

The airway epithelium in asthma

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Asthma is a T lymphocyte-controlled disease of the airway wall caused by inflammation, overproduction of mucus and airway wall remodeling leading to bronchial hyperreactivity and airway obstruction. The airway epithelium is considered an essential controller of inflammatory, immune and regenerative responses to allergens, viruses and environmental pollutants that contribute to asthma pathogenesis. Epithelial cells express pattern recognition receptors that detect environmental stimuli and secrete endogenous danger signals, thereby activating dendritic cells and bridging innate and adaptive immunity. Improved understanding of the epithelium's function in maintaining the integrity of the airways and its dysfunction in asthma has provided important mechanistic insight into how asthma is initiated and perpetuated and could provide a framework by which to select new therapeutic strategies that prevent exacerbations and alter the natural course of the disease.

INTRODUCTION

Asthma is a chronic inflammatory airway disorder that leads to symptoms such as coughing, wheezing and chest tightness. Airway obstruction runs a variable course, with symptom-free periods interrupted by periods of exacerbations, often caused by viral infection. A typical feature is bronchial hyperreactivity—the tendency of the airway to constrict in response to stimuli such as cold air and exercise. Allergic sensitization to inhaled allergens such as house dust mites (HDMs), animal dander, pollen or fungal spores is often found in children with asthma, and this is due to atopy, which is a predisposition to develop allergic hypersensitivity reactions and produce IgE in response to allergens. Only half of adults who have asthma are atopic, but the disease otherwise presents similarly in the clinic¹.

In bronchial biopsies or lung-resection samples, asthma is characterized by accumulation of eosinophils, mast cells and CD4⁺ T lymphocytes producing interleukin-4 (IL-4) and/or IL-5 in the epithelium and lamina propria, even in individuals who have non-atopic asthma^{2–4}. Animal models in which T_H2-type cytokines such as IL-4, IL-5 or IL-13 have been individually knocked out have provided important evidence that the T_H2 axis can drive eosinophilic airway inflammation and bronchial hyperreactivity⁵. A T_H2-biased response also seems to be detectable in 50% of individuals with asthma⁶. This finding of distinct clinical subsets of asthma is also reflected in clinical trials employing biologicals aimed at blocking T_H2 cytokines, wherein drugs targeting IL-5 are effective, but only in a subset of patients⁷. In some individuals with asthma, particularly those who respond poorly to steroids, airway infiltrates are composed primarily of neutrophils, which are probably recruited to the airways by IL-17-producing cells

such as T_H17 lymphocytes or $\gamma\delta$ T cells^{1,8}. Despite these differences in the underlying etiology of disease, in almost all forms of asthma there are structural changes to the airway wall, most often called airway remodeling. These changes consist of an increase in the smooth-muscle mass surrounding the airway wall, a deposition of extracellular matrix components under the epithelial basement membrane that causes a thickened appearance, a breach in the integrity of the airway epithelium and an increase of mucus-producing goblet cells in the epithelium or submucosal glands. Initially, these structural changes were considered a consequence of sustained airway inflammation, akin to a scarring reaction in response to chronic injury. However, recent data have led to a paradigm shift, with the structural components of the lung being shown to respond to environmental risk factors for asthma⁹ and to have profound upstream effects in initiating and sustaining the allergic cascade¹⁰.

Epithelial cells as frontline activators of dendritic cells

Airway epithelial cells (ECs) lie at the interface between the host and the environment and represent the first line of defense against microorganisms, gases and allergens¹¹. In their exposed position, ECs express many pattern recognition receptors (PRRs) to rapidly detect and respond to pathogen-associated molecular patterns (PAMPs) found in microbes or to damage-associated molecular patterns (DAMPs) released upon tissue damage, cell death or cellular stress (**Box 1**). The activation of epithelial PRRs leads to the release of cytokines, chemokines and antimicrobial peptides that attract and activate innate and adaptive immune cells (**Fig. 1**). Recent studies have demonstrated that EC activation is a key triggering event in the recognition of inhaled allergens that activates the local network of dendritic cells (DCs), which coordinate the subsequent immune response¹².

DCs are professional antigen-presenting cells of the lung immune system and have the ability to sample the inhaled air for antigens by extending dendritic processes across the epithelial layer while maintaining barrier function by formation of tight junctions¹³.

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They subsequently migrate to the draining mediastinal lymph nodes and present antigen to recirculating naive and memory T cells¹⁴ (Fig. 1). When lung DCs were isolated from the airways of allergen-exposed mice and transferred to naive mice, they induced allergen-specific T_H2 cells¹⁵. Conversely, when DCs were depleted from the airways of naive or sensitized mice, T_H2 sensitization or development of allergic inflammation in response to allergen exposure did not

BOX 1 Expression of pattern recognition receptors by lung epithelium

TLRs. Like cells of the innate immune system, respiratory ECs express cell-surface receptors and endosomal TLRs. Upon ligand engagement, TLRs signal via a series of adaptor proteins, resulting in the activation of NF- κ B or the interferon regulatory factors 3 and 7 (IRF-3 and IRF-7), and to the production of several pro-inflammatory cytokines, interferons, chemokines, prostaglandins and defensins¹³⁷. Human bronchial EC lines or primary cells express large numbers of TLRs and have been shown to react functionally to various TLR ligands. However, expression levels differ among TLRs: TLRs 2–5 are highly expressed and TLRs 7–8 are expressed at low levels¹³⁸. Apart from TLR-2 and TLR-4, no formal expression profile of TLRs by airway ECs in mice has been made, but functional studies showed that the ECs of mice react to almost all TLR agonists¹³⁹.

NOD-like receptors. Members of the NOD-like receptor (NLR) family share a C-terminal leucine-rich repeat domain that interacts with PAMPs, a central nucleotide oligomerization domain (NOD) and one of three N-terminal signaling domains. Although mostly expressed by leukocytes, NOD1 and NOD2 are expressed by human airway ECs¹⁴⁰. The activation of NODs results in MAPK and NF- κ B-dependent production of inflammatory mediators. Some NLRs promote the assembly of the inflammasome, a molecular complex that activates caspases to convert pro-IL-1 β and pro-IL-18 (and possibly pro-IL-33) into their mature secreted forms. The best known NLR in this regard is NLRP3, which was shown to induce T_H2 immune responses to crystals and the adjuvant alum^{141,142}. NLRP3 is involved in responses to serum amyloid A, a mediator and biomarker of inflammation in asthma, by promoting a T_H17-type immune response¹⁴³. The involvement of NLRP3 in pulmonary responses to environmental allergens or pollutants is not entirely clear, but in our hands, allergic airway inflammation induced by HDM, cigarette smoke or diesel did not involve the NLRP3 signaling pathway^{68,144}.

C-type lectins. Epithelial expression of the C-type lectin receptor dectin-1 was shown to mediate recognition of β -glucan motifs in the inhaled fungus *Aspergillus fumigatus* and in several other environmental allergens such as pollens, HDMs and animal dander^{32,145}. On DCs, dectin-2 seems to be the major C-type lectin receptor involved in recognition of HDM allergen¹⁴⁶.

Protease-activated receptors. ECs secrete cytokines and chemokines in response to proteolytic allergens such as Der p 1 (refs. 147,148) and protease allergens from pollen and cockroach¹⁴⁹ through activation of protease-activated receptor 2 (PAR-2). Particularly when allergens are administered mucosally, PAR-2 is required for the induction of T_H2 immunity, suggesting an important role for PAR-2 in the epithelial programming of the adaptive immune response by proteolytic allergens¹⁵⁰.

develop^{15,16}. As DCs express most PRRs, it was originally assumed that DCs directly recognize inhaled foreign substances. However, experiments using radiation-chimera mice lacking Toll-like receptor 4 (TLR4) on either radiosensitive hematopoietic or radioresistant structural cells revealed that the recruitment, activation and intraepithelial migration of DCs in response to inhaled endotoxin requires only epithelial TLR4 triggering¹⁷. When studying the response to inhaled HDM allergen, we similarly found a crucial requirement for epithelial TLR4 expression in promoting recruitment and activation of DCs and subsequent development of allergy to HDM¹⁷, findings that have been extended to other model allergens¹⁸.

Epithelial activation threshold influences allergic sensitization

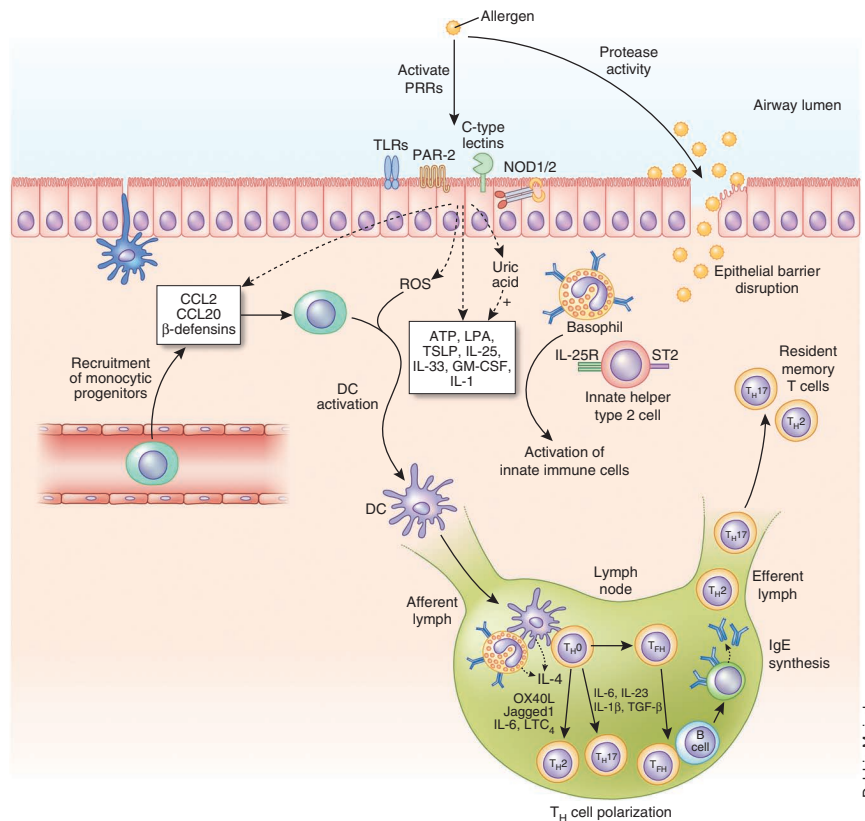
These studies^{17,18} highlighted the importance of a single TLR in generating a response to the complex allergen HDM, which contains multiple (proteolytic) allergens and microbial contaminants. Many allergens, such as Der p 2 and Der p 7 from HDM and Fel D 1 from cats, activate TLR4 signaling, which may explain why they are so potent at inducing allergic responses¹⁹. TLR4-mediated responses require the coordinated action of several molecules, including soluble CD14 and the TLR4 cofactor MD2. Previous studies have reported that, in the resting state, airway ECs are hyporesponsive to TLR4 agonists in comparison to myeloid cells as a result of either low expression of MD2 or intracellular localization of TLR4 (refs. 20,21). Inhaled allergens can simultaneously trigger other PRRs (Box 1), and it will be a challenge to untangle the intersections of the various signaling pathways involved.

The activation threshold of ECs and their resulting ability to trigger innate and adaptive responses could be promoted by prior exposure to DAMPs and PAMPs, which either increase the expression of PRRs themselves or alter the expression of signaling proteins downstream of PRRs. Viral infection of airway epithelia with respiratory syncytial virus (RSV) or exposure to cigarette smoke (which causes damage to cells and the release of reactive oxygen species (ROS)) upregulates TLR4 expression and promotes its localization to the cell membrane, thus increasing endotoxin responsiveness^{22,23}. This increased sensitivity of airway ECs might explain how RSV infections early in life or exposure to cigarette smoke act as predisposing factors for allergic sensitization and inflammation.

The activation threshold of ECs can also be regulated at the level of downstream signaling cascades. Most PRR signaling pathways activate the transcription factor nuclear factor- κ B (NF- κ B), which controls the expression of an array of inflammatory cytokine genes²⁴. The central role of NF- κ B in the control of pulmonary inflammation was elucidated in mice lacking the NF- κ B subunits p50 or p65, which showed reduced responses to endotoxin and allergens²⁵. Later studies revealed that constitutive activation of NF- κ B in airway ECs was sufficient to activate DCs, breach inhalational tolerance and promote sensitization to the harmless inhaled allergen ovalbumin (OVA)²⁶, whereas inhibition of epithelial NF- κ B reduced T_H2 cell recruitment and airway remodeling²⁷.

One of the early-response genes induced by NF- κ B nuclear translocation is the ubiquitin-editing enzyme A20 (also known as TNFAIP3), which deubiquitinates crucial signaling intermediates of TLR-induced activation of NF- κ B and thus acts as a negative regulator of inflammatory gene induction in the epithelium and DCs^{28,29}. Lung-specific overexpression of the A20 binding protein ABIN-1 suppressed allergic inflammation³⁰. Additional studies are needed to address the precise mechanisms whereby NF- κ B activation and deactivation are regulated in airway ECs. In particular, these control mechanisms

Figure 1 Influence of ECs on the induction of allergic sensitization by DCs. Inhaled allergens such as HDMs can activate PRRs on ECs and DCs and can cleave epithelial tight junctions, gaining access to the DC network. Some allergens also induce production of ROS that activate DCs or ECs through NF- κ B activation. The net result of PRR activation and ROS production in ECs is the production of endogenous danger signals such as uric acid, ATP and lysophosphatidic acid (LPA), as well as the DC-activating cytokines TSLP and GM-CSF and several interleukins, including members of the IL-1 family. ECs also produce CCL2 and CCL20, which attract more DC progenitors, such as monocytes, to the lung. Under the influence of these signals, DCs migrate to the T cell area of the draining lymph nodes, where they interact with naive T cells and induce T_H2 , T_H17 and T follicular helper (T_{FH}) differentiation. This T_H polarization is heavily influenced by secreted cytokines and membrane-expressed molecules such as OX40 ligand. T_{FH} cells can then promote B cell differentiation and IgE synthesis. Basophils are an important source of IL-4, which further supports T_H2 development initiated by DCs and promotes IgE synthesis by B cells. NOD 1/2, nucleotide oligomerization domain; PAR-2, protease-activated receptor 2.



may be susceptible to epigenetic alterations induced by environmental factors such as microbial infection and environmental pollutants. These epigenetic changes might, in turn, alter the threshold for epithelial activation in response to allergens and viruses.

Molecular crosstalk between ECs and immune cells

There has been great progress in understanding the mechanism by which ECs influence DC function³¹ (Fig. 1). Airway ECs produce CC chemokine ligand 2 (CCL2) and CCL20 in response to HDM inhalation, which attracts monocytes and immature DCs to the lung^{17,32}. Bronchial ECs also release homodimers of the cytokine subunit IL-12p40 that are chemotactic to monocytic cells and DCs³³. Triggering of TLR4 on ECs by HDM also induces the production of thymic stromal lymphopoietin (TSLP), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-25 and IL-33. These innate cytokines have pleiotropic effects, yet they share the propensity to activate DCs that prime T_H2 responses by inhibiting the production of the T_H1 -polarizing cytokine IL-12, inducing chemokines that attract T_H2 cells or upregulating expression of surface molecules such as OX40L that can instruct T_H2 cell development.

IL-33 and other members of the IL-1 family of cytokines, such as IL-1 α , IL-1 β and IL-36 γ (IL-1F9), are released from bronchial ECs in response to allergen exposure^{34,35}. It is unclear, however, whether all of these cytokines activate DCs to promote T_H2 immune responses³⁶. IL-33 is normally sequestered in the nucleus of ECs, but it can be secreted upon epithelial activation. IL-33 activates lung DCs and promotes their induction of T_H2 responses by acting on the T1 (ST2) receptor^{37,38}. Blocking IL-33 during sensitization to inhaled antigens by DCs abolishes T_H2 development³⁹. The expression of IL-33 is higher in bronchial ECs of people with asthma⁴⁰. Although IL-25 promotes T_H2 immunity in the lung^{41,42}, its potential to activate DCs remains unclear. The proteolytic enzyme MMP7, which is released from bronchial ECs, is necessary for optimal production of IL-25

(ref. 43). A recent study demonstrated that epithelial-derived IL-25 induced Jagged 1 expression on DCs and promoted T_H2 responses in the lung tissue of RSV-infected mice⁴⁴.

TSLP and GM-CSF stimulate DCs to promote T_H2 immunity. Overexpression of these cytokines in the lungs of mice induces spontaneous T_H2 sensitization to the inhaled innocuous protein OVA^{45,46}. Conversely, neutralization of GM-CSF abolishes sensitization to HDM and attenuates the adjuvant effects of diesel particles on allergic sensitization^{47–49}. When ECs from humans who have asthma are cultured, they continually overproduce GM-CSF, suggesting that its production could be epigenetically regulated in asthma⁵⁰. The expression of TSLP is also increased in bronchial biopsies and sputum from humans who have asthma, particularly in severe disease^{51,52}. Genetic polymorphisms in the promoter region of TSLP are associated with increased risk of asthma⁵³. Proteolytic allergens, diesel-exhaust particles and cigarette smoke induce epithelial production of TSLP, which causes DC activation^{54,55}. The TSLP receptor (TSLPR) is expressed not only in DCs but also in human bronchial ECs. TSLP stimulates the proliferation of bronchial ECs and EC IL-13 production⁵². One caveat regarding therapeutic targeting of TSLP is that TSLP can contribute to epithelial repair by induction of secretory leukocyte protease inhibitor (SLPI) and other mechanisms^{52,56}. Expression of SLPI has the potential to dampen allergic airway inflammation; therapeutic interference with TSLP might abolish this protective pathway⁵⁷.

Epithelial-derived cytokines also activate innate immune cells such as basophils, mast cells, eosinophils and innate non-T and non-B lymphoid cells (innate lymphoid cells or nuocytes)^{58–61}. TSLP promotes the growth and differentiation of basophils from the bone marrow⁶². Basophils provide an early source of polarizing IL-4 to further enhance T_H2 development initiated by DCs¹⁵ (Fig. 1), whereas nuocytes are an IL-33- and IL-25-responsive population

Debbie Maizels

that mainly produce IL-5 and IL-13 and could thus contribute to eosinophilia, goblet-cell metaplasia and virus-induced AHR^{63,64}.

Once initiated, epithelial responses to allergens and pollutants are maintained through the action of T_H2 effector cytokines, providing an important feedback loop that can perpetuate disease. Airway ECs respond to the T_H2 cytokines IL-4 and IL-13 by producing GM-CSF and the chemokines IL-8, CCL11 and CCL17, which attract neutrophils, eosinophils and CD4 T_H2 cells, respectively^{65,66}. These effects of IL-4 and IL-13 on ECs are counteracted by the T_H1-type cytokine interferon- γ (IFN- γ)⁶⁷.

Epithelial DAMPs promote airway inflammation

In addition to producing cytokines, ECs may also regulate the allergic immune response by producing DAMPs. We have previously reported that a single administration of HDM in the airways of naive mice or of people with atopic asthma induces the release of both ATP and uric acid, which activate DCs and other innate immune cells^{68–70}. HDM-induced uric acid promoted T_H2 sensitization by amplifying EC production of TSLP, GM-CSF and IL-25. In particular, airway ECs were a source of uric acid, and the release of uric acid was TLR4 dependent. Production of uric acid is the result of an antioxidant response to ROS, which are often produced by airway ECs in response to inhalation of allergens, virus infection or environmental pollutants^{71,72}.

ROS comprise another type of DAMP that can activate DCs by promoting NF- κ B activation⁷³. The transcription factor NRF2 controls the expression of a set of antioxidant genes. Mice lacking *Nrf2* are hypersensitive to ragweed allergen and ambient particulate matter, and DCs isolated from these mice are hyperactivated⁷². The ECs of subjects with asthma produce fewer antioxidants, which could explain their increased sensitivity to allergens and environmental pollutants¹¹. Exposure to allergens and pollutants can cause plasma extravasation, leading to tissue hypoxia and immediate tissue edema. In response to hypoxia, ECs upregulate the hypoxia-inducible factor-1 α (HIF-1 α), which leads to gene transcription and production of vascular endothelial growth factor (VEGF). Neutralization of HIF-1 α or VEGF suppresses established allergic inflammation^{74,75}.

Other danger signals released in response to allergen exposure include lysophosphatidic acid, which activates EC release of TSLP in a process requiring the adaptor molecule CARMA3 (ref. 76) and low-molecular-weight (200-kDa) hyaluronan⁷⁷, but it is unclear at present whether expression of these DAMPs is upregulated in ECs from subjects with asthma.

EC-derived signals can also dampen inflammation

In response to allergen exposure, ECs produce prostaglandin E₂ (PGE₂), which suppresses DC reactivity by acting on the EP4 receptor⁷⁸. Several other lipid mediators (such as lipoxin A4, resolvins and protectins) that suppress lung inflammation are reduced in the airways of subjects with asthma⁷⁹. After airway allergen challenge, airway ECs redistribute the heparan sulfate proteoglycan syndecan-1 from the basolateral space to the airway lumen, thus sequestering inflammatory chemokines that attract eosinophils and T_H2 cells⁸⁰. The IL-1 family member cytokine IL-37 is also produced by bronchial ECs and is an inhibitor of endotoxin-mediated innate immune lung inflammation⁸¹. However, it remains unclear whether IL-37 expression is reduced in subjects with asthma⁸¹. An additional regulatory mechanism that warrants further study is gene regulation by microRNAs. Although certain microRNAs are induced by allergen challenge in mice and have the potential to dampen inflammation⁸², their precise localization has not been studied.

Undoubtedly, other anti-inflammatory mechanisms are employed by ECs to keep immune activation in check, and future work is essential to understand the mechanisms involved in resolution of allergic inflammation.

Barrier function of the airway epithelium in asthma

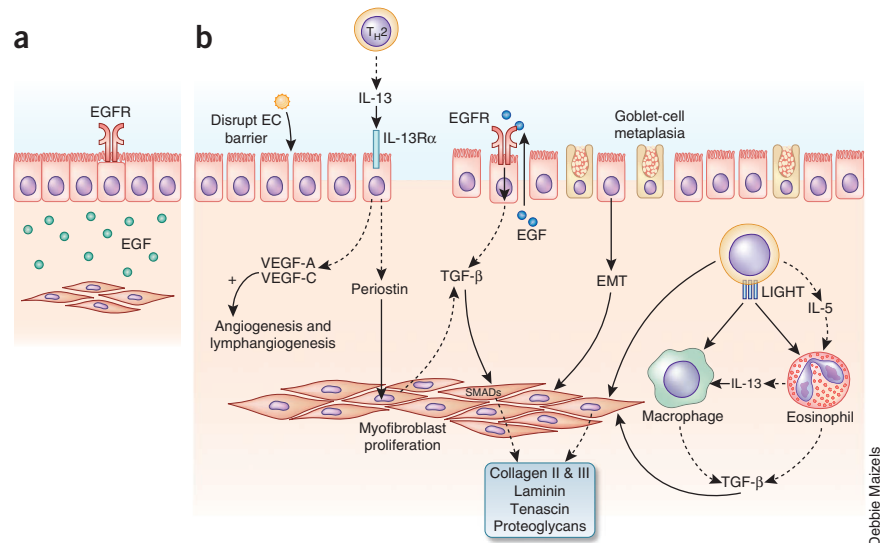
The airway epithelium has typically been thought to function mainly as a physical barrier and in mucociliary clearance by impeding the access of allergens to lung DCs. It has therefore been proposed that the allergenicity of inhaled substances depends on their potential to disrupt epithelial barriers⁸³. In the airways of individuals who have asthma, the epithelia are fragile, and some areas of epithelial basement membrane seem to be denuded of ciliated cells⁸⁴. The integrity of the epithelial barrier depends on apical tight junctions and adherens junctions that keep bronchial ECs together and maintain their apicobasal polarity¹¹. E-cadherin is a major component of adherens junctions, and its expression is reduced in biopsies from subjects with asthma, possibly as a result of epithelial-to-mesenchymal transition^{85,86}. DCs receive a tonic inhibitory signal through homotypic E-cadherin interactions between DCs and ECs^{87,88}. Loss of E-cadherin expression could release this inhibitory signal and facilitate allergic sensitization by activating DCs. Loss of E-cadherin in cultured ECs also enhances production of TSLP⁸⁹ and could therefore be a primary driving force in breaching inhalational tolerance to allergens in individuals with asthma.

It is difficult to address whether loss of E-cadherin is a cause or effect of allergic sensitization. However, in a linkage-analysis study in individuals with asthma, variants in the gene encoding protocadherin 1 were associated with bronchial hyperresponsiveness⁹⁰. Protocadherins, adhesion molecules related to cadherins, are extensively regulated and expressed during differentiation of bronchial ECs, but their function has yet to be determined. The loss of E-cadherin and/or tight-junction proteins and the consequent breach in barrier function could also be due to proteolytic activity of inhaled allergens originating in HDM⁸³, cockroach⁹¹, pollen⁹² or fungi⁹³; by exposure to environmental pollutants such as cigarette smoke⁹⁴ and ozone; or by respiratory infection with RNA viruses⁹⁵. HDM-induced disruption of junctional proteins and barrier function may also be due to the binding of epidermal growth factor (EGF) and/or TGF- α to apically expressed EGF receptor (EGFR) on ECs^{89,96} (Fig. 2). Recently, another molecule, septin-2, was found to regulate apicobasal polarization of ECs and to prevent disruption of lung epithelial integrity⁹⁷. Whether septin-2 expression differs between individuals with asthma and healthy controls remains to be elucidated. It is striking that IL-4 and IL-13, some of the prominent effector molecules of T_H2 cells, also disrupt epithelial barrier function and might, in this way, perpetuate allergic inflammation⁹⁸.

ECs and basement-membrane thickening

Some patients with chronic asthma show a progressive decline in lung function that is thought to be caused by structural remodeling of the airways, characterized by subepithelial fibrosis and smooth-muscle hyperplasia. Changes to the epithelial basement membrane and deposition of extracellular matrix components in the lamina reticularis lead to pseudothickening of the basement membrane, a common feature of airway remodeling (Fig. 2). This response is the result of communication between structural cells and immune cells. ECs release EGF, and eosinophils and myofibroblasts produce TGF- β to promote synthesis of extracellular matrix components and collagen^{99,100}. This interaction between ECs and fibroblasts

Figure 2 Airway remodeling in the epithelial mesenchymal trophic unit. **(a)** In healthy ECs, EGF is secreted on the basolateral side, where it is sequestered from EGFR on the apical side. The sequestration is ensured by an intact epithelial barrier function. In the healthy state, the basement membrane is thin and there are few fibroblasts. **(b)** When ECs are chronically triggered by proteolytic allergens, environmental pollutants or T_H2 cytokines, they can produce cytokines and growth factors such as TGF- β and TGF- α . Under the influence of EC-derived VEGF-A and VEGF-C, there is neoangiogenesis and lymphangiogenesis. New lymphatics might provide a means for DCs to reach the lymph nodes, whereas newly formed vessels allow for extravasation of more inflammatory cells. Epithelial tight junctions are disrupted by proteolytic enzymes or cytokines, allowing basolateral EGF to reach the apical EGFR, inducing EC activation. ECs release periostin, which further boosts TGF- β production, activating the underlying fibroblasts to differentiate into myofibroblasts that synthesize more collagen and extracellular matrix components. Another source of TGF- β is the eosinophil, which is induced under the influence of IL-5 derived from T_H2 lymphocytes. Inflammatory T cell-expressed LIGHT is able to interact with eosinophils and macrophages, driving further production of cytokines. There is also some evidence for epithelial mesenchymal transition (EMT), a process whereby ECs acquire characteristics of fibroblasts and downregulate expression of E-cadherin.



is sometimes called the epithelial-mesenchymal trophic unit¹⁰¹. ECs from individuals with asthma produce high amounts of periostin, which stimulates TGF- β production and modifies collagen synthesis by airway myofibroblasts¹⁰². A study employing mepolizumab, an antibody that blocks IL-5, demonstrated that the ensuing reduction in eosinophils was associated with reduced basement membrane deposition of tenascin, lumican and procollagen type III (ref. 99). Studies in mice genetically deficient in eosinophils or depleted of eosinophils through the use of antibodies to Siglec-F support the idea that eosinophils control airway remodeling¹⁰³. However, eosinophils are not always involved in remodeling. The tumor necrosis factor (TNF)-family member LIGHT is expressed on lung inflammatory cells after allergen exposure, and pharmacological inhibition of LIGHT reduces lung fibrosis, smooth-muscle hyperplasia and airway hyperresponsiveness in mouse models of chronic asthma, despite having little effect on airway eosinophilia¹⁰⁴. Lack of TNFR-I (p55) or TNFR-II (p75) reduces features of airway remodeling¹⁰⁵. Subepithelial fibrosis is also influenced by regulation of the coagulation cascade. The plasminogen activator inhibitor-1 (PAI-1) promotes features of airway remodeling, and an oral PAI-1 antagonist prevents airway remodeling induced by chronic allergen exposure¹⁰⁶.

It has been hypothesized that the thickened basement membrane constitutes a secondary barrier that compensates for the increased epithelial fragility and leakiness, thus halting entry of allergens and pathogens and their access to DCs. We have recently tested this hypothesis and found that remodeling of the epithelial basement membrane did not impair recognition of inhaled antigens by DCs; rather, it promoted sensitization to inhaled neoallergens by causing persistent activation of DCs and facilitated DC migration to the draining lymph node by promoting lymphangiogenesis¹⁰. In our view, the changes in the basement membrane are not the end result of chronic allergic inflammation, but could be a driving force contributing to allergic sensitization. This theory would fit with the observation that basement membrane thickening is detectable in children younger than three years old with persistent wheezing before the diagnosis of asthma^{107,108}.

EC differentiation and increased mucus production

The normal airway epithelium is made up of ciliated cells, Clara cells, goblet cells and basal cells that can transdifferentiate. In individuals with asthma, some groups have reported an increase in basal ECs that express high levels of cytokeratin 5 and the cell cycle-control protein p63 but do not express E-cadherin¹⁰⁹. In asthma, there is also an increase in the number of goblet cells. This increase, termed goblet-cell metaplasia (GCM), is not due to the proliferation of pre-existing goblet cells but rather to the transdifferentiation of ciliated and Clara cells to goblet cells, resulting in more mucus production. Goblet cells produce the mucins MUC5B and MUC5AC, which are responsible for the viscoelasticity and hydration of the mucus covering the ciliary escalator. In asthma, the sputum is often so dry that it can lead to airway obstruction. How GCM is regulated has been the subject of numerous studies, but several molecular signaling pathways converging on a common set of transcription factors have been identified¹⁰⁰ (Box 2 and Fig. 3).

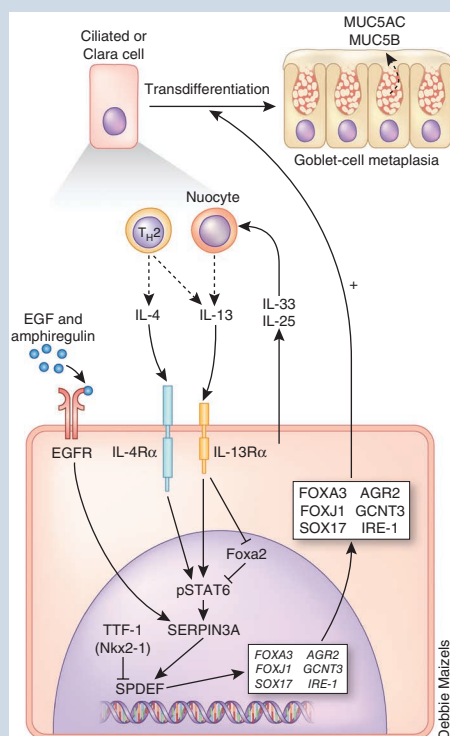
There is a link between altered epithelial barrier function and the induction of GCM. EGF is a key EC-derived cytokine that promotes GCM. EGF is normally secreted on the basolateral side of the epithelium and sequestered from its receptor on the apical side through intact adherens junctions and tight-junction barriers between ECs¹¹⁰. The EGFR is also activated by amphiregulin, which is released from damaged ECs¹¹¹. Conversely, it seems that epithelial-barrier repair mechanisms impede GCM. Trefoil factors are upregulated with damage and are important for epithelial repair in the gut mucosa. Deficiency of trefoil factor 2 increased GCM in a mouse model of chronic asthma¹¹². Increased production of mucus can, therefore, be seen as another form of compensation for a disrupted epithelial barrier.

The induction of GCM is regulated by cytokines secreted from cells of the innate and adaptive immune system (Fig. 3). GCM can be induced *in vitro* when air-liquid interface cultures of human bronchial ECs are exposed to the T_H2 -type cytokines IL-4 or IL-13 or when mice receive an intratracheal injection of IL-13 (refs. 113–115).

T_H2 effector cells are not the only source of IL-13; innate lymphoid cells (or nuocytes) can release IL-13 in response to EC-derived IL-33 and/or IL-25 as well. Similarly to ECs, innate lymphoid cells also release amphiregulin that can bind the EGF receptor and promote airway remodeling^{64,116}. Activation of the innate

BOX 2 Molecular control of goblet-cell metaplasia in asthma

The genetic program controlling lung development is well characterized. An emerging concept is that this same transcriptional network can also be turned on during adult life in response to lung injury or inflammation^{151,152}. This is illustrated by the molecular process controlling goblet-cell metaplasia (GCM) in asthma.



This increase is not due to proliferation of pre-existing goblet cells but rather to the transdifferentiation of ciliated and Clara cells to goblet cells, hence the term GCM. A key discovery was that IL-13, EGF and amphiregulin induce SPDEF in a process requiring serpin 3A (the mouse homolog of human serpin B3 and serpin B4)¹⁵³. Transgenic overexpression of *Spdef* in mouse Clara cells causes severe GCM and induces genes involved in epithelial- and goblet-cell differentiation and protein glycosylation, including members of the forkhead box family (*Foxa3* and *Foxj1*), *Sox17*, anterior gradient 2 (*Agtr2*) and *N*-acetyltransferase 3 mucin type (*Gcnt3*)¹⁵⁴; whereas deficiency of SPDEF abolished IL-13- and allergen-induced GCM¹⁵⁵. SPDEF binds Nkx2-1 (TTF-1), a developmental transcription factor involved in lung budding from the primitive foregut. The expression of Nkx2-1 is downregulated in mucosal biopsies of patients who have asthma, whereas transgenic overexpression of Nkx2-1 in mice inhibited GCM by suppressing the expression of SPDEF¹⁵⁶. *Foxa2*, another forkhead-box family transcription factor whose expression is restricted to bronchial ECs, also suppresses SPDEF and GCM¹¹⁷.

and adaptive arms of the immune system and induction of GCM are closely linked at the molecular level. The transcription factor Nkx2-1 suppresses the GCM master-switch transcription factor SAM pointed domain-containing Ets transcription factor (SPDEF) and suppresses the production of the proinflammatory chemokines CCL17 and CCL26 in ECs. Along the same lines, *Foxa2*, a forkhead box-family transcription factor involved in suppression of the GCM program, suppresses epithelial production of IL-13, IL-33 and the chemokines CCL20 and CCL17 (ref. 117). Inflammatory DCs accumulate in the lungs of *Foxa2*^{-/-} mice and promote T_H2 immunity¹⁵. These findings suggest that the epithelial GCM differentiation program is closely linked to the regulation of innate and adaptive T_H2 immunity and that these epithelial responses co-evolved as protective mechanisms to restore homeostasis in response to (infectious) insults. Protection from parasitic infection could have been the driving pressure behind this evolution. Helminth infection of the gut induces a similar program of goblet-cell metaplasia, which is driven by SPDEF and leads to changes in the composition of the mucus barrier, recruitment of inflammatory DCs, innate lymphoid cells and eosinophils, and a T_H2 response leading to parasite expulsion^{60,118–120} (Box 2).

The production of mucus is a complicated process involving multiple glycosylation reactions taking place within the endoplasmic reticulum (ER). Goblet cells in the gut activate the unfolded protein response (UPR) to deal with the increased biosynthetic demands of increased mucus production on the ER, leading to activation of the ER resident kinases inositol-requiring endonuclease-1 α (IRE-1 α) and IRE-1 β and of the downstream transcription factor X-box-binding protein-1 (XBP-1)¹²¹. IRE activity is also increased in lung ECs in response to GCM induction¹²², and downstream XBP-1 could influence the secretion of proinflammatory cytokines, as the XBP-1 and NF- κ B pathways act synergistically to activate inflammatory gene expression¹²³. The gene encoding ORMDL3 was identified as a susceptibility locus for childhood asthma¹²⁴. ORMDL3 is a regulator of the UPR that could modify IRE-1 activity and XBP-1 induction, but it remains unclear how this influences epithelial cell biology or the GCM differentiation program¹²⁵.

Viral exacerbations

Viral infection with rhinovirus is one of the most common triggers for asthma exacerbations, causing an increase in symptoms, mucus secretion and airway obstruction¹²⁶. Rhinovirus normally replicates only in the ECs of the upper airways, but bronchial ECs from the lower airways of people with asthma are uniquely sensitive to viral replication. Virus can be recovered from the deeper airways during an exacerbation. This greater sensitivity could be caused by reduced production of type I IFN- β and type III IFN- λ by ECs, resulting in defective EC apoptosis upon viral infection^{127,128}. More recently, studies have also focused on TLR3, a receptor for double-stranded RNA. IL-13 can inhibit production of double-stranded RNA (dsRNA)-induced IFN- λ by airway ECs, which contributes to the impaired antiviral response observed in individuals who have asthma¹²⁹. IFN- λ not only has antiviral effects but also controls allergic inflammation directly. When administered to mice, IFN- λ 2 (IL-28A) suppressed T_H2 cytokine release and protected mice from allergic airway inflammation, a result of its capacity to downregulate OX40L and promote IL-12 production in DCs¹³⁰. Viral infection or dsRNA exacerbate asthma by increasing the production of IL-8 and other chemokines that attract eosinophils, T_H2 cells and DCs^{131–133}. The epithelial barrier is also compromised after respiratory viral infection in a process that involves EGFR signaling and leads to enhanced leakiness and GCM⁹⁵.

Conclusion and therapeutic implications

Current standard therapy for asthma consists of inhaled corticosteroids combined with a long-acting bronchodilator. However, these treatments only manage symptoms and do not alter the natural course of disease. Although inhaled steroids have shown superior anti-inflammatory effects in clinical trials with carefully selected subjects, clinical experience with these drugs in asthma has shown that many patients do not respond favorably. Patients with a T_H2 signature in bronchial ECs that is characteristic of IL-13 activation and comprised of the genes encoding Serpin B2, periostin and CLCA1 respond best to inhaled steroids¹³⁴. This implies that biomarkers can be used to stratify patients before initiating therapy and increase the likelihood of a favorable clinical response using samples derived from the airways as well as from serum.

Finding new drugs that selectively inhibit crucial aspects of the epithelial-immune or the epithelial-mesenchymal interaction could potentially inhibit mechanisms driving sustained allergic inflammation. Therapeutic targeting of the cytokines involved in this network, such as TSLP, GM-CSF, IL-25, IL-33, IL-1, EGF, TGF- β and LIGHT, could reduce the risk of allergic sensitization and prevent airway remodeling. Given the importance of epithelial PRRs in promoting sensitization, therapeutic modulation of PRRs could also prove beneficial. Repeated mucosal delivery of TLR7 or TLR9 agonists has been shown to reduce airway eosinophilia and features of chronic airway remodeling in a process that resembles endotoxin tolerance^{135,136}. Chronic exposure to PRR agonists could also potentially reprogram ECs to react less to environmental triggers by engaging negative regulatory mechanisms within the PRR signaling cascade or by epigenetically silencing gene expression. It is also imperative that we find ways to restore the innate epithelial response to respiratory viral infection to prevent asthma exacerbations.

Fifteen years ago, Stephen Holgate was the first to suggest, on the basis of observations in asthma biopsies and cultured bronchial ECs¹⁰¹, that ECs are a major culprit in asthma. Since these initial studies, it is now clear that the airway epithelium controls many aspects of allergic sensitization and is an important player in allergic inflammation, remodeling and bronchial hyperreactivity. Thus, strategies aimed at targeting the ECs could prove beneficial in altering the natural course of disease and decreasing the burden of disease on patients.

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