ASBAI

Brazilian Journal of **Allergy *and* Immunology** [BJAI]

Review Article

Current perspectives on airway inflammation and remodelling in asthma and allergic rhinitis

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ABSTRACT

The development of AR and asthma requires an interaction between the environment, immune system and genetic susceptibility. While pollen-induced rhinitis is the most characteristic IgE-mediated allergic disease, in perennial allergic rhinitis the allergic triggers are more continuous, and lead to ongoing inflammation. Several cells and mediators orchestrate and maintain this inflammation. Although histamine is still one of the major mediators of the allergic reaction, many other mediators produced by different cell types are involved. Thus, the intricate interaction amongst these mediators, cytokines, chemokines, neuropeptides, adhesion molecules and various cells in the form of a complex network leads to the development of specific symptoms and the non specific hyperreactivity of allergic rhinitis. Asthma is characterized by variable degrees of chronic inflammation and structural alterations in the airways which include epithelial denudation, goblet cell metaplasia, subepithelial thickening, increased airway smooth muscle mass, bronchial gland enlargement, angiogenesis, and alterations in extracellular matrix components, involving large and small airways. Chronic inflammation is thought to initiate and perpetuate cycles of tissue injury and repair in asthma, although remodeling may also occur in parallel with inflammation. While AR and asthma share several similarities in the inflammatory cell and mediator profiles and responses, remodeling as seen in asthma is not characteristic of AR. In asthma, the relationships of airway inflammation, remodeling and lung function are becoming better understood. A variety of inflammatory cells and structural cells play a role in orchestrating the inflammation and structural changes in asthma. Increased airway responsiveness is a surrogate marker of inflammation and may reflect the development of structural changes in the airways. Such persistent increased bronchial responsiveness indicates remodeling which is partly resistant to therapy.

Keywords: Asthma, allergic rhinitis, airway remodeling, airway inflammation, cytokines.

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No conflicts of interest declared concerning the publication of this article.

Submitted May 26 2014,
accepted Jun 30 2014.

INTRODUCTION

Allergic diseases like allergic rhinitis (AR) and asthma are increasing to epidemic proportions worldwide and are major public health issues in both the developed and developing countries. Considering the focus of global bodies like the United Nations on non-communicable diseases and efforts to control these diseases, allergic diseases are amongst the earliest manifestations in early childhood of non-communicable diseases. While AR is an IgE-mediated chronic inflammation of the nasal mucosa characterized by symptoms of sneezing, rhinorrhea, nasal itching and nasal obstruction, asthma is a chronic, inflammatory condition of the lower airways characterized by largely reversible airflow obstruction, airway hyperresponsiveness, and episodic respiratory symptoms, including wheezing, productive cough, and sensations of breathlessness and chest tightness.[1]

Clinically, AR is a Th2-type inflammatory disease whereas asthma may be allergic or nonallergic,

distinguished by the presence or absence of IgE antibodies to common environmental allergens. About 80% of childhood and 50% of adult asthma is allergic (i.e., IgE associated). In the latter, the triggers are not so well defined, but are probably microbes or microbial components, viruses etc. However, both forms of asthma are characterized by T helper cell 2 (Th2) type inflammation, stimulation of inflammatory cells like mast cells, causing eosinophilia, leukocytosis, and enhanced B-cell IgE production, leading to airway hyperresponsiveness and tissue remodeling. The determinants for an individual to develop an asthmatic phenotype require both exposure to appropriate stimuli and a genetic predisposition.[2,3]

Airway remodeling involves structural changes in the airway wall characterized by epithelial alterations (epithelial shedding or denudation as a result of toxic proteins released especially by eosinophils), goblet cell metaplasia (their numbers increase first), bronchial mucous gland enlargement (excess production of mucus), subepithelial fibrosis and extracellular matrix deposition, increased smooth muscle mass and increased vascularization.[2] Chronic inflammation causes tissue injury, which is partly repaired between inflammatory exacerbations. Airway remodeling is associated with poorly-reversible or non-reversible airway narrowing, more severe airflow limitation and airway hyperresponsiveness and may be involved in component of adult severe asthma.[4] These changes involve both large and small airways, but the individual variation is large. Airway secretions also contribute to the air flow limitation in asthma, and increase in amount and viscosity of the secretions plays a crucial role especially in acute, life-threatening exacerbations.[5] However, remodeling is not a characteristic feature of AR.

Although much more is known in terms of the inflammatory mechanisms underlying AR and asthma, there is still a gap in our understanding of the cellular and molecular pathways involved in remodeling. Both inflammation and remodeling occur in the tracheobronchial tree of patients with asthma. Persistent, eosinophilic inflammation seems to be a prerequisite for the development of remodeling.[6] Increased vascularity and expression of vascular endothelial growth factor (VEGF) are features of the asthmatic airway, but little is known of their contribution of airway remodeling. Siddiqui et al.[7] showed that vascular remodeling is a feature of asthma, and is inversely correlated with postbronchodilator Forced Expiratory Volume in One Second (FEV_1) indicating a role in airflow obstruction.

Until recently, airway remodeling was considered to be a secondary phenomenon, developing later in the disease process as a consequence of persistent inflammation. The presence of airway inflammation and remodeling in children[8] with asthma indicates that the process of remodeling begins early in the disease process of asthma and synchronously with ongoing and repeated airway inflammation rather than as consequence of airway inflammation. Structural changes of the airways are already present and maximal in severely asthmatic school children,[9] indicating that changes begin early in life between the ages of 1 and 3 years; and in good association with tissue eosinophilia and reticular basement membrane thickening.[10] This gives a window of opportunity for early intervention that can possibly modify the natural history of asthma.[11]

CHRONIC INFLAMMATION IN ALLERGIC RHINITIS AND ASTHMA

Effector cells of inflammation

The upper and lower airways have a similar mucosal structure, and exhibit similar inflammatory reactions to irritants and allergens. Increasing evidence indicates that there is marked similarity in the degree of cell infiltration or cytokine expression during allergic inflammation between the nasal mucosa and bronchial mucosa.[12,13] However, there are minimal structural changes of the nasal mucosa in AR unlike that in asthma.

In allergic asthma and AR, inhaled allergens penetrate the mucociliary lining and enter the airway epithelium either via the tight junctions that surround the apical zone of epithelial cells or by direct uptake by the cells per se. Allergens are presented to T cells that then react by forming a pool of Th2 cells (IL-4, IL-5 and IL-13 secreting T cells) that drive the production of IgE via interaction with B cells. Subsequently the IgE binds to the high affinity IgE receptor on mast cells, and cross-linking of allergen specific mast cell surface IgE by allergens causes the release of mediators such as histamine and leukotrienes. They increase vascular permeability and initiate a cascade of recruitment of more inflammatory cells and further release of pro-inflammatory mediators.

Mast cells and basophils

Mast cells are critical in mediating the early phase of inflammatory response in AR and asthma. However, more recently it is known that mast cells are not only effector cells of the immediate phase response, but also play a role in ongoing allergic inflammation. They store and produce Th2 type cytokines, and chemokines, induce IgE synthesis in B cells,[14,15] express the Cysteinyl Leukotriene 1 Receptor ($CysLT_1$),[16] Glucocorticoid Receptor (GR),[17] and SAF-2,[18] and upregulate the production of cytokines/ chemokines in epithelial cells and fibroblasts.[19]

Accumulation of mast cells into the nasal and bronchial epithelium, a feature of AR and atopic

asthma, is also reported in idiopathic rhinitis.[20] RANTES (Regulated on Activation Normal T-Cell Expressed and Secreted chemokine) and TGF-beta (Transforming Growth Factor beta) are implicated in the intraepithelial migration of mast cells.[19,21] Increase in mast cells and basophils occurs within 1 hr of nasal allergen challenge (NAC).[22] Patients with AR with or without asthma have similar number of intraepithelial mast cells,[23] and segmental bronchial challenge (SBC) induces an increase in basophils and eosinophils wich predominate in nasal and bronchoalveolar lavage (BAL).[24-27]

Airway mast cells stimulated via the FcεRI are an important source of Th2 cytokines, proinflammatory cytokines like TNF-alpha (Tumor Necrosis Factor alpha, preformed in mast cells) and chemokines like Thymus and Activation-Regulated Chemokine (TARC), as well as the IL-7 like cytokine Thymic Stromal Lymphopoietin TSLP.[28,29] FcεRI-mediated TSLP production from mast cells is further enhanced in an autocrine manner by IL-4.[30] Moreover, mast cells can interact with structural cells like epithelial cells to enhance the production of cytokines and chemokines from epithelial cells. TNF-alpha in concert with IL-4 and IL-13 enhances the production of TARC, TSLP and eotaxin from epithelial cells.[19] These result in increased infiltration of Th2 cells, eosinophils and differentiation of dendritic cells. Moreover, tryptase and chymase from mast cells can upregulate RANTES and GMCSF (Granulocyte-Macrophage Colony-Stimulating Factor) production from epithelial cells.[19] TNF-alpha also promotes antigen- and Th17 cell-dependant neutrophilia after allergenic stimulation and induces dendritic cell migration.[31,32] Murine mast cells have been shown to induce CD4+T-cell migration in vitro, but down-regulate FcεR1 expression in T reg cells, while activated Treg cells suppress mast cell FcεR1 expression.

Mast cells can induce IgE synthesis in B cells and local IgE synthesis has been demonstrated in the nasal mucosa of patients with AR.[33,34] Both IL-4 and IgE can upregulate FcεRI expression in mast cells suggesting a crucial role for the mast cell-IgE-IgE receptor cascade in ongoing inflammation in AR and asthma.[34] A role for mast cells in antigen presentation is also suggested.[35] While classically mast cell activation occurs following the allergen crosslinking of FcεR1-bound, allergen-specific IgE, mast cells may also be activated through multiple other mechanisms like via the complement receptors and Toll-Like Receptors (TLRs) even in the absence of FcεR1 stimulation.[36-40]

Basophils are increased in the nasal secretions and bronchoalveolar lavage of patients with AR and asthma. Like mast cells, basophils can also initiate allergic inflammation through the binding of allergen-specific IgE antibodies to the FcεR1 on basophil cell surface.[41] Basophils also drive Th2 cell differentiation of activated naive CD4+T cells via production of IL-4 and direct cell-cell contact.[42] Basophils in AR and atopic asthmatics also express high levels of the FcεRI.[43] Down regulation of FceRI in basophils and mast cells comprises one of the mechanisms of anti-IgE monoclonal antibody (Omalizumab).

Eosinophils

Eosinophils increase in both the early and late phase of AR[44] and correlate with nasal flow, IL-4, IL-5, IL-8 and IFN-gamma, spirometric values, methacholine test positivity, percentage of predicted FEV_1 and BHR.[45-54] Eosinophils are increased in symptomatic atopics and increase in nasal as well as bronchial epithelium and lamina propria after NAC[22,55-57]. Eosinophils are a major source of Macrophage Migration Inhibitory Factor (MIF)[58] and Nerve Growth factor (NGF),[59] express 5-Lipoxygenase (5-LO), Five-Lipoxygenase Activating Protein (FLAP), Leukotriene C4 Syntase (LTC_4S), and $CysLT_1$ and $CysLT_2$ receptors,[60] are involved in the loss of epithelial integrity[61] and are also seen in the esophageal mucosa of symptomatic patients with respiratory allergy.[62]

IL-5 has a key role modulating eosinophil differentiation and survival. Targeting IL-5 as a therapeutic strategy for allergic asthma with anti- IL-5 therapy has demonstrated marked reduction of peripheral blood eosinophils, but only partial abrogation of the pulmonary eosinophilic response and minimal impact on clinical outcomes.[63,64] More recently, in selected patients with refractory, eosinophilic asthma anti-IL-5 monoclonal antibody (mepolizumab) reduced both peripheral blood and sputum eosinophil levels, asthma exacerbations, and resulted in a reduction in oral corticosteroid dose.[65,66]

Eosinophil chemoattractants include eotaxin, macrophage/monocyte chemotactic protein 4 (MCP4), RANTES, and CysLT among others.[67,78] They act on distinct cell surface receptors (e.g., CC chemokine receptor 3 [CCR3] and $CysLT_1$) present on the eosinophil, but not exclusively so. Challenge with leukotriene E_4 (LTE_4) or LTD_4 results in increased nasal secretion and nasal obstruction in AR and greatly increased numbers of eosinophils in the bronchial wall.[67,68] $CysLT_1$ receptor is expressed on a variety of nasal and bronchial inflammatory cells including eosinophil, neutrophils, mast cells, macrophages, B lymphocytes, and plasma cells.[69] These numbers of inflammatory cells expressing the $CysLT_1$ receptor are also increased in AR and asthma and there is a further, significant increase among patients experiencing a severe exacerbation of asthma leading to hospitalization.[70]

Activated eosinophils release highly toxic granules, the evolutionary function of which has probably been killing the potentially dangerous invader. Especially in

asthma, eosinophil derivatives damage the surface epithelial cells, loosening their attachments and resulting in shedding of cells into the airway lumen, where they admix with eosinophils, neutrophils and mucus.

T lymphocytes

In perennial AR (PAR) patients, CD3+, CD25+ (activated) and CD45RA+ (naïve) T lymphocytes are increased in the nasal mucosa. In PAR, memory T cells and in idiopathic rhinitis CD8+ T cells correlate with mucosal mast cells.[71] While CD86 is expressed on CD19, CD1a, CD14 and CD3 T cells in PAR, CD80, CD28, and CD152 are expressed after NAC.[72] Moreover, CCR4+ CD4 cells are increased in AR.[73] Besides Th2 cytokines and chemokines, T cells in AR express IL-16,[74] CXCR1[75] and CX(3)CR(1).[76] Mucosal gamma delta T cells in PAR and asthma are increased, induce IgE synthesis in B cells and induce proliferation of T cells.[77] CD23+ B cells increase in PAR, but do not correlate with the mucosal Th2 cells.[73]

Although conventionally both AR and allergic asthma have been considered to be due to a disruption in the normal Th1/Th2 balance, more recently, there is new evidence on the emerging roles of Th17 cells which are a distinct subpopulation of CD4+ T cells that produce IL-17A, IL-17F, IL-22, TNF-alpha, and IL-21.[78] Th17 cells were found in the nasal mucosa of AR patients[79] and in bronchial biopsies of asthmatics.[80] IL-17 induces the release of proinflamatory cytokines / chemokines from a variety of cell types,[79] is linked to the development of airway neutrophilia, and its presence in the asthmatic airway correlates with increased severity of disease. Treg cells play roles in the determination of self tolerance and the regulation of immune responses. Th17 and Treg cells have opposing actions, and T reg cells secrete IL-10 and TGF-beta and are increased in patients after immunotherapy.

Neutrophils

Although neutrophils are predominantly increased in non-allergic infective rhinitis and chronic rhinosinusitis, the increased expression of activation markers on neutrophils and Myeloperoxidase (MPO) levels in AR and increase in neutrophils in broncheoalveolar lavage after nasal allergen challenge suggests roles for neutrophils in AR.[25,81] In acute, severe exacerbations of asthma, there are increased eosinophils and neutrophils within the airway, and the increase in neutrophils is proportionately greater.[82] Inhaled corticosteroids reduce airway eosinophils but increase airway neutrophils and neutrophil chemoattractant IL-8, with loss of asthma control.[83]

Epithelial cells

Conventionally, epithelial cells placed at the interface between the external environment and the host have been considered to play a role as a defense barrier against environmental agents. However, over the past several years the roles of epithelial cells as effector cells have become more evident, directly via the action of inflammatory mediators as well as via cell-cell interaction with immune cells. Moreover, its pivotal position in orchestrating airway remodeling and fibroblast proliferation is also crucial.[84]

Airway epithelial cells are an important source of a variety of inflammatory mediators including multifunctional cytokines and chemokines like IL-1, IL-6, IL-8, TNF-alpha, GMCSF, RANTES, Eotaxin, TARC leading to their crucial role in the migration and activation of immune cells like eosinophils, basophils and Th2 cells.[85,86] More recently, TSLP derived from epithelial cells is increased in the nasal mucosa of AR patients and in the asthmatic airway.[87] TSLP can activate dendritic cells, promote Th2 responses, and activate mast cells.[30] Epithelial cells also express co-stimulatory molecules like CD86 and HLA-DR, CD86 and the FcεRI can present antigen to T cells.[88,89] Particulate matter like diesel exhaust particles can induce the release of proinflammatory mediators and enhance the expression of co-stimulatory molecules on epithelial cells. The expression of IL-17F in the the airways of asthmatics correlates with disease severity and induces several asthma-related molecules such as CCL20 that can attract Th17 cells into the airway thus amplifying airway inflammation. A recent study demonstrated that bronchial epithelial cells express IL-17F in response to IL-33 via ST2-ERK1/2-MSK1 signaling pathway.[90] Moreover, IL-17F is involved in airway remodeling and steroid resistance. Hence, IL-17F may be a valuable therapeutic target for development of novel strategies. Recently the importance of serum periostin measurements and the role of anti-IL-13 as a treatment in those with high periostin levels and antiIL-5 in those who are steroid sensitive with high eosinophils demonstrated that there are phenotypes and endotypes of asthma.

REMODELING IN ASTHMA

Remodeling is defined as a change in structure that is inappropriate to maintain normal airway function.[91,92] Some features of remodeling are evident, even in newly diagnosed or mild asthmatics and is characterized by epithelial fragility and reticular basement membrane thickening. With increasing severity of asthma, the changes are more pronounced and clear: increases of airway smooth muscle mass, vascularity, numbers

of fibroblasts, and interstitial collagen, as well as mucous gland hypertrophy.[93] These changes appear to be greatest in the larger, more proximal airways. Thickening of the reticular basement membrane occurs early in asthma, even before diagnosis, and is detected in children with mild asthma.[94] In school children between the ages of 6 and 16 with severe asthma there is already maximally thickening but with no relation to the age or symptom duration.[9] These changes appear in preschool wheezy children by the age of 29 months.[7] Bourdin et al.[95] showed recently that reticular basement thickness is a hallmark of severe asthma, but not of mild asthma or Chronic Obstructive Pulmonary Disease (COPD).

Airway smooth muscle surrounds the airways as two opposing helices, i.e., a geodesic pattern and as muscle shortens, it constricts and also tends to shorten the airway against an elastic load. Airway smooth muscle mass is increased in the asthmatic airway.[96] Airway smooth muscle cells can secrete mediators that may promote mast cell chemotaxis, proliferation, and survival, while cell-cell interaction between airway smooth muscle cells and mast cells enhances activated complement-induced mast cell degranulation.[97-99] Human lung mast cells migrate toward Th2 cytokine-stimulated airway smooth muscle cells from asthmatics, while supernatants obtained from airway smooth muscle cell cultures of non-asthmatics inhibit this chemotaxis.[100] Large numbers of mast cells have been found located within bronchial smooth muscle of asthmatics and mast cell mediators, such as tryptase and cytokines, can modulate airway smooth muscle cell function. Mast cells also express MMP9 and contribute to multiple features of chronic asthma and they play an important role in tissue remodeling.[19]

Bronchial epithelial cell experiments have demonstrated a role for TLR signaling in the activation of epidermal growth factor receptor, suggesting a role for TLRs in potentiating remodeling.[101] In the asthmatic airway, there are increased numbers of subepithelial myofibroblasts, and allergen challenge in people with asthma leads to increased accumulation of myofibroblasts in the airway mucosa.[84,102] Histamine can induce the transition from fibroblasts to myofibroblasts (as measured by alpha- smooth muscle actin expression), and induce connective-tissue-growth-factor expression in fibroblasts, suggesting the ability to participate in the process of remodeling.[102,103] Fibroblastic infiltration of the lung may be secondary to the recruitment of circulating bone marrow-derived progenitors of fibrocytes to the airway and to the proliferation and expansion of resident fibroblasts, or possibly, epithelial cells may undergo phenotypic change to effector fibroblasts through a process termed epithelial-mesenchymal transition. Airway epithelial cells derived from asthmatics demonstrated increased susceptibility to TGFβ-induced epithelial-mesenchymal transition than those derived from normal subjects.[104] In addition to stimulation of epithelial cells and extracellular matrix synthesis, TGFβ can elicit other responses in bronchial fibroblasts, including stimulating their proliferation and synthesis of a range of growth factors. It also has effects on the asthma susceptibility gene, a disintegrin and metalloprotease (ADAM)[33], which has been implicated as an asthma remodeling gene.

Measuring airway function (e.g. FEV_1) can give indirect information on the long-term airway inflammation and structural changes, but cannot detect the early inflammatory processes. Such inflammatory changes can exist even in patients with normal lung function but with symptoms indicative of asthma.[105] On the other hand, symptomatic infants, who have reversible airflow obstruction may not have bronchial mucosal eosinophilia or remodeling.[18]

How early can remodeling start

Airway biopsy studies in children suggest that pathologic changes such as epithelial loss, basement membrane thickening, and angiogenesis occur early in the asthmatic airway. In children with difficult asthma (mean age of 13, age range of 6-16) recruited to investigate whether the thickening of reticular basement membrane could occur in childhood asthma[9] have shown that the thickening of reticular basement membrane was seen in the airway. In another study, Barbato et al. have examined the biopsy specimens of airway in children that is nine children with asthma (age of 4-12 yr), six children with atopy without asthma (age of 4-12 yr), and eight control children without asthma or atopy to elucidate whether airway inflammation and remodeling could occur even in mild childhood asthma,[92] demonstrated that airway eosinophilia and basement membrane thickness were present in children with mild asthma, and even in children with atopy without asthma. This indicates that 1) airway inflammation shown by airway eosinophilia occurs even in mild as well as in difficult asthma, 2) airway inflammation occurs in the airway before developing episodic wheezing, although asthma symptom is difficult to establish in children, 3) the presence of both airway inflammation and remodeling indicate that remodeling process begins early in the disease process of asthma and occurs synchronously in ongoing and repeated airway inflammation rather than as subsequent event of airway inflammation.

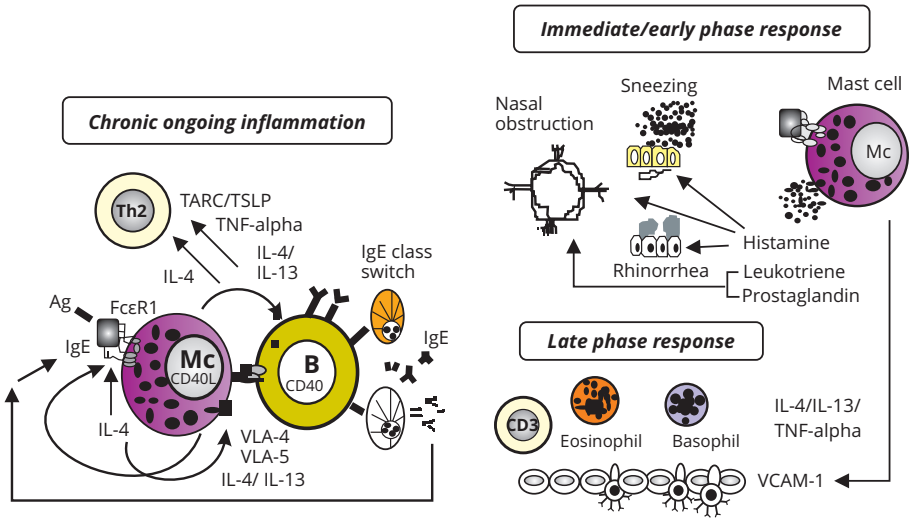

Figure 1 - Inflammatory mechanisms in allergic rhinitis (Modified from Pawankar et al. Allergic Rhinitis pathomechanisms)

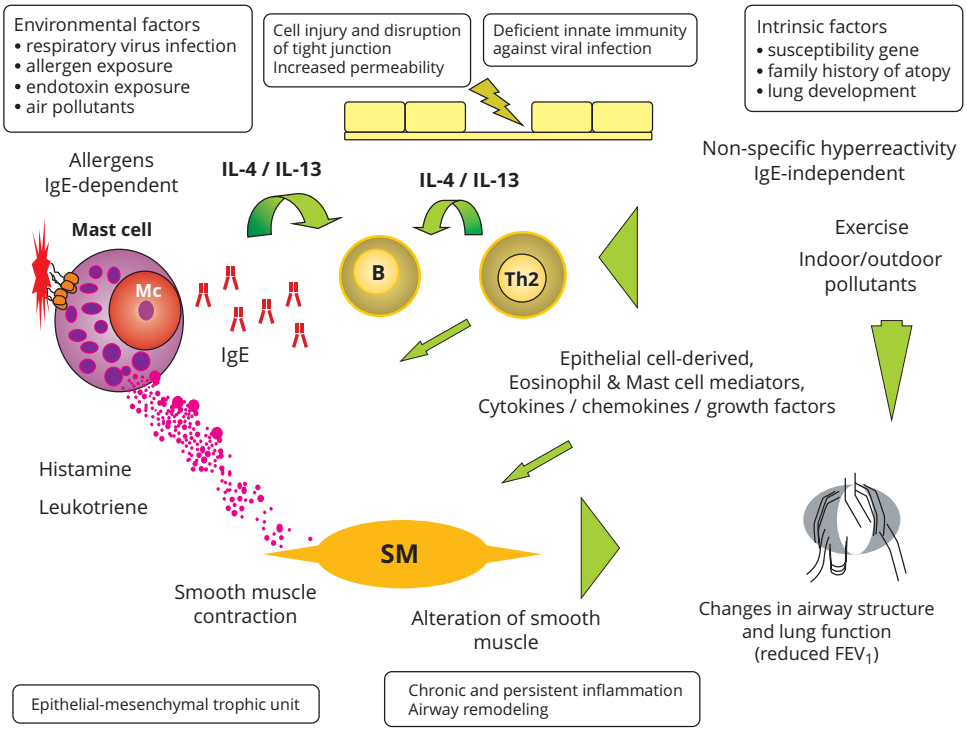

Figure 2 - Pathomechanisms of asthma

Summary

With the increasing evidence on the links between allergic rhinitis and asthma from epidemiologic, immunologic and clinical studies, early intervention and downregulation of inflammation are key to better control of both AR and asthma. New modes of immunomodulatory therapies and biologics that target specific phenotypes like IgE, IL-5, IL-13 and the IL4 receptor alpha chain have shown some efficacy in phenotyped patients. For those without evidence of Th2 inflammation, no specific therapies have been identified. Anti-IgE - and anti-IL-5, anti-IL-13, anti-IL4 R antagonists, and therapies that target TSLP, IL-33, IL-17 as well as those that induce tolerance hold more promising outcomes, but their effect on halting airway remodeling in severe asthma is not known. Clinical cluster analysis from the Severe Asthma Research Program (SARP) identified 5 asthma subphenotypes that represent the severity spectrum of early-onset allergic asthma, late-onset severe asthma, and severe asthma with chronic obstructive pulmonary disease characteristics. Analysis of induced sputum from a subset of SARP subjects showed 4 sputum inflammatory cellular patterns. Subjects with concurrent increases in eosinophil (≥2%) and neutrophil (≥40%) percentages had characteristics of very severe asthma. This multivariate approach identified 4 asthma subphenotypes representing the severity spectrum from mild-to-moderate allergic asthma with minimal or eosinophil-predominant sputum inflammation to moderate-to-severe asthma with neutrophil-predominant or mixed granulocytic inflammation.[106]

Given the immunological similarities that exists between the patterns of inflammation in asthma and allergic rhinitis as well as chronic rhinosinusitis and asthma and the impact of AR on asthma, treatment should focus to have a global approach to treat both the upper and lower airways for better outcomes.

REFERENCES

1. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med. 2000;161(5):1720-45.
2. Kabesch M, Schedel M, Carr D, et al. IL-4/IL-13 pathway genetics strongly infl uence serum IgE levels and childhood asthma. J Allergy Clin Immunol. 2006;117(2):269-74.
3. Loza MJ, Chang BL. Association between Q551R IL4R genetic variants and atopic asthma risk demonstrated by meta-analysis. J Allergy Clin Immunol. 2007;120(3):578-85.
4. Holgate ST. The mechanisms diagnosis, and management of severe asthma in adults. Lancet. 2006;368:780-93.
5. 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(2 Pt 1):663-9.
6. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, et al. A critical role for eosinophils in allergic airways remodeling. Science. 2004;305(5691):1776-9.
7. Siddiqui S, Sutcliffe A, Shikotra A, Woodman L, Doe C, McKenna S, et al. Vascular remodeling is a feature of asthma and nonasthmatic eosinophilic bronchitis. J Allergy Clin Immunol. 2007;120:813-9.
8. Saglani S, Malmstrom K, Pelkonen AS, Malomberg P, Lindahl H, Kajosaari M, et al. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. Am J Respir Crit Care Med. 2005;171;722-77.
9. Payne DN, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, et al. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med. 2003;167(1):78-82.
10. Saglani S, Payne DN, Nicholson AG, Jeffery PK, Bush A. Thickening of the epithelial reticular basement membrane in pre-school children with troublesome wheeze. In: American Thoracic Society. 2005;2005:A515.
11. Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, Jeffery PK. Early detection of airway remodeling and eosinophilic inflammation in preschool wheezers. Am J Respir Crit Care Med. 2007;176:858-64.
12. Pawankar R. Allergic rhinitis and asthma: are they manifestations of one syndrome? Clin Exp Allergy. 2006 Jan;36(1):1-4.
13. Cruz AA, Popov T, Pawankar R, Annesi-Maesano I, Fokkens W, Kemp J, et al. ARIA Initiative Scientific Committee. Common characteristics of upper and lower airways in rhinitis and asthma: ARIA update, in collaboration with GA(2)LEN. Allergy. 2007;62 Suppl 84:1-41.
14. Wilson AM, Duong M, Crawford L, Denburg J. An evaluation of peripheral blood eosinophil/basophil progenitors following nasal allergen challenge in patients with allergic rhinitis. Clin Exp Allergy. 2005;35(1):39-44.
15. Pawankar R, Yamagishi S, Yagi T. Revisiting the roles of mast cells in allergic rhinitis and its relation to local IgE synthesis. Am J Rhinol. 2000;14(5):309-17.
16. Shirasaki H, Kanaizumi E, Watanabe K, Matsui T, Sato J, Narita S, et al. Expression and localization of the cysteinyl leukotriene 1 receptor in human nasal mucosa. Clin Exp Allergy. 2002;32(7):1007-12.
17. Shirasaki H, Watanabe K, Kanaizumi E, Konno N, Sato J, Narita S, et al. Expression and localization of steroid receptors in human nasal mucosa. Acta Otolaryngol. 2004;124(8):958-63.
18. Kikly KK, Bochner BS, Freeman SD, Tan KB, Gallagher KT, D'alessio KJ, et al. Identification of SAF-2, a novel siglec expressed on eosinophils, mast cells, and basophils. J Allergy Clin Immunol. 2000;105(6 Pt 1):1093-100.
19. Pawankar R. Mast cells in allergic airway disease and chronic rhinosinusitis. Chem Immunol Allergy. 2005;87:111-29.
20. Powe DG, Hiskisson RS, Carney AS, Jenkins D, Jones NS. Idiopathic and allergic rhinitis show a similar inflammatory response. Clin Otolaryngol Allied Sci. 2000;25(6):570-6.
21. Salib RJ, Kumar S, Wilson SJ, Howarth PH. Nasal mucosal immunoexpression of the mast cell chemoattractants TGF-beta, eotaxin, and stem cell factor and their receptors in allergic rhinitis43: J Allergy Clin Immunol. 2004;114(4):799-806.
22. KleinJan A, McEuen AR, Dijkstra MD, Buckley MG, Walls AF, Fokkens WJ. Basophil and eosinophil accumulation and mast cell degranulation in the nasal mucosa of patients with hay fever after local allergen provocation. J Allergy Clin Immunol. 2000;106(4):677-86.
23. Braunstahl GJ, Fokkens WJ, Overbeek SE, KleinJan A, Hoogsteden HC, Prins JB. Mucosal and systemic inflammatory changes in allergic rhinitis and asthma: a comparison between upper and lower airways. Clin Exp Allergy. 2003;33(5):579-87.
24. Braunstahl GJ, Overbeek SE, Fokkens WJ, Kleinjan A, McEuen AR, Walls AF, et al. Segmental bronchoprovocation in allergic rhinitis patients affects mast cell and basophil numbers in nasal and bronchial mucosa. Am J Respir Crit Care Med. 2001a;164(5):858-65.

25. Gorski P, Krakowiak A, Ruta U. Nasal and bronchial responses to flour-inhalation in subjects with occupationally induced allergy affecting the airway. Int Arch Occup Environ Health. 2000;73(7):488-97.

26. Krakowiak A, Ruta U, Gorski P, Kowalska S, Palczynski C. Nasal lavage fluid examination and rhinomanometry in the diagnostics of occupational airway allergy to laboratory animals. Int J Occup Med Environ Health. 2003;16(2):125-32.

27. Palczynski C, Walusiak J, Krakowiak A, Szymczak W, Wittczak T, Ruta U, et al. Nasal lavage fluid examination in diagnostics of occupational allergy to chloramine. Int J Occup Med Environ Health. 2003;16(3):231-40.

28. Bradding P, Holgate ST. The mast cell as a source of cytokines in asthma. Ann N Y Acad Sci. 1996;Oct 31;796:272-81.

29. Pawankar R, Okuda M, Hasegawa S, Suzuki K, Yssel H, Okubo K, et al. Interleukin-13 expression in the nasal mucosa of perennial allergic rhinitis. Am J Respir Crit Care Med. 1995 Dec;152(6 Pt 1):2059-67.

30. Okayama Y, Okumura S, Sagara H, Yuki K, Sasaki T, Watanabe N, et al. FcepsilonRI-mediated thymic stromal lymphopoietin production by interleukin-4-primed human mast cells. Eur Respir J. 2009 Aug;34(2):425-35.

31. Nakae S, Suto H, Berry GJ, Galli SJ. Mast cell-derived TNF can promote Th17 cell-dependent neutrophil recruitment in ovalbumin-challenged OTII mice. Blood. 2007;109(9):3640-8.

32. Suto H, Nakae S, Kakurai M, Sedgwick JD, Tsai M, Galli SJ. Mast cell-associated TNF promotes dendritic cell migration. J Immunol. 2006;176:4102-12.

33. Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitics exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. J Clin Invest. 1997;99(7):1492-9.

34. Smurthwaite L, Durham SR. Local IgE synthesis in allergic rhinitis and asthma. Curr Allergy Asthma Rep. 2002;2(3):231-8.

35. Kambayashi T, Baranski JD, Baker RG, et al. Indirect involvement of allergen-captured mast cells in antigen presentation. Blood. 2008;111(3):1489-96.

36. Yang Z, Yan WX, Cai H, et al. S100A12 provokes mast cell activation: a potential amplification pathway in asthma and innate immunity. J Allergy Clin Immunol. 2007;119(1):106-14.

37. Kojima T, Obata K, Mukai K, et al. Mast cells and basophils are selectively activated in vitro and in vivo through CD200R3 in an IgE-independent manner. J Immunol. 2007;179(10):7093-100.

38. Ho LH, Ohno T, Oboki K, et al. IL-33 induces IL-13 production by mouse mast cells independently of IgE-FcepsilonRI signals. J Leukoc Biol. 2007;82(6):1481-90.

39. Iikura M, Suto H, Kajiwara N, et al. IL-33 can promote survival, adhesion and cytokine production in human mast cells. Lab Invest. 2007;87(10):971-8.

40. Allakhverdi Z, Smith DE, Comeau MR, Delespesse G. Cutting edge: the ST2 ligand IL-33 potently activates and drives maturation of human mast cells. J Immunol. 2007;179(4):2051-4.

41. Obata K, Mukai K, Tsujimura Y, et al. Basophils are essential initiators of a novel type of chronic allergic infl ammation. Blood. 2007;110(3):913-20.

42. Oh K, Shen T, Le Gros G, Min B. Induction of Th2 type immunity in a mouse system reveals a novel immunoregulatory role of basophils. Blood. 2007;109(7):2921-7.

43. MacGlashan D Jr. IgE and FcepsilonRI regulation. Clin Rev Allergy Immunol. 2005;29(1):49-60.

44. Milanese M, Ricca V, Canonica GW, Ciprandi G. Eosinophils, specific hyperreactivity and occurrence of late phase reaction in allergic rhinitis. Eur Ann Allergy Clin Immunol. 2005;37(1):7-10.

45. Ciprandi G, Vizzaccaro A, Cirillo I, Tosca M, Massolo A, Passalacqua G. Nasal eosinophils display the best correlation with symptoms, pulmonary function and inflammation in allergic rhinitis. Int Arch Allergy Immunol. 2005;136(3):266-72.

46. Ciprandi G, Marseglia GL, Klersy C, Tosca MA. Relationships between allergic inflammation and nasal airflow in children with persistent allergic rhinitis due to mite sensitization. Allergy. 2005b;60(7):957-60.

47. Ciprandi G, Cirillo I, Vizzaccaro A, Milanese M, Tosca MA. Nasal obstruction in patients with seasonal allergic rhinitis: relationships between allergic inflammation and nasal airflow. Int Arch Allergy Immunol. 2004;134(1):34-40.

48. Kurt E, Bavbek S, Aksu O, Erekul S, Misirligil Z. The effect of natural pollen exposure on eosinophil apoptosis and its relationship to bronchial hyperresponsiveness in patients with seasonal allergic rhinitis. Ann Allergy Asthma Immunol. 2005;95(1):72-8.

49. Di Lorenzo G, Pacor ML, Mansueto P, Esposito Pellitteri M, Lo Bianco C, Ditta V, et al. Determinants of bronchial hyperresponsiveness in subjects with rhinitis. Int J Immunopathol Pharmacol. 2005;18(4):715-22.

50. Sale R, Silvestri M, Battistini E, Defilippi AC, Sabatini F, Pecora S, et al. Nasal inflammation and bronchial reactivity to methacholine in atopic children with respiratory symptoms. Allergy. 2003;58(11):1171-5.

51. Jang AS. Nasal eosinophilic inflammation contributes to bronchial hyperresponsiveness in patients with allergic rhinitis. J Korean Med Sci. 2002;17(6):761-4.

52. Silvestri M, Battistini E, Defilippi AC, Sabatini F, Sale R, Pecora S, et al. Early decrease in nasal eosinophil proportion after nasal allergen challenge correlates with baseline bronchial reactivity to methacholine in children sensitized to house dust mites. J Investig Allergol Clin Immunol. 2005;15(4):266-76.

53. Ciprandi G, Cirillo I, Vizzaccaro A, Milanese M, Tosca MA. Correlation of nasal inflammation and nasal airflow with forced expiratory volume in 1 second in patients with perennial allergic rhinitis and asthma. Ann Allergy Asthma Immunol. 2004;93(6):575-80.

54. Ciprandi G, Cirillo I, Vizzaccaro A, Milanese M, Tosca MA. Airway function and nasal inflammation in seasonal allergic rhinitis and asthma. Clin Exp Allergy. 2004c;34(6):891-6.

55. Tatar M, Petriskova J, Zucha J, Pecova R, Hutka Z, Raffajova J, Brozmanova M. Induced sputum eosinophils, bronchial reactivity, and cough sensitivity in subjects with allergic rhinitis. J Physiol Pharmacol. 2005;56(Suppl 4):227-36.

56. Braunstahl GJ, Overbeek SE, Kleinjan A, Prins JB, Hoogsteden HC, Fokkens WJ. Nasal allergen provocation induces adhesion molecule expression and tissue eosinophilia in upper and lower airways. J Allergy Clin Immunol. 2001;107(3):469-76.

57. Braunstahl GJ, Kleinjan A, Overbeek SE, Prins JB, Hoogsteden HC, Fokkens WJ. Segmental bronchial provocation induces nasal inflammation in allergic rhinitis patients. Am J Respir Crit Care Med. 2000;161(6):2051-7.

58. Nakamaru Y, Oridate N, Nishihira J, Takagi D, Furuta Y, Fukuda S. Macrophage migration inhibitory factor in allergic rhinitis: its identification in eosinophils at the site of inflammation. Ann Otol Rhinol Laryngol. 2004;113(3 Pt 1):205-9.

59. Kobayashi H, Gleich GJ, Butterfield JH, Kita H. Human eosinophils produce neurotrophins and secrete nerve growth factor on immunologic stimuli. Blood. 2002;99(6):2214-20.

60. Figueroa DJ, Borish L, Baramki D, Philip G, Austin CP, Evans JF. Expression of cysteinyl leukotriene synthetic and signalling proteins in inflammatory cells in active seasonal allergic rhinitis. Clin Exp Allergy. 2003;33(10):1380-8.

61. Amin K, Rinne J, Haahtela T, Simola M, Peterson CG, Roomans GM, et al. Inflammatory cell and epithelial characteristics of perennial allergic and nonallergic rhinitis with a symptom history of 1 to 3 years' duration. J Allergy Clin Immunol. 2001;107(2):249-57.

62. Onbasi K, Sin AZ, Doganavsargil B, Onder GF, Bor S, Sebik F. Eosinophil infiltration of the oesophageal mucosa in patients with pollen allergy during the season. Clin Exp Allergy. 2005;35(11):1423-31.

63. Flood-Page P, Swenson C, Faiferman I, et al. International Mepolizumab Study Group. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. Am J Respir Crit Care Med. 2007;176(11):1062-71.
64. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. Am J Respir Crit Care Med. 2003;167(2):199-204.
65. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med. 2009;360(10):973-84.
66. Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med. 2009;360(10):985-93.
67. Pawankar R. Inflammatory mechanisms in allergic rhinitis. Curr Opin Allergy Clin Immunol. 2007 Feb;7(1):1-4.
68. Nonaka M, Pawankar R, Fukumoto A, Ogihara N, Sakanushi A, Yagi T. Induction of eotaxin production by interleukin-4, interleukin-13 and lipopolysaccharide by nasal fibroblasts. Clin Exp Allergy. 2004 May;34(5):804-11.
69. Parnes SM. Targeting cysteinyl leukotrienes in patients with rhinitis, sinusitis and paranasal polyps. Am J Respir Med. 2002;1(6):403-8.
70. Zhu J, Qiu YS, Figueroa DJ, Bandi V, Galczenski H, Hamada K,et al. Localization and upregulation of cysteinyl leukotriene-1 receptor in asthmatic bronchial mucosa. Am J Respir Cell Mol Biol. 2005 Dec;33(6):531-40.
71. Powe DG, Huskisson RS, Carney AS, Jenkins D, McEuen AR, Walls AF, et al. Mucosal T-cell phenotypes in persistent atopic and nonatopic rhinitis show an association with mast cells. Allergy. 2004;59(2):204-12.
72. Hattori H, Okano M, Yoshino T, Akagi T, Nakayama E, Saito C, et al. Expression of costimulatory CD80/CD86-CD28/CD152 molecules in nasal mucosa of patients with perennial allergic rhinitis. Clin Exp Allergy. 2001;31(8):1242-9.
73. Horiguchi S, Okamoto Y, Chazono H, Sakurai D, Kobayashi K. Expression of membrane-bound CD23 in nasal mucosal B cells from patients with perennial allergic rhinitis. Ann Allergy Asthma Immunol. 2005;94(2):286-91.
74. Karaki M, Dobashi H, Kobayashi R, Tokuda M, Ishida T, Mori N. Expression of interleukin-16 in allergic rhinitis. Int Arch Allergy Immunol. 2005;138(1):67-72.
75. Francis JN, Jacobson MR, Lloyd CM, Sabroe I, Durham SR, Till SJ. CXCR1+CD4+ T cells in human allergic disease. J Immunol. 2004;172(1):268-73.
76. Rimaniol AC, Till SJ, Garcia G, Capel F, Godot V, Balabanian K, et al. The CX3C chemokine fractalkine in allergic asthma and rhinitis. J Allergy Clin Immunol. 2003;112(6):1139-46.
77. Pawankar R. Gamma-delta T cells in allergic airway diseases. Clin Exp Allergy. 2000;30(3):318-23.
78. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. N Engl J Med. 2009;361(9):888-98.
79. Han D, Wang C, Lou W, Gu Y, Wang Y, Zhang L. Allergen-specific IL-10-secreting type I T regulatory cells, but not CD4(+)CD25(+)Foxp3(+) T cells, are decreased in peripheral blood of patients with persistent allergic rhinitis. Clin Immunol. 2010 Aug;136(2):292-301.
80. Pène J, Chevalier S, Preisser L, et al. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. J Immunol. 2008;180(11):7423-30.
81. Kinhult J, Egesten A, Benson M, Uddman R, Cardell LO. Increased expression of surface activation markers on neutrophils following migration into the nasal lumen. Clin Exp Allergy. 2003;33(8):1141-6.
82. Qiu Y, Zhu J, Bandi V, Guntupalli KK, Jeffery PK. Bronchial mucosal infl ammation and upregulation of CXC chemoattractants and receptors in severe exacerbations of asthma. Thorax. 2007;62(6):475-82.
83. Maneechotesuwan K, Essilfi-Quaye S, Kharitonov SA, Adcock IM, Barnes PJ. Loss of control of asthma following inhaled corticosteroid withdrawal is associated with increased sputum interleukin-8 and neutrophils. Chest. 2007;132(1):98-105.
84. Holgate ST. Epithelium dysfunction in asthma. J Allergy Clin Immunol. 2007;120(6):1233-44.
85. Takizawa H. Bronchial epithelial cells in allergic reactions. Curr Drug Targets Inflamm Allergy. 2005 Jun;4(3):305-11.
86. Pawankar R. Epithelial cells as immunoregulators in allergic airway diseases. Curr Opin Allergy Clin Immunol. 2002 Feb;2(1):1-5.
87. Ying S, O'Connor B, Ratoff J, Meng Q, Fang C, Cousins D, et al. Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. J Immunol. 2008 Aug15;181(4):2790-8.
88. Gereke M, Jung S, Buer J, Bruder D. Alveolar type II epithelial cells present antigen to CD4+ T cells and induce Foxp3(1) regulatory T cells. Am J Respir Crit Care Med. 2009;179(5):344-55.
89. Takizawa R, Pawankar R, Yamagishi S, Takenaka H, Yagi T. Increased expression of HLA-DR and CD86 in nasal epithelial cells in allergic rhinitics: antigen presentation to T cells and up-regulation by diesel exhaust particles. Clin Exp Allergy. 2007 Mar;37(3):420-33.
90. Ota K, Kawaguchi M, Matsukura S, Kurokawa M, Kokubu F, Fujita J, et al. Potential involvement of IL-17F in asthma. J Immunol Res. 2014;2014:602846.
91. Mauad T, Bel EH, Sterk PJ. Asthma therapy and airway remodeling. J Allergy Clin Immunol. 2007 Nov;120(5):997-1009.
92. Jeffery PK. Remodeling and inflammation of bronchi in asthma and chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2004;1(3):176-83.
93. Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Tura M, et al. Airway inflammation in childhood asthma. Am J Respir Crit Care Med. 2003,168(7):798-803.
94. Bourdin A, Neveu D, Vachier I, Paganin F, Godard P, Chanez P. Specificity of basement membrane thickening in severe asthma. J Allergy Clin Immunol. 2007;119:1367-74.
95. Panettieri RA Jr, Kotlikoff MI, Gerthoffer WT, Hershenson MB, Woodruff PG, Hall IP, Banks-Schlegel S; National Heart, Lung, and Blood Institute. Airway smooth muscle in bronchial tone, infl ammation, and remodeling: basic knowledge to clinical relevance. Am J Respir Crit Care Med. 2008;177(3):248-52.
96. El-Shazly A, Berger P, Girodet PO, et al. Fraktalkine produced by airway smooth muscle cells contributes to mast cell recruitment in asthma. J Immunol. 2006;176(3):1860-8.
97. Hollins F, Kaur D, Yang W, et al. Human airway smooth muscle promotes human lung mast cell survival, proliferation, and constitutive activation: cooperative roles for CADM1, stem cell factor, and IL-6. J Immunol. 2008;181(4):2772-80.
98. Thangam EB, Venkatesha RT, Zaidi AK, et al. Airway smooth muscle cells enhance C3a-induced mast cell degranulation following cell-cell contact. FASEB J. 2005;19(7):798-800.
99. Sutcliffe A, Kaur D, Page S, et al. Mast cell migration to Th2 stimulated airway smooth muscle from asthmatics. Thorax. 2006;61(8):657-62.
100. Koff JL, Shao MX, Ueki IF, Nadel JA. Multiple TLRs activate EGFR via a signaling cascade to produce innate immune responses in airway epithelium. Am J Physiol Lung Cell Mol Physiol. 2008;294(6):L1068-L1075.
101. Schmidt M, Sun G, Stacey MA, Mori L, Mattoli S. Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. J Immunol. 2003;171(1):380-9.
102. Vancheri C, Gili E, Failla M, et al. Bradykinin differentiates human lung fibroblasts to a myofibroblast phenotype via the B2 receptor. J Allergy Clin Immunol. 2005;116(6):1242-8.
103. Kunzmann S, Schmidt-Weber C, Zingg JM, et al. Connective tissue growth factor expression is regulated by histamine in lung fibroblasts: potential role of histamine in airway remodeling. J Allergy Clin Immunol. 2007;119(6):1398-407.

104. Hackett TL, Warner SM, Stefanowicz D, et al. Induction of epithelial-mesenchymal transition in primary airway epithelial cells from patients with asthma by transforming growth factorbeta1. Am J Respir Crit Care Med. 2009;180(2):122-33.

105. Rytila P, Metso T, Heikkinen K, Saarelainen P, Helenius IJ, Haahtela T. Airway inflammation in patients with symptoms suggesting asthma but with normal lung function. Eur Respir J. 2000,16(5):824-30.

106. Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, Wenzel SE, Peters SP, Meyers DA, Bleecker ER; National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. J Allergy Clin Immunol. 2013 Dec 8.pii:S0091-6749(13)01563-7.