

Cross-roads in the lung: immune cells and tissue interactions as determinants of allergic asthma

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Abstract Allergic asthma is a chronic disease of the lung characterized by underlying Th2- and IgE-mediated inflammation, structural alterations of the bronchial wall, and airway hyperresponsiveness. Initial allergic sensitization and later development of chronic disease are determined by close interactions between lung structural cells and the resident and migratory immune cells in the lung. Epithelial cells play a crucial role in allergic sensitization by directly influencing dendritic cells induction of tolerant or effector T cells and production of type 2 cytokines by innate immune cells. During chronic disease, the bronchial epithelium, stroma, and smooth muscle become structurally and functionally altered, contributing to the perpetuation of tissue remodeling. Thus, targeting tissue-driven pathology in addition to inflammation may increase the effectiveness of asthma treatment.

Keywords Allergic asthma · Allergic sensitization · Lung remodeling · Immune tolerance · Chronic asthma

The lung surface is constantly exposed to a multitude of inhaled substances. In normal individuals, tolerance mechanisms ensure that most antigen encounters resolve without deleterious consequences. Susceptible individuals may however become sensitized and develop asthma. Asthma is a chronic inflammatory disease of the airways that affect millions of people around the world. It is a multifactorial disease that involves immune cells and the structural cells of the lung and occurs as a result of the interactions of environmental factors with the genetic predisposition of the individual [1]. Allergic asthma is associated with an underlying Th2 immune response that mediates the production of IgE antibodies, and mast cell and eosinophilic inflammation. Asthma manifests by bronchospasm and

exaggerated mucus production in a background of chronic inflammation and tissue remodeling [2, 3].

The development of asthma can be viewed as the breach of several immunological checkpoints culminating in a pathological outcome. The epithelial layer is the first barrier encountered by allergens. The local innate responses constitute the first important factor determining tolerance or allergic sensitization (1st checkpoint). Tissue cells and resident hematopoietic cells shape a normal tolerogenic environment that imprints on migratory dendritic cells (DCs) the ability to drive the differentiation of allergen-specific naïve cells into Foxp3⁺ regulatory T cells (iTregs). These iTregs can effectively prevent allergic sensitization [4, 5]. Exaggerated epithelial responses to allergens and environmental factors can however induce an inflammatory phenotype in DCs [6]. In an atopy susceptible genetic background, or in the presence of Th2-driving environmental cues, allergen presentation by inflammatory DC may lead to Th2 differentiation, with subsequent IgE production and allergic sensitization (2nd checkpoint). Not all sensitized individuals suffer from allergic diseases, and only a fraction of all allergic patients suffer from asthma [7]. Interactions of inflammatory cells with structural tissue

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cells result, in some individuals, in allergic asthma (3rd checkpoint).

Thus, the nature of the cross-talk between resident tissue cells and immune cells determines the initial response to allergens and the development of tissue pathology during chronic inflammation. The current review focuses on the involvement of the structural components of the lung in conjunction with immune factors, in initiating allergic sensitization and in perpetuating chronic lung remodeling in asthma.

How tissue responses influence allergic sensitization

The epithelium as a sensing portal and a response hub

Evolution of the respiratory function resulted in the complex structure of the airways and lung where the gas exchange surface is maximized through extensive branching and alveolarization. The upper airways have air conducting function and are also responsible for mechanical elimination of microbes and particles. The lower airways are responsible for gas exchange through tight association of alveolar epithelial cells with blood capillaries. Since the large respiratory epithelium is continuously exposed to airborne microorganisms and substances that can penetrate deep into the lung, the physical integrity of the epithelium and homeostatic epithelial responses is essential to prevent inflammation.

The respiratory epithelium is heterogeneous, consisting of several types of epithelial cells tightly associated with patrolling macrophages and DCs [8]. The upper airway epithelium is pseudostratified and formed by ciliated cells, goblet cells, Clara cells, and basal cells. The alveolar epithelium consists of type I and type II cells. Basal cells and Clara cells have progenitor and self-renewal potential. Basal cells can give rise to ciliated cells, goblet cells, and granular secretory cells [9, 10], whereas Clara cells can develop into secretory cells and neuroendocrine cells [11–13]. Type II alveolar cells have a regenerative capacity forming both type I and type II cells [14, 15]. In addition to offering a scaffold for sampling and response by patrolling cells of the immune system, the airway epithelium can play an active role in shaping the course of the sensitization and onset of allergic responses.

The epithelium expresses a large array of innate immune receptors such as TLRs, NLRs, and C-type lectins [16]. In steady state, epithelial cells secrete microbicidal small cationic peptides, lysozyme, lactoferrin, and mucins. Upon inflammation, stress, or injury, a large array of cytokines, chemokines, and growth factors can also be produced [17]. There is strong evidence that epithelial cells can skew the immune response to Th2 by the production of the cytokines

IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) [4, 18–21].

In asthma, the airway epithelium is thought to be structurally and functionally disordered [22]. Exaggerated epithelial responses to allergens, infections, chemical, or mechanical stress may precede and facilitate allergic sensitization and development of asthma. It has been proposed that asthma in its origin is an epithelial disease of impaired barrier function [7].

Epithelial responses to allergens

Size and surface composition of allergen particles, including charge and enzymatic properties, determines binding, uptake, and penetrance into the deeper regions of the lung. In addition, the ability of allergen molecules to activate innate immune responses at mucosal surfaces appears to be important for the development of allergies [23].

Experimental evidences demonstrate that protease activity present in many allergens may facilitate allergic sensitization. Allergens like the mite antigen Der p 1, plant and grass pollen antigens, proteins from the fungus *Aspergillus* and from German cockroaches were found to have protease activity. Proteolytic activity can disrupt epithelial–epithelial tight junctions as well as DC–epithelial junctions, increasing permeability, inducing DC activation and triggering epithelial repair. Degradation of surfactant proteins by mite proteases may impair clearing mechanisms in the airways [23].

Activation of pattern recognition receptors by allergens has been implicated in allergic sensitization. The role of TLR4 expressed by structural lung cells in house dust mite (HDM)-mediate airway allergic sensitization was studied using BM chimeras in which irradiated WT or TLR4-deficient mice were reconstituted with either WT or TLR4-deficient cells. Expression of TLR4 in structural cells (presumably epithelial cells) but not on hematopoietic cells was necessary for allergic sensitization. A single administration of HDM extract induced the secretion of the cytokines GM-CSF, TSLP, IL-25, and IL-33. In this case, TLR4 signaling appeared to be dependent on trace amounts of LPS in the mite extract [24]. However, allergens can also activate innate responses through molecular mimicry. For instance, Der p 2, a lipid-binding allergen, was found to have structural homology with MD-2, the LPS-binding component of the TLR4 complex. As such, Der p 2 facilitates LPS signaling through TLR4 in the absence of MD-2 [25].

Endogenous damage-associated molecular patterns (DAMPs) such as uric acid and ATP can promote inflammation through the activation of the inflammasome and release of IL-1 β [26]. Airborne pollutants like asbestos and

silica can also activate the inflammasome [27]. Intra-tracheal administration of HDM to mice led to the production of uric acid by epithelial cells, and uricase treatment reduced allergic inflammation and airway hyperresponsiveness (AHR), thus demonstrating that uric acid can promote initiation and persistence of allergic inflammation [28]. Epithelial injury results in the release of ATP [29]. Extracellular ATP was shown to promote and maintain allergic inflammation by increasing recruitment of DCs that mediated the differentiation of Th2 cells. In this model, neutralization of ATP in established disease suppressed all the main features of asthma [30]. Further evidences for the role of ATP on allergic sensitization derive from experiments with the fungus *Alternaria alternata*. After fungal airway exposure, airway epithelial cells released IL-33 in the airway lumen, which promoted a Th2 response. It was found that IL-33 release was induced by acute extracellular accumulation of ATP and autocrine ATP signaling through P2 purinergic receptors [31].

The examples above illustrate how innate pathways of microbial recognition and detection of endogenous danger signals can be co-opted by allergens at mucosal surfaces. Activation of innate inflammatory pathways may then act on the adaptive immune response to promote allergic sensitization.

Mediators of epithelial origin and cellular cross-talk

Epithelial cells can release a number of inflammatory cytokines and chemokines upon activation by allergens or other environment stimuli [32–34]. These secreted products may either contribute to create an inflammatory environment that favors effector T-cell differentiation rather than tolerance or directly promote Th2 responses. An important cellular interaction occurring during sensitization of the airways is that of DCs and epithelial cells. Pulmonary DCs can establish direct contact with epithelial cells through tight junctions and reach the airway lumen through transepithelial expansions [35]. Disruption of tight junctions through proteases in allergens may not only increase permeability by impairing the epithelial barrier, but can also result in DC activation through the E-cadherin/ β -catenin pathway [32].

On allergen exposure, epithelial cells can release CCL20, a chemokine that attracts DCs, as well as TNF α and GM-CSF, cytokines that drive DC maturation [6, 36]. TNF α serves as an amplifier of inflammatory responses by activating NF- κ B [37]. Administration of TNF α has been shown to overcome inhalation tolerance in a mouse model, an effect mediated by DCs [38]. GM-CSF is a pleiotropic cytokine produced by airway epithelial cells in vitro in response to the proteolytic activity of Der p 1 [39, 40] and in response to airway particulate matter [41]. Adenovirus-induced

expression of GM-CSF in mouse airways inhibited tolerance and promoted allergic sensitization [42]. In addition, increased GM-CSF expression was reported in several studies that analyzed gene expression changes after segmental challenge in asthma [43].

IL-6, an important early pro-inflammatory cytokine, is upregulated in lung epithelial cells on exposure to particulate pollutants like asbestos [44]. IL-6 is also upregulated on exposure to fungal antigens as seen by in vivo and in vitro studies [45, 46]. IL-6 is increased in asthma [47], and higher levels of IL-6 were associated with loss of lung function [48].

Epithelial cells can produce several chemokines that may directly promote Th2-mediated inflammation or tissue remodeling. For instance, CCL17 (TARC) which chemottracts Th2 cells is expressed by epithelial cells in response to TNF α , Der p 1, IL-4, and TGF β . [49], whereas IL-8, a CXC chemokine with potent pro-inflammatory activity, is upregulated in epithelial cells by a variety of stimuli including mechanical stretch, air pollutants, and infections [50–52]. IL-8 stabilizes Muc5ac RNA in a post-transcriptional manner and contributes to increased mucus production [53]. Elevated levels of CCL17 and IL8 (refs) are found in the BAL fluid of allergen-challenged asthmatic individuals [54, 55].

IL-33, IL-25 (IL-17E), and TSLP are epithelial cytokines that can be released by airway cells under a variety of stimuli. IL-33 is a recent member of the IL-1 cytokine family that is released by necrotic cells and functions as an alarmin. IL-33 is expressed by epithelial cells, fibroblasts, and endothelial cells and can be induced in macrophages. The IL-33 receptor ST2 (IL-1RL1) is expressed on Th2 cells, mast cells, and the recently described natural helper cells or nuocytes. IL-25, also known as IL17E, is produced by Th2 cells, and by epithelial cells, mast cells, and macrophages upon activation. Both IL-33 and IL-25 induced Th2 responses when administered to mice [18, 19].

Allergens and environmental pollutants can stimulate the production of TSLP by epithelial cells. Epithelial production of TSLP is NF- κ B-dependent and has been shown in vivo to induce features of airway inflammation including Th2 cytokine and chemokine production, AHR, fibrosis, goblet cell hyperplasia, and mucus production [56, 57]. TSLP directly acts on DCs, promoting DC maturation and conditioning DCs to drive Th2 differentiation through OX-40/OX40L interactions with naïve T cells [58]. Exposure of human bronchial epithelial cells in vitro to diesel exhaust particles induced the production of TSLP that drove DC maturation and upregulation of OX40 and Jagged-1 [36, 59]. TSLP treatment for myeloid DCs was also shown to induce the production of IL-8, CCL11 (eotaxin-1), CCL17, and CCL22 [20]. Recently, Nakano and collaborators showed that among the lung DCs, a subset of myeloid DCs,

the CD103⁺ DCs, are mainly responsible for priming Th2 responses to a variety of inhaled allergens [60].

Within the innate lymphoid cells (ILCs), a novel subsets of innate cells have been identified in various tissues including the gut, the fetal, and adult lung. Fort and collaborators first identified a lineage negative population of IL-25-responsive, Th2 cytokine-producing cells in mice [61]. These cells increase in an IL-25- and IL-33-dependent fashion and produce Th2 cytokines [62–64], hence the name ILC2 cells. While ILC2s appear to be important for helminth immunity, their role in asthma is yet to be demonstrated. Interestingly, it was found that lung ILC2s express amphiregulin, an EGF family member protein that is involved in epithelial repair. Depletion of ILC2s in an influenza model, impaired epithelial remodeling and repair, a phenotype that could be rescued by the administration of amphiregulin [65]. Additionally, increased cell numbers of ILC2s were found in nasal polyps from chronic rhinosinusitis patients suggesting a “helper” function that is deleterious [66].

Mechanical stress as an initiator of asthma

The epithelium reacts to the alteration of the physical environment such as mechanical stretch or cold temperature by upregulating molecules that are recognized to be present in inflammation [50, 67]. Bronchoconstriction generated compressive mechanical stress in bronchial epithelial cells that triggered EGFR-mediated fibrotic response and production of MUC5A and the chitinase-like molecule YKL-40 [68]. These responses required metalloprotease activity mediated by TACE/ADAM17 in mouse cells [69]. Allahverdian and collaborators showed that IL-13 is expressed by epithelial cells in response to mechanical injury as part of a normal repair program. Dysregulated repair and increased IL-13 contributes to the long-term structural changes seen in chronic asthma [70].

A recent study evaluated the influence of repeated allergen or methacholine induced bronchoconstriction on airway structural changes in patients with asthma. Only allergen challenge caused eosinophilic inflammation, but both treatments resulted in increased TGF- β production, subepithelial collagen band width, and in the number of mucus-secreting epithelial cells [71]. This study in patients, together with previous studies in vitro, strongly suggests that bronchial smooth muscle constriction per se is sufficient to initiate remodeling in the airways. Inflammation-independent bronchoconstriction effects on remodeling may explain the observed airway remodeling in young children, as well as the failure of corticosteroid treatment to modify the natural history of asthma [72]. Exaggerated or chronic mechanical stress responses may thus contribute to the onset of allergic sensitization and asthma.

Viruses and development of asthma

Early life exposure to respiratory viral infection is thought to predispose an individual to later onset of asthma. Almost all children are infected with respiratory syncytial virus (RSV) at least once before their second birthday. Factors that have a predictive value for later onset of asthma are the severity of bronchitis, bronchiolitis, and wheezing observed during an acute infection and the timing of the infection itself. The correlation observed in humans between primary RSV infections followed by subsequent development of asthma has been corroborated by experimental infection of animal models [73]. Since pneumoviruses are highly restricted to their natural hosts, a very high inoculum is required to infect other species. And when they do, not all features of the human infection can be recapitulated. This disadvantage notwithstanding, mice have been infected with human RSV and shown to develop AHR and produce a multitude of chemokines and cytokines including IL-4, IL-5, IL-13, KC, and IFN γ [74]. Allergen exposure following infection of BALB/c mice with RSV showed increased Th2 cytokine levels, influx of eosinophils and neutrophils, and higher AHR [75]. While similar observations have been reported in other animal models [73], some other studies have shown that pulmonary infection with RSV followed by allergen exposure actually protected against allergic inflammation and AHR [76]. These differences could be due to the genetic background of mice, age at infection, Th1/Th2 balance, and viral strains used, indicating that the final outcome is due to complex interactions.

RSV infection of the lung epithelium drives the production of inflammatory mediators by epithelial cells, influx of effector cells, and also leads to epithelial damage. While this response facilitates pathogen elimination, the mediators also impact on the host epithelium. As the lung continues to mature during early life, exposure to an infection and an inflammatory milieu during its developmental stage could have long-lasting effects on lung function. RSV infection during the first 3 months of life seems to have a profound effect of inducing a Th2 type response [77]. Primary airway epithelial cells infected with RSV upregulate TSLP, a cytokine that has been shown to drive Th2 polarization of DCs and T cells [78]. Since the lung epithelium is the primary target of RSV, early infection could drive polarization of responses to a Th2 type likely mediated via TSLP. Persistent infection or re-infection with RSV compounds these epithelial responses and might prime the individual to developing asthma later in life.

Influenza virus forms another group of pulmonary pathogens that impact on asthma. Chang and collaborators have shown that influenza infection during early life might

actually protect mice from allergen-induced airway inflammation. Infection of suckling mice with influenza A led to the expansion of an NKT cell subset that had a protective role against OVA-induced AHR. Adoptive transfer of this subset associated with the expansion of an allergen-specific Treg population [79]. On the other hand, Dahl et al. showed that influenza A infection of mice induced a robust IFN γ response and a DC phenotype that was polarized to Th1. This infection, however, actually worsened the subsequent Th2 immunity [80]. The mice used in these studies were much older (6–8 week old), and these results cannot be directly compared to those obtained from the neonatal mice. Nonetheless, with the emergence of increasingly virulent strains of influenza and with infants as a particularly vulnerable group, it remains to be seen how these interactions would modulate subsequent lung function in affected individuals. And more importantly, whether intervention and therapeutic approaches employed against infections also ameliorate subsequent development of asthma in susceptible individuals.

How chronic inflammation and tissue responses alter lung structure and function

Asthma is associated with chronic inflammation of the airways and alterations in lung tissue structure and lung function. Tissue remodeling in asthma affects primarily the bronchial wall and involves the activation of the epithelium, thickening of the basal membrane, changes in the composition of the extracellular matrix (ECM), increase smooth muscle mass, vascularity, and edema. Asthma is also characterized by an exacerbated broncho-contractile response and impaired lung function. Airway obstruction due to the accumulation of mucus and broncho-constriction contributes to increased resistance to airflow and the stiffening of the airways decreases lung elasticity. It is believed that tissue remodeling contributes to AHR and to loss of lung function in chronic asthma.

The epithelium in asthma

Repeated exposure to allergens in asthmatic individuals results in chronic activation of the bronchial epithelium rendering it fragile and susceptible to invading pathogens [7]. Chronic inflammation induces dysregulated epithelial repair with alterations in cellular composition and loss of epithelial barrier function. The epithelium is also responsible for the effects on subepithelial structural cells such as stroma cells and smooth muscle cells.

Shedding of the epithelial layer is one of the pathologies observed in asthma. An observation made in both rat and

mice is the alteration in the composition of tight junction proteins [81, 82]. In vitro studies showed from epithelial cells from asthmatic patients had impaired ability to form tight junctions and displayed greatly reduced transepithelial resistance compared to cultures from healthy individuals [83]. Recently, it was shown that the addition of EGF to the cultures of primary epithelial cells from asthmatic patients decreased epithelial permeability, an effect likely due to protection against tight junction disruption [84, 85]. Keratinocyte growth factor was also able to limit inflammation and improve the epithelial barrier in a rat model of asthma [82]. Thus, therapies that improve epithelial repair and function may have clinical benefits in asthma patients.

Activation of developmental pathways changes epithelial composition in asthma

Molecules involved in cell-type determination during lung development participate in the adult lung responses to inflammation and repair and may be involved in pathological responses in asthma. One of the most pronounced compositional changes in the epithelium during chronic allergic inflammation is goblet cell hyperplasia. Murine studies indicated that the notch pathway [86], the transcription factor Foxa2 [87], and Sam-pointed domain-containing ETS transcription factor (SPDEF) [88, 89] tightly regulate goblet cell development. Foxa2 expression suppresses, while SPDEF promotes differentiation of goblet cells.

Foxa2 expression was decreased or absent in airway epithelial cells of mice in which goblet cell hyperplasia was induced through ovalbumin sensitization, IL-4 or IL-13 treatment, or deletion of the gene-encoding surfactant protein C [87]. Furthermore, selective deletion of Foxa2 in respiratory epithelial cells led to eosinophilic inflammation, goblet cell hyperplasia, recruitment of myeloid DC and Th2 cells to the lung, and production of CCL20, eotaxins, IL-4, IL-13, IL-33, and SPDEF. Pulmonary inflammation and goblet cell hyperplasia were suppressed by neonatal treatment for Foxa2-deficient mice with anti-IL-4R antibody [90]. Consistent with the mouse experiments, Foxa2 expression was inversely correlated with goblet cell hyperplasia and Muc5ac and Clca1 levels in lung tissue from patients with pulmonary diseases [87] and asthma [91]. In studies using primary human bronchial epithelial cells, IL-13 and the EGFR pathway positively regulated mucus production and negatively regulated Foxa2 expression [92]. Both findings in mouse models and humans demonstrate that the epithelium, through Foxa2 expression, plays a critical role in controlling Th2 inflammation and epithelial remodeling [90].

Ttf-1/Nkx2.1 is a critical transcription factor in regulating gene expression during development and perinatal adaptation to air breathing [93]. Nkx2.1 target genes

include those related to pulmonary surfactant production, innate defense proteins, and fluid homeostasis [94, 95]. Haplo insufficiency in *Nkx2.1*^{+/−} mice led to increased mucus metaplasia after allergen challenge. Conversely, transgenic expression of *Nkx2.1* prevented allergen-induced decrease of *Foxa2* expression, and increased SPDEF and *Muc5A* expression. Studies of bronchial brushes from asthmatic and control individuals found reduced expression of *Nkx2.1* and increased expression of SPDEF [96]. Thus, *Nkx2.1* and *Foxa2* play complementary roles to inhibit mucous metaplasia and pulmonary inflammation by suppressing expression of SPDEF. On the other hand, SPDEF induction by IL-13/STAT6 in Clara cells or basal cells suppresses *Nkx2.1* and *Foxa2* expression, inducing goblet cell differentiation and increased mucus production [97, 98].

In addition to increased goblet cell numbers, there is a decrease in ciliated cells in the bronchial epithelium in asthma. The possibility that ciliated cells may also transition into mucus-producing cells is supported by cell fate tracing experiments [99, 100]. Treatment with IL-13 showed a reversible increase in goblet cells that previously expressed FOXJ1, a transcription factor of ciliated cells [101].

In sum, elevated SPDEF levels along with reduced *Nkx2.1* and *Foxa2* correlates with mucus metaplasia and altered ciliated/goblet cell balance in asthma. These epithelial changes contribute to airway occlusion and impaired clearance function.

Cytokines that induce goblet cell hyperplasia and mucus hypersecretion

Increased production of mucus in asthma may be caused by epithelial cell differentiation into the goblet cell lineage as described above or by the activation of existing goblet cells. Th2-derived cytokines IL-4 and IL-13 have been shown to increase mucus metaplasia in overexpression experiments in mice [102, 103]. Induction of SPDEF by IL-13 was found at sites of goblet cell hyperplasia in non-ciliated epithelial cells [89]. It is not yet clear which epithelial cells directly respond to Th2 cytokines in vivo. Studies on the expression of IL-4 and IL-13 receptors in differentiating human epithelial cells (containing basal cells, goblet cells, and ciliated cells) showed increased receptor expression and cytokine response in the more differentiated cells. In addition, subtypes of epithelial cells showed differences in receptor expression [104].

IL-9 is a Th2 cytokine [105] involved in the recruitment of mast cells. Studies of experimental asthma in IL-9 deficient mice showed decreased goblet cell hyperplasia [106]. In human epithelial cell cultures, addition of IL-9 induced goblet cell hyperplasia and increased mucus and lysozyme production under injury/repair conditions, but IL-9 did not affect fully differentiated ciliated cells [107].

Recently, it was shown the blocking IL-9 in a chronic model of asthma reduced the number and activation of mast cells with concurrent decrease in TGF β , VEGF, and FGF [108]. Thus, IL-9 may regulate remodeling through direct effects on the epithelium and on mast cells.

IL-1 β and TNF α can induce MUC5A in human airway epithelial cells [109]. Perinatal expression of IL-1 β in airway epithelium disrupted lung morphogenesis, induced goblet cell and smooth muscle hyperplasia, and infiltration of neutrophils and macrophages [110]. IL-1 β can be produced by a wide variety of cells including fibroblasts, bronchial, and alveolar epithelial cells [111]. Another IL-1 family member, IL-33, is increased in asthma and has been shown to induce AHR and goblet cell hyperplasia [112], [113]. IL-33 induces the production of Th2 cytokines IL-4 and IL-13 in ST2-expressing cells such as Th2 cells and innate lymphocytes and thus may promote mucus metaplasia through indirect effects. Lastly, the IL-1 family member IL-18 has also been shown to induce goblet cell hyperplasia and mucus hypersecretion [114]. IL-18 promoter polymorphisms were associated with predisposition [115] and severity of asthma [116]. However, like IL-33, the effect of IL-18 may be indirect since the administration of IL-18 to RAG2-deficient mice did not result in allergy-associated pathology [114].

Signaling pathways in epithelial activation

The JAK-STAT pathway, the EGFR pathway, and the NF- κ B pathway have been implicated in epithelial activation in asthma. STAT6-mediated signal transduction is the main mechanism behind IL-13-induced epithelial remodeling, as demonstrated in experiments with STAT6-deficient mice [117, 118]. Epithelial restricted expression of STAT6 in these mice was sufficient to drive IL-13-dependent AHR and mucus production independent of other lung pathology [117]. IL-13/STAT6 signaling was found to be necessary for SPDEF upregulation and mucus metaplasia in epithelial cells [119]. IL-13 can also signal through STAT6-independent mechanisms involving the ERK1/2 pathway [120, 121] and induce AHR and peribronchial fibrosis [122]; however, the relevance of STAT6-independent IL-13 signaling for disease is not fully understood.

The EGFR pathway is the primary repair pathway activated by injury in the epithelium [123]. Elevated EGFR expression by the lung epithelium in severe asthma may indicate a chronic repair response that leads to epithelial remodeling [124]. Consistent with this, inhibition of EGFR signaling in a mouse model of chronic asthma reduced goblet cell hyperplasia and AHR [125].

The NF- κ B pathway is activated in the airways of asthmatic individuals [126], and many pro-inflammatory molecules that are expressed in the asthmatic epithelium

are NF- κ B-responsive genes [127]. Glucocorticoids, the main drugs used in asthma treatment, inhibit NF- κ B activation [128]. The contribution of epithelial NF- κ B activation has been studied using different models of tissue-specific expression. Expression of a constitutively active form of the activating kinase subunit IKKB in the airway epithelium was sufficient to drive hallmark features of airway inflammation [129, 130]. Conditional ablation of NF- κ B signaling in the airway epithelium diminished responses to ovalbumin sensitization and reduced NF- κ B-regulated chemokines eotaxin-1 and TARC [131]. Inhibition of NF- κ B through various approaches consistently resulted in reduced tissue inflammation, fibrosis, and alveolar remodeling but not mucus production [132]. Inhibition of NF- κ B by the administration of a small molecule inhibitor of IKKB, IMD-0354, in a chronic murine asthma model resulted in reduced eosinophilia, and features of lung remodeling, including goblet cell hyperplasia, collagen deposition, and smooth muscle hypertrophy. IMD-0354 inhibited the production of TGF β , IL-13, and IL-1 β but not IFN γ and ameliorated AHR [133]. Thus, increased epithelial NF- κ B activation could drive both the pro-inflammatory phenotype as well as some of the structural changes seen in asthma.

In sum, the Th2 cytokine-dependent STAT6 pathway, the repair-induced EGFR pathway, and innate immune-induced NF- κ B pathways are thought to play crucial roles in airway activation in asthma.

Epithelial-to-mesenchymal transition

Epithelial-to-mesenchymal transition (EMT) is postulated to be an important mechanism affecting lung remodeling and function, but the extent to which EMT is responsible for asthma pathogenesis is still unknown. EMT involves disruption of epithelial tight junctions through expression of Snail and Zeb family members and downregulation of E-cadherin. Transcription of Snail-1 in the lung epithelium during allergy is induced by TGF β [134]. Downregulation of E-cadherin releases beta-catenin and allows the activation of Wnt pathway [135, 136] resulting in the inhibition of proteasomal degradation of Snail-1 [137]. Experimental evidences point to the activation of EMT in chronic experimental asthma. Loss of epithelial E-cadherin and occludin, increased expression of mesenchymal proteins, and increased expression and nuclear translocation of Snail-1 were observed in epithelial cells of HDM-exposed mice. Furthermore, fate-mapping experiments demonstrated the migration of airway epithelial cells into the subepithelial regions of the airway wall [138].

TGF β induced EMT in air-liquid interface cultures of primary epithelial cells from normal and asthmatic individuals. While EMT induction was restricted to basally

located cells in cultures of normal subjects, it was wide spread in cultures of subjects with asthma. Thus, there appears to be a higher propensity of epithelial cells from asthmatic patients to undergo EMT [139], suggesting that EMT might contribute to the tissue remodeling in asthma.

Eosinophils are believed to be the main source of TGF β in asthma. Corticosteroid treatment is effective in decreasing eosinophilia in asthma but did not inhibit TGF β -induced EMT [140]. Thus, other sources of TGF β may maintain tissue remodeling in the absence of overt inflammation. It was recently found that MMP9 could induce TGF β production in epithelial cells [141]. MMP9 plays an important role in the pathology of asthma and drives the breakdown of tight junction proteins among other effects [142]. MMP9 is produced by a variety of immune and non-immune cells in the lung including epithelial cells, airway smooth muscle cells, stromal cells, and lung resident alveolar macrophages [143]. Thus, non-immune lung cells may propagate airway remodeling even in the conditions of therapeutic control of inflammation.

Changes in extracellular matrix in asthma

An important remodeling change in asthma is an increase in the thickness and composition of the subepithelial basement membrane [144]. Decrease in collagen IV and increase in collagen I and tenascin are the characteristic changes of the extracellular matrix in asthma. Elastic fibers and the collagen matrix counteract the bronchoconstrictive effects of the ASM. As such changes in either of these components could have a detrimental effect on lung function. Two layers of elastic fibers are found in the airways: a superficial one attached to the basement membrane and a deeper one closer to the smooth muscle. Studies of lungs from fatal asthma patients showed decreased and fragmented superficial elastic layer, and elastosis of the deep, smooth muscle-associated layer [145]. Elastic fibers were also decreased in the alveolar attachments of the distal lung in fatal asthma [146]. Elastase fibers can be degraded by macrophage elastase (MMP12) and neutrophil elastase (ELA2). Genetic polymorphisms in MMP12 have been associated with severity of asthma and COPD [147].

Bronchial smooth muscle in asthma

Pathophysiology of the bronchial smooth muscle in asthma involves increase in mass and acquisition of an inflammatory phenotype

One of the main pathological features of asthma is the increase in bronchial smooth muscle mass. In the healthy

human lung, a balance is maintained between bronchoconstrictive airway smooth muscle cells (ASM) and the bronchodilating ECM elastic fibers and a subepithelial layer of collagen, which provides rigidity to the airways. In asthma, the smooth muscle bundles are thickened and the composition and amount of the associated extracellular matrix proteins are also altered. In addition, smooth muscle cells acquire a secretory phenotype in asthma, producing a variety of mediators such as cytokines, chemokines, and growth factors that may impact inflammation and remodeling. ASM express the high affinity receptor for IgE in asthma and can be activated by receptor crosslinking [148]. Expression of immune receptors, such as cytokine receptors, TLR receptors, and adhesion molecules render ASM cells responsive to the inflammatory environment and enable them to interact with immune cells [149–151].

The most convincing evidence that the increase in bronchial smooth muscle mass negatively impacts lung function comes from the studies of ablation of smooth muscle fibers by bronchial thermoplasty [152, 153]. Follow-up studies in treated severe asthma patients showed that lung function improvement lasted for at least 5 years post-ablation [154]. Thermoplasty may benefit patients by reducing airway obstruction as well as inflammation.

ASM can switch from a contractile phenotype to a proliferative, migratory, or synthetic phenotype, and these changes are greatly influenced by the extracellular matrix [155] [151]. As discussed before, a decreased in elastic fibers may promote a hypercontractile phenotype of the ASM. The basal lamina of the smooth muscle was found to be thicker in asthmatic patients than in controls and extracellular matrix accumulated between ASM cells [156]. Fibronectin and collagen I, which are increased in asthma, promote ASM proliferation in vitro [157] while reducing the contractile response [158, 159], and several ECM proteins increased the survival of ASM cells in vitro [160]. ECM proteins can also affect the secretory response of ASM cells. For example, collagen I deposition can induce the release of eotaxin, RANTES, and GM-CSF [161–163]. In both asthmatic and non-asthmatic ASM cells in vitro, TGF β and connective tissue growth factor led to the production of fibronectin and collagen I. Secretion of collagen I by the smooth muscle cells in asthma may thus generate a feed back loop that further increases pathogenic ASM responses [164].

Cellular hypertrophy and hyperplasia due to increased proliferation contribute to the increase in smooth muscle mass [165–168]. It is also possible that differentiation from progenitor cells or through EMT will play a role [156]. Smooth muscle proliferation can be induced by mechanical stress [169, 170] and directly by tissue growth factors such as FGF2 [171], EGF [172], BDNF [173], and PDGF [174].

TGF β alone did not induce proliferation but potentiated FGF2 activity [171].

Experiments in IL-4-deficient mice demonstrated that Th2, but not Th1 inflammation, induced bronchial remodeling [175]. IL5-deficient mice did not develop eosinophilia after sensitization, and this was associated with decreased TGF β and lower bronchial smooth muscle mass [176]. Inhibition of the tumor necrosis family member LIGHT in chronic experimental asthma in mice reduced lung fibrosis, smooth muscle hyperplasia, AHR, and TGF β and IL-13 production without affecting eosinophilia. Similar results were obtained using LIGHT-deficient mice. Furthermore, exogenous administration of LIGHT caused fibrosis and smooth muscle hyperplasia [177]. In humans, levels of LIGHT in sputum of asthmatic patients were found to be correlated to decreased lung function [178].

In sum, the increase in bronchial smooth muscle mass and the inflammatory phenotype of bronchial smooth muscle cells in asthma are believed to play a crucial role in pathogenesis. Th2 inflammation is the primary trigger of smooth muscle remodeling through direct effects on ASM cells and by affecting immune cells and structural cells, ultimately leading to ECM perturbations, increased cytokine, and tissue growth factor production. The ability of ASM cells to become pro-inflammatory may contribute to perpetuating remodeling.

Cross-talk of smooth muscle cells and mast cells

Mast cells are found in the airway epithelium, the submucosal glands, and infiltrating the bronchial smooth muscle bundles in asthma [179]. The presence of mast cells in the ASM is the characteristic of asthma, as it was not found in the airways of eosinophilic bronchiolitis patients or in healthy controls. Eosinophilic bronchitis and asthma are both Th2-mediated diseases; however, there is no variable airflow obstruction or AHR in eosinophilic bronchitis [180]. These observations raised the intriguing possibility that direct contact of mast cells with ASM cells have a crucial role in smooth muscle pathology in asthma.

A significant correlation was found between ASM, mast cell number, and AHR in patients with asthma. The majority of the mast cells found in the smooth muscle bundles contained both tryptase and chymase, and expressed IL-4 and IL-13 but not IL-5 [179]. Electron microscopy studies revealed direct interaction of ASM cells with mast cells and lymphocytes in bronchial tissue of asthmatic patients [156].

Consistent with the presence of mast cells in the ASM of asthmatic subjects, mast cells were chemoattracted by Th2-stimulated ASM from subjects with asthma but not from ASM from non-asthmatics [181]. In vitro, ASM

contributed to increased longevity, proliferation, and constitutive activation of human lung mast cells. These effects required cell–cell contact and occurred through a cooperative interaction between membrane-bound stem cell factor (SCF) expressed on ASM, soluble IL-6, and CADM1 expressed on mast cells [182].

IgE-mediated degranulation of mast cells leads to the release of a wide variety of effector molecules such as histamine, proteases, cytokines, leukotrienes (LT), and prostaglandins (PG) [183]. Histamine, PGD₂, and (LT)C₄ are known to induce a strong bronchial contraction and might influence hypertrophy of the ASM in patients with moderate to severe asthma [179]. Histamine signaling over the H1 receptor on the ASM enhances IL-1 β -induced GM-CSF release but suppresses the release of TNF α -induced RANTES [184]. GM-CSF production is also enhanced by mast cell tryptase [184].

Mast cells can release large amounts of TNF α especially after IgE-mediated activation [185]. TNF α induces the upregulation of CD40 and OX40L on ASM and a higher expression has been observed in asthmatics [186]. Expression of these co-stimulatory molecules by ASM could contribute to T-cell proliferation, cytokine production, and increased survival. Moreover, OX40–OX40L interaction can block Treg generation, activation, and functionality even in the presence of TGF β [187–189].

In sum, the presence of mast cells in the bronchial smooth muscle bundles in asthma suggests an important role of mast cells in the pathophysiology of this disease. Interactions between mast cells and ASM cells may directly promote bronchoconstriction and immune activation of ASM cells on one hand, and mast cell survival and activation on the other. As long as allergen-specific IgE antibodies are present, disease-promoting mast cell–ASM interactions may persist even when inflammation is reduced by corticosteroid treatment.

Lung microbes and asthma

Virus and asthma exacerbations

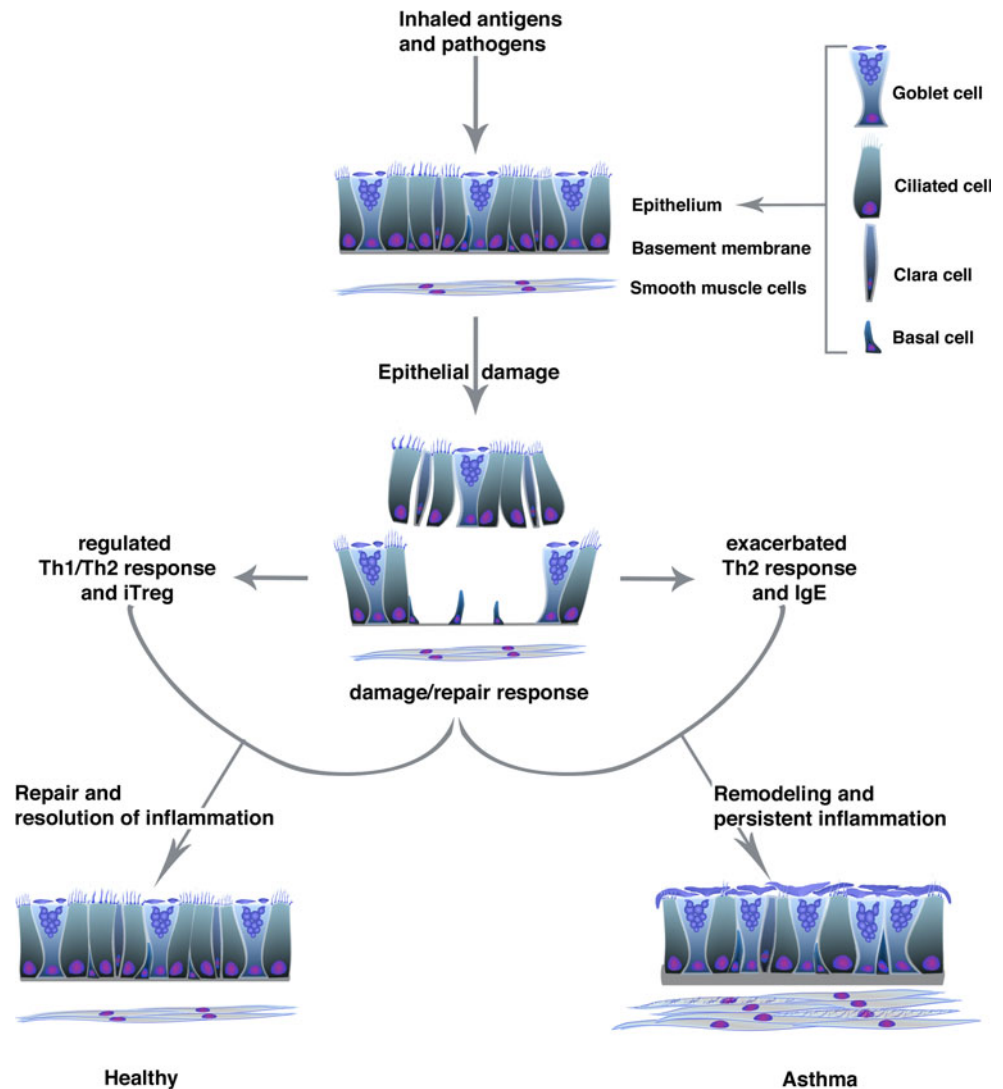
Frequent early childhood respiratory infections are shown to be associated with later development of asthma in children. Whether early infections predispose a susceptible individual to later onset of asthma or whether early infections just mark the populations that would develop asthma later in life is debatable [190]. Infection of the asthmatic airways by common respiratory pathogens, especially viruses, leads to the exacerbation of asthma [191]. Pathogen-driven exacerbation imposes a heavy toll on lifestyle and productivity of an individual and is an important area of active research.

The majority of asthma exacerbations are caused by rhinoviruses (RV). Exacerbations could be due to the virus accessing the lower airways in asthmatics or due to inflammatory mediators induced by the infection reaching the lower airways in conjunction with a compromised barrier function. However, it should be noted that individuals with asthma are not more prone to infections with respiratory viruses, but that their responses are more severe and prolonged than non-asthmatics [192]. The contribution of viral infections to asthma has been the subject of extensive reviews examining clinical data and animal studies [73, 192, 193]. Virus-induced pro-inflammatory cytokines upregulate matrix metalloproteinases in epithelial cells. This could lead to extracellular matrix damage followed by aberrant repair in asthma. Epithelial cell infection of RSV can induce the production of IL-8 in an EGFR-dependent manner contributing to exacerbations [194]. RSV infection has also been shown to synergize with Th2 cytokines driving the expression of the chemokine TARC (CCL17), which can further recruit T cells to the lung [195]. RV infection upregulates its own receptor ICAM-1 in an NF- κ B-dependent manner. ICAM-1 drives leukocyte infiltration in addition to enhanced viral particle attachment to the epithelial cell. Virus-infected cells also produce growth factors like fibroblast growth factor (FGF) and VEGF [191], which could potentially drive extracellular matrix changes.

Changes in the lung microbiota

The idea of the lung microbiome is recent as the lower airways were thought to be essentially sterile. However, efforts are ongoing to score the lung microbiota as part of the Human Microbiome Project (HMP) initiated in 2008 (<http://www.commonfund.nih.gov/hmp/>). The approach is mainly culture-independent microbiota profiling based on 16S ribosomal RNA polymorphisms. There is evidence to the existence of a core pulmonary bacterial microbiome that includes *Pseudomonas*, *Streptococcus*, *Prevotella*, *Fusobacterium*, *Haemophilus*, *Veillonella*, and *Porphyromonas* in a lung with normal function [196]. The normal lung microbiome is disturbed in asthmatic lungs [197]. The bacterial diversity was significantly higher in asthmatic bronchial brushings and correlated with hyperresponsiveness [198]. Commensal microbiota of the gut has also been shown to contribute to immune regulation against respiratory viruses [199]. Given that the epithelial barrier hosts a resident population of microbiota, studying interactions with the underlying epithelial cells opens an entire new avenue for study. Akin to the gut microflora and its influence on the immune status of the host, it is tantalizing to imagine the role of our lung microbiota in shaping immune responses of the lung in health and disease.

Fig. 1 Dysregulated immune response and tissue repair in asthma. The airways are continuously exposed to inhaled antigens, pollutants, and pathogens. These insults may cause epithelial damage and broncho-contractile responses and trigger inflammation and tissue repair. In healthy individuals, an initial effector T-cell response can be counterbalanced by regulatory mechanisms that include iTreg. This balance leads to the restoration of the epithelium and resolution of inflammation. In susceptible individuals, an exacerbated Th2 and IgE response together with a defective tissue repair response drives chronic inflammation (not shown) and tissue remodeling of the bronchial wall. Remodeling results in goblet cell hyperplasia and excessive mucus production, reduced number of ciliated cells and Clara cells in the epithelium, increased basement membrane thickness, increased smooth muscle mass and edema. Inflammation and structural tissue alterations are the characteristics of the asthmatic lung



Summary and conclusions

The airway epithelium is at the boundary of the organism with the environment. Owing to their location, epithelial cells are the first ones exposed to allergens, viruses, microbial products, and environmental chemicals. These encounters may directly elicit secretion of inflammatory cytokines and chemokines or induce epithelial injury leading to the loss of barrier function and release of danger molecules. Homeostatic tissue repair and self-limiting regulated immune responses may successfully establish tolerance and prevent inflammation (Fig. 1). In susceptible individuals, allergic sensitization may ensue. Chronic allergic inflammation and repair in the lung result in permanent and self-perpetuating alterations of the bronchial epithelium, stroma, and smooth muscle bundles that drive respiratory dysfunction. Thus, pharmacologic targeting of pathways involved in tissue remodeling may add a considerable benefit to current anti-inflammatory treatments.

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