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The sentinel role of the airway epithelium in asthma pathogenesis

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Summary: The adoption of the concept that asthma is primarily a disease most frequently associated with elaboration of T-helper 2 (Th2)-type inflammation has led to the widely held concept that its origins, exacerbation, and persistence are allergen driven. Taking aside the asthma that is expressed in non-allergic individuals leaves the great proportion of asthma that is associated with allergy (or atopy) and that often has its onset in early childhood. Evidence is presented that asthma is primarily an epithelial disorder and that its origin as well as its clinical manifestations have more to do with altered epithelial physical and functional barrier properties than being purely linked to allergic pathways. In genetically susceptible individuals, impaired epithelial barrier function renders the airways vulnerable to early life virus infection, and this in turn provides the stimulus to prime immature dendritic cells toward directing a Th2 response and local allergen sensitization. Continued epithelial susceptibility to environmental insults such as viral, allergen, and pollutant exposure and impaired repair responses leads to asthma persistence and provides the mediator and growth factor microenvironment for persistence of inflammation and airway wall remodeling. Increased deposition of matrix in the epithelial lamina reticularis provides evidence for ongoing epithelial barrier dysfunction, while physical distortion of the epithelium consequent upon repeated bronchoconstriction provides additional stimuli for remodeling. This latter response initially serves a protective function but, if exaggerated, may lead to fixed airflow obstruction associated with more severe and chronic disease. Dual pathways in the origins, persistence, and progression of asthma help explain why anti-inflammatory treatments fail to influence the natural history of asthma in childhood and only partially does so in chronic severe disease. Positioning the airway epithelium as fundamental to the origins and persistence of asthma provides a rationale for pursuit of therapeutics that increase the resistance of the airways to environmental insults rather than concentrating all effort on suppressing inflammation.

Keywords: asthma, epithelium, inflammation, remodeling, environmental injury, origins, progression, phenotypes

Introduction

Asthma is an inflammatory disorder of the conducting airways, which adopt a hyperresponsive profile leading to variable airflow obstruction both spontaneously and in response to a wide range of exogenous stimuli. Almost all asthma is characterized by infiltration of the airway walls with mast

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cells, eosinophils, and T-helper 2 (Th2)-type T cells that define the inflammatory response. Since exposure to and challenge with inhaled allergens in genetically at risk subjects as well as in sensitized animals evokes an almost identical Th2 type immune response in the respiratory mucosa, it has been widely assumed that the cellular pattern of chronic asthma is driven largely by allergen (1). Indeed, this concept has dominated modern thinking about the pathophysiology of the disease and the way anti-asthma drugs are selected and trialed in proof of concept studies, even though non-allergic human asthma is a clearly recognizable entity with almost identical pathology to the more classical allergic form of the disease (2, 3). While undoubtedly allergen exposure is one key driver of airway inflammation in asthma, asthma not associated with allergy both in adults and children has an almost identical pathological phenotype, thereby questioning the obligatory requirement of allergen sensitization for the immunopathology of asthma to occur. Similar issues arise when considering the role of immunoglobulin E (IgE) in asthma. IgE is the triggering antibody for the allergic response through activation of mast cells and basophils, and an association between serum total IgE or allergen-specific IgE and asthma at a population level is clearly established (4, 5). However, in certain types of asthma, e.g. late onset 'intrinsic' asthma or asthma triggered by some occupational chemicals, e.g. toluene di-isocyanate, as well as non-allergic asthma in children, Th2 inflammation occurs in the absence of increased circulating levels of IgE (6), although does not exclude increase mucosal 'local' IgE production (7).

Airway inflammation if fundamental to asthma pathogenesis

Airway inflammation in asthma is a multicellular process involving mainly eosinophils, neutrophils, CD4⁺ T lymphocytes, monocytes, mast cells, and basophils, with eosinophilic infiltration being the most striking feature (8). The inflammatory process is largely restricted to the conducting airways (i.e. the first 15 or so generations), but as the disease becomes more chronic, the inflammatory infiltrates spread both proximally to involve the trachea and larynx and distally to the small airways and occasionally adjacent alveoli (9). The inflammatory response in the small airways appears to be predominantly outside the airway smooth muscle, whereas in the large airways, inflammation of the submucosa dominates (10). Factors that determine the pattern of airflow obstruction, responses to treatment, and natural history of asthma are highly variable, giving rise to the concept of disease heteroge-

neity. In carefully phenotyped asthmatic populations, cluster and factorial analyses are revealing different subtypes of disease that extend beyond the simple classifications of allergic or non-allergic and mild, moderate, and severe (11, 12). With this in mind, it is increasingly likely that multiple manifestations of asthma exist and that response to treatments will differ. This leads to the concept of stratified or personalized medicine as a future therapeutic approach to chronic and difficult asthma with a view of identifying key pathways involved in the different forms of the disease (13).

The role of atopy in asthma

A fundamental feature of asthma associated with allergic sensitization is the ability of the airway to recognize common environmental allergens and to generate a Th2 cytokine response to them (14). Recognizing that in excess of 40% of the western population is atopic (i.e. have elevated IgE to common environmental allergens), only about 7% of these express their atopy in the form of asthma (15). An important question to ask is why in some patients atopy expresses itself in the conducting airways as the disease asthma, whereas in others in whom an equivalent degree of atopy maybe present, asthma does not occur or the atopy manifests in other ways such as rhinoconjunctivitis, food allergy, atopic dermatitis, or rhinoconjunctivitis. To explain this paradox, there must be important factors that translate the atopic phenotype to manifest in the lower airways as Th-2 type airway inflammation. Careful analysis of birth cohort studies reveal that atopy has little effect on the occurrence of childhood wheezing in the first 3 years of life, but by 5 years, atopy takes over as a major driver for persistent childhood wheeze in parallel with acquisition of specific sensitization to aeroallergens such as those from dust mites, animal danders, and pollens (16, 17).

Early onset atopy is also a powerful driver for acquisition of childhood asthma. In a population-based birth cohort commenced in 1995 in Manchester, UK (MAAS), 1185 newborn children were recruited *in utero* and reviewed for the occurrence of atopy and respiratory symptoms and lung function at age 1, 3, 5, and 8 years. One thousand and thirty (87%) children were available for review at age 8 years, and skin prick tests and IgE to eight common inhalant and food allergens were measured at each follow-up visit (18). Using non-supervised clustering, it was early within the first 3 years of life sensitization to multiple allergens that afforded by far the greatest risk for developing asthma at aged 8 years rather than atopy as a general trait (odds ratio 29.3, 95% CI 11.1–77.2). It also suggests that early life events are critical for the

induction of asthma in childhood. Research should be focused on what is different about the early sensitized children compared with those who develop atopic markers later in childhood (19).

From a mechanistic standpoint, early aeroallergen sensitization presumably indicates a series of early life cellular events in the airways mucosa to initiate the recruitment of Th2 cells with their capacity to secrete pro-allergic cytokines and chemokines accompanied by isotype switching of B cells to IgE synthesis. It is well known that these events requires allergen uptake and processing by a subpopulation of mucosal dendritic cells (DCs) with subsequent peptide epitope presentation to naive T cells in association with major histocompatibility complex class II (20). Of special interest is the propensity of biologically active allergens with proteolytic activity such as those derived from house dust mites (*Dermaphagoides pteronyssinus* and *D. farinae*), pollen grains, and fungi (21) as well as certain occupational sensitizers, e.g. enzymes used in detergents (22), to cleave protease activated receptors on DCs (23) and disrupt epithelial tight junctions (TJs) (24).

Dust and airborne particulates contain components from microorganisms such as cell wall and nucleic acid fragments that act through a growing number of pattern recognition receptors including Toll-like receptors (TLRs) on DCs (25) to enhance and direct their response by serving as 'danger' signals that can influence the balance of T cells toward Th1, Th2, Th17, or T-regulatory (Treg) subtypes (26). Microbial products also activate TLR3, TLR4, TLR7, and TLR8 on airway epithelial cells, leading to the release of the interleukin-7 (IL-7)-like cytokine, thymic stromal lymphopoietin (TSLP) (27) that, on interacting with its receptor on DCs, upregulates their expression of the costimulatory molecules CD40, OX40, and CD80 to enhance Th2 polarization (28) as well as activating mast cells for selective cytokine secretion (29).

Transition from airway inflammation to structural changes

As in other chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis, enhanced immune and inflammatory responses in asthma are associated with structural changes (remodeling) in the target tissue that have an important influence over disease expression (30) (Fig. 1). In the case of asthma, the extent of inflammation and structural changes seen in the airways are related to disease severity but not on the presence or extent of atopy or duration of symptoms (31). In early life, the primacy of airway inflam-

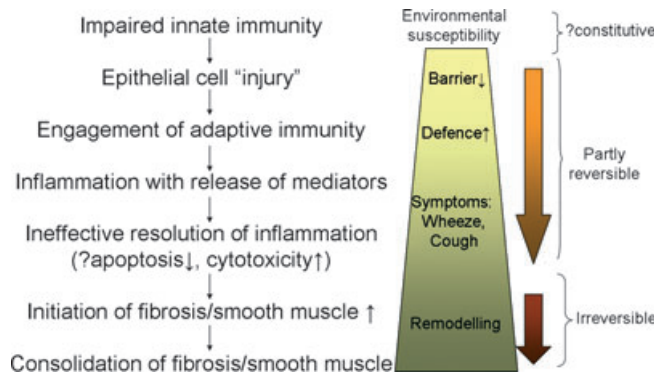


Fig. 1. Pathophysiological processes implicated in the life course of asthma. Combinations of these give rise to the different asthma phenotypes and responses to interventions.

mation as being fundamental to the origins of asthma is being challenged with the discovery that at its onset, asthma is associated with the presence of structural changes in the airways often in the relative absence of airway inflammation (32–34). Thus, the increasingly popular concept of 'remodeling' of the airways as observed in chronic adult asthma has marked similarities with airway modeling during branching morphogenesis in the fetal lung and aberrant modeling linked to the origins of asthma (35, 36). Indeed, interactions between organ morphogenesis and wound healing responses have lead to a new concept of chronic airway inflammation being supported by the structural components of remodeling through activation of the epithelial-mesenchymal trophic unit (EMTU) (37) (Fig. 2).

Stated differently, the tissue environment provided by an activated EMTU in asthma has the capacity to provide the 'soil' for the Th2-related inflammation ('seed') to persist. The interdependency of structure, altered immunity, and inflammation involving the epithelium, fibroblasts, myofibroblasts, smooth muscle and their secreted matrix, microvasculature, and neural networks helps explain why chronic airway inflammation could persist in the absence of obvious environmental stimuli and, at the more severe and chronic end of the asthma spectrum, for the incomplete therapeutic response to anti-inflammatory drugs (38).

The epithelium as in the orchestration of the asthmatic response

Building further on this concept, a new paradigm for persistent asthma is emerging of a damaged epithelium that repairs incompletely and leads to a chronic wound scenario with the secretion of a range of growth factors capable of driving structural changes linked to airway remodeling (39–41).

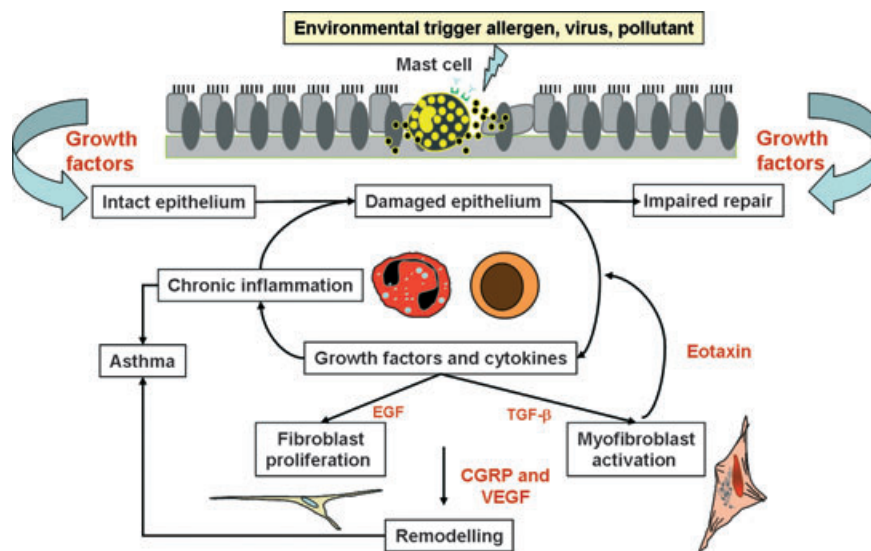


Fig. 2. The relationship of epithelial-mesenchymal communication (epithelial-mesenchymal trophic unit) to persistent inflammation and airway wall remodeling. EGF, epidermal growth factor; TGF, tumor growth factor; CGRP, calcitonin gene related peptide; VEGF, vascular endothelial growth factor.

The human lung is the largest surface that is in continuous contact with the outside world. It presents an estimated area of 100 m² that comes into contact with approximately 10 000 l of inhaled air every day containing numerous potentially harmful physical, chemical, and biological agents. The lung epithelium is the interface with which this inhaled environment interacts and when functioning normally forms an efficient physical barrier complemented by the mucociliary escalator to provide a first line of defense (42). The stratified epithelium of the major conducting airways is covered by surface liquid approximately 10 µm thick, which comprises a periciliary layer around the microvilli and an overlying mucus layer onto which inhaled particles become trapped.

Under normal circumstances, the epithelium containing ciliated columnar, mucus-secreting goblet and surfactant secreting Clara cells forms a highly regulated and impermeable barrier made possible through the formation of TJs localized the apical aspect of the columnar cells and a series of other adhesion mechanisms along the basal and basolateral epithelial cell borders. TJs are comprised of a series of interacting proteins and receptors including ZO 1–3, occludin, claudins 1–5, as well as trans-membrane adhesion proteins (β-catenin, E-cadherin, and JAM) that enable communication between adjacent cells as well as regulating intercellular transport (43, 44) (Fig. 3). Structural integrity of the epithelium is further maintained through cell–cell and cell extracellular matrix interactions involving adherens junctions, cell adhesion molecules, desmosomes and hemidesmosomes (45). It

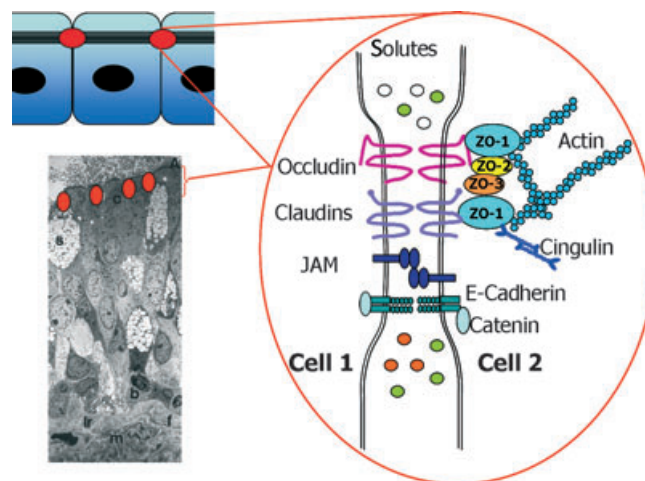


Fig. 3. Schematic representation of principle components that comprise the epithelial tight junctions.

follows that disruption of the columnar epithelium by breaking TJs or not reforming them efficiently enables tissue-damaging agents (e.g. air pollutant particles, tobacco smoke particles) and infectious particles to penetrate the airway wall to facilitate toxic, immune, and inflammatory responses and accompanying tissue damage (24, 46, 47).

In asthma there is evidence *in vivo* and *in vitro* that the barrier function of the airways epithelium is impaired (48). Although epithelial damage in asthma has been considered as being artefactual (49), molecular based biomarker studies have found evidence for a stressed and damaged airway epithelium both in established asthmatic of adults (50) and children

(51). Using immunohistochemistry applied to thin resin-embedded sections and whole airway tissue mounts viewed by confocal microscopy, epithelial TJs in asthma are severely disrupted (41, 52) (Fig. 4). When compared to normal epithelium cultured *in vitro* and differentiated at an air-liquid interface (ALI), the asthmatic counterpart is unable to fully form TJs, even though the cells have been passaged several times since being brushed from the airways and therefore separated from any airway inflammatory cells or mediators for many weeks. When fully differentiated, measurement of transepithelial resistance (TER) across the epithelium as well as increased transfer of high molecular weight markers at baseline indicates marked 'leakiness' (41). Furthermore, apical exposure of the asthmatic cultures to injurious agents such as tobacco smoke extract resulted in a further reduction in TER at much lower concentrations than was required to achieve a similar response in normal epithelium.

Since, many of the injurious chemical, particulate, and biological insults to the airways mediate their tissue-damaging effects through the generation of reactive oxygen species, the normal airway epithelium is well equipped with anti-oxidant enzymes as well as active radical traps that helps maintain integrity and resist injury (53–55). In asthma the anti-oxidant pathways such as superoxide dismutase and glutathione peroxidase are defective (56–58) as well as exhibiting polymorphic genetic association especially in relation to oxidant

exposures such as air pollution and environmental tobacco smoke (59). Both in asthmatic biopsies and in tissue culture, there is evidence of increased basal apoptosis assessed as tunnel staining, annexin V expression or activation of the caspase cascade (60, 61).

For many years 'epithelial desquamation' has been described as a characteristic pathological feature in asthma death (62, 63), but until recently, its significance has not been appreciated. Bronchial biopsy studies from patients with asthma of increasing severity not only demonstrates physical damage to the columnar cell layer but also evidence for injury through the expression of cell stressors such as heat shock protein 70 (64), evidence for activation of the caspase enzyme cascade involved in apoptosis (60, 61), and enhanced surface expression and phosphorylation of epidermal growth factor receptors (EGFRs) (50, 65, 66). These markers of stress and injury expressed even in the mildest forms of adult asthma but also increase in proportion to disease severity and chronicity. Almost identical changes have been observed in moderate and severe childhood asthma (51), suggesting that epithelial injury and aberrant repair is involved in the origins of the disease and not simply present as a consequence of longstanding inflammation. Under normal circumstances, injury to the airways epithelium should stimulate the intrinsic repair pathways with engagement of EGFRs by autocrine secretion of appropriate ligands (EGF, amphiregulin, and HB-EGF) to drive

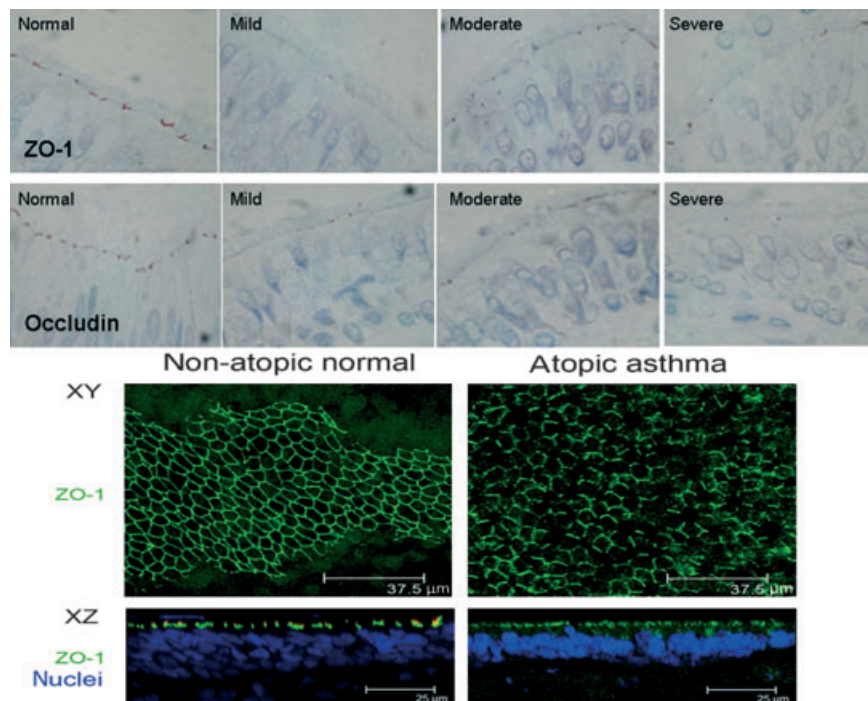


Fig. 4. Immunohistochemical demonstration of tight junctions in normal and asthmatic bronchial biopsies (top – glycol methacrylate 2 μ m sections, bottom – confocal microscopy).

cell migration, proliferation, and differentiation - all features of a primary mucosal repair response (healing by 'primary intention') (67).

In asthma, impaired proliferation of basal and Clara cells is revealed as reduced nuclear expression of cell cycle markers such as Ki67 and proliferating cell nuclear antigen and increased nuclear expression of the cell cycle inhibitors P21^{waf} (51, 68). It is proposed that this incomplete repair response to injury is what leads to the chronic wound scenario, i.e. healing by 'secondary intention'. This idea has received recent support from studies in mice in which acute Clara cell ablation caused squamous metaplasia of ciliated cells and induced proliferation of residual progenitor cells, while ciliated cells in the bronchioles and pro-surfactant protein C-expressing cells in the bronchiolar alveolar duct junctions failed to proliferate (69). Epithelial cell proliferation occurred at multiple sites along the airways and was not selectively associated with regions around neuroepithelial bodies. With chronic Clara cell depletion in mice, there occurred ineffective repair and associated with peribronchiolar fibrosis resulting from proliferation in subepithelial fibroblasts. This appearance resembles that of the asthmatic airway (see below). An abnormally functioning epithelium in asthma is also the source of a wide range of cytokines and chemokines as well as autacoid mediators that include eicosanoids (PGE₂, 15-HETE) (70, 71). Airway epithelial cells cultured from the airways of children with asthma demonstrate increased production of mediators and cytokines which, in being preserved in culture after several passages, indicates their adoption of a secretory profile intrinsic to the origin of the disease rather than occurring secondary to airway inflammation (72, 73).

Kicic (74) has also suggested that the impaired epithelial wound healing response observed *in vitro* with monolayers of epithelial cells from the airways of asthmatic children results in part from reduced fibronectin production, a key molecule for epithelial cell migration and effective proliferation (75). Possible mechanisms accounting for these intrinsic differences between asthmatic and normal airway epithelial cells include primary genetic, secondary genetic (e.g. somatic mutations), or epigenetic (e.g. altered miRNA, histone acetylation/deacetylation, or DNA CpG methylation) changes to epithelial cell control pathways.

Asthma-related changes to the epithelial basal lamina

In adults and children a highly characteristic feature of asthma is the hyalinization and thickening of the basal lamina (*lamina reticularis*) beneath a seemingly 'normal' epithelial basement membrane (76) (Fig. 5). This unique pathological change in

asthma is accompanied by an increase in the number and activity of subepithelial myofibroblasts with their capacity to lay down new matrix including tenascin C, fibronectin, and types I, III, and VI collagens (77, 78). What is less clear is whether the accumulation of repair-type matrix proteins precedes or is intrinsic to the thickening of the *lamina reticularis*. That these changes are also seen in childhood asthma close to disease inception (32, 34, 79, 80) indicates that they probably occur as part of the initiation of asthma and are not secondary to the influence of chronic inflammation.

It is worth noting that this change to the basal lamina only occurs in one other airway disease, *bronchiolitis obliterans syndrome* associated with a cytotoxic T-cell attack on small airway epithelial cells in lung allograft rejection (81). Airway biopsies obtained from infants with severe intermittent wheezing reveal little difference in the *lamina reticularis* from that of normal airways, but after the age of about 3 years, the characteristic asthmatic change is seen (79). A detailed electron microscopic assessment of the basal lamina in adult and children's asthmatic and normal epithelium showed that collagen fibrils in the basal lamina were significantly thinner and fewer of the fibrils were banded than observed in the interstitial collagen of the adjacent submucosa, suggesting a different matrix structure (82). The ratio of fibrils to matrix in the thickened basal lamina of asthmatics also did not differ from that of their respective controls (82). This finding suggests that thickening of the *lamina reticularis* precedes the laying down of repair collagens as observed in adult asthma.

The mechanisms involved in this modeling/remodeling of the *lamina reticularis* is not known, although, in a range of animal models, similar changes can be induced when the epithelium is chronically injured or is transfected with profibrotic cytokines such as IL-11 and IL-13 to stimulate the release of

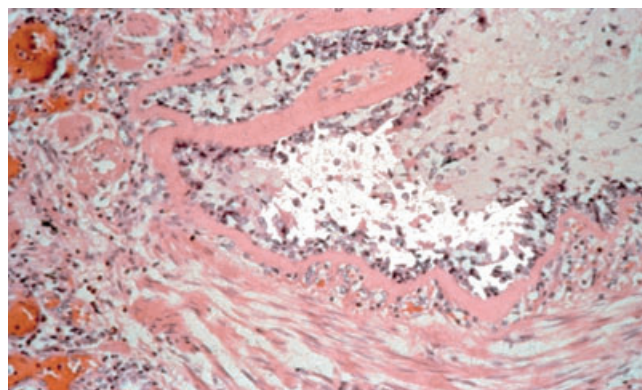


Fig. 5. Cross-section of asthmatic airway showing thickened subepithelial *lamina reticularis* and a mucus plug occluding the airway lumen.

growth factors such as TGF- β (83, 84). The airway epithelium also has the capacity to generate EGF (85), HB-EGF (86), amphiregulin (87), fibroblast growth factors, platelet-derived growth factors (88), insulin-like growth factors (89), neurotrophins (e.g. NGF) (90), and vascular endothelial growth factors (VEGFs) (91), all of which are capable of interacting with structural cells to either cause their proliferation and/or differentiation. Because these growth factors are over-expressed in the airway epithelium of patients with active asthma, they are well placed to contribute to the remodeling of the epithelial *lamina reticularis* in asthma (92, 93). Factors that are known to augment the enhanced secretion of these growth factors include mechanical stress (94, 95), chemical and physical injury (92, 96) virus infection (91, 97), and interactions with inflammatory leukocytes (98–100).

Interactions between eosinophils and the epithelium

Eosinophils are considered to be fundamental to the inflammatory response of asthma and in many ways are used as a biomarker of the disease, e.g. in induced sputum. There is an extensive literature incriminating eosinophils in epithelial injury through the release of their cytotoxic granule-derived basic proteins and generation of reactive oxygen (reviewed in 101). IL-5, in particular, is a Th2 cytokine that drives eosinophil differentiation, maturation, and supports their survival (i.e. anti-apoptotic). However, despite having a major impact in reducing circulating and sputum eosinophils, systemic administration of the anti-IL-5 blocking humanized IgG monoclonal antibody mepolizumab to patients with asthma only reduced airway tissue eosinophils by approximately 50% (102). It was suggested that this incomplete response might be due to loss of IL-5 receptors from a proportion of the eosinophils as they entered the airways from the circulation and, as a consequence, losing their dependence upon IL-5 for survival (103, 104).

There is also evidence that in asthmatic airways enhanced secretion of GM-CSF by epithelial cells, fibroblasts, smooth muscle, as well as inflammatory cells is an alternative pathway for prolonging eosinophil survival (105). In a separate study, anti-IL-5 also had no overall effects on the allergen-induced early or late phase responses, methacholine bronchial hyperresponsiveness, or symptoms and baseline lung function manifestations of chronic asthma (106). It is worth noting, however, that three infusions of mepolizumab over a period of 10 weeks resulted in a marked reduction in immunoreactive of tenascin, collagen III, and lumican present in the *lamina reticularis* (107), suggesting that eosinophils contribute to

remodeling of the *lamina reticularis*. It seems increasingly likely that eosinophils are capable of driving remodeling of this structure but, based on the studies in infants with early onset asthma, not in the initiation of its thickening (82). Thickening and hyalinization of the *lamina reticularis* of the epithelial basement membrane is also a feature in cough-variant asthma (eosinophilic bronchitis) (108) indicating that while being a marker of epithelial injury and aberrant repair, this pathological feature of asthma need not be associated with bronchial hyperresponsiveness and variable airway obstruction that are defining features of classical asthma. An important issue that requires careful study is whether the altered *lamina reticularis* in asthma can be extrapolated to remodeling of the entire airway wall. Asthma in remission is accompanied by loss of markers of eosinophil activation but the basement membrane changes persist (109).

Remodeling of the airway wall in asthma progression

High resolution computed tomography (HRCT) has revealed substantial evidence that chronic and severe asthma in children and adults is associated with thickening of the airway walls (110, 111). Pathological analysis has also shown the deposition of matrix proteins such as fibronectin, collagens I, III, and VI as well as the proteoglycans biglycan, lumican, and versican in the submucosa as well as an increase in smooth muscle that are in large part responsible for airway wall thickening (112, 113). Using either HRCT (114) or an ultrasound bronchoscopic probe (115) to examine airway dimensions, bronchial hyperresponsiveness to methacholine appears to be inversely related to airway wall thickness. This might be interpreted in some asthmatics as a compensatory response to maintain airway patency in the presence of repeated bronchoconstriction, whereas in others in whom hyperresponsiveness persists, it is thickening of smooth muscle that dominates. It follows that in highly unstable or 'brittle' asthma absence of airway wall remodeling will lead to rapid onset and unopposed bronchoconstriction that can be catastrophic (105).

Over time, exuberant airway wall collagen deposition in those patients where this dominates the response could be an important factor responsible for irreversible airflow obstruction and an accelerated decline in lung function that has been observed in some patients with chronic severe asthma. This possibility has recently been confirmed in asthmatics *in vivo*. It has been largely assumed that restructuring of the airway wall in asthma is driven predominantly by inflammation. Acute allergen challenge causing early and late phase bronchoconstriction is associated with deposition of new matrix beneath

the epithelium that can be partially inhibited by blocking the action of IL-5 (107). However, recently we have shown that deposition of matrix at this site as well as secretion into the airways of TGF- β is no different whether the equivalent amount of bronchoconstriction is produced by allergen or methacholine (116). Moreover, if the airway narrowing provoked by these challenges is prevented by prior administration of the bronchodilator albuterol, the remodeling markers are inhibited. This leads to the conclusion that airway narrowing irrespective of cause is a sufficient stimulus for initiating remodeling and suggests that in addition to current advice on controlling airway inflammation in asthma management, maintaining airway patency, and preventing bronchoconstriction, e.g. with a long-acting β_2 -agonist, might also be an additional therapeutic objective.

While resident airway fibroblasts are likely to be the principle drivers of overall airway wall remodeling, the recent description of epithelial cells themselves undergoing transdifferentiation into fibroblasts by 'epithelial-mesenchymal transition' driven by profibrotic cytokines such as TGF- β or that primitive 'fibrocytes' or mesenchymal stem cells may be recruited from CD34⁺ progenitors in the bone marrow via the circulation into the asthmatic airway (117, 118) provide new mechanisms to help explain airway wall remodeling. While there is strong evidence for epithelial-mesenchymal transition in interstitial pulmonary fibrosis (119) and in animal models of pulmonary fibrosis (120), evidence that this mechanism is a major driver of remodeling in asthma is not strong (121, 122). It seems more likely that resident airway fibroblasts are the most likely source of the remodeling response with these cells being especially sensitive to both spontaneous and growth factor (e.g. TGF- β) and cytokine (e.g. IL-13) transition into collagen secreting myofibroblasts (123–125).

Whatever the underlying mechanisms for remodeling in chronic asthma it is important to emphasize that this is a complex process that also involves microvascular proliferation as a key component. Members of the VEGF family of growth factors from injured epithelial and activated inflammatory cells are strong candidates for driving this component of remodeling (126, 127). However, following allergen exposure abnormally high levels of calcitonin gene related peptide (CGRP) have also been found in the airways in association with the late phase of bronchoconstriction (128). CGRP is a potent stimulus of microvascular leakage and endothelial proliferation (129). A dimer comprising the receptor activity modifying protein 1 (RAMP1) and the calcitonin receptor-like receptor combine to form the CGRP1 receptor. RAMP1 has been shown to be highly expressed in airway epithelial cells

which, upon CGRP binding to CGRP1, results in internalization of RAMP1 and enhanced IL-6 production, both of which are inhibited by the CGRP antagonist, CGRP (8–36) (130). The immunoreactivity and RAMP1 mRNA expression in the epithelium of bronchial biopsies from subjects with asthma were significantly lower than in normal subjects. Additionally, inhalational challenge of atopic subjects with asthma with allergen-derived peptides led to a decrease in RAMP1-positive epithelial cells, but only in those who developed a lower airways bronchoconstrictor response. It is suggested that RAMP1 is dysregulated in the asthmatic epithelium, suggesting continuous stimulation of pathways involving CGRP. The novel asthma susceptibility gene, ADAM33, is also associated with asthma chronicity and severity (131, 132), and its soluble secreted form has been shown as a powerful stimulus for microvascular proliferation (133).

Altered mucus production: a key feature of asthma

An important and consistent feature of chronic asthma is the production of excess and altered mucus that blocks peripheral airways and is difficult to expectorate (Fig. 5). In asthma there is overwhelming evidence for goblet cell metaplasia involving the conducting airways (134), but in chronic persistent disease, goblet cells also spread down to the more peripheral airways where they normally do not exist (135, 136). Submucosal glands in asthma are also larger and contain a greater proportion of mucin as compared to serous cells. In more severe disease the submucous glands contain increased numbers of myoepithelial cells containing contractile proteins to provide structural support to mucous cells and actively facilitate glandular secretion (137). The net result is the secretion of abnormally large amounts of the highly viscous mucins 5AC and 5B (138), which, together with DNA and eosinophil basic proteins, contribute to the tenacious and viscous mucus characteristic of asthma (139). Genetic association studies reveal evidence for the role of four highly polymorphic genes (MUC-6, -2, 5AC and 5B) encoding gel forming mucins on chromosome 11p15.5 (140). The recognition that IL-4, IL-9, and IL-13 not only induce selective mucin gene expression in airway epithelial cells but also the enhanced expression of the calcium-activated chloride channel 1 (hCLC A1; Gob 5) (141). This channel is tightly associated with goblet cell differentiation and increased mucus secretion (142, 143).

Factors contributing to goblet cell metaplasia have been extensively studied and include signaling pathways involving EGF, Notch, and IL-4/IL-13 receptors that control genes that

drive mucus cell differentiation. In the mouse, mucus metaplasia is dependent upon the expression of the transcription factor Sam pointed domain Ets-like factor (Spdef) (144, 145), which in turn is induced by airways exposure to aeroallergens and IL-4 or IL-13. The ensuing increase in goblet cells and mucus secretion is also linked to the loss of expression of thyroid transcription factor-1 [(TTF-1) or Nk2 homeobox1 (Nkx2-1)]. Normally, TTF-1 expression is localized to type II alveolar cells, where it regulates surfactant protein expression (146, 147). In mice, mucus metaplasia following aeroallergen sensitization and repeated challenge was markedly reduced when Nkx2-1 was conditionally expressed in the respiratory epithelium (148). This suggested that mucous metaplasia followed downregulation of TTF-1 and parallel upregulation of Spdef. Conversely, with allergen challenge the number of goblet cells increased in the airways of heterozygous TTF-1^{+/-} gene-targeted mice. Genome-wide transcription analysis of antigen challenged lungs also revealed that increased TTF-1 expression inhibited signals linked to Th2-type inflammation and mucus metaplasia, including CCL17, IL-4/IL-13, and Spdef (144). Moreover, ectopic expression of TTF-1 in epithelial cells *in vitro* directly inhibited Spdef, while siRNA downregulation of TTF-1 induced expression of Spdef and Th2 signals. We have recently found almost identical findings of reduced TTF-1 and increased expression of Spdef in asthmatic epithelial cells and in the epithelium of airway biopsies (149). Taken together, these observations point to an integrated gene network in the asthmatic epithelium involving critical tran-

scription factors such as TTF-1 and Spdef that are capable of controlling both mucous cell differentiation as well as initiating Th2-type inflammation (Fig. 6).

The epithelium and its role in controlling inflammation

While, considerable advances continue to be made into the immunological and inflammatory components of asthma, increasingly the airway epithelium is being seen as both a functional component of the chronic disease. Increased susceptibility to injury and altered repair and considered to be important in both the airway remodeling and mucous metaplastic responses of chronic asthma. The asthmatic airway epithelium is a major source of other cytokines and chemokines including IL-13, TARC, IL-33, RANTES, eotaxins, and MCP-3. TSLP is unique in being such a powerful stimulus of Th2 immune polarization (150) (Fig. 6). The recognition of TSLP generated in large amounts by airway epithelial cells in response to selective TLR stimulation is a good example of how the epithelium can provide a microenvironment to sustain ongoing Th2-like inflammation (150, 151). Following allergen challenge and other types of epithelial injury, both CCL17 and CCL22 are key cytokines that drive Th2-type T-cell chemotaxis by interacting with the common CCR4 receptor (152). Greater than 90% of the Th2 chemoattractant capacity of isolated asthmatic mucosal explants exposed to specific allergens can be explained by activation of the CCR4 receptor making this an attractive therapeutic target (153, 154).

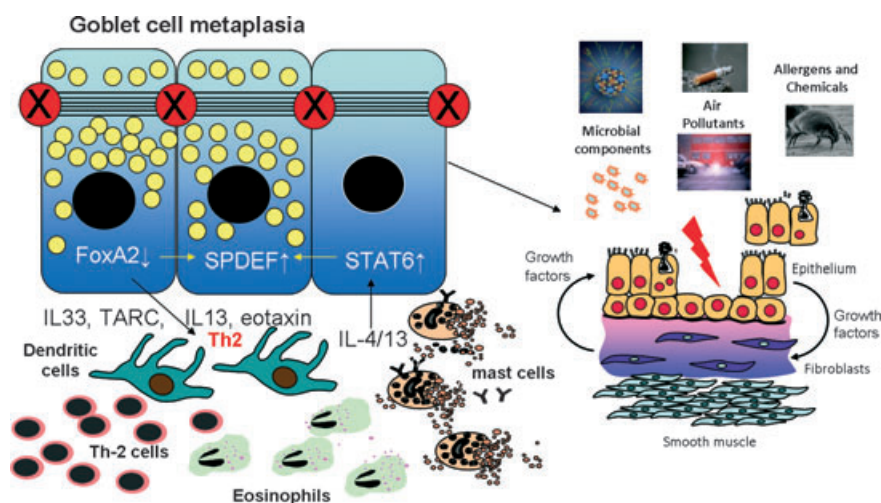


Fig. 6. Schematic representation of the sentinel role of the airway epithelium in coordinating mucous metaplasia and chronic airway inflammation. It is proposed that multiple environmental factors interact with an epithelium whose set point is altered in asthma to generate an exaggerated response to injury and impaired repair. To create this response, a network of transcription factors including FoxA2 and Spdef direct the epithelium toward a Th2 immune response, goblet cell differentiation, and impaired innate immunity. In this way, the epithelium orchestrates much of the disordered pathology and airway dysfunction in chronic asthma. Spdef, Sam pointed domain Ets-like factor; Th2, T-helper 2.

Virus infection and asthma

In addition to allergen and air pollutants, the asthmatic airway is particularly susceptible to respiratory virus infection as a cause of 40–80% of asthma exacerbations both in children and adults (reviewed in 155). Of particular significance is the finding in asthma that the common cold viruses (e.g. rhinovirus), which usually causes only upper respiratory tract symptoms, causes exacerbations especially in the spring, fall, and winter months. These exacerbations can be prolonged and severe, are associated with neutrophil as well as eosinophil influx into the airways (156, 157), are refractory to the commonly used practice of doubling the dose of inhaled corticosteroids (158, 159), and are associated with persistence of virus in the respiratory tract (160). Bronchial biopsy and lavage has revealed that in asthma human rhinovirus preferentially infects airway epithelial cells utilizing the ICAM-1 and LDL receptors to gain entry in the case of the major and minor virus subtypes, respectively (161). A crucial question is why the epithelium in asthma is so susceptible to infection with respiratory viruses.

The ability to culture asthmatic epithelial cells *in vitro* either as confluent monolayers or differentiated at an ALI has revealed impaired virus elimination and increased cytotoxic cell death as a consequence of defective interferon (IFN)- β and IFN- λ production (162, 163). IFN- β and IFN- λ are cytokines responsible for initiating apoptosis and viral clearance by normal epithelial cells as well as activating a large range of protective antiviral pathways (164). One possibility to account for these findings that persist in epithelial cultures many days after the cells have been brushed from asthmatic airways is that a genetic deficiency in IFN production may underlie the increased susceptibility of the asthmatic airways to common cold virus infection (Fig. 7). This idea is further supported by showing in asthmatic children that impaired IFN signaling is a feature of airway cells obtained from sputum (165). Most recently we have shown that when compared to normal epithelial cells, those from asthmatic airways release more pro-inflammatory mediators (166) that are selectively suppressed *in vitro* by exogenously added IFN- β (167).

One interesting idea that may, in part, account for the onset of asthma in early life in genetically susceptible children is that the airway epithelium is fundamentally abnormal both in its to environmental insults such as virus infection followed by emergence of an asthma phenotype. A study (COAST) in children born of asthmatic and atopic parents has shown that those who develop more persistent wheezing at the age of

4 years are the same children whom had frequent symptomatic virus infections in early infancy. Of importance in this study is the primacy of rhinovirus in dominating in the emergence of the asthma phenotype, particularly rhinovirus (168). Impaired innate immunity, possibly as a result of a genetic defect in IFN production in response to virus infection, may initiate the chronic injury-repair cycle associated with the onset of chronic disease (38). Early life rhinovirus-induced wheezing illness is also associated with the preferential development of allergen sensitization both in magnitude and range (169). Since the airways at birth are essentially devoid of DCs with recruitment and programming being stimulated by exposure to microorganisms including viruses (170, 171), it seems possible the lack of primary IFN production when lower airway epithelial cells first encounter common respiratory viruses such as rhinovirus could be a factor that deviates DC differentiation away from anti-microbial protection more toward the pro-allergic pathways (172). This would place a two hit model at the center of asthma pathogenesis is involving viral infections followed by allergen sensitization (173) (Fig. 8). Such a model helps explain that early life multiple allergen sensitization is strongly driven by frequent rhinovirus exposures manifesting in the lower respiratory tract very early in life (174).

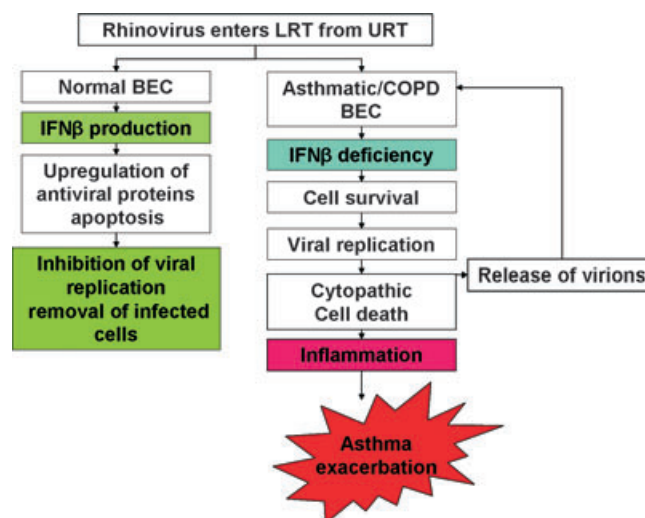


Fig. 7. The airway epithelium is the principle target for common respiratory viruses such as rhinovirus. Rhinovirus infection of the upper respiratory tract (URT) is followed by infection of the lower respiratory tract (LRT). Entry of virus into normal epithelial cells initiates a vigorous innate immune response through Toll-like receptor driven induction of primary interferons such as IFN- β which in turn sets in train antiviral defense and initiates apoptosis. In asthma, this primary interferon response is attenuated resulting in viral replication, cell cytotoxicity, enhanced virion shedding, and mediator release associated with an asthma exacerbation.

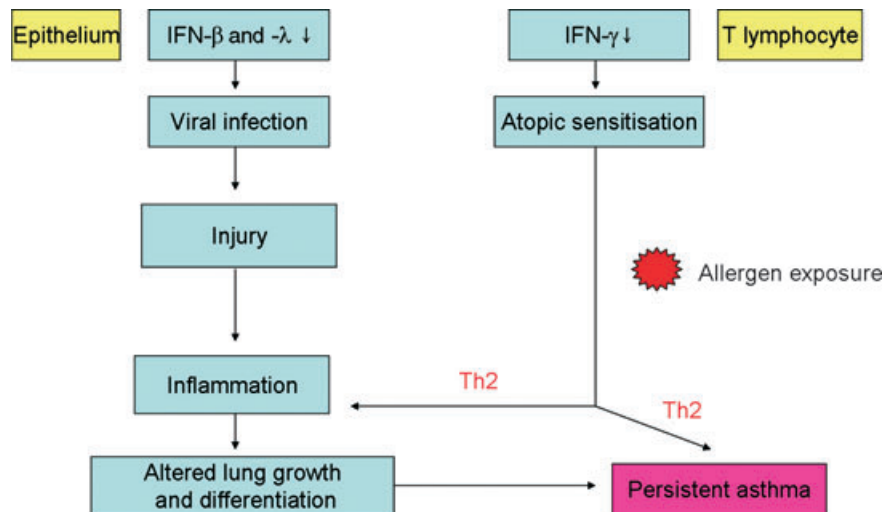


Fig. 8. 'Two hit' model for the induction of asthma in childhood. In genetically susceptible individuals, impaired interferon defense leads to exaggerated lower respiratory tract responses in early life that provides the 'danger signal' for maturation and activation of dendritic cells toward allergen sensitization and Th2-mediated inflammation characteristic of asthma. Th2, T-helper 2.

Concluding comments

While asthma is an inflammatory disorder of the conducting airways, inflammation itself does not explain the origin(s) of this disease nor why the airways are so susceptible to a range of different environmental factors. Placing the airway epithelium at the center of asthma origin, progression, and exacerbation is a paradigm shift away from considering Th2-type inflammation as primary more toward defects in epithelial innate immunity and responses to injury. This conceptual change in asthma pathogenesis raises the interesting possibility that the susceptibility to this disease in its various forms is dictated by disordered epithelial signaling pathways that not

only drives susceptibility to environmental insults, but the inflammation and remodeling responses that follow. In terms of the origins of childhood asthma, defective innate immunity against common rhinoviruses may be especially important in setting the stage for allergen sensitization. Equally, defective innate immunity maybe the principle factor behind asthma exacerbation beyond exposure to allergens. Directing therapeutics more toward raising the airways' resistance to environmental insults rather than concentrating all efforts on suppressing adaptive immunity and its associated inflammatory response might raise the stakes of treating asthma to one of curing this disease.

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