Immunologic and inflammatory mechanisms that drive asthma progression to remodeling

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Abstract

Although histologic features of airway remodeling have been well characterized in asthma, the immunologic and inflammatory mechanisms that drive progression of asthma to remodeling are still incompletely understood. Conceptually, airway remodeling may be due to persistent inflammation and/or aberrant tissue repair mechanisms. It is likely that several immune and inflammatory cell types and mediators are involved in mediating airway remodeling. In addition, different features of airway remodeling are likely mediated by different inflammatory pathways. Several important candidate mediators of remodeling have been identified including TGF-β and Th2 cytokines (including IL-5 and IL-13), as well as VEGF, ADAM-33, and MMP-9. Mouse models of airway remodeling have provided important insight into potential mechanisms by which TGF-β activation of the Smad 2/3 signaling pathway may contribute to airway remodeling. Human studies have demonstrated that anti-IL-5 reduces levels of airway eosinophils expressing TGF-β, as well as levels of airway remodeling as assessed by bronchial biopsies. Further such studies confirming these observations, as well as alternate studies targeting additional individual cell types, cytokines, and mediators are needed in human subjects with asthma to determine the role of candidate mediators of inflammation on the development and progression of airway remodeling.

PROGRESSION OF ASTHMA TO AIRWAY REMODELING: EPIDEMIOLOGY

Asthma is a chronic inflammatory disease of the airway which affects approximately 7% of the population of the USA[1]. The chronic inflammatory response in the airway in asthma is characterized by the presence of increased numbers of Th2 lymphocytes, eosinophils, and activated mast cells[2]. In addition to the presence of inflammatory cells in the airway, the airways of patients with asthma exhibit varying levels of structural changes termed airway remodeling[3–5]. Characteristic structural changes of airway remodeling include epithelial cell mucus metaplasia, smooth muscle hypertrophy/hyperplasia, subepithelial fibrosis, and increased angiogenesis[3–5]. Studies of lung function over time have demonstrated that lung function in adult asthmatics declines at a greater rate than non-asthmatic controls[6]. In a study of the change in FEV_1 in a general adult population of 17,506 subjects, asthmatics demonstrated a greater decline in FEV_1 (38 ml per year), as compared to those without asthma (22 ml per year) over the fifteen year duration of the study (Figure 1). While such epidemiologic studies point out the significant potential for populations of asthmatics to progress with an accelerated decline in lung function over time, it is likely that both genetic and environmental factors contribute to differing rates of decline in lung function in individual asthmatic subjects (Figure 1). The potential for a subset of asthmatics to develop a more rapid disease progression to non-

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reversible airflow obstruction (defined as a β-agonist response <9%) was noted in 23% of 92 adult lifelong non-smoking subjects with moderate to severe asthma after 10 years[7]. At present there are no reliable clinical characteristics, genotypes, or biomarkers to accurately identify subsets of asthmatics that are more prone to airway remodeling or progression of their asthma (Figure 1). An improved understanding of the immune and inflammatory mechanisms which mediate the progression of asthma may provide important insight into biomarkers or genotypes to identify such patients, as well as suggest novel therapeutic interventions to prevent or reverse disease progression.

IS THERE A LINK BETWEEN IMMUNE CELLS, AIRWAY INFLAMMATION, AND AIRWAY REMODELING ?

Although it is well recognized that airway inflammation is a prominent feature of asthma, the relationship between individual components of airway inflammation and the progression of inflammation to remodeling of the airways in asthma is not well understood. Evidence that immune mechanisms and inflammation are important in the pathogenesis of airway remodeling are derived either from studies in animal models of airway remodeling in asthma or from human studies of asthmatics with remodeled airways. Each of these approaches has strengths as well as limitations. For example, studies of airway remodeling in mice subjected to repetitive allergen challenge demonstrate that there is an association between sustained airway inflammation and airway remodeling[8–10]. Insights into which immune or inflammatory cells are important in mediating specific aspects of airway remodeling in mice can be determined from studies in mutant mice lacking either specific cell types, cytokines, or mediators[8–10]. The limitation of using murine models of airway remodeling is the uncertainty regarding the translation of findings in murine models to human disease. Studies in human asthmatics utilizing bronchial biopsies have the advantage of being able to characterize the presence of immune and inflammatory cells and the cytokines and mediators they express in the remodeled airway of asthmatics, as well as correlate levels of individual cell types, cytokines or mediators with levels of airway remodeling[11–15]. A limitation of these correlative human studies is that a cause and effect relationship between expression of a particular cell, cytokine, or mediator and the pathogenesis of airway remodeling cannot be established until specific cells have been depleted, and/or specific cytokine/mediators neutralized. In human studies of airway remodeling in asthma, only anti-IL-5 has thus far been used as a specific intervention (in this case to deplete eosinophils) and demonstrate that such a targeted intervention reduced both levels of a specific cell type (i.e. eosinophils) as well as levels of airway remodeling as assessed in bronchial biopsies[16]. Additional such specific intervention studies in humans with asthma targeting particular cells, cytokines, or mediators coupled with bronchial biopsies are needed to determine the relationship between individual components of the immune and inflammatory response and the pathogenesis of airway remodeling in asthma.

EVIDENCE FOR INDIVIDUAL CELLULAR COMPONENTS OF THE IMMUNE AND INFLAMMATORY RESPONSE CONTRIBUTING TO AIRWAY REMODELING

T cells

Although Th2 cells play an important role in the pathogenesis of allergic inflammation and asthma[17] (Figure 2), the contribution of Th2 cells to the pathogenesis of airway remodeling requires further investigation. Insight into the role of T cell subsets in allergen induced airway remodeling has been derived from the study of transgenic mice with enhanced expression of transcription factors that direct lineage commitment of T cells to either Th1 or Th2 cells. For example, studies of airway remodeling in mice with increased numbers of Th2 cells (GATA-3

transgenic mice) demonstrate that the level of subepithelial fibrosis and airway smooth muscle hyperplasia after repeated allergen exposure were significantly enhanced[18]. In contrast, mice with increased numbers of Th1 cells (T-bet transgenic mice) had reduced levels of allergen-induced goblet cell hyperplasia and mucus hypersecretion[18]. Interestingly, human subjects with asthma and remodeled airways express increased levels of GATA-3 and reduced levels of T-bet[19,20], suggesting an association between increased numbers of Th2 cells and remodeled airways of asthmatics. In addition to studies suggesting a role for Th2 cells in remodeling, there are also studies demonstrating a role for several Th2 cell derived cytokines (i.e. IL-5, IL-13) in aspects of airway remodeling[8,21]. Studies using either IL-5 deficient mice (reduced airway remodeling)[8], or IL-13 transgenic mice (increased airway remodeling)[21], support a role for Th2 cytokines in airway remodeling in vivo.

Regulatory T cells

While Th2 cells may play a role in promoting airway remodeling, regulatory T cells have the ability to down-regulate Th2 cell function and thus potentially reduce levels of airway remodeling (figure 2). A deficiency in Treg cell function in asthma could thus theoretically play an important role in asthma progression. There are two broad categories of Tregs, a) natural Tregs and b) inducible or adaptive Tregs[22–26]. Natural Tregs constitute 1–5% of the peripheral blood CD4+ T cell population and are defined as CD4+, CD25+, a phenotype not specific for Tregs alone. Expression of CD127 (the IL-7 receptor) is inversely correlated with Treg suppressive function and may discriminate between Tregs and activated T cells[22–26]. Natural Treg develop in the thymus and may expand in the periphery upon antigen exposure. Antigen specific adaptive Treg cells are induced by immunization with antigen or by natural exposure to antigen in the environment. Adaptive Tregs have many features in common with natural Tregs, but exhibit marked cytokine dependent suppressive mechanisms in vitro which are mediated through the secretion of IL-10 and TGF-β, which may suppress Th2 cells. In contrast, in vitro natural Tregs suppress through cell contact dependent mechanisms and do not secrete significant quantities of inhibitory cytokines such as IL-10 or TGF-β.

Studies have attempted to define whether Treg function is impaired in allergy and asthma. Some but not all studies of peripheral blood have reported that Treg function is impaired in some allergic patients with active disease[27–29], whereas one study of pulmonary natural Tregs obtained by BAL in pediatric asthma demonstrated that these Tregs had an impaired capacity to suppress both responder T cell proliferation and Th2 cytokine synthesis[30]. Further studies are needed to determine whether Treg function is impaired in asthma and if so whether this impaired Treg function contributes to airway remodeling.

Eosinophils

Persistent airflow limitation has been used as a physiologic surrogate for airway remodeling. Studies of severe asthmatics have demonstrated that persistent airflow limitation (defined as either post-bronchodilator FEV_1 <75% predicted, or FEV_1/FVC ratio <0.75) occurred almost nine times more often in patients with sputum eosinophilia[31]. More recent studies suggest that eosinophil derived mediators such as TGF-β (figure 3)[8,16] may play an important role in the pathogenesis of airway remodeling.

IL-5—The potential importance of eosinophils to airway remodeling has been demonstrated in mouse models of allergen induced airway remodeling[8] as well as in human studies[16]. Studies have examined levels of airway remodeling in IL-5 deficient mice which lack the ability to generate eosinophils. These studies demonstrated that IL-5 deficient mice chronically challenged with allergen had significantly lower levels of peribronchial fibrosis as well as significantly reduced thickness of the peribronchial smooth muscle layer[8]. The mechanism by which eosinophils contribute to airway remodeling in mice is likely due to eosinophil

expression of TGF-β as levels of TGF-β were significantly reduced in the remodeled airways of IL-5 deficient mice. The importance of IL-5 and eosinophils to airway remodeling in humans with asthma is suggested from studies in which anti-IL-5 was administered to asthmatics in a double blind placebo controlled study[16]. Asthmatics enrolled in the study had a baseline bronchial biopsy prior to receiving a single dose of anti-IL-5 or placebo and had a repeat bronchial biopsy three months later. Anti-IL-5 significantly reduced levels of BAL eosinophils and levels of remodeling as assessed by reduced deposition of the extracellular matrix associated remodeling proteins pro-collagen and tenascin. The reduced levels of total TGF-β as well as the reduced levels of TGF-β+ eosinophils in anti-IL-5 treated subjects suggested that eosinophil expression of TGF-β contributed to airway remodeling in asthma.

The potential importance of eosinophils to tissue remodeling in humans has also been demonstrated in patients with eosinophilic esophagitis (EE)[32]. EE is characterized by extensive eosinophilia of the esophagus. A subset of EE subjects develop esophageal strictures[32]. Recent studies have demonstrated evidence of remodeling in the esophagus in subjects with EE. The remodeling changes include increased levels of collagen deposition and increased numbers of angiogenic blood vessels[32]. The increased number of eosinophils in EE are associated with increased levels of TGF-β as well as increased activation of the phosphorylated-Smad 2/3 signaling pathway which is activated by TGF-β[32]. Thus there is evidence in at least two human diseases associated with eosinophilic inflammation (asthma and EE) that eosinophils and TGF-β may play an important role in tissue remodeling.

CCR-3—Although targeting IL-5 significantly reduces levels of eosinophils in mice, in human studies airway eosinophils were only reduced approximately 50–60% by anti-IL-5[33] suggesting that other strategies were needed to more completely deplete tissue eosinophils. An alternative strategy to targeting IL-5 to reduce levels of eosinophils, is to inhibit recruitment of eosinophils into the airway by targeting chemokine receptors expressed by eosinophils. One such important chemokine receptor expressed by eosinophils is CCR3 which mediates eosinophil chemotaxis in response to chemokines including eotaxin and RANTES both of which are expressed in the airway in asthma. Evidence that targeting CCR3 reduces eosinophilic inflammation are derived from studies of CCR3 deficient mice, or eotaxin deficient mice, which have significantly decreased levels of airway eosinophilia and associated decreased mucus production[10]. Studies with low molecular weight antagonists of CCR3 in a mouse model of airway remodeling have demonstrated reduced subepithelial fibrosis and goblet cell hyperplasia[34]. Similarly, administration of a bi-specific antibody recognizing CCR3 and the inhibitory receptor CD300a expressed on eosinophils and mast cells, reversed allergen induced airway inflammation and significantly inhibited goblet cell hyperplasia, mucus production, deposition of collagen, and smooth muscle thickness in a murine model[35]. Thus, targeting CCR-3 may provide a complementary approach to targeting IL-5 to reduce levels of eosinophilic inflammation and associated airway remodeling.

Siglec-8—While much is known about the pathways which induce eosinophilic airway inflammation (i.e. IL-5, CC chemokines), there is more limited information regarding the pathways which mediate resolution of eosinophilic inflammation. Knowledge of the pathways that mediate resolution of eosinophilic tissue inflammation could theoretically be harnessed to treat eosinophil diseases associated with tissue remodeling. One such candidate molecule to mediate resolution of eosinophilic inflammation is Siglec-8 which is highly expressed on human eosinophils[36,37] (figure 3). In vitro studies have demonstrated that cross-linking Siglec-8 receptors on eosinophils induces an apoptotic signal[37]. Interestingly, neither IL-5 nor GM-CSF (eosinophil survival-promoting cytokines) are able to counteract the ability of Siglec-8 cross-linking to induce eosinophil apoptosis[37].

Siglecs (sialic-acid-binding immunoglobulin-like lectins) are a family of thirteen human and nine mouse molecules involved in innate immunity[38]. Siglecs are transmembrane receptors characterized by an extracellular N terminal V-set domain important in ligand binding to sialic acid containing ligands, a variable number of extracellular immunoglobulin like C2-set domains, and a cytosolic tyrosine based signaling motif (ITIM/ITAM) which is present in several Siglecs. Because mouse Siglec-F shares many properties with human Siglec-8, including predominant expression on eosinophils, and shared unique ligand specificity[39,40], studies of mouse Siglec-F have provided insight into the potential role of Siglec-8 in human allergic disease. Studies using Siglec-F deficient mice have demonstrated an important role for Siglec-F in mediating the resolution of eosinophilic inflammation in the airway following allergen challenge[41]. Siglec-F deficient mice challenged with inhaled allergen have significantly enhanced levels of eosinophilic airway inflammation as well as delayed resolution of eosinophilic airway inflammation suggesting that Siglec-F normally functions to down-regulate eosinophilic inflammation[41]. At present there are no studies demonstrating whether targeting Siglecs on eosinophils reduces levels of airway remodeling in asthma.

Mast cells

In studies of asthmatics, mast cells have been noted to infiltrate airway smooth muscle in particular suggesting a potential mast cell smooth muscle cell remodeling interaction[42]. Several mast cell derived mediators including tryptase, histamine, and cytokines such as TNF have been postulated to contribute to airway remodeling[43]. In addition to effects on smooth muscle, mast cells may also play a role in other features of remodeling including angiogenesis. Endobronchial biopsy specimens from asthmatic patients have demonstrated increased numbers of chymase positive mast cells associated with both increased numbers of blood vessels, and increased numbers of cells expressing the pro-angiogenic cytokine VEGF compared to healthy controls subjects[44]. Co-localization immunohistochemistry studies demonstrated that mast cells were a cellular source of VEGF. Recent studies of airway remodeling in mast cell deficient mice chronically challenged with allergen have also supported a role for mast cells in mediating chronic airway inflammation (including infiltration by eosinophils and lymphocytes), as well as features of remodeling including increased airway mucus expression, and increased pulmonary collagen levels[45]. Thus, mast cells may potentially modulate levels of airway remodeling either directly through effects on structural cells, or indirectly through effects on levels of eosinophilic and T cell mediated inflammation.

Epithelium

Epithelial cells are an example of a structural cell that can undergo both remodeling changes as well as contribute to inflammation by producing pro-inflammatory molecules. Epithelial cells are a significant source of pro-inflammatory molecules which modulate the recruitment and function of immune and inflammatory cells[46,47]. Thus, epithelial cell signaling molecules and mediators have the potential to play a significant role in airway remodeling.

NF-kb—Studies utilizing cre/lox molecular techniques have examined whether inhibiting NF-κB expression only in airway epithelial cells would reduce levels of airway remodeling in a mouse model[48]. Transcription factors such as NF-κB are important regulators of the expression of several genes (e.g. cytokines, chemokines, and adhesion molecules) important to the pathogenesis of airway inflammation in asthma[48]. Mice with selective airway epithelial ablation of the inhibitor of kappa B kinase β (Ikkβ), which is required for activation of NF-κB, had significantly decreased peribronchial fibrosis, levels of BAL TGF-β, and numbers of peribronchial TGF-β1 positive cells[48]. Levels of airway mucus, airway eosinophils, and peribronchial CD4+ cell were also reduced significantly upon airway epithelial Ikk-β ablation. The diminished airway inflammatory response was associated with reduced expression of NF-κB-regulated chemokines, including eotaxin-1 and thymus- and activation-regulated

chemokine, which attract eosinophils and Th2 cells, respectively, into the airway. These results support the potential important role of the bronchial epithelium, and specifically the epithelial NF-κB pathway, in the process of remodeling.

Thymic stromal lymphopoieten (TSLP)—Transgenic mice which overexpress TSLP develop increased levels of allergic inflammation[49–51]. Lung epithelial cells are an important source of TSLP[49,52] and increased levels of TSLP have been detected in asthmatic airway[50]. One mechanism by which TSLP increases allergic inflammation is through its role in enhancing the capacity of dendritic cells to induce the development of Th2 cells[51]. Stimuli which are known to induce epithelial secretion of TSLP include IL-4, dsRNA, and rhinovirus.

IL-25 (IL-17E)—Following initial studies demonstrating an important role for IL-25, a member of the IL-17 cytokine family, in Th2 cell-mediated immunity to parasitic infection[53], subsequent studies have suggested an important role for IL-25 in the initiation of asthma[54]. IL-25 is expressed by lung epithelial cells as a result of innate immune responses to allergens[54] Transgenic overexpression of IL-25 by these cells leads to mucus production and airway infiltration of macrophages and eosinophils, whereas blockade of IL-25 conversely reduces the airway inflammation and Th2 cytokine production in an allergen-induced asthma model[54].

At present there are no studies demonstrating whether either TSLP or IL-25 are important in the progression of asthma as opposed to the initiation of asthma.

SELECTED CANDIDATE MEDIATORS OF AIRWAY REMODELING

Although multiple mediators are likely to participate in the pathogenesis of airway remodeling, several mediators (TGF-β, MMP-9, ADAM33, VEGF) have been the focus of considerable investigation.

TGF-β

Evidence to support the contribution of TGF-β to airway remodeling in asthma is derived from animal model studies with either mice deficient in Smad-3 (Smad3 mediates signaling in response to TGF-β)[55], or studies with wild type mice administered an anti-TGF-β Ab[56]. Neutralizing either TGF-β, or TGF-β signaling through Smad-3, significantly reduces peribronchial fibrosis, airway smooth muscle proliferation, and mucus production without an associated significant change in levels of airway inflammation[55,56]. Interestingly, Smad-3 deficient mice chronically challenged with allergen have reduced numbers of peribronchial myofibroblasts suggesting an important role for TGF-β in vivo in the differentiation of fibroblasts to myofibroblasts[55].

TGF-β, which is expressed in the airway in asthma[57], stimulates fibroblasts to produce extracellular matrix proteins (collagen, fibronectin)[58]. In addition, TGF-β decreases the production of enzymes that degrade the extracellular matrix (collagenase), and increases the production of proteins that inhibit enzymes that degrade the extracellular matrix (Tissue inhibitor of metalloprotease, or TIMP)[58] The net effect of TGF-β acting on fibroblasts is to increase the production of extracellular matrix proteins. There is evidence that the subepithelial fibrosis component of airway remodeling in asthma is mediated through induction of TGF-β expression with consequent activation of myofibroblasts to produce extracellular matrix proteins such as collagen. In addition to effects on fibrosis, TGF-β also plays an important role in airway smooth muscle proliferation through paracrine as well as potential autocrine pathways of TGF-β acting on smooth muscle[59].

An important role for eosinophil expressed TGF-β in airway remodeling in humans with asthma is also suggested from studies in which TGF-β levels in the lung of asthmatics were reduced by depleting eosinophils which express TGF-β[16]. In these studies utilizing anti-IL-5 (which depletes eosinophils), there was a parallel decrease in levels of airway TGF-β, eosinophil TGF-β expression, and associated airway remodeling[16]. These studies suggest that eosinophil expression of TGF-β is an important contributor to airway remodeling in human asthma. In subjects with asthma increased levels of TGF-β have been reported in BAL[60] and biopsy specimens[57]. TGF-β expression correlates with the degree of subepithelial fibrosis, and levels of TGF-β are significantly increased in patients with severe asthma who have prominent airway eosinophilic inflammation[61].

MMP-9

Matrix metalloproteinase-9 (MMP-9) belongs to a family of extra-cellular proteases that are responsible for the degradation of the extracellular matrix during tissue remodeling[62]. Levels of MMP-9 (gelatinase B) are significantly increased in bronchoalveolar lavage (BAL) fluid, blood, and sputum from allergic asthmatic patients[63,64,65]. In addition, allergen challenge in asthmatics induces expression of MMP-9 in the airway[63]. Studies in MMP-9 deficient mice demonstrate that when challenged with allergen they have slightly less peribronchial fibrosis and total lung collagen compared wild type mice[66]. However, MMP-9 deficient mice do not have reductions in mucus expression, smooth muscle thickness or airway responsiveness[66]. Thus, therapies targeting MMP-9 to reduce airway remodeling in asthma may result in modest reductions in the levels of peribronchial fibrosis and may not influence levels of mucus expression, thickness of the smooth muscle layer, or airway responsiveness.

ADAM33

Like MMP-9, ADAM33 (A Disintegrin And Metaloproteinase 33) is a metalloprotease that is expressed in asthma and has been investigated for its potential role in airway remodeling[67]. A genetic polymorphism in ADAM33 has been associated with an accelerated decline in lung function over time in asthma, suggesting a role for ADAM33 in chronic airway injury and repair[68]. ADAM33 mRNA expression is significantly higher in both moderate and severe asthma compared with mild asthma and controls[69]. Immunostaining for ADAM33 is increased in the epithelium, submucosal cells, and smooth muscle in severe asthma compared with mild disease and controls[69]. However, in a mouse model of allergic asthma, ADAM33 deficient mice showed normal allergen-induced airway hyperreactivity, immunoglobulin E production, mucus metaplasia, and airway inflammation[70]. Studies of peribronchial fibrosis and smooth muscle remodeling were not evaluated in ADAM33 deficient mice in this acute allergen challenge protocol[70].

VEGF

Increased angiogenesis, as well as increased levels of the pro-angiogenic cytokine VEGF and its receptors have been noted in asthmatic airway[71,72] and animal models of asthma[73–75]. VEGF is elevated in induced sputum, BALF and bronchial biopsies of asthmatic patients[71,72]. Lung-targeted VEGF165 transgenic mice have an asthma-like phenotype with not only vascular remodeling, but also inflammation, edema, mucus metaplasia, myocyte hyperplasia and airway hyperresponsiveness[73,74]. Studies with VEGF lung transgenic mice suggest that VEGF is a mediator of both vascular remodeling as well as extravascular remodeling and inflammation. Interestingly, studies with inhibitors of nitric oxide (NO) suggest that NO is an important mediator of the extravascular VEGF remodeling effects[74].

ASTHMA GENES AND AIRWAY REMODELING

While over 100 genes have been linked to asthma[76], these studies have generally focused on identifying linkage of genes to airway responsiveness, IgE, atopy, and asthma, rather than linkage of genes to airway remodeling. The identification that a genetic polymorphism in ADAM33 is associated with an accelerated decline in lung function over time in asthma[68], suggests that there may be a subset of genes associated with airway remodeling. Further studies are needed to confirm this observation and determine which genes may have linkage to airway remodeling.

ASTHMA EXACERBATIONS, IMMUNE AND INFLAMMATORY RESPONSE, AND RATE OF AIRWAY REMODELING

Acute exacerbations of asthma and airway remodeling

Although patients with asthma exhibit a greater decline in lung function compared to non-asthma controls, analysis of the rate of decline in lung function in asthmatics as a group may obscure a greater decline in a subset of asthmatic subjects. Thus, it is possible that asthmatics who have inadequate airway repair mechanisms and/or inadequately treated episodes of inflammation may have prolonged episodes of airway inflammation which lead to significantly remodeled airways in a subset of asthmatics. In this model, airway remodeling would not be a linear decrease in adult lung function over time but rather a step wise decrease in lung function following acute exacerbations in a subset of genetically predisposed individuals. Studies have started to examine whether acute exacerbations of asthma associated with increased levels of airway inflammation may account for increased levels of airway remodeling and an accelerated decline in lung function. A study examining the rate of decline in lung function in a cohort of 93 non-smoking asthmatics with moderate to severe asthma followed for at least five years demonstrated that those asthmatics with the highest frequency of acute exacerbations had a significantly greater rate of decline in lung function compared to asthmatics with the lowest frequency of acute exacerbations[77] One severe asthma exacerbation/year was associated with an excess decline in FEV_1 of approximately 30 ml/year[77]. No airway biopsies were obtained in this study so it is not possible to determine the relationship between the increased decline in lung function in patients with frequent exacerbations and changes in levels of airway remodeling.

Studies of endobronchial biopsies derived from mild asthmatics challenged with an inhaled allergen have also provided insight into the effect of such an "allergen induced exacerbation" on inflammatory responses and airway remodeling. Allergen challenge was associated with increased airway inflammation as well as increased expression of remodeling genes (including procollagen, tenascin, α-smooth muscle actin)[78]. Interestingly, while the increase in airway inflammation after allergen challenge resolved by seven days, the increases in markers of remodeling persisted[78]. Thus, acute increases in airway inflammation may contribute to more prolonged periods of airway remodeling.

Although these studies suggest that acute exacerbations of asthma may be important in mediating airway remodeling there are other studies which dissociate the number of acute exacerbations from the decline in lung function. For example, in the START trial (Steroid Treatment As Regular Therapy), low-dose inhaled corticosteroid therapy in patients with mild persistent asthma (of less than two years duration) reduced asthma exacerbations but did not reduce the decline in lung function (post-bronchodilator FEV_1 percent predicted)[79]. Differences in results between studies[77,79] may be due to differences in severity of asthma in the different study populations. However, more studies are needed to address whether acute exacerbations drive asthma progression in a subset of asthmatics.

Viruses and airway remodeling

As viruses are the most common cause of acute exacerbations of asthma[80], if acute exacerbations of asthma were an important trigger of remodeling then viruses could play an important role in airway remodeling. Studies in mouse models have demonstrated that a single paramyxovirus infection causes both acute airway inflammation/hyperreactivity and chronic airway remodeling/hyperreactivity phenotypes (the latter by a hit-and-run strategy, since viral effects persist after viral clearance)[81]. Further human studies are needed to determine the role of viruses in airway remodeling and whether particular viruses are more likely to play a role in inducing airway remodeling.

Environmental tobacco smoke and airway remodeling

Epidemiologic studies in asthma demonstrate that asthmatics who smoke have a greater decline in forced expiration volume in 1 s when followed over a 15-year period compared with asthmatics who do not smoke[6], suggesting that tobacco smoke may contribute to the decline in lung function and airway remodeling. Studies using a mouse model of allergen-induced airway remodeling have demonstrated that chronic co-exposure of mice to the combination of ETS and allergen induces significantly increased levels of airway remodeling compared with levels of airway remodeling noted in mice from chronic exposure to either stimulus alone[82]. In particular, the combination of chronic ETS and chronic allergen co-exposure significantly increased the thickness of the peribronchial smooth muscle layer and this was associated with a significant increase in AHR to Mch. Constituents of ETS in combination with allergen stimulate airway remodeling by inducing increased expression of eosinophil chemoattractants such as eotaxin-1 by airway epithelial cells, and increased Th2 cytokines[82], which recruit larger numbers of TGF-$\beta 1^+$ eosinophils to the airway than that induced by allergen alone. The increased levels of peribronchial eosinophils and cells expressing TGF-$\beta 1$ are likely to contribute to airway remodeling.

IS AIRWAY REMODELING REVERSIBLE ? ENDOBRONCHIAL BIOPSY STUDIES

Corticosteroids are the most effective anti-inflammatory therapy in asthma. Several studies using bronchial biopsies have also demonstrated that corticosteroids reduce airway inflammation in asthma[83]. Although the ability of corticosteroids to improve features of airway inflammation is well established, the ability of corticosteroids to reduce airway remodeling based on airway biopsy studies is not as well established. There are airway biopsy studies that support as well as refute a role for corticosteroids in reducing airway remodeling[84–92]. Although the majority of studies which have taken biopsies of the airway demonstrate that corticosteroids reduce features of airway remodeling in asthma[86,88,89,90,91,92], several other studies do not support a role for corticosteroids in reducing levels of airway remodeling [84,85,87]. The differences in the results may be due to differences in the dose, duration, and compliance with corticosteroid therapy, as well as the severity of asthma and remodeling end points studied.

Studies in corticosteroid resistant asthmatics have also suggested a potential role for corticosteroids in airway remodeling[93]. For example, corticosteroids are unable to enhance TIMP-1 production in corticosteroid resistant asthmatics and this may promote proteolytic activity in the airways of such patients with corticosteroid resistant asthma contributing to chronic airway remodeling[93].

The role of leukotriene inhibitors in airway remodeling has also been investigated. Studies in mouse models demonstrate that a CysLT Receptor 1 inhibitor reverses features of airway remodeling[94]. Subsequent studies in human asthmatics have demonstrated that treatment of

asthmatics with a CysLT receptor 1 antagonist inhibits the number of myofibroblasts in the airway following allergen challenge[95]. Further human studies are needed to determine whether CysLT receptor 1 antagonists inhibit cardinal features of remodeling (fibrosis, smooth muscle changes, angiogenesis, etc) in subjects with persistent asthma not undergoing allergen challenge.

LIMITATIONS OF CURRENT APPROACHES TO QUANTITATING AIRWAY REMODELING IN ASTHMA

In general, studies of airway remodeling in asthma utilize either non-invasive or invasive methods to quantitate levels of airway remodeling. Each of these approaches has strengths and weaknesses which need to be taken into account when interpreting the results of such studies.

Non-invasive methods used to assess airway remodeling

The decline in the post-bronchodilator FEV_1 and FEV_1/FVC ratio is frequently used in studies as a surrogate end-point for structural changes of airway remodeling in asthma. Although intuitively appealing, no studies have directly demonstrated that a decline in lung function with time is synonymous with histologic changes in the degree of airway remodeling in airway biopsies. Thus, further studies are needed to determine whether particular histologic changes in levels of airway remodeling correlate with a decline in lung function. Although imaging techniques such as CT scans have been used to quantitate airway wall thickness[96,97] as a surrogate for airway remodeling, further improvements in imaging techniques especially of the small airways are needed for this to be of practical utility in the clinic.

Invasive methods used to assess airway remodeling

Although bronchial biopsy studies have an advantage over non-invasive studies in directly being able to quantify different aspects of airway remodeling there are also limitations with this approach. Bronchial biopsies are frequently taken from large proximal airways in asthma that might not reflect the site of disease activity and airway remodeling in small airways in asthma[98]. For example, postmortem studies of central cartilaginous airways and peripheral membranous small airways in the same asthmatic subject suggest that reticular basement membrane thickness measured in a bronchial biopsy from the central airways reflects airway remodeling in central, but not peripheral, airways[98]. Transbronchial biopsies have been utilized in a small number of studies[99–103] to sample the distal lung, including the distal airway wall and the alveolar tissues. The primary reason for limited studies is the concern about major complications, such as bleeding and pneumothorax[103]. Interestingly, studies of fibroblasts obtained from the proximal and distal airway of asthmatics demonstrate phenotypic differences[104] which may contribute to the variable fibroblast response to injury and repair between proximal airways and distal lung/parenchyma.

Relationship between airway inflammation, airway remodeling, and airway responsiveness

Although there is evidence to support the relationship between airway inflammation and airway remodeling, as well as between airway inflammation and airway hyperresponsiveness, the relationship between airway remodeling and airway responsiveness may be more complex than we initially appreciated. For example, airway remodeling with increased smooth muscle mass is likely to lead to increased airway responsiveness[105]. However, studies in human asthmatics have also demonstrated that airway wall thickening in asthma is associated with reduced rather than increased airway reactivity to MCh[106]. In these human asthma studies, noninvasive high-resolution computerized tomography scanning methods were used to assess airway wall thickening and, surprisingly, demonstrated that airway wall thickening in asthma is associated with reduced rather than increased airway reactivity to MCh[106]. One potential explanation

suggested by Pare[107] for the discrepancy in results between the mathematical modeling studies in asthma and the computerized tomography scan studies in asthma evaluating airway wall thickness and airway responsiveness is that the mathematical modeling studies were primarily based on altered airway geometry and did not fully take into account the potential effect of airway wall thickening on the mechanical properties of the airway, e.g., stiffness of the airway[107].

KEY CONCEPTS AND THERAPEUTIC IMPLICATIONS

- Airway remodeling may be due to persistent inflammation and/or aberrant tissue repair mechanisms. Different features of airway remodeling are likely mediated by different inflammatory pathways.

- Several important candidate mediators of remodeling have been identified including TGF-β and Th2 cytokines (including IL-5 and IL-13), as well as VEGF, ADAM-33, and MMP-9.

- Although decline in lung function is used as a very important surrogate for airway remodeling in asthma, longitudinal studies measuring decline in lung function and features of airway remodeling on airway biopsies have not yet been performed.

- Asthmatics remodel their airways at different rates suggesting that certain genetic factors and/or environmental factors (exacerbations, viral infections, tobacco smoke, etc) may contribute to enhanced remodeling in a subset of asthmatics.

- Human studies have demonstrated that anti-IL-5 reduces levels of airway eosinophils expressing TGF-β, as well as levels of airway remodeling as assessed by bronchial biopsies. Further such studies confirming these observations, as well as alternate studies targeting additional individual cell types, cytokines, and mediators are needed in human subjects with asthma to determine the role of candidate mediators of inflammation on the development and progression of airway remodeling.

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Glossary

ANTI-IL-5, Biologic agent blocking IL-5 induced eosinophilopoesis and tissue trafficking to lungs, GI tract, and skin. Therapeutic trials using anti-IL-5 include asthma, hypereosinophilic syndrome, and eosinophilic esophagitis treatment.

CD25, The α chain of the IL-2 receptor, can be expressed on IL-10 producing T regulatory cells

CD300a, Also known as IRp60, expressed on eosinophils and mast cells, suppresses IL-5, GM-CSF, and eotaxin effects

CHYMASE, Mast cell subsets can be characterized by protease production, e.g. as tryptase, or chymase/tryptase; chymase can play a role in airway remodeling by activating MMP-9 and inducing VEGF.

EOTAXIN-1, Eotaxins are a family of CC chemokines that bind to CCR3 and induce eosinophil trafficking. Eotaxins-1, -2 target eosinophils to the lung and lower gastrointestinal tract while eotaxin-3 functions as a chemoattractant for esophageal eosinophils.

GATA-3, Member of the GATA family of transcription factors, increases transcription of the IL-5, -13, and -4 genes. The *Gata3* gene is activated by IL-4, STAT-6, and GATA-3.

ITIM, ITAM (Immunoreceptor Tyrosine Activation/Inhibitory Motifs), Immunoglobulin receptors have activating and inhibiting motifs that interact with cellular kinases and phosphatases to regulate cell signaling.

MMP (Matrix Metalloproteinase), Activity of MMPs is balanced by Tissue Inhibitors of Metalloproteinases (TIMPs); while MMPs increase breakdown of extracellular matrix and promote tissue remodeling, TIMPs inhibit their function. In asthmatic airway remodeling, the balance is tipped in favor of MMP function.

NFkB, A family of proteins that contain a Rel DNA binding domain, that form homo- and hetero-dimers and are inactivated when bound to IkB proteins. Phosphorylation of IkB proteins by IkB kinases (IKK) allows release of NFkB from the cytoplasm

REPEATED AIRWAY CHALLENGE, Murine asthma models involve a sensitization period (often intraperitoneal on days 0, 12) followed by allergen challenge. Short allergen challenge (often 3 challenges over 1 week) results in an "acute" model of Th2 and eosinophilic airway inflammation; longer allergen challenges cause chronic inflammation and airway remodeling.

Smad3, One of a family of Smad proteins, forms heteromeric protein complexes that regulate gene transcription; TGFβ, activins, and bone morphogenetic proteins utilize the phosphorylated Smad signaling pathway; Smad3 complexes with Smad2 and Smad4 while Smad7 inhibits signaling.

T regulatory cells, Regulatory CD4^{+} or CD8^{+} cells, can express the transcription factor FoxP3 and/or TGFβ, CD25, and IL-10. Function to dampen the immune response; therapies that successfully treat autoimmunity or allergy can be associated with elevated numbers of Treg cells.

T-bet, Transcription factor essential for Th1 cell induction and IFNγ expression; expression of T-bet can cause shift of Th2 to Th1 cells.

TGFβ (Transforming growth factor β), A pro-fibrotic agent, inactive form bound to LAP, can be activated by mast cell proteases, e.g. chymase, tryptase. TGFβ mediated fibrosis is implicated in many diseases including pulmonary and renal fibrosis; TGFβ gene SNPs can be associated with asthma.

TSLP, (Thymic stromal lymphopoeitin): Expressed in activated epithelium, e.g. bronchial epithelial cells in asthmatic patients, promotes antigen presentation by dendritic cells by inducing expression of second signal molecules such as OX40, CD40, and CD80

VEGF, (Vascular Endothelial Growth Factor): Required for angiogenesis, VEGF deficient mice have an embryonic lethal phenotype; the balance between pro- and anti-angiogenic factors (e.g. endostatin) is tipped toward increased vascularity in asthma
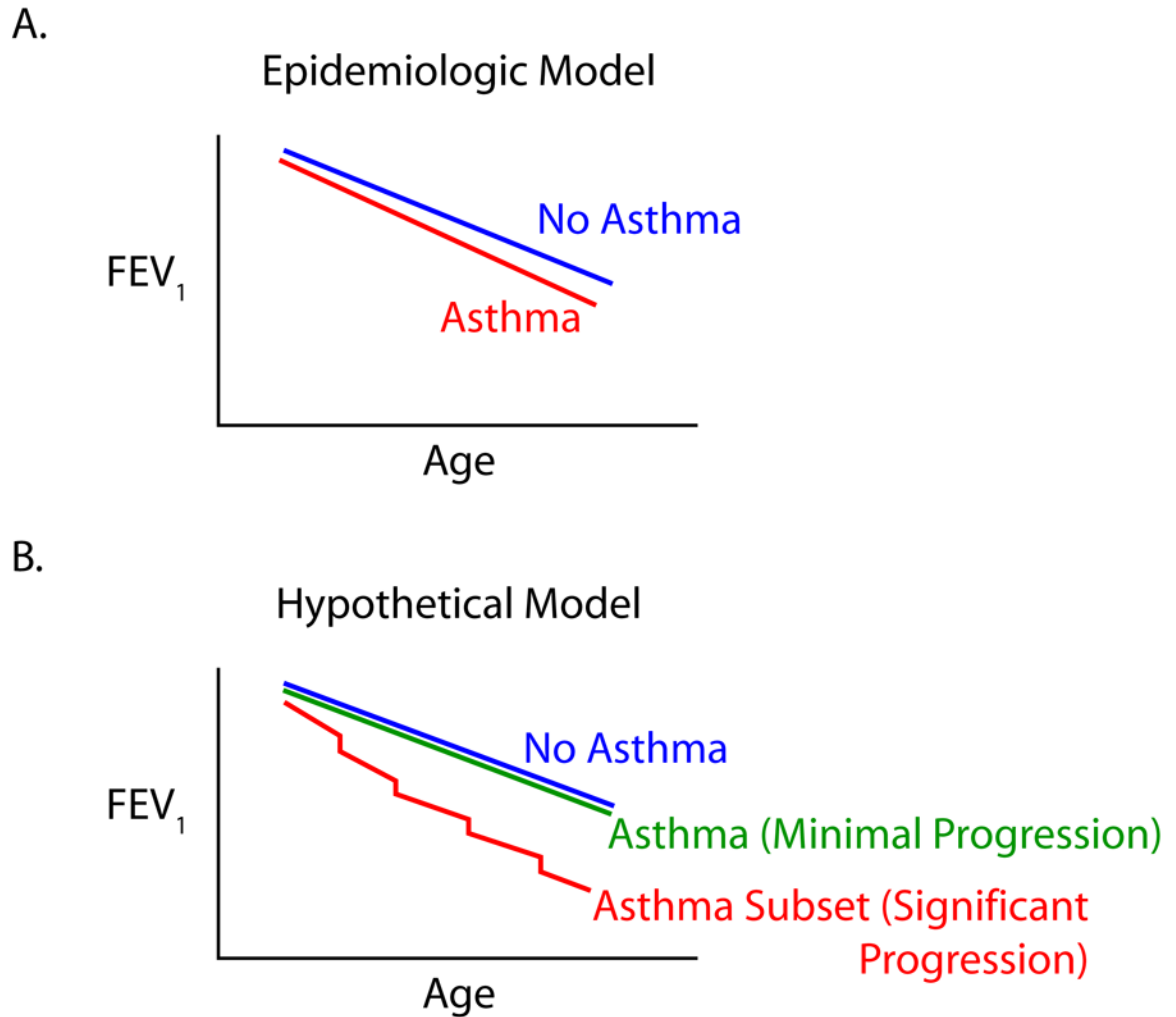
Asthma Progression in Adults

Figure 1. Asthma Progression in adults
Population based studies of asthma progression in adults (e.g. reference 6)_shows an increased rate of decline in lung function compared to non-asthma controls (Panel A).Asthmatics may remodel their airways at different rates (Hypothetical Model) suggesting that certain genetic factors and/or environmental factors (exacerbations, viral infections, tobacco smoke, etc) may contribute to enhanced remodeling in a subset of asthmatics (Panel B)

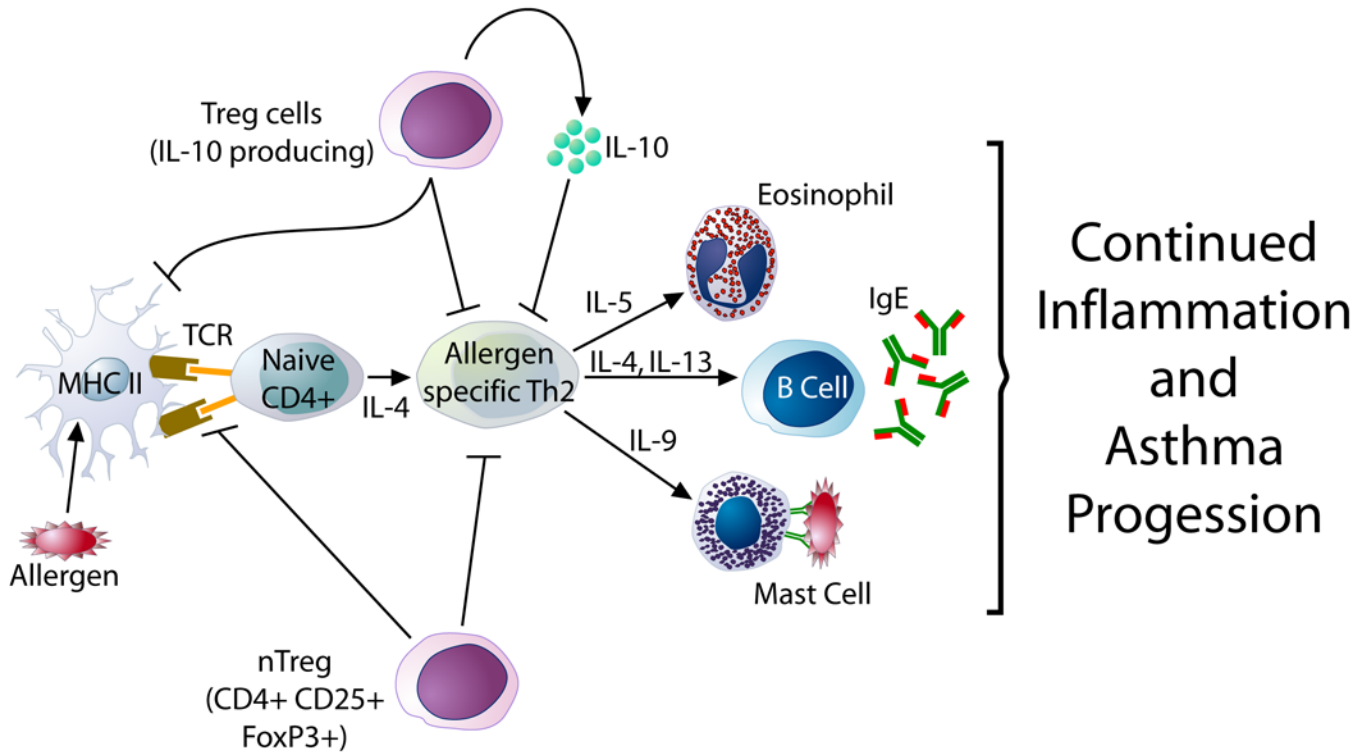# Immune and Inflammatory Mechanisms and Asthma Progession

Figure 2. Immune and inflammatory mechanisms and asthma progression
Asthma progression may be due to persistent airway inflammation and/or impaired repair mechanisms. Allergen inhalation induces activation of Th2 cells which express cytokines including IL-5 which generates TGF-β+ eosinophils that promote features of remodeling. Treg cells (natural and adaptive) have the ability to inhibit Th2 responses. Theoretically a deficiency of Treg function in asthma could promote continued Th2 mediated inflammation.
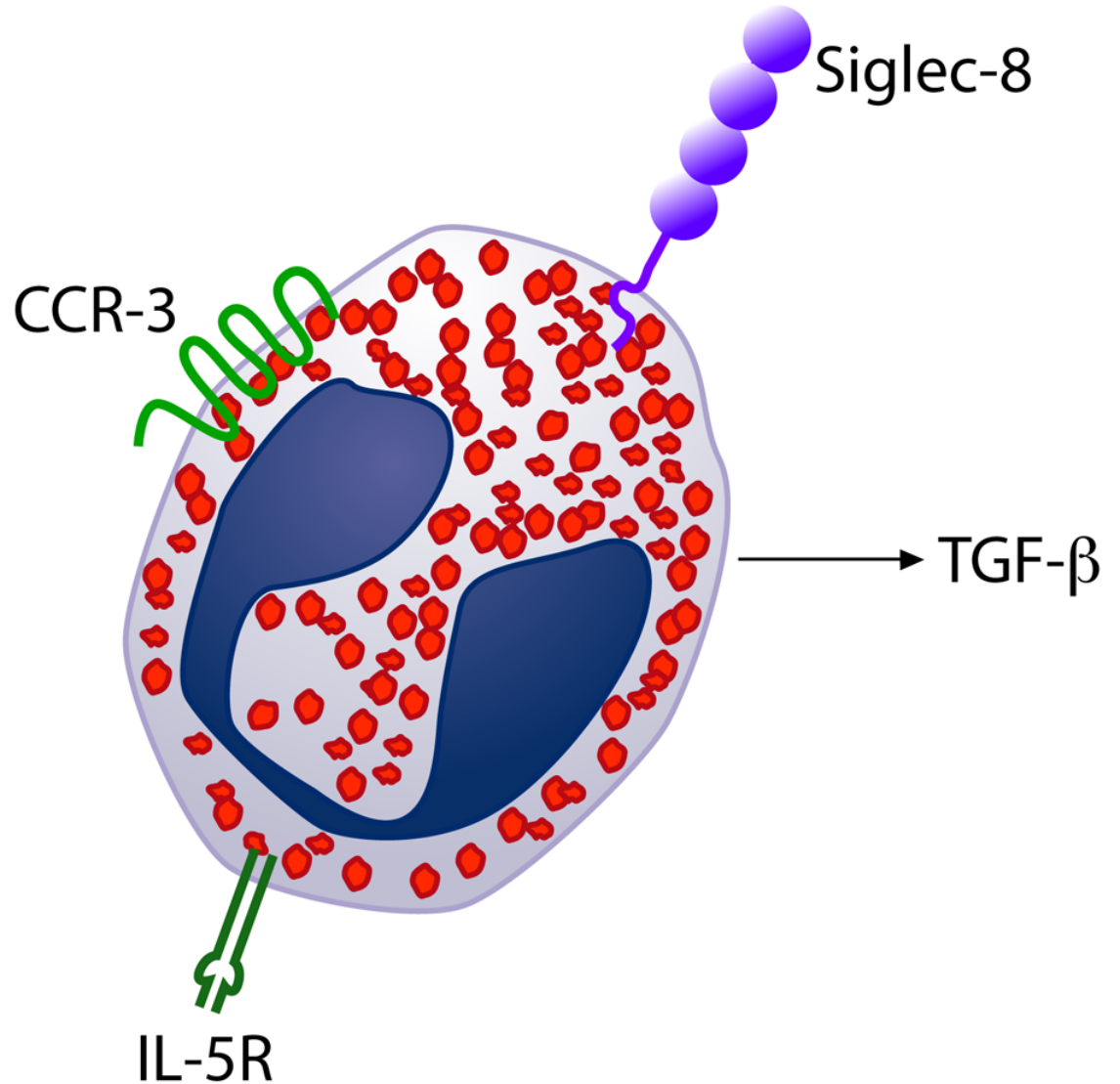
Eosinophil and Asthma Progession

Figure 3. Eosinophil and asthma progression
Eosinophils express receptors which induce their proliferation (IL-5R), chemo-attraction 1003
into tissues (CCR-3), and apoptosis (Siglec-8). Targeting such receptors can modulate levels
of eosinophilic inflammation and the ability of eosinophils to express TGF-β an 1005 important
pro-remodeling cytokine.