase C (PKC), and IP₃ induces the release of stored Ca⁺⁺ from endoplasmic reticulum (ER). The two stimuli together induce cell proliferation. There is also an evi-

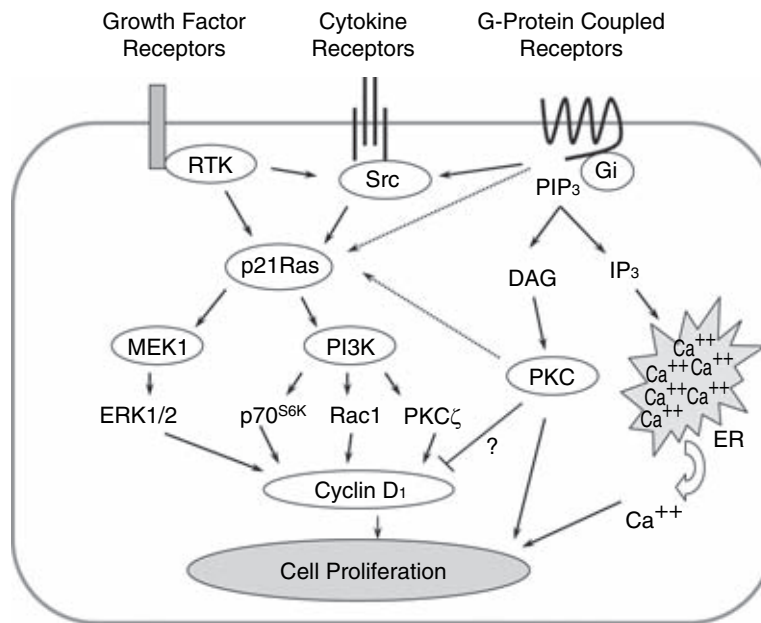


Fig. 1 Schematic presentation of signal transduction mechanisms regulating airway smooth muscle cell proliferation through major three receptors systems (see text).

dence of link between GPCRs and the activation of p21ras in human airway smooth muscle.⁶² Cytokines signal through cell surface glycoprotein receptors that function as oligomeric complexes consisting of typically two to four receptor chains coupled to Src family non-receptor tyrosine kinase, and stimulate p21ras pathway. Recent study reported by Krymskaya and associates indicated that Src is a key molecule even in human airway smooth muscle proliferation and migration.⁶³ In addition, other interactions among these three major pathways are also recognized.^{47,64,65}

PREVENTION AND TREATMENT OF AIRWAY SMOOTH MUSCLE REMODELING IN ASTHMA

Strategy to control airway smooth muscle remodeling in asthma has been studied intensively. Recent *in vitro* experiments revealed that glucocorticoids inhibit some but not all growth factor induced proliferation of airway smooth muscle.⁶⁶ Glucocorticoids arrest human airway smooth muscle cells in the G1 phase. They reduce thrombin-stimulated increases in cyclin D1 and also attenuate phosphorylation of retinoblastoma protein, via a pathway either downstream or parallel to the mitogen-activated protein kinase (MAPK) pathway.⁶⁶ However, they are not effective to inhibit airway smooth muscle cell mitogenesis in response to RTK-activating mitogens.⁴² Another action of glucocorticoids might be an inhibition of chemokine and cytokine secretion induced by inflammatory mediators. In experimental model of asthma,

several studies have shown evidences that glucocorticoids may inhibit airway smooth muscle remodeling. We have examined the effect of salbutamol and methylprednisolone (mPSL) on airway remodeling induced by continuous exposure of antigen to Brown-Norway Rats. MPSL inhibited airway remodeling almost completely, however, mono-therapy with salbutamol worsened airway responsiveness to methacholine and also enhanced airway epithelial remodeling, such as goblet cell hyperplasia.⁶⁷ These *in vitro* and *in vivo* experimental results suggest possible usefulness of glucocorticoids to regulate airway smooth muscle remodeling in asthma.

The important question for pulmonary physicians is whether airway smooth muscle remodeling observed in asthmatic patients could be regulated by inhaled glucocorticoids. Haatela and associates have done the double blind crossover study to examine the effect of inhaled glucocorticoids (budsonide) and β_2 -agonist on pulmonary function including peak expiratory flow rate (PEFR). In this study, they compared the improvement of PEFR by budesonide between the group in which budesonide was introduced in the first line and the group in which budesonide was used in the second line, meaning two years delay in the treatment with inhaled glucocorticoids. The improvement of PEFR was significantly attenuated in the subjects with two years delay in the treatment with inhaled glucocorticoids.⁶⁸ Selroose and associates have examined the relation between the duration of asthma symptom before the beginning of inhaled glucocorticoids therapy and the degree of improvement

in PEFR and FEV₁.⁶⁹ They clearly demonstrated the negative correlation between the degree of pulmonary functional improvement and the duration of symptom before the induction of inhaled glucocorticoids. These studies suggest that early intervention with inhaled glucocorticoids may be important to improve pulmonary functions and to control airway remodeling in asthma. Recently, the first results of randomized control trial to examine the effect of early intervention with inhaled glucocorticoids on airway remodeling in adult asthmatics have been published.⁷⁰ In this study, after 3 years of treatment, there is a significant improvement in post-bronchodilator FEV₁ in adult asthmatics treated with inhaled glucocorticoids compared to those without it. However, in children with asthma, early interventions with inhaled glucocorticoids did not improve post-bronchodilator FEV₁, suggesting the lack of inhibitory effect on airway remodeling. Same negative result was obtained in the previous large scale study by the Childhood Asthma Management Program (CAMP) Research Group.⁷¹ In children, airway caliber is getting larger during their growth, and the effect of early intervention might be observed as an attenuation of airway growth. This shows a clear contrast to the adult airways in which constant decline in the caliber is observed during aging. That might be a cause of the difference observed between children and adults asthmatics in early intervention studies.^{70,71}

Anti-asthmatics drugs other than inhaled glucocorticoids, such as leukotriene modifiers, theophylline, and β_2 -agonist (especially long-acting β -agonist ; LABA) have not studied extensively yet. When considering the mechanisms of airway smooth muscle proliferation, there seems to be a good reason to expect usefulness of these drugs on airway remodeling. In experimental asthma model, there are a few studies demonstrated the suppression of airway remodeling by leukotriene modifiers.⁷² Although such experimental models seem to be useful as models of acute allergic airway inflammation, there are fair amount of debates whether these models could be applied for more chronic airway inflammation observed in severe intractable asthma. Further attempts to obtain higher level of clinical evidence for the effectiveness of these anti-asthmatic drugs on airway remodeling, and to develop new strategies to prevent or reverse airway remodeling have been awaited.

REFERENCES

1. Asthma prevention and management guidelines. Ministry of Health and Welfare, Japan. *Int. Arch. Allergy Immunol.* 2000;**121** (Suppl 1):1-77.
2. *Global Initiative for Asthma, Global Strategy for Asthma Management and Prevention.* NHLBI/WHO, 2003.
3. Apostol GG, Jacobs DR Jr, Tsai AW *et al.* Early life factors contribute to the decrease in lung function between ages 18 and 40: the Coronary Artery Risk Development in Young Adults study. *Am. J. Respir. Crit. Care Med.* 2002;**166**:166-172.
4. James AL, Palmer LJ, Kicic E *et al.* Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. *Am. J. Respir. Crit. Care Med.* 2005;**171**:109-114.
5. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. *N. Engl. J. Med.* 1998;**339**:1194-1200.
6. Rijcken B, Weiss ST. Longitudinal analyses of airway responsiveness and pulmonary function decline. *Am. J. Respir. Crit. Care Med.* 1996;**154**:S246-249.
7. Munakata M, Nasuhara Y, Sato A *et al.* Meanings of the clinical parameters of airway hyperresponsiveness. *Nihon Kyobu Shikkan Gakkai Zasshi* 1996;**34**(Suppl): 42-47 (in Japanese).
8. Amishima M, Munakata M, Nasuhara Y *et al.* Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. *Am. J. Respir. Crit. Care Med.* 1998;**157**:1907-1912.
9. Niimi A, Matsumoto H, Minakuchi M *et al.* Airway remodelling in cough-variant asthma. *Lancet* 2000;**356**:564-565.
10. James AL, Pare PD, Hogg JC. The mechanics of airway narrowing in asthma. *Am. Rev. Respir. Dis.* 1989;**139**:242-246.
11. Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study. *Am. Rev. Respir. Dis.* 1993;**148**:720-726.
12. Ebina M, Yaegashi H, Chiba R, Takahashi T, Motomiya M, Tanemura M. Hyperreactive site in the airway tree of asthmatic patients revealed by thickening of bronchial muscles. A morphometric study. *Am. Rev. Respir. Dis.* 1990;**141**:1327-1332.
13. Woodruff PG, Dolganov GM, Ferrando RE *et al.* Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression. *Am. J. Respir. Crit. Care Med.* 2004;**169**:1001-1006.
14. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am. J. Respir. Crit. Care Med.* 2003;**167**:1360-1368.
15. Demoly P, Simony-Lafontaine J, Chanez P *et al.* Cell proliferation in the bronchial mucosa of asthmatics and chronic bronchitics. *Am. J. Respir. Crit. Care Med.* 1994;**150**:214-217.
16. Bai TR, Cooper J, Koelmeyer T, Pare PD, Weir TD. The effect of age and duration of disease on airway structure in fatal asthma. *Am. J. Respir. Crit. Care Med.* 2000;**162**:663-669.
17. Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;**1**:520-524.
18. Altraja A, Laitinen A, Virtanen I *et al.* Expression of laminins in the airways in various types of asthmatic patients: a morphometric study. *Am. J. Respir. Cell Mol. Biol.* 1996;**15**:482-488.
19. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N. Engl. J. Med.* 2002;**346**:1699-1705.
20. Brightling CE, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID, Bradding P. Interleukin-4 and -13 expression is co-localized to mast cells within the airway smooth muscle in asthma. *Clin. Exp. Allergy* 2003;**33**:1711-1716.
21. Vincenc KS, Black JL, Yan K, Armour CL, Donnelly PD,

- Woolcock AJ. Comparison of *in vivo* and *in vitro* responses to histamine in human airways. *Am. Rev. Respir. Dis.* 1983;**128**:875-879.
22. Armour CL, Lazar NM, Schellenberg RR *et al.* A comparison of *in vivo* and *in vitro* human airway reactivity to histamine. *Am. Rev. Respir. Dis.* 1984;**129**:907-910.
 23. Roberts JA, Raeburn D, Rodger IW, Thomson NC. Comparison of *in vivo* airway responsiveness and *in vitro* smooth muscle sensitivity to methacholine in man. *Thorax* 1984;**39**:837-843.
 24. De Jongste JC, Mons H, Bonta IL, Kerrebijn KF. *In vitro* responses of airways from an asthmatic patient. *Eur. J. Respir. Dis.* 1987;**71**:23-29.
 25. Bai TR. Abnormalities in airway smooth muscle in fatal asthma. *Am. Rev. Respir. Dis.* 1990;**141**:552-557.
 26. Cerrina J, Le Roy Ladurie M, Labat C, Raffestin B, Bayol A, Brink C. Comparison of human bronchial muscle responses to histamine *in vivo* with histamine and isoproterenol agonists *in vitro*. *Am. Rev. Respir. Dis.* 1986;**134**:57-61.
 27. Bai TR, Mak JC, Barnes PJ. A comparison of beta-adrenergic receptors and *in vitro* relaxant responses to isoproterenol in asthmatic airway smooth muscle. *Am. J. Respir. Cell Mol. Biol.* 1992;**6**:647-651.
 28. Dulin NO, Fernandes DJ, Dowell M *et al.* What evidence implicates airway smooth muscle in the cause of BHR? *Clin. Rev. Allergy Immunol.* 2003;**24**:73-84.
 29. Brown RH, Mitzner W, Wagner E, Permutt S, Togias A. Airway distension with lung inflation measured by HRCT. *Acad. Radiol.* 2003;**10**:1097-1103.
 30. Ma X, Cheng Z, Kong H *et al.* Changes in biophysical and biochemical properties of single bronchial smooth muscle cells from asthmatic subjects. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2002;**283**:L1181-1189.
 31. Niimi A, Matsumoto H, Takemura M, Ueda T, Chin K, Mishima M. Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. *Am. J. Respir. Crit. Care Med.* 2003;**168**:983-988.
 32. Hirst SJ, Walker TR, Chilvers ER. Phenotypic diversity and molecular mechanisms of airway smooth muscle proliferation in asthma. *Eur. Respir. J.* 2000;**16**:159-177.
 33. Ma X, Wang Y, Stephens NL. Serum deprivation induces a unique hypercontractile phenotype of cultured smooth muscle cells. *Am. J. Physiol.* 1998;**274**:C1206-1214.
 34. Halayko AJ, Camoretti-Mercado B, Forsythe SM *et al.* Divergent differentiation paths in airway smooth muscle culture: induction of functionally contractile myocytes. *Am. J. Physiol.* 1999;**276**:L197-206.
 35. Roth M, Johnson PR, Borger P *et al.* Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells. *N. Engl. J. Med.* 2004;**351**:560-574.
 36. Van Eerdewegh P, Little RD, Dupuis J *et al.* Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002;**418**:426-430.
 37. Zhang Y, Leaves NI, Anderson GG *et al.* Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nat. Genet.* 2003;**34**:181-186.
 38. Allen M, Heinzmann A, Noguchi E *et al.* Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nat. Genet.* 2003;**35**:258-263.
 39. Vendelin J, Pulkkinen V, Rehn M *et al.* Characterization of GPR4, a novel G protein-coupled receptor related to asthma. *Am. J. Respir. Cell Mol. Biol.* 2005;**33**:262-270.
 40. Lee JY, Park SW, Chang HK *et al.* A disintegrin and metalloproteinase 33 protein in patients with asthma: relevance to airflow limitation. *Am. J. Respir. Crit. Care Med.* 2006;**173**:729-735.
 41. van Diemen CC, Postma DS, Vonk JM, Bruinenberg M, Schouten JP, Boezen HM. A disintegrin and metalloproteinase 33 polymorphisms and lung function decline in the general population. *Am. J. Respir. Crit. Care Med.* 2005;**172**:329-333.
 42. Ammit AJ, Panettieri RA Jr. Invited review: the circle of life: cell cycle regulation in airway smooth muscle. *J. Appl. Physiol.* 2001;**91**:1431-1437.
 43. Chanez P, Vignola M, Stenger R, Vic P, Michel FB, Bousquet J. Platelet-derived growth factor in asthma. *Allergy* 1995;**50**:878-883.
 44. Aubert JD, Hayashi S, Hards J, Bai TR, Pare PD, Hogg JC. Platelet-derived growth factor and its receptor in lungs from patients with asthma and chronic airflow obstruction. *Am. J. Physiol.* 1994;**266**:L655-663.
 45. Hoshino M, Nakamura Y, Sim JJ. Expression of growth factors and remodelling of the airway wall in bronchial asthma. *Thorax* 1998;**53**:21-27.
 46. Puddicombe SM, Polosa R, Richter A *et al.* Involvement of the epidermal growth factor receptor in epithelial repair in asthma. *Faseb. J.* 2000;**14**:1362-1374.
 47. Nasuhara Y, Munakata M, Sato A, Amishima M, Homma Y, Kawakami Y. Mechanisms of epidermal growth factor-induced contraction of guinea pig airways. *Eur. J. Pharmacol.* 1996;**296**:161-168.
 48. Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J. Allergy Clin. Immunol.* 2000;**105**:193-204.
 49. Redington AE, Madden J, Frew AJ *et al.* Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid. *Am. J. Respir. Crit. Care Med.* 1997;**156**:642-647.
 50. Ohno I, Nitta Y, Yamauchi K *et al.* Transforming growth factor beta 1 (TGF beta 1) gene expression by eosinophils in asthmatic airway inflammation. *Am. J. Respir. Cell Mol. Biol.* 1996;**15**:404-409.
 51. Goldsmith AM, Bentley JK, Zhou L *et al.* Transforming growth factor-beta induces airway smooth muscle hypertrophy. *Am. J. Respir. Cell Mol. Biol.* 2006;**34**:247-254.
 52. Chen G, Khalil N. TGF-beta 1 increases proliferation of airway smooth muscle cells by phosphorylation of map kinases. *Respir. Res.* 2006;**7**:2.
 53. Bosse Y, Thompson C, Stankova J, Rola-Pleszczynski M. FGF2 and TGF{beta}1 Synergism in Human Bronchial Smooth Muscle Cell Proliferation. *Am. J. Respir. Cell Mol. Biol.* 2006;**34**:746-753.
 54. Redington AE, Springall DR, Ghatei MA *et al.* Endothelin in bronchoalveolar lavage fluid and its relation to airflow obstruction in asthma. *Am. J. Respir. Crit. Care Med.* 1995;**151**:1034-1039.
 55. De S, Zelazny ET, Souhrada JF, Souhrada M. Interleukin-1 beta stimulates the proliferation of cultured airway smooth muscle cells via platelet-derived growth factor. *Am. J. Respir. Cell Mol. Biol.* 1993;**9**:645-651.
 56. De S, Zelazny ET, Souhrada JF, Souhrada M. IL-1 beta and IL-6 induce hyperplasia and hypertrophy of cultured guinea pig airway smooth muscle cells. *J. Appl. Physiol.* 1995;**78**:1555-1563.
 57. Stewart AG, Tomlinson PR, Fernandes DJ, Wilson JW, Harris T. Tumor necrosis factor alpha modulates mitogenic responses of human cultured airway smooth muscle. *Am. J. Respir. Cell Mol. Biol.* 1995;**12**:110-119.

58. Laporte JC, Moore PE, Baraldo S *et al.* Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. *Am. J. Respir. Crit. Care Med.* 2001;**164**:141-148.
59. Shore S. Airway smooth muscle: new tricks for an old dog. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2002;**282**:L518-519.
60. Howarth PH, Knox AJ, Amrani Y, Tliba O, Panettieri RA Jr, Johnson M. Synthetic responses in airway smooth muscle. *J. Allergy Clin. Immunol.* 2004;**114**:S32-50.
61. Panettieri RA Jr. Cellular and molecular mechanisms regulating airway smooth muscle proliferation and cell adhesion molecule expression. *Am. J. Respir. Crit. Care Med.* 1998;**158**:S133-140.
62. Emala CW, Liu F, Hirshman CA. G α but not gq α is linked to activation of p21 (ras) in human airway smooth muscle cells. *Am. J. Physiol.* 1999;**276**:L564-570.
63. Krymskaya VP, Goncharova EA, Ammit AJ *et al.* Src is necessary and sufficient for human airway smooth muscle cell proliferation and migration. *Faseb. J.* 2005;**19**:428-430.
64. Pascual RM, Billington CK, Hall IP *et al.* Mechanisms of cytokine effects on G protein-coupled receptor-mediated signaling in airway smooth muscle. *Am. J. Physiol. Lung Cell Mol. Physiol.* 2001;**281**:L1425-1435.
65. Ravasi S, Citro S, Viviani B, Capra V, Rovati GE. CysLT1 receptor-induced human airway smooth muscle cells proliferation requires ROS generation, EGF receptor transactivation and ERK1/2 phosphorylation. *Respir. Res.* 2006;**7**:42.
66. Fernandes D, Guida E, Koutsoubos V *et al.* Glucocorticoids inhibit proliferation, cyclin D1 expression, and retinoblastoma protein phosphorylation, but not activity of the extracellular-regulated kinases in human cultured airway smooth muscle. *Am. J. Respir. Cell Mol. Biol.* 1999;**21**:77-88.
67. Kamachi A, Munakata M, Nasuhara Y *et al.* Enhancement of goblet cell hyperplasia and airway hyperresponsiveness by salbutamol in a rat model of atopic asthma. *Thorax* 2001;**56**:19-24.
68. Haahtela T, Jarvinen M, Kava T *et al.* Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N. Engl. J. Med.* 1994;**331**:700-705.
69. Selroos O, Pietinalho A, Lofroos AB, Riska H. Effect of early vs late intervention with inhaled corticosteroids in asthma. *Chest* 1995;**108**:1228-1234.
70. Pauwels RA, Pedersen S, Busse WW *et al.* Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial. *Lancet* 2003;**361**:1071-1076.
71. Long-term effects of budesonide or nedocromil in children with asthma. The Childhood Asthma Management Program Research Group. *N. Engl. J. Med.* 2000;**343**:1054-1063.
72. Henderson WR Jr, Chiang GK, Tien YT, Chi EY. Reversal of allergen-induced airway remodeling by CysLT1 receptor blockade. *Am. J. Respir. Crit. Care Med.* 2006;**173**:718-728.