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Epithelium dysfunction in asthma

Stephen T. Holgate, MD, DSc *Southampton, United Kingdom*

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Although asthma is an inflammatory disorder of the conducting airways involving T_H2-type T cells, there is increasing evidence for an important role played by the epithelium in orchestrating the inflammatory response by interacting with multiple environmental factors to produce a chronic wound scenario involving tissue injury and aberrant repair. Part of this abnormal response is the consequence of impaired barrier function caused by a primary disruption of epithelial tight junctions that allows inhaled substances to pass more easily into the airway wall to interact with immune and inflammatory cells. Aberrant communication between the damaged and stressed epithelium leads to the generation of growth factors that interact with the underlying mesenchyme to promote airway remodeling responses and a more chronic and persistent inflammatory phenotype. Disordered epithelial function with reduced antioxidant defense and impaired capacity to produce primary IFNs may also account for asthmatic susceptibility to air pollution and respiratory virus infection, respectively. Considering asthma as a disease of impaired barrier function opens new opportunities for therapeutic intervention or prevention by agents that could increase the airways resistance to the inhaled environment rather than suppressing the immune or inflammatory response. (*J Allergy Clin Immunol* 2007;120:1233-44.)

Key words: *Asthma, environment, epithelium, epithelial mesenchymal trophic unit, airway remodeling, origins of asthma, progression of asthma, tight junctions, impaired barrier function*

Asthma behaves as a spectrum of disorders initiated at different stages throughout life by a range of environmental factors interacting with a susceptible genetic background.¹ At its simplest, asthma is divided into allergic (extrinsic) or nonallergic (intrinsic) subtypes, but even

Abbreviations used

DC: Dendritic cell
EGF: Epidermal growth factor
EGFR: Epidermal growth factor receptor
EMT: Epithelial-mesenchymal transition
EMTU: Epithelial-mesenchymal trophic unit
TJ: Tight junction
TLR: Toll-like receptor
TSLP: Thymic stromal lymphopoietin
ZO: Zonula occludens

within each of these 2 broad categories, there exists considerable heterogeneity with respect to underlying mechanisms, clinical and physiological manifestations, response to treatment, and natural history.² The majority of asthma is associated with T_H2-type T-lymphocyte-driven cell recruitment and mediator release involving mast cells, eosinophils, basophils, and macrophages that contribute to the chronic, subacute, and acute inflammatory responses. T_H2-mediated inflammation is the target against which much of the current therapy for asthma is directed, especially corticosteroids, mast cell stabilizing agents, antileukotrienes, immunosuppressants, and the mAb directed to IgE, omalizumab.

IMPORTANCE OF LOCAL AIRWAY FACTORS IN ASTHMA PATHOGENESIS

Implicit in the chronic mucosal inflammatory response is the bidirectional interaction between formed elements of the airways such as the epithelium, smooth muscle, vasculature, and nerves and the inhaled environment as exemplified by exposure to allergens, viruses, bacteria, and fungi, environmental tobacco smoke (ETS), and a range of indoor and outdoor chemicals and pollutants³ (Fig 1). The great majority of asthma is associated with atopy, especially that form associated with the rising disease trends over the period of the last 30 years, but although more than 40% of the population in the developed world is atopic, only 7% to 11% expresses this as asthma.⁴ To explain this paradox, there are likely to be important factors

From Allergy and Inflammation Research, Division of Infection, Inflammation and Repair Division, School of Medicine, Southampton General Hospital and the University of Southampton.

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Reprint requests: Stephen T. Holgate, MD, DSc, Allergy and Inflammation Research, MP810, Level F, South Block, Southampton General Hospital, Southampton SO16 6YD United Kingdom. E-mail: sth@soton.ac.uk.

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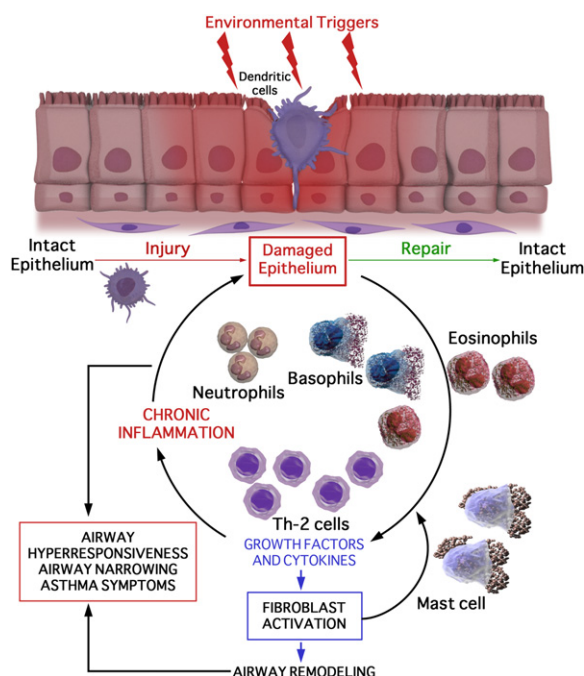


FIG 1. Schematic representation of how epithelial injury and aberrant repair interact with inflammatory components in chronic asthma.

that translate the atopic phenotype into the lower airways. Sensitization of the airways to selective aeroallergens that initiates the recruitment of T_H2 cells and subsequent isotype switching of B cells to IgE requires allergen uptake and processing by a subpopulation of mucosal dendritic cells (DCs) with subsequent antigen presentation to naive T cells in association with MHC class II.⁵ Of special interest is the propensity of biologically active allergens with proteolytic activity such as those derived from dust mites, pollen grains, and fungi as well as certain occupational sensitizers (eg, enzymes used in detergents) to cleave protease activated receptors on DCs and increase their capacity to interact with T cells.⁶ In addition, airborne particulates that contain biologic components such as micro-organism cell wall and nucleic acid fragments have the capacity to activate pattern recognition receptors including Toll-like receptors (TLRs) on DCs to enhance and direct their interaction with T cells further. Together these interactions serve as danger signals that can influence both the intensity and the balance of T cells toward T_H1 , T_H2 , T_H17 , or T-regulatory subtypes. Proteolytic allergens and microbial products such as lipopolysaccharide and double-stranded RNA also activate TLR-3, TLR-4, TLR-7, and TLR-8 expressed on airway epithelial cells leading to the release of the IL-7-like cytokine, thymic stromal lymphopoietin (TSLP). This IL-7-like cytokine represents an interesting example of an epithelial product that, on interacting with its receptor on DCs, upregulates the expression of the costimulatory molecules CD40, OX40, and CD80 to enhance T_H2 polarization⁷ as well as directly activating mast cells for selective cytokine secretion (Fig 2).⁸

As in other chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, and psoriasis, enhanced immune and inflammatory responses are associated with structural changes (remodeling) in the target tissue that have an important influence over disease expression. In the case of asthma, the extent of inflammatory and structural changes observed in the airways is related to disease severity but not to the presence or extent of atopy or duration of symptoms.⁹ In early life, the primacy of airway inflammation as 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 that can occur in the relative absence of inflammation.^{10,11} The increasingly popular concept of *remodeling* of the airways described in established adult asthma has similarities with airway *modeling* that occurs during branching morphogenesis in the fetal lung and the structural changes observed in childhood asthma at its onset.¹² Interactions between organ morphogenesis and wound healing responses are leading to a new concept of chronic airway inflammation supported by the structural components of remodeling through activation of epithelial cells that interact with the underlying fibroblast sheath (Fig 1). This concept was first introduced by Plopper et al^{13,14} by showing that, in nonhuman primates, air pollutant and allergen interactions with airway epithelial cells during early life initiated airway remodeling events and associated hyperresponsiveness along with T_H2 -type inflammation that persisted when the exposure was removed.^{13,14} The bidirectional interaction between the epithelium and underlying mesenchyme involving the release of selective growth factors and cytokines was called the epithelial-mesenchymal trophic unit (EMTU). Thus, the tissue environment provided by an activated EMTU has the capacity to provide the “soil” to sustain T_H2 -related inflammation (“seed”; reviewed in Holgate et al¹⁵). Although in this review it is not possible to detail the many cellular interactions that are being uncovered between airway structural cells and the immune and inflammatory responses, the principle of interdependency of the epithelium, fibroblasts, smooth muscle (and their secreted matrix), microvasculature, and neural networks goes some way to explain why chronic airway inflammation might persist in the absence of obvious environmental stimuli and, at the more severe and chronic end of the asthma spectrum, why there is an incomplete therapeutic response to anti-inflammatory drugs.^{14,15}

THE EPITHELIUM IN ORCHESTRATION OF THE ASTHMATIC RESPONSE

Building on this concept further, 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 secondary growth factors capable of driving structural changes linked to airway remodeling.¹⁶

The human lung is the largest surface that is in continuous contact with the outside world. It presents an

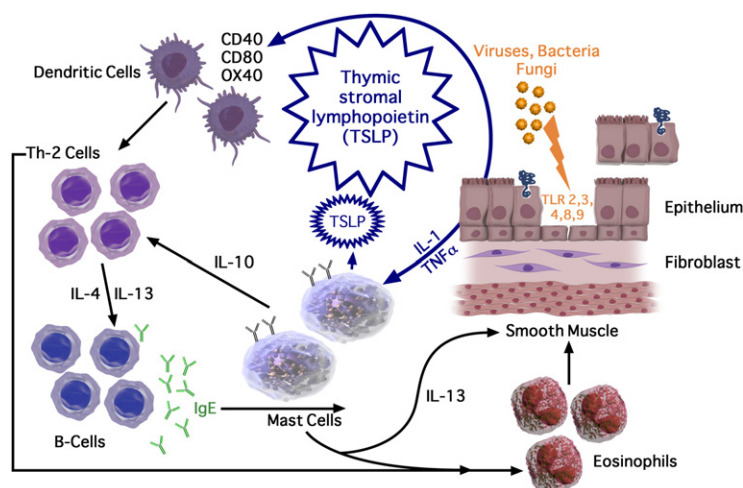


FIG 2. Schematic representation of the interrelationships between epithelial injury and aberrant repair and chronic inflammation in moderate-severe asthma. Immune and inflammatory cells interact with structural cells both to maintain the inflammatory response and to drive airway wall remodeling.

estimated area of 100 m² that comes into contact with approximately 10,000 L inhaled air each day containing numerous potentially harmful physical, chemical, and biological agents. The airway epithelium is the interface with the inhaled environment and, when functioning normally, forms a physical barrier complemented by the mucociliary escalator to provide the first line of defense. The stratified epithelium of the major conducting airways is covered by surface liquid approximately 10 μm thick that is made up of a periciliary layer around the microvilli and an overlying mucus layer onto which inhaled particles are 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 tight junctions (TJs) localized to the apical aspect of the columnar cells. TJs are made up of a series of interacting proteins and receptors including zonula occludens (ZO) 1-3, occludin, claudins 1-5, and transmembrane adhesion proteins (β-catenin, E-cadherin, and junctional adhesion molecules) that enable communication between adjacent cells as well as regulating intercellular transport¹⁷ (Fig 3). Structural integrity of the epithelium is further maintained through cell-cell and cell-extracellular matrix interactions involving adherens junctions, desmosomes, and hemidesmosomes.¹⁸ Disruption of the columnar epithelium enables tissue-damaging agents and infectious particles to penetrate the airway wall, thereby facilitating toxic, immune, and inflammatory responses with ensuing tissue damage.¹⁹

In asthma, there is evidence *in vivo* and *in vitro* that the barrier function of the airways epithelium is impaired.^{17,20,21} Although epithelial damage in asthma has been considered to be artefactual resulting from damage incurred at bronchoscopy,²² other studies have consistently reported evidence for a more fragile airway epithelium in adults and children with asthma (see review²³).

Using immunohistochemistry applied to whole airway tissue mounts viewed by confocal microscopy, epithelial TJs in asthma are severely disrupted (Fig 4). Compared with normal, epithelial cells brushed from asthmatic airways, cultured *in vitro* and differentiated on an air liquid interface, are unable to form effective TJs fully even though the cells have been passaged several times and separated from any airway inflammatory cells or mediators for 6 to 8 weeks. At this time, measurement of transepithelial resistance across the differentiated epithelial cell cultures at baseline is markedly reduced, indicating increased leakiness. Apical exposure of the asthmatic cultures to injurious agents such as tobacco smoke extract revealed a further reduction in transepithelial resistance at much lower concentrations than required to achieve a similar response in differentiated normal epithelium. As observed in the asthmatic biopsies, whole mount immunofluorescence confocal microscopy with antibodies directed to ZO-1 and occludin confirmed poorly developed TJs in asthmatic compared with normal cultures. Environmental factors such as respiratory viruses and proteolytically active allergens (eg, *Dermatophagoides pteronyssinus* allergen cysteine protease) have the capacity to disrupt TJs further and increase epithelial permeability.²⁴

Because 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 antioxidant enzymes as well as active radical traps that help maintain integrity and resist injury.²⁵ In asthma, the antioxidant pathways such as superoxide dismutase, catalase, and glutathione peroxidase are also defective^{26,27} as well as exhibiting polymorphic genetic association with the disease.^{28,29} Oxidants are known to decrease epithelial barrier function by disrupting perijunctional actin, which affects the distribution of TJ proteins and predisposes to apoptosis. Both in

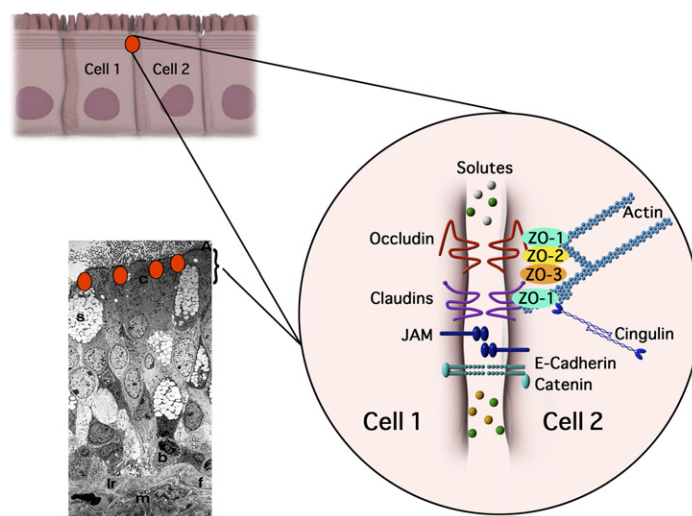


FIG 3. Epithelial TJs localized to the apex of the epithelium that control paracellular transport of inhaled material. TJs are complex structures composed of multiple transmembrane proteins (occludin, claudins, junctional adhesion molecules [*JAMs*] and E-cadherin) and adapter proteins (catenin, ZO 1-3) to the cytoskeleton (actin and cingulin). *b*, Basal cell; *f*, fibroblast; *lr*, lamina reticularis; *m*, macrophage.

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.^{26,30-32}

For many years, “epithelial desquamation” has been described as a pathological feature in asthma death,³³ but until recently, its significance has not been fully appreciated.^{34,35} Bronchial biopsy studies from patients with asthma of increasing severity demonstrate not only physical damage to the columnar cell layer but also evidence for injury through the expression of cell stressors such as heat shock protein 70,³⁶ evidence for activation of the caspase enzyme cascade involved in apoptosis both in asthma^{26,37-39} and in a murine model of airway inflammation,⁴⁰ and enhanced surface expression of epidermal growth factor receptors (EGFRs),⁴¹⁻⁴³ as well as other receptors involved in innate immunity, including CD40 and TLRs.^{44,45} These epithelial markers of stress and injury increase in proportion to disease severity and chronicity. Almost identical changes have also been observed in moderate and severe childhood asthma,¹¹ suggesting that epithelial injury and aberrant repair may be involved in the origins of the disease and may not be simply a consequence of long-standing inflammation.⁴⁶ Under normal circumstances, injury to the airways epithelium should stimulate the intrinsic repair pathways with engagement of EGFRs by autocrine secretion of appropriate ligands (epidermal growth factor [EGF], amphiregulin, and heparin-binding EGF-like growth factor) to drive cell migration, proliferation, and differentiation—all features of a primary mucosal repair response (healing by “primary intention”; see review⁴⁷). In asthma, there occurs impaired proliferation of basal cells revealed as reduced nuclear expression of cell cycle markers such as Ki67 and proliferating cell nuclear antigen and increased nuclear

translocation of the cell cycle inhibitor, P21^{waf} (Fig 5).^{11,48} It is this incomplete repair response to injury that could provide the basis for a chronic wound scenario—that is, healing by “secondary intention.”

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 (prostaglandin E₂, 15-hydroxyeicosatetraenoic acid) and the ability to activate the plasminogen and kininogen cascades.⁴⁹⁻⁵¹ Airway epithelial cells cultured from the airways of children with asthma demonstrate increased production of prostaglandin E₂ and IL-6 that is preserved after several passages, indicating a secretory primary defect rather than being secondary to airway inflammation.⁵² Possible mechanisms responsible for this epithelial cell phenotype include primary genetic, secondary genetic (eg, somatic mutations) or epigenetic (eg, altered micro-RNA, histone acetylation, or CpG dinucleotide methylation) influences. What would be helpful to explore this further is a comprehensive assessment of gene regulation comparing asthmatic with normal epithelial cells using genomic, proteomic, and metabolomic technologies.^{53,54}

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. This unique pathological change 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,

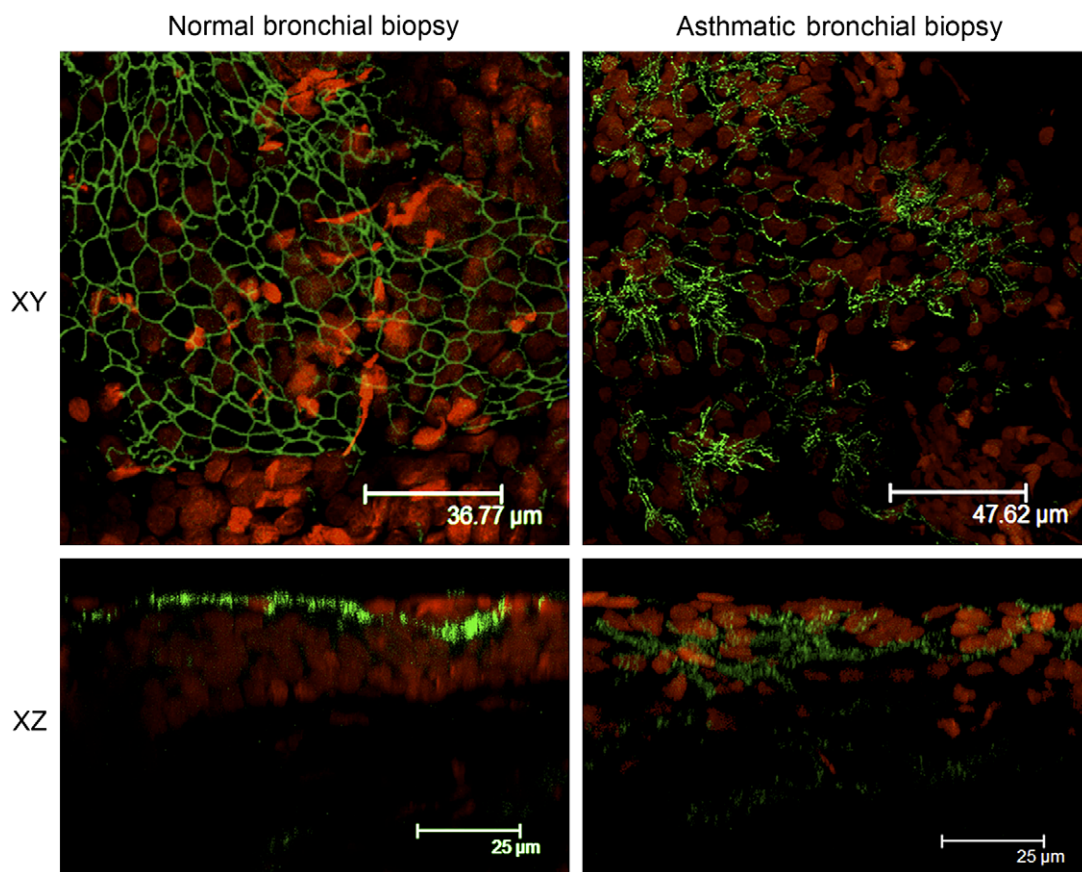


FIG 4. Immunofluorescent confocal microscopy of whole mounts of bronchial biopsies from a normal (*left*) and asthmatic (*right*) airway. The section has been stained with an antibody that identifies the TJ protein ZO-1 (*green*), with the epithelial cell nuclei stained *red*. Note the lack of TJ organization in the asthmatic epithelium when viewed in the horizontal and vertical planes.

and types I, III, and VI collagen.^{55,56} The fact that these changes are also seen in childhood asthma close to disease inception indicates that, like abnormalities in the epithelium itself, these changes in matrix deposition are initiated close to or at disease onset and are not a result of chronic inflammation over time.⁵⁷⁻⁵⁹ It is worth noting that this change to the basal lamina occurs in only 1 other airway disease, bronchiolitis obliterans associated with a cytotoxic immunologic attack on small airway epithelial cells in lung transplant rejection.^{60,61} Airway biopsies obtained from infants with severe intermittent wheezing reveal little difference in the basal lamina from that of normal airways, but after the age of about 3 years, the characteristic thickening associated with asthma is seen.⁶² A detailed electron microscopic assessment of the basal lamina in adult and childhood asthma compared with normal epithelium has shown that the collagen fibrils were significantly thinner, and fewer of the fibrils were banded as found in the interstitial collagen of the adjacent submucosa, suggesting a different matrix structure.⁶³

The mechanisms involved in this remodeling of the basal lamina are not known, although in a range of animal models, similar changes have been 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 growth factors such as TGF- β .^{64,65} Similarly elegant use of IL-13-overexpressing transgenic mice with signal transducer and activator of transcription 6 (STAT6) expression limited to epithelial cells has shown that IL-13 has direct effects on airway epithelial cells to increase mucus secretion and increase airway hyperresponsiveness in the absence of inflammation.⁶⁶⁻⁶⁸ The airway epithelium also has the capacity to generate EGF, heparin-binding EGF-like growth factor, amphiregulin, fibroblast growth factors 1 and 2, platelet derived growth factors, insulinlike growth factors, neurotrophins (eg, nerve growth factor), and vascular endothelial growth factors, most of which are capable of interacting with fibroblasts to cause their proliferation and/or differentiation into myofibroblasts.⁶⁹ Because these growth factors are all overexpressed in the airway epithelium of patients with active asthma, they are likely to contribute to the remodeling of the epithelia basal lamina in asthma. Factors that are known to enhance the secretion of these growth factors include mechanical stress, chemical and physical injury, virus infection, and interactions with inflammatory cells, especially eosinophils and neutrophils.^{70,71}

INTERACTIONS BETWEEN EOSINOPHILS AND THE EPITHELIUM

Eosinophils are considered to be fundamental to the inflammatory response of asthma. There is an extensive literature (that will not be reviewed here) incriminating these cells in epithelial injury through the release of their cytotoxic granule-derived basic proteins and generation of reactive oxygen.⁷² However, despite having a major effect in reducing circulating and sputum eosinophils, systemic administration of the anti-IL-5 blocking humanized IgG mAb mepolizumab to patients with asthma reduced airway tissue eosinophils by only ~50%.⁷³ It was suggested that this incomplete response might be a result of 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 on IL-5 for survival.⁷⁴ There is also evidence that in asthmatic airways, enhanced secretion of GM-CSF by epithelial cells, fibroblasts, smooth muscle, and inflammatory cells is an alternative pathway for prolonging eosinophil survival.⁷⁵ In separate studies, anti-IL-5 had no observable effect on the allergen-induced early or late-phase responses or, using another anti-IL-5 antibody (SCH55700), on baseline lung function and clinical manifestations of chronic asthma.^{76,77} However, 3 infusions of mepolizumab over a period of 10 weeks did result in a marked reduction in immunoreactive of tenascin, collagen III, and lumican in the epithelial basal lamina.⁷⁸ On the basis of this observation, it has been suggested that airway eosinophils play a role in airway remodeling.^{79,80} In addition to macrophages, lymphocytes, and mast cells, human eosinophils are an important source of TGF- β that has the capacity to drive differentiation of fibroblasts into myofibroblasts. After inhalation allergen challenge to produce both early and late bronchoconstrictor responses, the ensuing T_H2-type airway inflammation resolves within 7 days, whereas biomarkers of matrix production (procollagens I and III, fibronectin, and tenascin) persist much longer in parallel with an increase in myofibroblast numbers.⁸¹

Thickening and hyalinization of the basal lamina of the epithelial basement membrane is also a feature in cough-variant asthma (sometimes called *eosinophilic bronchitis*),⁸² indicating that, although it is 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 classic asthma, but has been associated with an accelerated loss of lung function over time.⁸³

REMODELING OF THE AIRWAY WALL

High-resolution computed tomography has revealed substantial evidence that chronic and severe asthma in children and adults is associated with thickening of the airway walls.^{84,85} Pathological analysis has also shown the deposition of matrix proteins such as fibronectin, collagens I, III, and VI, and the proteoglycans biglycan, lumican,

and versican in the submucosa as well as an increase in smooth muscle, which are in large part responsible for airway wall thickening.^{86,87} Using either high-resolution computed tomography^{88,89} or an ultrasound bronchoscopic probe⁹⁰ to examine airway dimensions, bronchial hyperresponsiveness to methacholine appears to be inversely related to airway wall thickness. The deposition of matrix in the airway wall of subjects with chronic persistent asthma might be indicative of a compensatory response designed to limit repeated bronchoconstriction and maintain airway patency by stiffening the airways. The demonstration that physical distortion of airway epithelium in culture is a potent stimulus for profibrogenic growth factor production⁹¹ adds support to this concept. It follows that in highly unstable or "brittle" asthma, absence of airway wall remodeling may be the reason these patients experience rapid onset and unopposed bronchoconstriction that is often severe and life-threatening.^{92,93} Conversely, over time, extensive airway wall remodeling could be an important factor responsible for irreversible airflow obstruction and an accelerated decline in lung function that has been observed in chronic severe asthma over time.⁹⁴

Although resident airway fibroblasts are likely to be the principal drivers of remodeling, the recent descriptions of epithelial cells themselves undergoing transdifferentiation into fibroblasts by a process called *epithelial-mesenchymal transition* (EMT) driven by profibrotic cytokines such as TGF- β ⁹⁵ and of primitive "fibrocytes" being recruited from CD34⁺ progenitors in the bone marrow via the circulation into the asthmatic airway⁹⁶ provide exciting new mechanisms that are being explored. Although there is some evidence for EMT in certain forms of interstitial lung disease⁹⁷ and also in animal models of pulmonary fibrosis,⁹⁸ evidence of a related process in asthma is preliminary, based on some evidence for EMT in stable lung transplantation.⁹⁹ In contrast, repeated allergen provocation of asthmatic airways has been shown to lead to the recruitment of CD34⁺ fibrocytes from the circulation into airway tissue and bronchoalveolar lavage fluid and could realistically contribute to remodeling,^{100,101} but it is not yet known whether these fibrocytes are capable of matrix deposition.

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. In asthma there is ample evidence for goblet cell metaplasia involving the conducting airways,¹⁰² but in chronic persistent disease, goblet cells also spread down to the more peripheral airways where they normally do not exist.¹⁰³ In addition, submucosal glands in asthma are larger and contain a greater proportion of mucin compared with serous cells. The net result is the secretion of abnormally large amounts of the highly viscous mucins 5AC and 5B which, along with DNA and eosinophil basic proteins, contribute

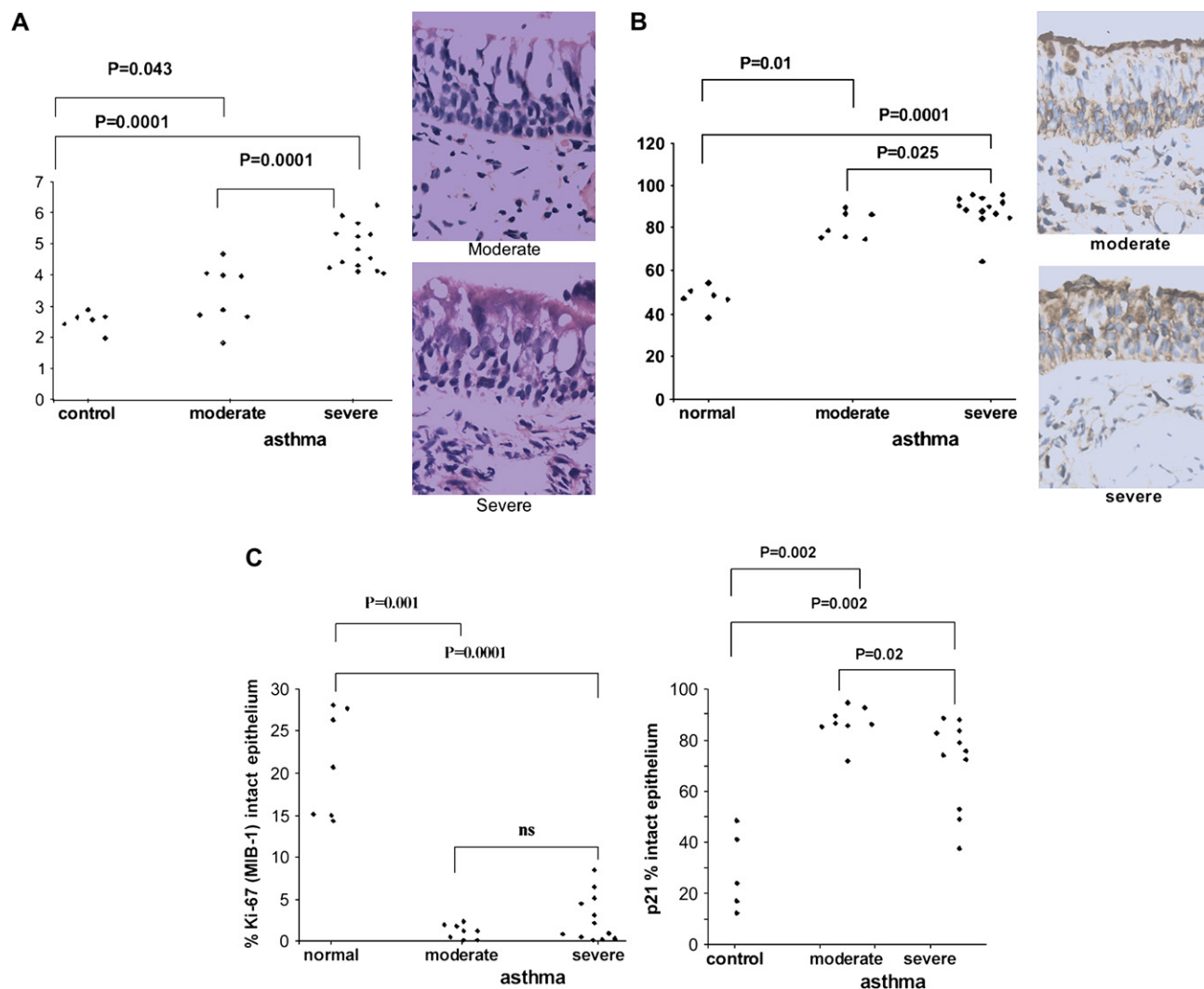


FIG 5. Involvement of the airway epithelium in childhood moderate and severe asthma compared with normal control as revealed in bronchial biopsies with quantification by image analysis shown in the dot plots. **A**, Goblet cell metaplasia and thickening of the basal lamina beneath the epithelium in moderate and severe asthma (hematoxylin and eosin stain). **B**, Increased expression of the EGFR (immunostaining using EGFR antibody). **C**, Dot plot to show reduced epithelial nuclear expression of the cell proliferation marker Ki67 identified with monoclonal antibody MIB-1 in childhood asthma (*left*) and parallel increase in P21^{waf} (*right*).

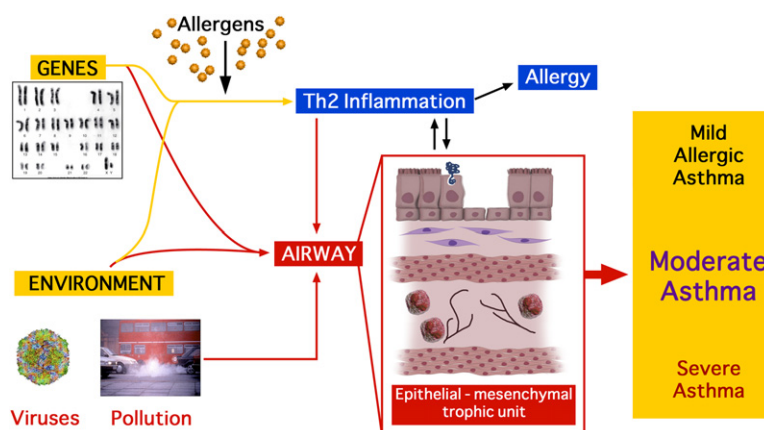


FIG 6. Schematic representation of the EMTU in providing epithelial interactions with the external environment and the T_H2 immune and inflammatory response to determine asthma phenotypes.

to the tenacious and viscous mucus characteristic of asthma.¹⁰⁴ Factors that contribute to goblet cell metaplasia have been extensively studied and include activation of the EGFRs on epithelial cells during repair by TGF- α released from its membrane precursor by a disintegrin and metalloprotease (ADAM) 17 TNF- α -converting enzyme (TACE).^{105,106} IL-4 and IL-13 also induce the production of TGF- α by epithelial cells that, through autocrine signaling, results in mucous metaplasia characteristic of T_H2 -mediated inflammation.¹⁰⁷ Finally, reactive oxygen species generated by epithelial damage through activation of dual oxidase 1 (Duox1, a homolog of glycoprotein p91^{phox}) stimulate TGF- α cleavage from its membrane precursor to promote mucous metaplasia.¹⁰⁸ Genetic association studies reveal evidence for the role of 4 highly polymorphic genes (*MUC-6*, *MUC-2*, *5AC*, and *5B*) encoding gel-forming mucins on chromosome cluster on 11p15.5.¹⁰⁹ The recognition that IL-4, IL-9, and IL-13 induce not only selective mucin expression in airway epithelial cells¹¹⁰ but also the calcium-activated chloride channel 1 (hCLC A1; Gob 5), which is itself implicated in regulating the expression of gel forming mucins,¹¹¹⁻¹¹⁴ suggests a potential therapeutic target.

THE EPITHELIUM AND CONTROL OF INFLAMMATION

The asthmatic airway epithelium is a major source of cytokines and chemokines that are strongly implicated in maintaining asthmatic inflammation including IL-5, RANTES (CCL5), eotaxin (CCL11), macrophage chemoattractant peptides and TSLP that are all powerful stimuli for T_H2 polarization. The recent 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 T_H2 -like inflammation.¹¹⁵ After allergen challenge and other types of epithelial injury, both CCL17 and CCL22 are key cytokines that drive T_H2 chemotaxis by

interacting with the common CCR4 receptor.^{116,117} Greater than 90% of the T_H2 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. By contrast, it has been difficult to reproduce the initial report¹¹⁸ of a high proportion of invariant natural killer T cells in chronic asthma.¹¹⁹

THE EPITHELIUM IN VIRUS-INDUCED ASTHMA

In addition to allergen and air pollutants, the asthmatic airway is particularly susceptible to respiratory virus infection as a cause of 40% to 80% of asthma exacerbations both in children and adults. Of particular significance is the finding in asthma that the common cold viruses (eg, rhinovirus), which usually cause 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,¹²⁰ are refractory to the commonly used practice of doubling the dose of inhaled corticosteroids,¹²¹ and are associated with persistence of virus in the respiratory tract.¹²² Bronchial biopsy and lavage has revealed that in asthma, human rhinovirus preferentially infects airway epithelial cells using the intercellular adhesion molecule 1 and very-low-density lipoprotein receptors to gain entry in the case of the major and minor virus subtypes, respectively.¹²³ A fundamental 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 air-liquid interface has revealed impaired virus elimination and increased cytotoxic cell death as a consequence of defective IFN- β production.¹²⁴ IFN- β is the cytokine responsible for initiating apoptosis and viral clearance by normal epithelial cells as well as activating a large range

of protective antiviral pathways. IFN- λ production is similarly reduced in asthmatic epithelium and monocyte/macrophages with virus infection,¹²⁵ suggesting that a generic deficiency in IFN production may underlie the increased susceptibility of the asthmatic airways to common cold virus infection. Inhaled IFN- β is now under development as a therapeutic for the prevention of severe virus exacerbations in asthma.

CONCLUSION

Although asthma is an inflammatory disorder of the conducting airways, inflammation itself does not explain the origins of this disease or why the airways are so susceptible to a range of different environmental factors. Two recent intervention studies with inhaled corticosteroids at the inception of asthma in early childhood in those genetically at risk,^{126,127} as well as prolonged use of inhaled corticosteroids later in childhood asthma, have failed to influence the natural history of this disease when assessed by a wide-range criteria.¹²⁸ These studies show that aggressive anti-inflammatory therapy appears suppress the disease as long as corticosteroids are used continuously, but presumably do not influence the factors required to initiate and maintain the disease. Abnormalities in the epithelium both structurally and functionally provide a final common pathway to explaining disease pathogenesis. The identification of a range of epithelial expressed susceptibility genes through positional cloning such as *ESE-1* and *ESE-2*, *DPP10*, *HLA-G*, *GPRA*, and *MUC8* adds further credence to the hypothesis that the origins of asthma lie in the epithelium/EMTU and not primarily in immune or inflammatory pathways (Fig 6). One exciting observation supporting a key role of the EMTU in the origins of asthma is the finding that frequent rhinovirus infection assessed by PCR in respiratory secretions sufficient to cause wheezing in young genetically at-risk infants over a period of 2 years is a major predictor of persistent wheezing at age 3 years.¹²⁹ This runs counter to the popular view that respiratory virus infection is protective in asthma,¹³⁰ although there is evidence it is protective against atopy (the hygiene hypothesis).¹³¹ This different view of asthma opens up a new approach to disease prevention and treatment focused more on increasing the resistance of the airways to environmental inability rather than simply suppressing inflammation or relaxing airways smooth muscle. A role for the epithelium in orchestrating T_H2-type disease in other organs such as atopic dermatitis¹³² and food allergy¹³³ is also attracting interest and maybe a principle that can be applied to a number of allergic diseases expressed at epithelial surfaces.

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Epithelium dysfunction in asthma

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Learning objectives: "Epithelium dysfunction in asthma"

1. To review current knowledge regarding the bronchoepithelium in patients with asthma.
2. To explore the reasons for dysfunctional epithelium in patients with asthma.
3. To consider explanations for desquamation of epithelium.

CME items

Question 1. In the developed world's atopic population, what percentage of subjects express their atopy as asthma?

- A. <5%
- B. 10%
- C. 30%
- D. 50%

Question 2. The extent of inflammation and structural changes seen in the airways of patients with asthma is related to the —

- A. presence of atopy.
- B. extent of atopy.
- C. severity of asthma.
- D. duration of asthma symptoms.

Question 3. There is evidence that barrier function of airway epithelium in patients with asthma is impaired in part by —

- A. overexpression of zona occludens (ZO-1) and occludin.
- B. decrease in mucus-secreting goblet cells.
- C. inactivation of the caspase cascade.
- D. disruption of epithelial tight junctions.

Question 4. Which of the following is an explanation for the "epithelial desquamation" that is described as a pathologic feature of severe asthma?

- A. impaired proliferation of basal cells as evidenced by reduced expression of Ki67 and proliferating cell nuclear antigen
- B. increased activity of superoxide dismutase and glutathione peroxidase pathways
- C. decreased activity of the caspase enzyme cascade
- D. increased autocoid mediators, such as prostaglandin E₂, that are secondary to airway inflammation

Question 5. Which of the following best describes the airway basal lamina in asthma?

- A. Infants with severe intermittent wheezing often show characteristic hyalinization and thickening of the basal lamina.
- B. The exact mechanism of basal lamina remodeling is unknown, though the elevation of various growth factors likely plays a role.
- C. The ratio of collagen fibrils to matrix in the basal lamina is increased in subjects with asthma when compared to normal subjects.
- D. The number and activity of subepithelial myofibroblasts are decreased.

Question 6. Which of the following has been reported after the administration of anti-IL-5 monoclonal antibody in subjects with asthma?

- A. minor decrease in circulating eosinophils
- B. complete eradication of airway tissue eosinophils
- C. marked reduction of tenascin, collagen III, and lumican in the basal lamina
- D. moderate decrease in methacholine bronchial hyperresponsiveness

Question 7. Therapies directed at which target would be most likely reduce mucin formation in airway epithelial cells?

- A. TGF- β
- B. IL-5
- C. thymic stromal lymphopoietin (TSLP)
- D. Gob-5 (hCLC A1)

Question 8. Which chemokines interact with the CCR4 receptor to drive T_H2 chemoattraction?

- A. CCL1 (I-309) and CXCL10 (IP-10)
- B. CCL1 (I-309) and CCL22 (MDC)
- C. CCL17 (TARC) and CCL22 (MDC)
- D. CCL1 (I-309) and CCL17 (TARC)

Question 9. In epithelial cells from patients with asthma, increased cytotoxic cell death and impaired viral clearance are a result of which of the following?

- A. defective production of IFN- β
- B. increased surface expression of epidermal growth factor receptors
- C. defective function of superoxide dismutase
- D. enhanced production of GM-CSF

Question 10. Which of the following is produced in airway epithelial cells and is involved in promoting T_H2-like inflammation?

- A. mucins 5AC and 5B
- B. TGF- β
- C. ZO 1-3
- D. TSLP