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Airway remodelling in asthma: role for mechanical forces

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Asthma is a chronic airway inflammatory disease with functional and structural changes, leading to bronchial hyperresponsiveness and airflow obstruction. Airway structural changes or airway remodelling consist of epithelial injury, goblet cell hyperplasia, subepithelial layer thickening, airway smooth muscle hyperplasia and angiogenesis. These changes were previously considered as a consequence of chronic airway inflammation. Even though inhaled corticosteroids can suppress airway inflammation, the natural history of asthma is still unaltered after inhaled corticosteroid treatment. As such there is increasing evidence for the role of mechanical forces within the asthmatic airway contributing to airway structural changes.

Key words: Asthma; Airway remodelling; Mechanical forces

INTRODUCTION

Asthma is a disease that defined by its typical clinical, physiological and pathological characteristics. The major feature of clinical history is episodic shortness of breath, cough and wheezing particularly at night or during exercise. The characteristic physiological feature of asthma is variable airway obstruction and its measure bronchial hyperresponsiveness. The main pathological findings are airway inflammation and structural airway changes namely airway remodelling.

Airway inflammation and asthma pathogenesis

The airway inflammation in asthma is typically eosinophilic and accompanied by elevation of Th_2 cytokines. Eosinophils are a key feature of Th_2 inflammation and are a useful biomarker in guiding treatment [1]. However, Th_2 inflammation alone cannot explain all features of asthma. For example airway hyperresponsiveness and tissue remodelling are not entirely linked to this inflammation [2]. There are a number of asthmatic patients in whom anti-inflammatory therapy does not lead to symptom control and who are considered treatment resistant. Furthermore whilst recognized to modify eosinophilic

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inflammation, inhaled corticosteroids treatment in atopic children with recurrent wheezing has shown to have no effect on declining in lung function and the natural history of asthma [3-5]. This irreversible airflow obstruction has been shown to develop despite appropriate use of inhaled corticosteroids, as advocated by international disease management guidelines [6].

Airway remodelling in asthma

Pathological repair of the airways leads to structural changes that are called airway remodelling. Airway remodelling which has been proposed to result in lower lung function is characterised by subepithelial thickening from collagen deposition, epithelial denudation with goblet cell metaplasia, increased airway smooth muscle mass, angiogenesis and alterations in the extracellular matrix components (ECM) such as collagens, proteoglycans and glycoproteins throughout the airway wall [7] (Fig. 1). Structural remodelling of the airways has been found in children with recurrent wheezing regardless their atopic status [8]. It has also been reported that airway epithelial cells in asthmatic children express makers of injury, such as the epidermal growth factor receptor (EGFR), even in the absence of significant eosinophilic inflammation [9]. In paediatric severe therapy resistant asthma, it was shown the increased subepithelial layer thickness without the evidence of mucosal Th_2 cytokine cell [10]. These studies suggest that remodelling can occur independently of Th_2 inflammation. Furthermore, evidence of airway remodelling, such as epithelial layer damage, thickening of basement membrane, angiogenesis has been demonstrated in children as early as 4 years of age in asthmatic subjects [8, 11, 12]. It is thus an early feature of the disease and not only a marker of long standing chronic disease. However, the subepithelial thickening was not demonstrated in wheezer infants [13]. These indicate that airway thickening begins early in the development of asthma and may play role in the disease progression in some patients.

Airway exposure to mechanical forces: what is the consequence?

Human airway development requires a mechanical environment to promote proliferation and airway elongation. The major structural cells of the airways (epithelial cells, fibroblasts, and smooth muscle cells) are responsible for these mechanical environments. During *in utero* development, mechanical stress results from epithelial fluid secretion to the airways, peristaltic movement of fluid and intermittent foetal breathing. At the time of birth, the mechanical environment alters suddenly as the air-liquid interface is a novel factor contributing to the dynamic balance between muscle contraction, airway lumen patency and wall structure [14]. Airway smooth muscle contraction produces mechanical force from compressive stress on the airway epithelium, fibroblasts and smooth muscle itself. Therefore, abnormal mechanical loading conditions may result in altered cellular activations and modify the composition of ECM leading to fibrosis in the airways [15].

Effect of mechanical forces on the airway epithelium and airway remodelling

Recent studies have shown that mechanical forces activates epithelial cells causing release of factors that are involved in airway remodelling. Savla and Waters [16] have shown that mechanical forces from cyclical mechanical strain and compressive stress

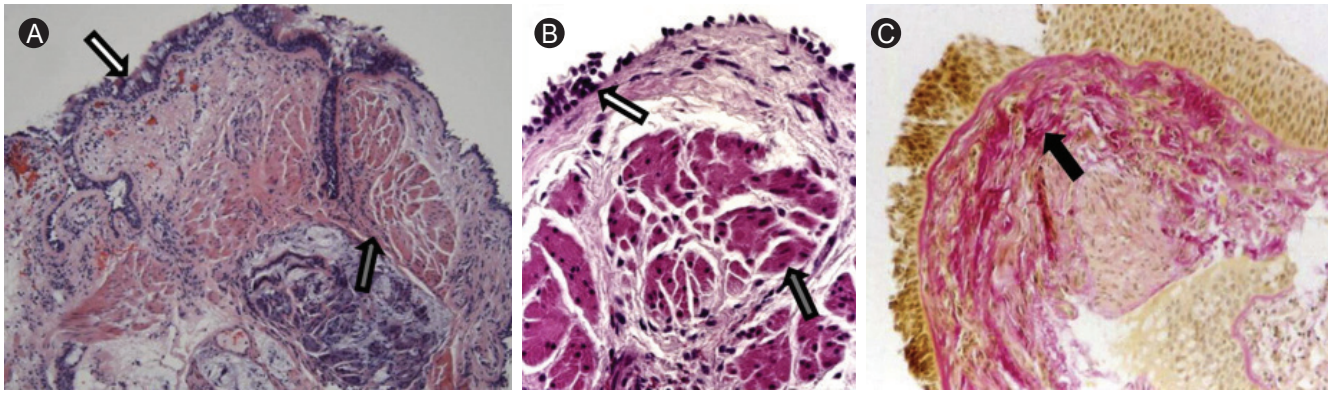

Fig. 1. Airway structural changes in asthma. Panels A and B demonstrate epithelial injuries (white arrows) and increased thickness of airway smooth muscle (grey arrows). Panel C demonstrates subepithelial collagen deposition (red stain; black arrow). Reprinted from Al-Muhsen et al. [7], with permission of Elsevier.

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to human and cat airway epithelium cells inhibited epithelial layer repair after wounded by scraping. A model of compressive stress on differentiated normal human bronchial epithelial cells cultured at air-liquid interface has been shown to promote airway remodelling by increasing the gene expression of transforming growth factor (TGF)-β, endothelin-1, and plasminogen activator gene [17, 18], enhancing the release of profibrotic cytokines: TGF-β2 and endothelin [17], increasing intracellular mucin-5AC (MUC5AC) levels [19], increasing expression of EGFR and EGFR ligand [20], enhancing the production of matrix metalloproteinase (MMP)-2 and MMP-9 [18], YKL-40 [21], a chitinase like protein which was recently shown to be associated with airway remodelling in children as well as tissue factor, a coagulation factor that was shown to enhance angiogenesis [22]. Cyclical mechanical strain of airway epithelium cells has also been shown to increase the production of reactive oxygen species (ROS) [23], and to down-regulate prostaglandin E_2 synthesis (PGE_2) [24]. PGE_2 was found to inhibit fibroblast proliferation and collagen production in vitro [25, 26]. Comparative studies have shown that mechanical strain enhances DNA synthesis in rat foetal epithelial cells and fibroblasts when cultured in three-dimensional (3D) organotypic cultures and in this respect has greater influence than monolayer mechanical strain [27]. Mechanical injury to guinea-pig epithelial cells, co-cultured with fibroblasts in the human amnion chamber, results in fibroblast differentiation to myofibroblast and the expression of procollagen I and III [28]. Compressive mechanical stress of human epithelial cell with fibroblasts has been shown to have a greater effect on increasing the MMP-9/tissue inhibitors of metalloproteinase (TIMP)-1 ratio than when epithelial cells or fibroblasts are cultured alone [29]. Similar effects have been seen on collagen production, where mechanical stress applied to 3D epithelial cells co-cultured with fibroblasts causes enhanced collagen expression more than if fibroblasts are cultured alone [30]. Application of 3D dynamic lateral compressive stress to foetal rat lungs cells in organotypic cultures has also been shown to increase the production of fibronectin in the culture supernatants [31]. These studies highlight the importance of epithelial-mesenchymal cross talk in airway remodelling

Effect of mechanical for forces on the airway fibroblasts and airway remodelling

Fibroblasts are the major cell that responds to mechanical signals, translating them into biological events especially in expression of ECM genes. As a result, fibroblasts play a pivotal role in tissue remodelling and wound healing [32]. Previous studies have highlighted the role of airway fibroblasts in the production of ECM in response to mechanical stress, including up-regulation of versican and decorin mRNA expression [33, 34], as well as up-regulation of procollagen mRNA expression [35]. Airway fibroblasts from normal and asthmatic subject have been shown to respond to mechanical stimuli differently. Mechanical strain increased versican mRNA expression only asthmatic bronchial fibroblasts but not in normal bronchial fibroblasts [33]. In contrast, Ludwig et al. [34] found that mechanical strain up-regulated versican mRNA expression both in normal and asthmatic bronchial fibroblasts, but decorin was up-regulated only in asthmatic bronchial fibroblasts. However, these investigators reported the mRNA expression using northern blot analysis without showing the house keeping gene, so the difference in gene expression may have been due to the difference in RNA content [34]. Le Bellego et al. [33] have reported that asthmatic bronchial fibroblasts secrete more IL-6 than fibroblasts from normal controls after 24 hours of mechanical strain. A recent study has shown that mechanical strain promoted airway fibroblasts to secret more soluble collagen [36]. Furthermore, mechanical strain has been found to up-regulate IL-8 mRNA expression and enhanced the secretion of IL-8 in culture supernatants in both normal and asthmatic fibroblasts [33, 36]. The impact of mechanical strain on fibroblast proliferation is controversial. Whilst Bishop et al. [37] reported an increase in foetal lung fibroblast cell numbers after mechanical strain, Sanchez-Esteban et al. [38] found that mechanical strain led to both an increase in apoptosis and a decrease in cell proliferation. In a study of the effect of mechanical stress on foetal rat lung fibroblasts, it has been found that a 3D model promoted more DNA synthesis than a monolayer model [27]. Therefore, the mechanical conditions are essential to the cellular responses.

Effect of mechanical forces on the airway smooth muscles

Mechanical strain has been reported to play a critical role in airway smooth muscle (ASM) proliferation and migration [39, 40], increase in stiffness and contractile function [41, 42], induction vascular endothelial growth factor (VEGF) expression and release [43] and ECM deposition [40]. These studies underline the possibility that mechanical stress to ASM participates in the pathogenesis of airway remodelling.

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Mechanical forces to the airway and airway remodelling: is there any evidence *in vivo*?

Human airways are exposed to a range of mechanical forces that may potentially arise in several ways, such as during inspiration-expiration, cough and bronchoconstriction from airway smooth muscle contraction during asthma exacerbation. The major structural cells of the airways (epithelial cells. fibroblasts, and smooth muscle cells) are responsible for these physical stimulations. Airway smooth muscle contraction in response to stimuli such as allergen produces a compressive stress on the airway epithelium, fibroblasts and smooth muscle itself. Previous reports have shown that tidal breathing produces a 4% strain of ASM and a deep inspiration causes a 25%–30% strain [44]. Therefore, abnormal mechanical loading to the airways may result in altered cellular activations and modify the composition of ECMs leading to airway structural changes or airway remodelling. Airway wall thickening as demonstrated by computed tomography (CT) [45] and bronchial biopsy [46] has been observed in patients with cough variant asthma and non-asthmatic chronic cough. A recent *in vivo* study which has shown increases in collagen deposition in the subepithelial layer, mucus secreting goblet cells and cell proliferation in both subepithelial layer and submucosal layer after bronchoconstriction using methacholine challenge, a stimulus that did not affect airway inflammation [47]. Formoterol-budesonide, a treatment targeting both airway inflammation and bronchoconstriction, has been shown to decrease subepithelial layer thickness in asthmatic subjects as assessed by high resolution computed tomography (HRCT) [48] and airway biopsy [49]. However, bronchial hyperresponsiveness has been shown to be inversely related with the airway wall thickness [50, 51]. It was also shown that asthmatic patients who have highly variable airway obstruction showed less airway wall thickening, while those who had less variable or fixed airway obstruction exhibited more thickened airways [52]. Thus the thickening with deposition of the matrix proteins may be a protective mechanism by increasing the stiffness of the airways to attenuate the force from smooth muscle contraction [53].

CONCLUSIONS

Airway remodelling in asthma consists of changes in epithelial layer, subepithelial layer thickening from increased in deposition of extracellular matrix proteins such as collagen, increase in smooth muscle layer and angiogenesis. Several *in vivo* and *in vitro* studies demonstrate provide new important insights on the impact of mechanical forces on pathogenesis of airway remodelling in asthma. Apart from, anti-inflammatory treatment, drugs that alleviate the effect of mechanical forces on the airways such as anti-bronchoconstrictors may have a role in airway remodelling.

REFERENCES

1. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.
2. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet 2008;372:1107-19.
3. Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F. Intermittent inhaled corticosteroids in infants with episodic wheezing. N Engl J Med 2006;354:1998-2005.
4. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, Bacharier LB, Lemanske RF Jr, Strunk RC, Allen DB, Bloomberg GR, Heldt G, Krawiec M, Larsen G, Liu AH, Chinchilli VM, Sorkness CA, Taussig LM, Martinez FD. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med 2006;354:1985-97.
5. Murray CS, Woodcock A, Langley SJ, Morris J, Custovic A; IFWIN study team. Secondary prevention of asthma by the use of Inhaled Fluticasone propionate in Wheezy INfants (IFWIN): double-blind, randomised, controlled study. Lancet 2006;368:754-62.
6. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Silva PA, Poulton R. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003;349:1414-22.
7. Al-Muhsen S, Johnson JR, Hamid Q. Remodeling in asthma. J Allergy Clin Immunol 2011;128:451-62.
8. Turato G, Barbato A, Baraldo S, Zanin ME, Bazzan E, Lokar-Oliani K, Calabrese F, Panizzolo C, Snijders D, Maestrelli P, Zuin R, Fabbri LM, Saetta M. Nonatopic children with multitrigger wheezing have airway pathology comparable to atopic asthma. Am J Respir Crit Care Med 2008;178:476-82.
9. Fedorov IA, Wilson SJ, Davies DE, Holgate ST. Epithelial stress and structural remodelling in childhood asthma. Thorax 2005;60:389-94.
10. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, Tsartsali L, Lloyd CM, Bush A, Saglani S. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. J Al-

http://dx.doi.org/10.5415/apallergy.2014.4.1.19

lergy Clin Immunol 2012;129:974-82.e13.
11. Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Panizzolo C, Zanin ME, Zuin R, Maestrelli P, Fabbri LM, Saetta M. Epithelial damage and angiogenesis in the airways of children with asthma. Am J Respir Crit Care Med 2006;174:975-81.
12. Payne DN, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, Jeffery PK. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med 2003;167:78-82.
13. Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, Jeffery PK. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med 2007;176:858-64.
14. Tschumperlin DJ, Drazen JM. Chronic effects of mechanical force on airways. Annu Rev Physiol 2006;68:563-83.
15. Wang JH, Thampatty BP. An introductory review of cell mechanobiology. Biomech Model Mechanobiol 2006;5:1-16.
16. Savla U, Waters CM. Mechanical strain inhibits repair of airway epithelium in vitro. Am J Physiol 1998;274:L883-92.
17. Tschumperlin DJ, Shively JD, Kikuchi T, Drazen JM. Mechanical stress triggers selective release of fibrotic mediators from bronchial epithelium. Am J Respir Cell Mol Biol 2003;28:142-9.
18. Chu EK, Cheng J, Foley JS, Mecham BH, Owen CA, Haley KJ, Mariani TJ, Kohane IS, Tschumperlin DJ, Drazen JM. Induction of the plasminogen activator system by mechanical stimulation of human bronchial epithelial cells. Am J Respir Cell Mol Biol 2006;35:628-38.
19. Park JA, Tschumperlin DJ. Chronic intermittent mechanical stress increases MUC5AC protein expression. Am J Respir Cell Mol Biol 2009;41:459-66.
20. Chu EK, Foley JS, Cheng J, Patel AS, Drazen JM, Tschumperlin DJ. Bronchial epithelial compression regulates epidermal growth factor receptor family ligand expression in an autocrine manner. Am J Respir Cell Mol Biol 2005;32:373-80.
21. Park JA, Drazen JM, Tschumperlin DJ. The chitinase-like protein YKL-40 is secreted by airway epithelial cells at base line and in response to compressive mechanical stress. J Biol Chem 2010;285:29817-25.
22. Park JA, Sharif AS, Tschumperlin DJ, Lau L, Limbrey R, Howarth P, Drazen JM. Tissue factor-bearing exosome secretion from human mechanically stimulated bronchial epithelial cells in vitro and in vivo. J Allergy Clin Immunol 2012;130:1375-83.
23. Chapman KE, Sinclair SE, Zhuang D, Hassid A, Desai LP, Waters CM. Cyclic mechanical strain increases reactive oxygen species production in pulmonary epithelial cells. Am J Physiol Lung Cell Mol Physiol 2005;289:L834-41.
24. Savla U, Sporn PH, Waters CM. Cyclic stretch of airway epithelium inhibits prostanoid synthesis. Am J Physiol 1997;273:L1013-9.
25. McAnulty RJ, Chambers RC, Laurent GJ. Regulation of fibroblast procollagen production: transforming growth factor-beta 1 induces prostaglandin E2 but not procollagen synthesis via a pertussis toxin-sensitive G-protein. Biochem J 1995;307:63-8.
26. McAnulty RJ, Hernández-Rodriguez NA, Mutsaers SE, Coker RK, Laurent GJ. Indomethacin suppresses the anti-proliferative effects of transforming growth factor-beta isoforms on fibroblast cell cultures. Biochem J 1997;321:639-43.
27. Liu M, Xu J, Souza P, Tanswell B, Tanswell AK, Post M. The effect of mechanical strain on fetal rat lung cell proliferation: comparison of two- and three-dimensional culture systems. In Vitro Cell Dev Biol Anim 1995;31:858-66.
28. Morishima Y, Nomura A, Uchida Y, Noguchi Y, Sakamoto T, Ishii Y, Goto Y, Masuyama K, Zhang MJ, Hirano K, Mochizuki M, Ohtsuka M, Sekizawa K. Triggering the induction of myofibroblast and fibrogenesis by airway epithelial shedding. Am J Respir Cell Mol Biol 2001;24:1-11.
29. Swartz MA, Tschumperlin DJ, Kamm RD, Drazen JM. Mechanical stress is communicated between different cell types to elicit matrix remodeling. Proc Natl Acad Sci U S A 2001;98:6180-5.
30. Choe MM, Sporn PH, Swartz MA. Extracellular matrix remodeling by dynamic strain in a three-dimensional tissue-engineered human airway wall model. Am J Respir Cell Mol Biol 2006;35:306-13.
31. Mourgeon E, Xu J, Tanswell AK, Liu M, Post M. Mechanical strain-induced posttranscriptional regulation of fibronectin production in fetal lung cells. Am J Physiol 1999;277:L142-9.
32. Wang JH, Thampatty BP, Lin JS, Im HJ. Mechanoregulation of gene expression in fibroblasts. Gene 2007;391:1-15.
33. Le Bellego F, Perera H, Plante S, Chakir J, Hamid Q, Ludwig MS. Mechanical strain increases cytokine and chemokine production in bronchial fibroblasts from asthmatic patients. Allergy 2009;64:32-9.
34. Ludwig MS, Ftouhi-Paquin N, Huang W, Pagé N, Chakir J, Hamid Q. Mechanical strain enhances proteoglycan message in fibroblasts from asthmatic subjects. Clin Exp Allergy 2004;34:926-30.
35. Breen EC. Mechanical strain increases type I collagen expression in pulmonary fibroblasts in vitro. J Appl Physiol 2000;88:203-9.
36. Manuyakorn W, Noto A, Haitchi H, Holgate S, Howarth P, Davies D. Cyclical mechanical stretch enhances the proinflammatory and profibrotic responses of bronchial fibroblasts. Allergy 2010;65(Suppl s92):544.
37. Bishop JE, Mitchell JJ, Absher PM, Baldor L, Geller HA, Woodcock-Mitchell J, Hamblin MJ, Vacek P, Low RB. Cyclic mechanical deformation stimulates human lung fibroblast proliferation and autocrine

growth factor activity. Am J Respir Cell Mol Biol 1993;9:126-33.

38. Sanchez-Esteban J, Wang Y, Cicchiello LA, Rubin LP. Cyclic mechanical stretch inhibits cell proliferation and induces apoptosis in fetal rat lung fibroblasts. Am J Physiol Lung Cell Mol Physiol 2002;282:L448-56.
39. Smith PG, Tokui T, Ikebe M. Mechanical strain increases contractile enzyme activity in cultured airway smooth muscle cells. Am J Physiol 1995;268:L999-1005.
40. Hasaneen NA, Zucker S, Cao J, Chiarelli C, Panettieri RA, Foda HD. Cyclic mechanical strain-induced proliferation and migration of human airway smooth muscle cells: role of EMMPRIN and MMPs. FASEB J 2005;19:1507-9.
41. Smith PG, Deng L, Fredberg JJ, Maksym GN. Mechanical strain increases cell stiffness through cytoskeletal filament reorganization. Am J Physiol Lung Cell Mol Physiol 2003;285:L456-63.
42. Fairbank NJ, Connolly SC, Mackinnon JD, Wehry K, Deng L, Maksym GN. Airway smooth muscle cell tone amplifies contractile function in the presence of chronic cyclic strain. Am J Physiol Lung Cell Mol Physiol 2008;295:L479-88.
43. Hasaneen NA, Zucker S, Lin RZ, Vaday GG, Panettieri RA, Foda HD. Angiogenesis is induced by airway smooth muscle strain. Am J Physiol Lung Cell Mol Physiol 2007;293:L1059-68.
44. Wang L, Paré PD. Deep inspiration and airway smooth muscle adaptation to length change. Respir Physiol Neurobiol 2003;137:169-78.
45. Matsumoto H, Niimi A, Tabuena RP, Takemura M, Ueda T, Yamaguchi M, Matsuoka H, Jinnai M, Chin K, Mishima M. Airway wall thickening in patients with cough variant asthma and nonasthmatic chronic cough. Chest 2007;131:1042-9.
46. Niimi A, Torrego A, Nicholson AG, Cosio BG, Oates TB, Chung KF. Nature of airway inflammation and remodeling in chronic cough. J Allergy Clin Immunol 2005;116:565-70.
47. Grainge CL, Lau LC, Ward JA, Dulay V, Lahiff G, Wilson S, Holgate S, Davies DE, Howarth PH. Effect of bronchoconstriction on airway remodeling in asthma. N Engl J Med 2011;364:2006-15.
48. Wang K, Liu CT, Wu YH, Feng YL, Bai HL, Ma ES, Wen FQ. Effects of formoterol-budesonide on airway remodeling in patients with moderate asthma. Acta Pharmacol Sin 2011;32:126-32.
49. Pavord ID, Jeffery PK, Qiu Y, Zhu J, Parker D, Carlsheimer A, Naya I, Barnes NC. Airway inflammation in patients with asthma with high-fixed or low-fixed plus as-needed budesonide/formoterol. J Allergy Clin Immunol 2009;123:1083-9.
50. Milanese M, Crimi E, Scordamaglia A, Riccio A, Pellegrino R, Canonica GW, Brusasco V. On the functional consequences of bronchial basement membrane thickening. J Appl Physiol 2001;91:1035-40.
51. 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;168:983-8.
52. Paganin F, Séneterre E, Chanez P, Daurés JP, Bruel JM, Michel FB, Bousquet J. Computed tomography of the lungs in asthma: influence of disease severity and etiology. Am J Respir Crit Care Med 1996;153:110-4.
53. Holgate ST. Pathogenesis of asthma. Clin Exp Allergy 2008;38:872-97.