

Respiratory epithelial imbalances in asthma pathophysiology

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ABSTRACT

The pathophysiology of asthma is complex and involves a number of factors including atopy and bronchial hyperreactivity. A strong body of evidence suggests that structural and functional respiratory epithelial alterations play a crucial role in both development and persistence of this condition. From the onset of symptoms the airways epithelium of asthmatic patients seems to be altered and unable to repair. The interactions between the epithelium and the underlying mesenchyma, which are jointly referred to as the epithelial–mesenchymal trophic unit (EMTU), are thought to result in a self-sustaining damage of the airways and, ultimately, in a chronic inflammatory scenario. A better understanding of the relationship occurring across EMTU, environmental noxae, and factors of susceptibility to epithelial damage is likely to pave the way to future new preventive and therapeutic strategies for this condition.

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Asthma pathophysiology is very complex and has historically been studied under two main points of view, *i.e.*, atopy and airway hyperreactivity. There are still a lot of gray areas when interpreting asthma as a merely IgE-mediated condition because most atopic patients are not affected by asthma,^{1–5} and at the onset of the disease asthma anatomic alterations can occur in the absence of inflammation.⁶

In addition, histopathological differences between atopic asthma and nonatopic asthma are not significant in both adults and children,^{1,2,7} allergen avoidance measures and immunotherapy have given quite disappointing results in asthma,^{1,2,8} and clinical trials with some biological drugs meant to counter eosinophilic inflammation (anti-interleukin [IL]-5 and mepolizumab) in asthma have not had a significant impact on most of the outcomes.⁹

By contrast, studies conducted on pediatric populations have reported that it is epithelial and basal membrane alterations and not eosinophilic infiltration that make the histopathological difference between the airways of asthmatic patients and those of healthy individuals.⁶ Figure 1 shows the complex interrelationship between inflammatory, immunologic, and epithelial structural elements of the airways thought to play a role in the pathogenesis of asthma.

At the beginning of the past 10 years a research group headed by Holgate advanced a hypothesis on asthma pathophysiology whereby respiratory epithelium dysfunctions are expected to result in a chronic inflammatory damage of the airways of predisposed individuals in a kind of “second-intention repair,” thus, introducing the notion of epithelial–mesenchymal trophic unit (EMTU). Under this hypothesis a functionally defective epithelium, unable to repair damage induced by exogenous noxae because of either environmental or genetic reasons, through a crosstalk of cytokines, chemokines, and growth factors between the epithelium and the underlying mesenchyma, would lead to the development of structural and functional alterations of the airways that would persist also when exposure to the causal noxa is discontinued.^{1,2,4,5}

As suggested by Holgate and coworkers,^{1,2,4,5} there are several theoretical grounds to substantiate this hypothesis because many of the genes involved in asthma pathogenesis are expressed in the epithelium and in mesenchymal cells.³ In addition, most asthma triggers such as viruses, cigarette smoke, allergens, pollutants act on the epithelium,^{1–3,10–12} epithelial damage is a feature of asthma, but not of other chronic inflammatory airway diseases such as chronic obstructive pulmonary disease and cystic fibrosis,^{1–3,10–13} and some epithelial dysfunction mechanisms seem also to occur in other hypersensitivity-mediated conditions characterized by chronic inflammation, including atopic dermatitis and chronic rhinosinusitis.^{1–3,13–15}

Furthermore, pioneer animal studies have been able to show that in primates airways, exposure to oxidants (O₃) or allergens (dust mites) in the earliest stages of life can result in the development of structural and functional alterations (including changes in both lu-

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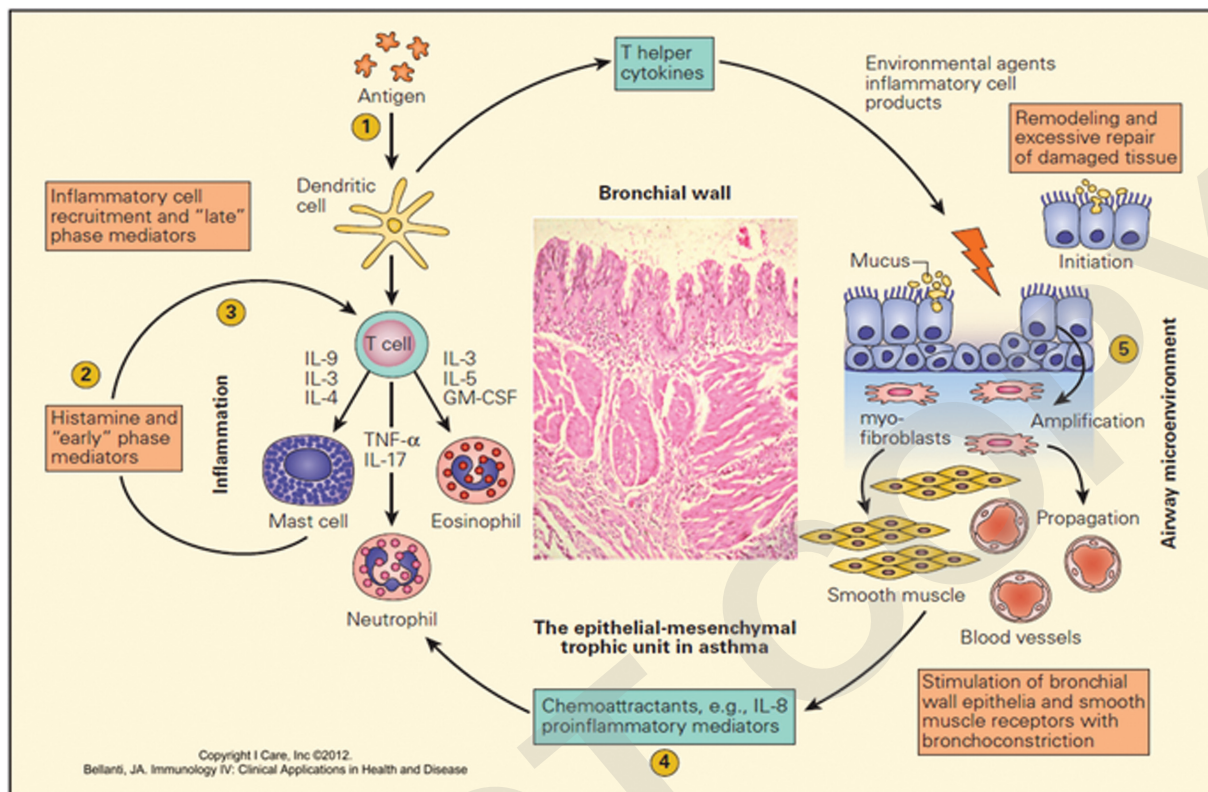


Figure 1. Schematic representation of the interrelationship between inflammatory, immunologic, and epithelial structural components of the airways involved in the pathogenesis of asthma. (Reproduced with permission from Luccioli S, Escobar-Gutierrez A, and Bellanti JA. Allergic diseases and asthma. In *Immunology IV: Clinical Application in Health and Disease*. Bellanti JA (Ed). Bethesda: I Care Press, 685–767, 2012).

Table 1 Main epithelial dysfunction of airways in asthma

Characteristics	References
TJ dysfunction and increased permeability	20–28
Inability to antagonize oxidative stress	29–32
Defective damage repair capability	15, and 33–41
Increase in mucin production	42
Altered mucociliary clearance	43
Classes I and III IFN deficiency in response to RV and bacterial LPS	45 and 46
Up-regulation of cytokines and growth factors	53–58
Altered handling of the respiratory microbiota	46, 47, and 60–63

IFN = interferon; LPS = lipopolysaccharide; RV = rhinovirus; TJ = tight junction.

men and length of the bronchial tree), which persist in time also when the causal factor has been removed.¹⁶

The main epithelial dysfunctions observed in asthma are reported in the following sections (see also Table 1).

Many data have been published by Holgate and other groups on this topic until now.^{1–5,17–19} We aimed to review the most recent literature in this field, paying special attention to the relationships among epithelial dysfunction, defective innate immunity, and airway microbiota in asthma.

TIGHT JUNCTIONS DYSFUNCTIONS AND INCREASED PERMEABILITY

Bronchial biopsy specimens in asthma patients consistently highlight the presence of a large number of exfoliated epithelial cells in the lumen of the respiratory tree. This feature seems to be typical of asthma in that it does not occur to the same extent in other inflammatory airway conditions. Electron microscopy studies have shown that in asthma (i) the epithelium is constitutively fragile and (ii) the function of the tight

junctions (TJs) is severely compromised.¹ This has been corroborated by some by now historical scintigraphic studies reporting an increased permeability of the airways in asthmatic patients.²⁰ Recently, Xiao *et al.* found that bronchial biopsy specimens from asthmatic patients presented with patchy disruption of TJs and concluded that this defect could facilitate the passage of allergens and other agents into the airways, leading to an immune response and likely contributing to the organ expression of the disease.²¹ Interestingly, claudin-1, which is a main structural component of TJs, has been shown to be expressed on airway smooth muscle and likely plays a role in airway remodeling.²²

Other authors go so far as to assume that this increased epithelial permeability accounts for a systemic phenomenon,² given that a similar occurrence at a gastrointestinal level has also been reported in pediatric asthma.²³ In addition, alterations in desmosome structure have been observed in upper airways of biopsy specimens of nasal polyps from patients with asthma and polyposis.²⁴ The site of disruption is likely to be the basal-columnar junction because the ratio of basal cells to columnar cells in the bronchoalveolar lavage of asthmatic patients is 40 times that of healthy individuals.²⁵

On the other hand, the cluster of susceptibility genes for asthma mapping on the long arm of chromosome 1 includes many genes involved in epithelium integrity with a recognized key role played by the filaggrin gene.²⁶

Among the factors known to increase epithelial permeability there are also oxidants and the proteases of some allergens, the best known being the *Dermatophagoides* cys-proteases that enable degradation of respiratory epithelium junctional proteins and favor the trans-epithelial transit of the same allergen.²⁷ As to the effect of oxidants on epithelial junctions, some authors have observed that after exposure to agents such as O₃ and NO₂, the cultured bronchial epithelial cells of asthmatic patients exhibit an increased permeability compared with that of healthy individuals.²⁸

INABILITY TO ANTAGONIZE OXIDATIVE STRESS

The occurrence of a certain level of imbalance between oxidants and antioxidants at the airway level has been well documented in asthmatic patients. This imbalance causes an increased airways oxidative stress that grows as asthma severity increases.²⁹ The asthma research group led by Holgate has shown that the epithelium of asthma patients is more susceptible to the proapoptotic action of oxidative agents such as H₂O₂, an effect that is not observed with other toxic agents.³⁰ It seems to depend on a deficit of activity on the part of detoxifying enzymes such as superoxide

dismutase or glutathione-peroxidase, an activity observed to be inversely correlated with respiratory function parameters.³¹ As a confirmation of the importance of these alterations in asthma pathogenesis, the polymorphisms of some enzymes such as glutathione-S-transferase, involved in the control of the redox state in the airways, have been shown to correlate with the prevalence of asthma in some populations.³²

DEFECTIVE DAMAGE REPAIR CAPABILITY

It has been suggested that the epithelium of the airways of asthmatic patients turns out to be chronically damaged and unable to repair.^{1,2} The repair process that follows respiratory epithelium cellular damage is a very complex phenomenon where a key role is played by the up-regulation of some cytokines and their relevant receptors, in particular, transforming growth factor (TGF) β and epidermal growth factor receptors (EGFRs), which are able to guide the process of cell migration, proliferation, and differentiation.^{1,2,15}

Holgate and coworkers have indicated that EGFR expression in respiratory epithelial cells, even in areas of apparent structural integrity, turns out to be increased in asthma patients. Moreover, the level of EGFR expression correlates with the thickness of the basal membrane.³³ Also, epithelial expression of p21^{waf}, a cell-cycle inhibitor with antiapoptotic activity, seems to be increased in asthmatic patients,³⁴ an occurrence that, however, does not seem to be matched by an increased expression of cell replication markers such as Ki67 or the proliferating cell nuclear antigen, thus, indicating that the epithelium of asthmatic patients is not able to give an appropriate response to cellular damage.^{2,35} These indirect pieces of evidence on the inability to repair the respiratory epithelium have been recently confirmed by studies on children with asthma, which have been able to show that in bronchial cultures the time of epithelial repair after mechanic damage is significantly longer than that of healthy subjects.³⁶ This phenomenon appears to be intrinsic to epithelial cells, given that it persists even after repeated passages in culture and seems to result from inability to produce fibronectin.³⁶

Defective repair processes can reasonably lead to phenomena of "epithelial-mesenchymal" transition. Although no conclusive evidence still exists for asthma in humans, recent researches on experimental murine models have reported that after exposure to dust mites, respiratory epithelial cells can cross the basal membrane, reducing the expression of junctional markers (caderins and occludins) and up-regulating other mesenchymal markers (vimentin), a phenomenon thought to be mediated by TGF- β .³⁷ On the other hand, TGF- β itself seems to be one of the major mediators of phenomena of delay in epithelial repair and promotion of differentiation of fibroblasts into myofibroblasts, *i.e.*,

into cells that, in their turn, play a pivotal role in airway remodelling processes.^{2,38–41}

INCREASE IN MUCIN PRODUCTION

The occurrence of aspects of mucipar metaplasia in asthma has long been known, a phenomenon that seems to involve also the most peripheral airways where goblet cells are not present under normal conditions.^{1,2} Goblet cell mucin content has been observed to be increased in asthma, an increase that seems to go in parallel with increased mucin MUC5AC gene mRNA expression.⁴² As a result, also, the amount of epithelium-synthesized mucin, especially of the 5AC and 5B types, increases, thus contributing to the excessive production of viscous mucus, which is typical of asthma.^{1,2}

EGFR activation by TGF- α and other Th2-type cytokines, including IL-4, l'IL-9, and l'IL-13,^{1,2} seems to play a key role in these processes.

ALTERED MUCOCILIARY CLEARANCE

Some recent studies have indicated that a ciliary beat rate reduction can be observed in asthma patients together with an increase in ciliary dyskinesia indices being directly proportional to the degree of asthma severity.⁴³ These dysfunctions are structurally matched by a decreased number of ciliary cells and a larger amount of ciliary disorientation phenomena, which are more evident in severe asthma.⁴³

DEFECTIVE INNATE IMMUNITY AGAINST MICROBES

Rhinoviruses (RVs) are known to account for the main viral agents able to favor asthma exacerbations.⁴⁴ Breakthrough studies conducted by the Southampton research group have been able to show that the respiratory epithelium and the alveolar macrophages of asthmatic patients are not able to produce appropriate amounts of interferons (IFNs) of classes I and III (IFN- β and IFN- λ) in response to RV infection,^{45,46} a phenomenon that seems to correlate with both exacerbation severity and lung viral load. Interestingly, this same dysfunction seems to occur in macrophage responses to bacterial lipopolysaccharide, a fact that can in part account for the higher susceptibility of asthmatic patients to some bacterial infections.⁴⁷

A defective Toll-like receptor 7 function, which might be involved in determining the increased proneness to viral airway infections, has also been shown in adolescents with asthma,⁴⁸ whereas a recent work from Johnston's group indicated that the IL-15 production from bronchoalveolar lavage macrophage after RV infection was impaired in asthmatic patients and inversely related to lower respiratory symptoms.⁴⁹

The key role of these imbalances in respiratory epithelium immune defenses is substantiated by studies that have shown that some inborn immunity polymorphic variants, including β -defensin, correlate with asthma prevalence in the general population.⁵⁰

Other defects in innate immunity genes, *i.e.*, mannose-binding lectin gene, have also been shown to play a role in conditioning the development of asthma in individuals infected with *Chlamydia pneumoniae*.^{51,52}

INCREASED PRODUCTION OF CYTOKINES AND GROWTH FACTORS

Many cytokines, chemokines, and growth factors produced by the epithelium are involved in asthma pathogenesis, including IL-5, eotaxin (CCL11), RANTES (CCL5), chemotactic factors for monocytes/macrophages, and thymic stromal lymphopoietin (TSLP). Among all of them, a key role is thought to be played by TSLP in which its increased expression in the airways of asthmatic patients has been shown to correlate with asthma severity.⁵³ As a matter of fact, this cytokine has been shown to play a unique role in causing and maintaining immunophlogosis because, on the one hand, it is able (i) to promote both survival and activation of dendritic cells, by Th2 polarization of the immune responses (dendritic cell-mediated mechanisms), and, on the other hand, (ii) to activate mast cells, thus promoting inflammation independently of Th2 responses (mast cell-mediated mechanisms).^{2,3} Recent studies suggest that the airways epithelium of asthmatic patients exhibits a kind of bias in terms of immune responses to viral dsRNA because it produces less IFN- β and more TSLP compared with healthy individuals.⁵⁴

Chemokines account for other major respiratory epithelium up-regulated molecules crucial to immunophlogosis. Among these chemokines special mention is deserved by thymus- and activation-regulated chemokine, one of the main ligands of CCR4 because, according to some authors, this latter is responsible for most of the allergen-induced Th2 chemoattracting activity.^{1,55}

Some epithelium-produced cytokines play a key role in myofibroblast proliferation processes as well as in airways remodelling. It has been shown that after allergen challenge, human bronchial epithelial cells stimulate the production of type III collagen by myofibroblasts independently of the eosinophilic inflammatory response.⁵⁶ TGF- β is expected to play a major role among these cytokines. A recent study seems to suggest that the mechanical stimulation of the airways, *e.g.*, by methacholine inhalation, can in itself result in basal membrane thickening and mucipar hyperplasia phenomena, which go in parallel with TGF- β and Ki67 up-regulation (as mentioned previously) in the epithelium.⁵⁷

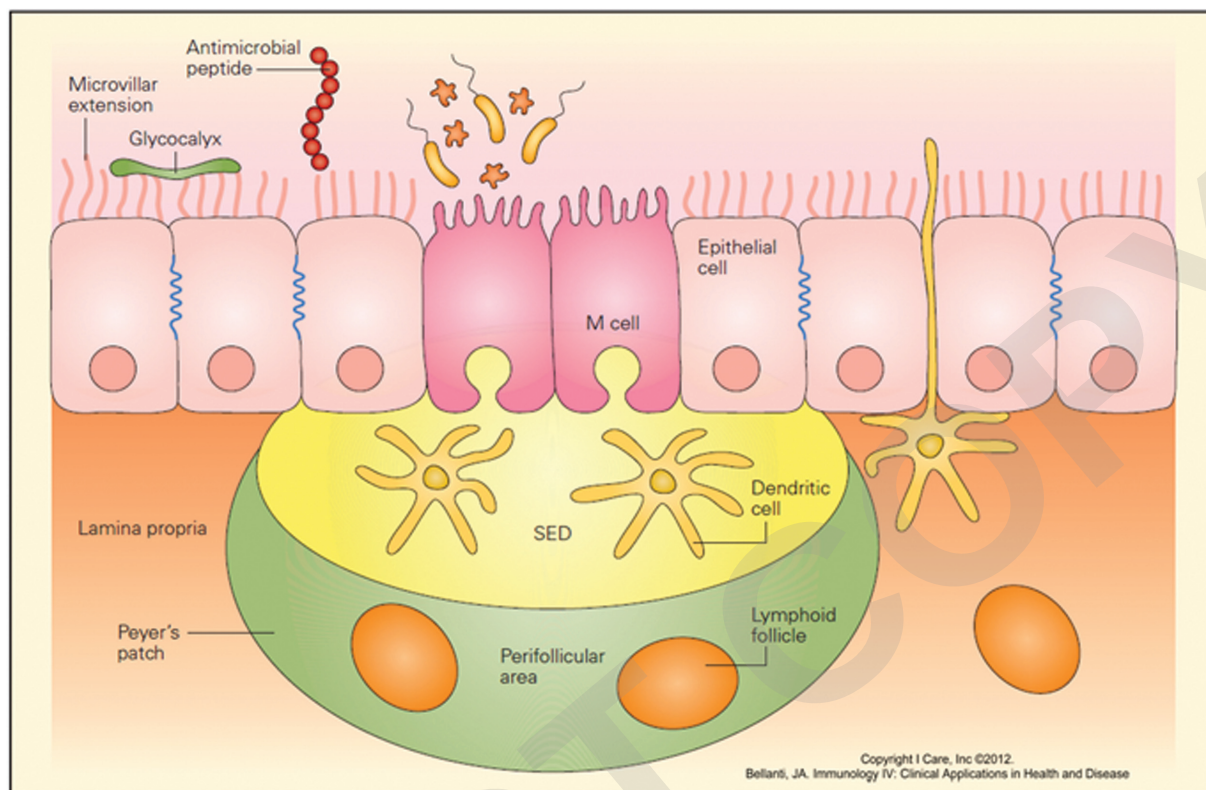


Figure 2. Schematic representation of the intracellular antimicrobial peptide trafficking and the mucosal regulatory system. (Reproduced with permission from Vega-Lopez M, Cole MF, and Bellanti JA. *The mucosal immune system in health and disease*. In *Immunology IV: Clinical Application in Health and Disease*. Bellanti JA (Ed). Bethesda: I Care Press, 255–286, 2012).

As a confirmation of the crucial role played by the epithelium in maintaining bronchial inflammation, some bioengineering experiments have shown that the “structural” cells of the airways (epithelium and fibroblasts) are able to inhibit bronchial T lymphocytes apoptosis (another TGF- β -mediated effect) in asthmatic patients.⁵⁸

ALTERED HANDLING OF THE RESPIRATORY MICROBIOTA

An altered interaction with the airway microbiota is to be included in the list of epithelial imbalances.⁵⁹ For example, asthmatic patients have long been known to be at a greater risk of invasive pneumococcal infections compared with healthy subjects.⁴⁷ A schematic representation of the intracellular antimicrobial peptides trafficking and their interaction with mucosal immune cells at the enterocytes level is shown in Fig. 2.

The airway microbial ecosystem has been reported to be profoundly altered in asthmatic patients by recent literature studies. Actually, the newborn studies performed by Bisgaard and colleagues show that early airway colonization (at 1 month of age) by normal extracellular bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*) is associated with an increased 5-year risk of developing asthma and

elevation in both total IgEs and blood eosinophils.⁶⁰ Furthermore, more recent studies by the same group have indicated that preschool children with a wheezing exacerbation exhibit a threefold risk of ongoing airways infection by the same bacterial agents.⁶¹ The association between bacterial infection and wheezing can, of course, be interpreted from the following dual perspective: some infections predispose to asthma or, most likely, asthma predisposes to some bacterial infections.^{46,47}

Other observations suggest that in the lungs of asthmatic patients bacterial flora differs from that of healthy subjects. In fact, when molecular techniques (bacterial 16S-rRNA characterization) are applied to samples of bronchial mucosa obtained by brushing, a flora mostly made of the phyla of the Proteobacteria group of bacteria (in particular *Haemophilus* spp.) has been shown to prevail in asthmatic patients compared with normal subjects who exhibit a prevalence of Bacteroidetes phylum (in particular *Prevotella* spp.).⁶² Some of the latest studies also suggest that both airway bacterial load and microbial “biodiversity” turn out to be increased in patients with poorly controlled asthma and that bronchial reactivity to provocative concentration of methacholine correlates with either parameter.⁶³

CONCLUSIONS AND FUTURE PERSPECTIVES

Asthma is a very complex disease with a number of areas of uncertainty regarding both the early initiation and the mechanisms of persistence of the inflammatory process. Recent insights suggest that interactions occurring very early in life between the EMTU and some noxae, above all viral and bacterial noxae, play a key role in the pathogenesis of this disease.^{60,61,64} The childhood origins of asthma study has, *e.g.*, shown that being affected by RV-induced wheezing illnesses in the first 3 years of life accounts for an important predictor of asthma in school age children, which seems much more important than any other known risk factor of asthma related to allergy sensitization.⁶⁴ The RV subtype involved in the wheezing illnesses of infancy is also likely to play a pivotal role in determining who will develop asthma among infected children, and some recent research seems to indicate that some strains of RV, *i.e.*, RV type C, are more “asthmogenic” than others.^{65,66} Understanding the relationships between EMTU, individual genetic and epigenetic factors, and early life infections will undoubtedly open new perspectives in asthma prevention and treatment.

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