The Airway Epithelium is Central to the Pathogenesis of Asthma

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ABSTRACT

Asthma is an inflammatory disorder principally involving the conducting airways and characterised by infiltration of the airway wall with a range of inflammatory cells driven in large part by activation of Th2-type lymphocytes, mast cells and eosinophils. However a key component of asthma is the structural change that involves all of the elements of the airway wall. Here evidence is presented to suggest that the airway epithelium in asthma is fundamentally abnormal with increased susceptibility to environmental injury and impaired repair associated with activation of the epithelial-mesenchymal trophic unit (EMTU). In addition to adopting an activated phenotype, the barrier function of the epithelium is impaired through defective tight junction formation thereby facilitating penetration of potentially toxic or damaging environmental insults. Activated and repairing epithelial cells generate a range of growth factors that are involved in the early life origins of this disease as well as its progression in the form of mucous metaplasia and airway wall remodeling. By placing the epithelium at the forefront of asthma pathogenesis, different approaches to treatment can be devised focused more on protecting vulnerable airways against environmental injury rather than focusing on suppressing airway inflammation or manipulating the immune response.

KEY WORDS

Airway remodeling, Asthma, Disease-susceptible genes, Epithelial mesenchymal trophic unit (EMTU), Thymic stromal Lymphopoietin (TSLP)

INTRODUCTION

The airway epithelium represents the interface between the external environment and tissue of the airway wall. In this review evidence will be presented to support a central role of the epithelium in asthma pathogenesis especially in more chronic and severe disease and to show that damage to this structure in a chronic wound scenario orchestrates both continued inflammation and remodeling.

IMPORTANCE OF LOCAL AIRWAY FACTORS IN ASTHMA PATHOGENESIS

Implicit in the chronic mucosal inflammatory response in asthma is the interaction between formed elements of the airways such as the epithelium, smooth muscle, vasculature and nerves and the inhaled environment that contains allergens, viruses, bacteria and fungi, tobacco smoke (ETS) and a range of indoor and outdoor chemicals and pollutants. The great majority of asthma is associated with atopy but it is important also to appreciate that, while over 40% of the population in the developed world are atopic, only 7–11% express this as asthma. In order to explain this paradox, there must be important factors that translate the atopic phenotype into the lower airways to manifest this as Th2-type airway inflammation. Sensitisation of the airways to selective aeroallergens is preceded by their uptake and processing by a subpopulation of mucosal dendritic cells that then present specific peptide epitopes to naive T cells in association with MHC Class II. The propensity of biologically active allergens with proteolytic activity or allergens that are associated with proteolytic enzymes such as those from dust mites, pollen grains and fungi to serve as strong sensitising agents probably relates to their capacity to act as danger signals by cleaving protease activated receptors (PARs) on DCs. Other aspects of the inhaled environment can also influence the maturational direction of the subse-

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quent T cell response by interacting with pattern recognition receptors on DCs. Dust and airborne particulates containing cell wall and nucleic acid fragments of microorganisms interact with up to 10 different toll like receptors (TLRs) to enhance and direct their response by in favour of Th-1, Th-2, Th-17 or Treg (regulatory T cell) subtypes, depending upon the composite stimulus. Precisely how activation of TLRs dictates T-cell differentiation is not known, but altering the expression of different co-stimulatory molecules on DCs is likely to be important because these are intimately involved in directing the T cell response to TCR/MHC Class II signalling.

Microbial products also activate TLR-3, 4, 7 and 8 on airway epithelial cells leading to the release of the IL-7-like cytokine, thymic stromal lymphopoietin (TSLP) that, on interacting with its receptor on DCs, upregulates their expression of the co-stimulatory molecules CD40, OX40, and CD80 to enhance Th-2 polarisation as well as directly activating mast cells for selective cytokine secretion (Fig. 1). TLRs are also involved in the activation of epithelial cells for chemokine release, but here there is the requirement for activation of the EGF receptor. An important environmental stimulus for asthma is respiratory virus infection with the presence of virus in nasal secretions and indications of allergy synergistically increasing the odds ratio for wheezing. It is therefore relevant that virus double strand RNA and IL-4 interact synergistically to induce the production of TSLP by bronchial epithelial cells.

**THE EPITHELIAL MESENCHYMAL TROPHIC UNIT (EMTU) IN ASTHMA PATHOGENESIS**

In early life the central role that airway inflammation plays in the origin of asthma is being questioned with the discovery that, at its onset, asthma is associated with the presence of marked structural changes in the Airways often in the relative absence of airway inflammation. The process of “remodelling” of the Airways as seen in adult asthma has many similarities with airway “modelling” observed during branching morphogenesis in the developing foetal lung and the structural changes seen early during the development of asthma in infancy. Similarities that exist between organ morphogenesis and wound healing have lead to a new concept of chronic inflammation, namely, one that is supported by structural components through activation of the epithelial mesenchymal trophic unit (EMTU) (Fig. 2). Recent studies by Plopper et al. have revealed that repeated exposure of infant rhesus monkeys to house dust mite allergen with or without ozone cased remodelling of the EMTU in the form of reduced airway number, epithelial cell hyperplasia, increased number of mucus secreting cells, reorganization of airway vascular and modified epithelial distribution of epithelial nerves as well as altered immunity towards a Th-2 phenotype favouring the development of airways obstruction. The significance of these observations in non-human primates is strengthened by showing that these remodelling events are not influenced by corticosteroids, and are likely to be central to the long term structural changes within the Airways.

Thus, the tissue microenvironment created by an activated EMTU has the capacity to provide the “soil” for the Th-2 related inflammation (“seed”) to persist. The interdependency of structure and inflammation involving the epithelium, fibroblasts, myofibroblasts, smooth muscle and their secreted matrix, microvasculature and neural networks helps explain why chronic airway inflammation could persists at the more severe and chronic end of the asthma spectrum and for the incomplete response to anti-inflammatory treatments. A new paradigm for persistent asthma is that of a damaged epithelium which repairs incompletely. The consequence of this is a chronic wound scenario characterised by the secretion of a range of secondary growth factors by epithelial cells and underlying fibroblasts capable of driving structural changes linked to airway remodelling.

**THE EPITHELIAL BARRIER FUNCTION IN ASTHMA**

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) localised the apical aspect of the columnar cells. Tight junctions 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 (Fig. 3). Structural integrity of the epithelium is further maintained through cell-cell and cell extra-cellular matrix interactions involving adherens junctions, desmosomes and hemidesmosones. 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.

For many years “epithelial desquamation” has been described as a pathological feature in asthma death, but until recently, its significance has not been appreciated. Bronchial biopsy studies from patients with asthma of increasing severity not only demonstrate physical damage to the columnar cell layer, but also evidence for injury through the expression of cell stressors such as heat shock protein (HSP) 70, evidence for activation of the caspase enzyme cascade involved in apoptosis both in asthma and, more recently in a murine model of airway inflammation. There is also enhanced surface expression of...
Fig. 1  Generation of thymic stromal lymphopoietin (TSLP) by epithelial cells on activation of toll-like receptors. TSLP interacts with both dendritic cells and mast cells to simulate cytokine release independent of allergen exposure. (Reproduced with permission from reference 5)

Fig. 2  Schematic representation of the interaction between immune, inflammatory and structural cells through the epithelial-mesenchymal trophic unit (EMTU) in asthma pathogenesis. (Reproduced with permission from reference 12)
Fig. 3 Components that form epithelial tight junctions at the apex of adjacent epithelial cells.

epidermal growth factor receptors (EGFRs)\textsuperscript{27-29} as well as other receptors involved in mucosal innate immunity including CD40 and toll-like receptors (TLRs).\textsuperscript{30,31} These markers of stress and injury are 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,\textsuperscript{11} suggesting that epithelial injury and aberrant repair is involved at the inception of the disease.

In asthma there is evidence that the barrier function of the airways epithelium is impaired.\textsuperscript{32,33} In vivo this was first shown by demonstrating increased passage into the systemic circulation of the inhaled impermeant probe Tc-99m-labelled DTPA.\textsuperscript{34} Confocal microscopy and immunohistochemistry applied to whole airway tissue mounts show that in asthma TJs are severely disrupted. When cultured in vitro and differentiated on an air liquid interface (ALI), asthmatic epithelial cells also fail to form effective TJs even though the cells have been passaged several times prior to their differentiation. Measurement of transepithelial resistance (TER) across the differentiated epithelial cell cultures at baseline is also markedly reduced indicating their increased “leakiness”. When the asthmatic cultures are exposed through the apical aspect to injurious agents such as tobacco smoke, a further reduction in TER occurs at much lower concentrations of smoke extract than that required to achieve a similar response in normal epithelium. As observed in the asthmatic biopsies, confocal microscopy applied to the epithelial cultures in association with antibodies directed to ZO-1 and occlud-including, constituents of TJs, confirmed inadequate TJ formation in asthmatic compared to normal cultures. In this respect it is of specific interest that respiratory viruses, air pollutants and proteolytically active allergens (e.g Der p1 cysteine protease) also have the capacity to further disrupt TJs and increase epithelial permeability.\textsuperscript{35}

**ASTHMA AS A CHRONIC WOUND OF THE AIRWAYS**

Under normal circumstances injury to the airway epithelium stimulates the intrinsic repair pathway (healing by “primary intention”) with engagement of EGFRs by autocrine secretion of their ligands (EGF, amphiregulin and HB-EGF) to drive cell migration, proliferation and differentiation, the features of a primary mucosal repair response. In asthma, there is impaired proliferation of basal cells as revealed by reduced nuclear expression of cell cycle markers such as Ki67 and proliferating cell nuclear antigen (PCNA) and the increased nuclear expression of cell cycle inhibitors such as P21\textsuperscript{waf}.\textsuperscript{11,36} It is this incomplete repair response to injury that could contribute to the chronic wound scenario \textit{i.e.} 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 (PGE\textsubscript{2}, 15-HETE) and the ability to activate the plasminogen and kininogen cascades.\textsuperscript{37,38} Airway epithelial cells cultured from the airways of children with asthma demonstrate increased production of PGE\textsubscript{2} and IL-6, which in being preserved in
culture after several passages indicates a secretory profile intrinsic to the origin of the disease rather than being secondary to airway inflammation. Possible mechanisms include primary genetic, secondary genetic (e.g., somatic mutations) or epigenetic (e.g., altered miRNA, histone acetylation or CpG methylation) changes involving epithelial cell control pathways.

**CHANGES TO THE EPITHELIAL BASAL LAMINA**

In adults and children a unique feature of asthma is hyalinisation and thickening of the basal lamina (basal lamina, lamina reticularis) beneath a seemingly normal looking epithelial basement membrane. This pathological change is accompanied by an increase in the number and activity of subepithelial myofibroblasts with their capacity to lay down new matrix including tenasin C, fibronectin and types I, III and V collagens. 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 begin close to or at disease onset and are not a result of chronic inflammation over time. A similar change in the lamina reticularis only occurs in bronchiolitis obliterans associated with a cytotoxic immununologic attack on small airway epithelial cells in lung transplant rejection. Airway biopsies 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 those with persistent wheezing develop the characteristic asthmatic change. A detailed electron microscopic assessment of the asthmatic compared to the normal basal lamina in adult and children shows that the collagen fibrils were significantly thinner, and fewer of these fibrils were banded when compared to the interstitial collagen of the adjacent submucosa, thereby suggesting a different matrix structure. In the thickened basal lamina of asthmatics the ratio of fibrils to matrix did not differ from that of their respective controls.

The mechanisms involved in this remodelling of the basal lamina are not known for certain, but there are clues from animal studies. In mice and rats similar changes in the subepithelial region are seen when the epithelium is chronically injured or is transfected with profibrotic cytokines such as TGF-β or IL-11 and IL-13 that have been shown to stimulate the secondary release of TGF-β. The airway epithelium also has the capacity to generate EGF, HB-EGF, amphiregulin, fibroblast growth factors -1 and -2, platelet derived growth factors (PDGFs), insulin-like growth factors (IGFs), neurotrophins (e.g., NGF) and vascular endothelial growth factors (VEGFs) the majority of which are capable of interacting with fibroblasts to either cause their proliferation and/or differentiation into myofibroblasts. Because these growth factors are all overexpressed in the airway epithelium of patients with active asthma, they probably contribute to the remodelling of the epithelia basal lamina in this disease. Factors known to enhance secretion of these growth factors include mechanical stress, chemical and physical injury, virus infection, and interactions with inflammatory cells especially eosinophils and neutrophils.

**THE ROLE OF EOSINOPHILS IN EPITHELIAL INJURY AND REPAIR**

Eosinophils are considered to be fundamental to the inflammatory response of asthma. There is an extensive literature incriminating these cells in epithelial injury through the release of cytotoxic granule-derived basic proteins and the generation of reactive oxygen. However, while having a major impact in reducing circulating and sputum eosinophils, systemic administration of the anti-IL-5 blocking humanised IgG monoclonal antibody mepolizumab to patients with asthma only reduced airway tissue eosinophils by ~50%. It was proposed that this incomplete response might be the result of loss of IL-5 receptors from a subset of eosinophils as they entered the airways from the circulation and, as a consequence, losing their dependence on IL-5 for survival and priming. There is also evidence that in asthmatic airways enhanced secretion of GM-CSF by epithelial cells, fibroblasts, smooth muscle and a range of inflammatory cells provides an alternative pathway for prolonging eosinophil survival. While anti-IL-5 also had no observable effect on the allergen-induced early, late phase responses or methacholine bronchial hyperresponsiveness, and in a large clinical trial in moderate/severe asthma no effect on symptoms, use of bronchodilators, three infusions of mepolizumab over a period of 10 weeks resulted in a marked reduction in immunoreactive of tenascin, collagen III and lumican in the basal lamina. Based on this observation it has been proposed that airway eosinophils play an important role in the remodelling of the basal lamina. Human eosinophils have proven to be an important source of TGF-β with the capacity to drive differentiation of fibroblasts into myofibroblasts. Following inhalation allergen challenge causing both early and late bronchoconstrictor responses, airway inflammation resolves within 7 days whereas biomarkers of matrix production (procollagens I and III, fibronectin and tenascin) persist much longer along with an increase in myofibroblasts.

Thickening and hyalinisation of the basal lamina of the epithelial basement membrane is also a feature in cough-variant asthma ( eosinophilic bronchitis). Thus, while being a marker of epithelial injury and aberrant repair, this pathological feature 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 basal lamina in asthma can be extrapolated to remodelling of the entire airway wall. Although frequently cited as an index of airway wall remodelling, it is now crucial to establish if this is the case or not or whether the airway thickening accompanying chronic asthma occurs independently. Remodelling of the airways in severe asthma is of great importance because it may account for part of the corticosteroid refractoriness that occurs in chronic and severe disease.

AIRWAY WALL REMODELLING

High resolution computed tomography (HRCT) has demonstrated that chronic and severe asthma in children and adults is associated with thickening of the walls of the large and intermediate conducting airways. Pathological analysis has also shown increased submucosal and adventitial deposition of matrix proteins that comprise fibronectin, collagens I, III and V as well as the proteoglycans biglycan, lumican and versican in the as well as an increase in smooth muscle that account for much of the airway wall thickness. Using both HRCT and an ultrasound bronchoscopic probe to examine airway dimensions, BHR is inversely related to airway wall thickness. This might be interpreted as a compensatory response designed to help maintain airway patency in the presence of repeated cycles of bronchoconstriction. It follows that in highly unstable or “brittle” asthma the relative absence of airway wall remodelling will lead to rapid onset and unopposed bronchoconstriction that could be catastrophic. A disadvantage of remodelling over time is the occurrence of a component of irreversible airflow obstruction and an accelerated decline in lung function that has been observed in chronic severe asthma over time.

While resident airway fibroblasts are likely to be the principle drivers of remodelling, the recent description of epithelial cells themselves undergoing transdifferentiation into fibroblasts by a process called epithelial-mesenchymal transition driven by profibrotic cytokines such as TGF-β or that primitive “fibrocytes” may be recruited from CD34 progenitors in the bone marrow via the circulation into the asthmatic airway provide exciting new mechanisms that are being explored. While there is some evidence for epithelia-mesenchymal transition in certain forms of interstitial lung disease and also in animal models of pulmonary fibrosis, evidence of a related process in asthma is preliminary based on some evidence for EMT in stable lung transplantation. 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 remodelling.

MUCUS PRODUCTION IN 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. 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 cluster on 11p15.5. In asthma there is ample evidence for goblet cell metaplasia involving the conducting airways, but in chronic persistent disease goblet cells also spread down to the more peripheral airways where they normally do not exist. In addition submucosal glands in asthma are larger and contain a greater proportion of mucin as opposed to 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 to the tenacious and viscous mucus characteristic of asthma. Factors that contribute to goblet cell metaplasia have been extensively studied and include activation of the EGF family of receptors on epithelial cells during repair by TGFα released from its membrane precursor by ADAM 17 (TACE). Interleukin-4 and IL-13 also induce the production of TGFα by epithelial cells that, through autocrine signalling, results in mucous metaplasia characteristic of Th-2 mediated inflammation. Finally, reactive oxygen species generated by epithelial damage through activation of dual oxidase 1 (Duox1, a homolog of glycoprotein p91phox) stimulates TGFα cleavage from its membrane precursor to promote mucous metaplasia. The recognition that IL-4, IL-9 and IL-13 not only induces selective mucin expression in airway epithelial cells, but also the calcium-activated chloride channel 1 (hCLC A1; Gob 5) that is itself implicated in regulating the expression of gel-forming mucins might point to a new therapeutic target for this aspect of asthma.

CONCLUDING COMMENTS

While undoubtedly, asthma is an inflammatory disorder of the conducting airways, inflammation itself does not fully explain the origin(s) of this disease nor why the airways are so susceptible to such a wide range of different environmental factors. Two intervention studies with inhaled corticosteroids at the inception of asthma in early childhood in those genetically at risk, as well as prolonged use of inhaled corticosteroids later in schoolchildren with symptomatic asthma have failed to influence the natural history of this disease when assessed by a wide range criteria. Taken together these longitudinal studies show that while aggressive anti-inflammatory therapy appears suppress the disease as long as they are used continuously, it appears not to influence those factors that are 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 ETS-2 and ETS-3, DPP10, HLA-G, GPRAs and MUC8\textsuperscript{90,91} (Fig. 4) adds further credence to the hypothesis that the origins of asthma lie in the epithelium/EMTU and not primarily in immune or inflammatory pathways. This different view of chronic asthma opens up a new approach to disease prevention and treatment focused more on increasing the resistance of the airways to environmental stimuli rather than simply suppressing inflammation or relaxing airways smooth muscle.

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