

# Rethinking the Pathogenesis of Asthma

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Asthma research has focused primarily on allergic pathways on the basis that the majority of asthma is associated with atopy, the recruitment of the Th2-type T cell, the cytokines, and the chemokines that are released on exposure to allergens, and the IgE pathway. However, despite considerable investment by industry, targeting these pathways has not resulted in new treatments being developed beyond blockade of cysteinyl leukotrienes and IgE and improvements in inhaled corticosteroids and  $\beta_2$ -adrenoceptor bronchodilators. Increasingly, it is recognized that asthma is a heterogeneous disorder, and while important, allergen sensitization is only one component of the disease, with many other environmental and genetic factors playing a role. In addition, these factors act locally on a susceptible airway epithelium that is both structurally and functionally deficient. It may be worthwhile to focus on increasing the resilience of the airways to environmental insults in addition to improving strategies that modify adaptive immunity or suppress inflammation.

## Current Shortfalls in Understanding Asthma Pathogenesis

Asthma is a common disease that afflicts all ages and can vary greatly in severity. It is primarily an inflammatory disorder of the conducting airways upon which is superimposed both an increase in smooth muscle and a change in its responsiveness to stimulation that manifests clinically as variable airflow obstruction. The disease is also characterized by varying degrees of airway-wall remodeling linked to more fixed airflow obstruction and a gradual decline in lung function over time. Another degree of complexity is the wide number of environmental factors associated with the inception of asthma, its persistence, and its acute deterioration (exacerbations) that include exposure to inhaled allergens, air pollutants, certain drugs, occupational chemicals, and environmental tobacco smoke. Most frequently, asthma begins in early childhood, although the factors that contribute to its origin are still not known. Its course can vary widely over time, with up to half the children entering into remission during the adolescent and young adult years but with a propensity to return later in life again through mechanisms that are poorly understood. One reason why we know so little about those factors that contribute to its origin and the different phenotypes that ensue is because, until relatively recently, asthma has not been studied across the life course. Even in late-onset asthma in adults, we know little about those factors that initiate the

disease other than to the form associated with exposure to occupational chemicals. In the case of nonallergic asthma, where there is a conspicuous absence of atopy, almost nothing is known about its origins or how and why it can persist.

From a therapeutic standpoint, asthma is mostly managed with a combination of short- and long-acting bronchodilators and inhaled corticosteroids that target the inflammatory and smooth muscle responses. Although effective in the majority of patients, this approach to management falls a long way short of being ideal, as reflected by low patient adherence to treatment, dependency on the use of inhaled drugs (sometimes for life), and the remaining unmet clinical need, especially from exacerbations, at the severe end of the disease spectrum (approximately 10%). Patient-based surveys of asthma conducted in many countries all reveal similar findings of a persisting disease burden and impaired quality of life (Holgate et al., 2008). This does not mean that progress has not been made over the last two decades. Indeed, the introduction of management guidelines initially focused on a stepwise approach to drug use but more recently focused on attaining complete control has reduced mortality and hospital admissions where such guidelines are adhered to (Haahtela et al., 2006). However, the question that needs to be asked is whether this dependence on suppressive drugs is an end in itself or whether we should be aspiring to higher gains for

asthma sufferers. As the editor of the *Lancet* highlighted in a recent issue of this journal dedicated to asthma, "Progress in understanding asthma and its underlying mechanisms is slow; treatment can be difficult and response unpredictable; and prevention and cure are still a pipedream" (The Lancet, 2008).

So where do we go from here? Over the last 50 years, there have only been two therapeutic targets introduced that have translated to patient benefit: the discovery of cysteinyl leukotrienes (CysLTs) and their receptors and immunoglobulin (Ig) E leading to leukotriene antagonists and synthesis inhibitors and the IgE blocking monoclonal antibody, omalizumab, both targets originally identified many years ago. Part of the problem in identifying novel ways to treat asthma upstream of the inflammatory and remodeling responses is the notion that these aspects of asthma and disordered smooth muscle function are driven primarily by exposure to allergen, sensitization with production of IgE, and the subsequent recruitment of T lymphocytes differentiated to secrete an array of cytokines encoded in the *IL4* gene cluster on chromosome 5q. Undoubtedly, allergen-driven inflammation plays a role in the clinical manifestations of most asthma, with recruitment of mast cells, basophils, macrophages, and eosinophils to the airways where they secrete an array of mediators, including CysLTs, that interact with the formed elements of the airways resulting in airflow obstruction. However,

a question that still needs to be addressed satisfactorily is whether these T helper 2 (Th2) responses are primary, or whether they are secondary to other events that render the airways susceptible to asthma. One might go one step further and ask whether adaptive immunologic events are at the center of asthma pathogenesis or whether the disease originates within the lung itself, leading to recruitment of immune-effector mechanisms. This is important not least because most of the “novel” therapeutic targets that have been identified from animal models have emerged from using short- or long-term variants of antigen (usually ovalbumen) challenge following peritoneal sensitization in conjunction with adjuvant, such as Freund's or alum (Wenzel and Holgate, 2006). Reliance on such models, especially in rodents, emerged from the more traditional use of animals in pharmacology that helped in the discovery and improvement of bronchodilators, cromone-like anti-allergic drugs (e.g., cromolyn sodium, nedocromil sodium, lodoxamide), anti-histamines, corticosteroids, cystLT modifiers, and anti-IgE.

### Considering Asthma as a Th2 Disease

In their review, Barrett and Austen (2009) point out some of the anomalies of considering asthma purely as a Th2 disease. A range of new inflammatory cells and cytokines may be involved, including invariant NKT cells, Th17 cells and their associated cytokines IL-17 A and IL-17 F, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), the latter three being derived from the epithelium and all capable of driving either an eosinophilic, neutrophilic, or combined inflammatory response. However, in looking at the supportive evidence for any one of these, the evidence has largely accrued through manipulating the gene encoding the cytokine or its receptor in mice coupled with a range of antigen-challenged rodent models. For each pathway, there is evidence of expression in asthmatic airways but difficulty in selecting any one as taking primacy. In some respects, this has been a problem with research that has underpinned the components of the Th2 pathway that, despite predictions from *in vitro* and animal models, have so far proved somewhat disappointing as asthma targets. In rodents and, in some

cases in nonhuman primates, blockade of IL-4, IL-5, IL-9, eotaxin, and IL-13 or attempts to manipulate the T cell response in favor of Th1 cells all produce remarkable inhibitory responses on antigen sensitization and challenge models, although with no or less effect on an ongoing established Th2 response associated with remodeling changes (Holgate and Polosa, 2008).

Despite considerable initial optimism, blocking antibodies or soluble receptors that target Th2 cytokines or their receptors have been disappointing. In the case of IL-5, the blocking monoclonal antibody mepolizumab was shown to be highly effective in almost abolishing circulating and sputum eosinophils in asthma but had no discernable effect on clinical outcome measures. Of importance, in two subsequent small studies of mepolizumab highlighted by Barrett and Austen (2009) conducted in patients with persistent eosinophilia despite oral corticosteroids, revealed efficacy mostly against exacerbation (but not BHR). Thus, the concept that within the population of asthmatics there are subpopulations of IL-5 mAb responders raises the important issue of different disease phenotypes (Wenzel, 2009). Thus, although at one time considered as a single-disease entity, asthma subphenotypes are now recognized with differing pathology, clinical expression, responses to treatment, and long-term outcomes. The initial promising results with the anti-IgE mAb in severe asthma with associated allergy has also been revisited recently with the recognition of responders and nonresponders requiring up to 12 weeks treatment before a decision regarding future therapy can be made on combined clinical grounds because no biomarker of efficacy has so far been found (Holgate et al., 2009).

The latest Th2 cytokine target to come under scrutiny is IL-13 because of its Th2 type inflammatory profile, its capacity to promote mucus formation and secretion, and its effect in promoting remodeling in rodent models. Abrogation of IL-13 in these models has a dramatic effect at reducing all aspects of the asthma-like features following single or repeated antigen challenge. This cytokine has, therefore, become the most popular recent target against which to direct a new asthma therapy. As a consequence, there

are at least 12 biologics that are in clinical development designed to block IL-13 or its receptors. Despite this enthusiastic response by the pharmaceutical and biotechnology industries, the evidence linking asthma to IL-13 is limited to *in vitro* studies on human cells, some genetic association studies, and a few observational studies in which IL-13 has been measured in induced sputum and airway biopsies. Notably, there are no functional studies using airway explants from different types of asthma to show that blockade of IL-13 has any useful effect in this complex disease. Most recently, there have been two allergen challenge studies and one phase 2 clinical trial in moderate-severe asthma that show efficacy that is limited and unfortunately no better than cromolyn sodium that has recently been removed from the WHO list of approved drugs for asthma on account of limited efficacy. With the Amgen IL-4R-IL-13R $\alpha$ 1 blocking monoclonal antibody (mAb), there appears to be a small subgroup with the severest disease where there was a suggestion of efficacy. As further phase 2 clinical studies are completed, it will be of great interest to see whether the animal model predictions for IL-13 translate into patient benefit or whether this is going to be yet another target that emphasizes our incomplete understanding of disease pathogenesis in humans.

### Attacking the Inflammatory Effector Cells

Rather than targeting individual cytokines, chemokines, and autacoid mediators, an alternative strategy has been to target the effector cells themselves to inhibit their recruitment or their activation. In many ways, this is an attractive approach because it makes no assumptions as to why the cells are there in the first place.

#### Mast Cells

The mast cell has long been known to be a sentinel cell of the allergen-induced early- and late-phase airway response through the release of granule-associated and newly formed mediators and cytokines. Indeed, the rationale behind the introduction of inhaled cromolyn sodium in asthma therapy was through inhibiting mast cells, but its mechanism of action has never been clearly established (Holgate, 1989). While these drugs were used for many years in asthma prophylaxis,

especially in children, they eventually fell out of favor on account of their limited efficacy across the asthma spectrum (van der Wouden et al., 2008). However, its efficacy and safety in a subset of allergic and exercise-induced asthma is unquestionable; the problem was how to identify those that best respond—another example of stratified medicine (Stevens et al., 2007).

The last decade has witnessed an explosion in knowledge of mast cell ontogeny, subtypes, and activation-secretion coupling involving both cell-bound IgE and other stimuli. Despite this, there have been no additional asthma treatments to emerge that block mast cell mediator secretion, although there have been many attempts. Mucosal mast cells are largely T cell dependent and are sensitive to being inhibited by cromolyns (MC<sub>T</sub>) but only account for a proportion of the mast cells that contribute to asthma. Of particular relevance here is the dominance of the connective tissue mast cell (MC<sub>TC</sub>) prominent in the airway's smooth muscle and more peripheral airways, and which is involved in more severe disease (Bradding, 2009). The finding of a high population of MC<sub>TC</sub> in asthmatic airway smooth muscle, as well as a unique set of signaling molecules involved in muscle and mast cell communication, raises the important issue of whether these unique cells are primarily involved in programming the asthmatic airways smooth muscle and vice versa. Mast cells are an important source of Th2 cytokines and tumor necrosis factor alpha (TNF $\alpha$ ). In severe corticosteroid refractory asthma associated with a mixed neutrophilic and eosinophilic inflammatory response, TNF $\alpha$  expression is greatly enhanced both at mRNA and protein levels. However, despite initial promising results of small trials blocking TNF $\alpha$  with etanercept (soluble TNF receptor fusion protein) (Howarth et al., 2005) or a mAb (infliximab) (Erin et al., 2006), two large randomized control trials (RCTs) using etanercept or golimumab, a fully human monoclonal antibody against TNF $\alpha$ , failed to confirm this, though in the latter trial, there may be a subgroup with upper airways disease and bronchodilator reversibility that may respond (Wenzel et al., 2009). As with selective IL-5 and IL-13 blockade, variation in response to inhibiting TNF $\alpha$  might be a further

example of subphenotypes (or endotypes) of severe asthma that respond to different interventions (Anderson, 2008).

#### **Eosinophils**

The eosinophil is the other effector cell associated with asthma and certainly in animal models of antigen-driven airway inflammation and BHR; it has been causally linked, although this has depended on the mouse strain and stimulus. In asthma, the evidence is correlative. Previous reference has been made to the negative results of IL-5 blockade apart from the few patients with highly eosinophilic asthma in the face of high-dose corticosteroids, but this still does not definitively answer the question about the role of eosinophils per se in this disease. The nearest we are likely to get to this is the upcoming trial of an antibody-dependent cell cytotoxic defucosylated IgG1 monoclonal antibody (MEDI-563) directed to all cells expressing the IL-5 $\alpha$ . Engineering of mAbs by removing fucose residues from the Fc fragment leads to greatly enhanced antigen-dependent cellular cytotoxicity (ADCC) activity as compared to a highly fucosylated conventional antibody (Yamane-Ohnuiki et al., 2004). Data from a completed Phase 1 study of MEDI-563 have demonstrated the antibody is well tolerated with substantial and prolonged depletion of blood eosinophils, thereby supporting its continued development.

#### **T Lymphocytes**

A similar approach is being taken to manipulate the Th2 T cell population. Th-2-type T cells are greatly enriched with CCR4, the principle chemokine receptor responsible for allergen-induced migration of these cells into asthmatic airways. AMG 761 (previously KW-0761) is a humanized IgG1 monoclonal antibody targeted to CCR4-positive T cells and leads to depletion of CCR4-positive T cells as a therapy for asthma. This antibody has already shown efficacy in relapsed patients with adult T cell leukemia-lymphoma and cutaneous T cell lymphoma, including *mycosis fungoides* and Sézary syndrome (Yano et al., 2007).

Another T cell subtype that has attracted much recent interest in asthma is the regulatory T cell (Treg) (Akdis and Akdis, 2009). In their review of a potential role for regulatory T cells, Lloyd and Hawrylowicz (2009) emphasize the importance of these cells in mediating allergen-

specific tolerance through the secretion of IL-10, a known suppressive cytokine. Treg cells have also been strongly implicated in the efficacy of both allergen subcutaneous and sublingual immunotherapy, including peptide immunotherapy. As a broad approach to treating asthma, it seems that clinical efficacy may be limited to mild-moderate allergic asthma, where one specific allergen is a major contributor, e.g., cat *Fel d1*. Another exciting development made by Hawrylowicz is the capacity of vitamin D3 to reverse corticosteroid refractoriness of circulating mononuclear cells from patients with difficult-to-treat asthma through induction of IL-10. Clinical trials are now in progress. TGF $\beta$  is also an immunosuppressive cytokine that promotes the differentiation of an "adaptive" subset of Tregs (Curotto de Lafaille and Lafaille, 2009), but as discussed by Lloyd and Hawrylowicz, this is also a profibrotic cytokine, so manipulation to enhance its production could enhance remodeling and promote epithelial-mesenchymal transition (Boxall et al., 2006).

Invariant natural killer T (iNKT) cells have aroused interest as potential mediators of asthmatic inflammation. These specialized T cells recognize endogenous and exogenous glycolipid antigens in the context of the CD1d receptor and secrete cytokines (especially interferons and CXCR3 chemokines) that amplify both innate and adaptive immunity. Although outside the Th2 hypothesis of asthma, in mice, these cells have been incriminated in neutrophilic inflammation. The initial high-profile interest in iNKT cells has largely come from mouse models and initial reports of their high numbers in asthmatic lavage fluid (Akbari et al., 2006). Application of more stringent analytical techniques for their detection by flow cytometry using CD1d tetramers loaded with alpha-galactosylceramide and antibodies specific to the invariant natural killer T cell receptor in samples of lavage fluid, induced sputum, and bronchial-biopsy specimens has revealed that <2% of T cells belong to this subtype, compared to initial claims of upwards of 60% (Vijayanand et al., 2007). Selective antagonists and blocking monoclonal antibodies for CD1d are being developed that will enable functional studies in humans.

### Does Asthma Originate in the Airway Structural Cells?

The up-to-date review by Lambrecht and Hammad (2009) focuses attention on the dendritic cell (DC) as a target for intervening to control asthma. These function in antigen recognition and can be programmed through innate mechanisms to shape the subsequent adaptive immune response. In addition to covering the different subsets and ontogeny of DCs, this review focuses attention on the role of the airway epithelium to instruct DCs along their differentiation pathway. It is surprising that it has taken so long to place the airway epithelium in the context of asthmatic inflammation and remodeling because in all subtypes of asthma, irrespective of age, evidence of epithelial activation and damage is one of the most prominent features. In 2000, this led us to suggest that asthma is a disorder of the epithelium, with connections both to the origin and sustenance of airway inflammation, as well as being the principle driver of remodeling (epithelial-mesenchymal trophic unit, EMTU) (Holgate et al., 2000). DCs extend their processes up between adjacent columnar epithelial cells and make intimate contact with the expression of tight junction proteins. As pointed out by Lambrecht and Hammad, crosstalk between these airway elements is likely to be fundamental to the origins and emergence of asthma subphenotypes across the life course. One mediator that has aroused interest in this respect is thymic stromal lymphopoietin (TSLP), whose release from the airway epithelium upon activation of selective toll-like receptors (TLRs) 2, 4, 8, and 9 lead to upregulation of OX40L on DCs and their increased capacity to drive a Th2 response (Holgate, 2007a). The crucial question is the nature of this epithelial-DC interaction. A clue has come from understanding the pathogenesis of exacerbations.

It is now clear that most exacerbations of asthma, both in adults and children, occur in relation to respiratory virus exposure following upper respiratory tract infections, although exposure to seasonal allergens and air pollutants are also causes (Sears, 2008). Rhinoviruses (RVs) seem to account for the majority of the causative organisms, especially with the recent identification of the RV C clade (Miller et al., 2009). In 2005, we reported

that asthmatic airway epithelial cells are deficient in their capacity to generate IFN $\beta$  in response to RV 16 infection in vitro (Wark et al., 2005). This was associated with an inability of the cells to limit viral replication by entering into apoptosis, with the consequence that the replicating virus caused cytopathic cell death with extensive virus shedding. Subsequently, a similar defect in IFN $\beta$  production has been shown with the minor subtype RV1B (Wark et al., 2009). Addition of exogenous IFN $\beta$  restored the asthmatic cells' ability to eliminate the virus. Human recombinant IFN $\beta$  by inhalation is being developed as a treatment to prevent asthma exacerbations in severe disease.

Early life exposure to respiratory viruses is now considered to be a major predisposing factor in the induction of asthma. A recent important discovery is that repeated infections with rhinovirus (RV) during the first 3 years of life increased the risk of developing asthma by age 6 years 26-fold compared to 3-fold for allergen sensitization (Jackson et al., 2008). The key role of early life virus infection extends into adult asthma in the European Community Respiratory Health Survey (Dharmage et al., 2009). In a U.S. 95,000 infant cohort study, the timing of birth in relationship to the winter virus season conferred a 30% increased risk of developing asthma by 6 years of age (Wu et al., 2008), while in a Perth cohort, respiratory virus infection (RV = 70% and RSV = 16%) positively interacted with atopy to promote later asthma at 5 years of age (Kusel et al., 2007). Importantly, the target for viruses and environmental stimuli such as ETS and other pollutants is the airway epithelium. Understanding why the airway epithelium of these children is so susceptible to these stimuli and how it affects allergic sensitization provides a potential new route to prevent asthma.

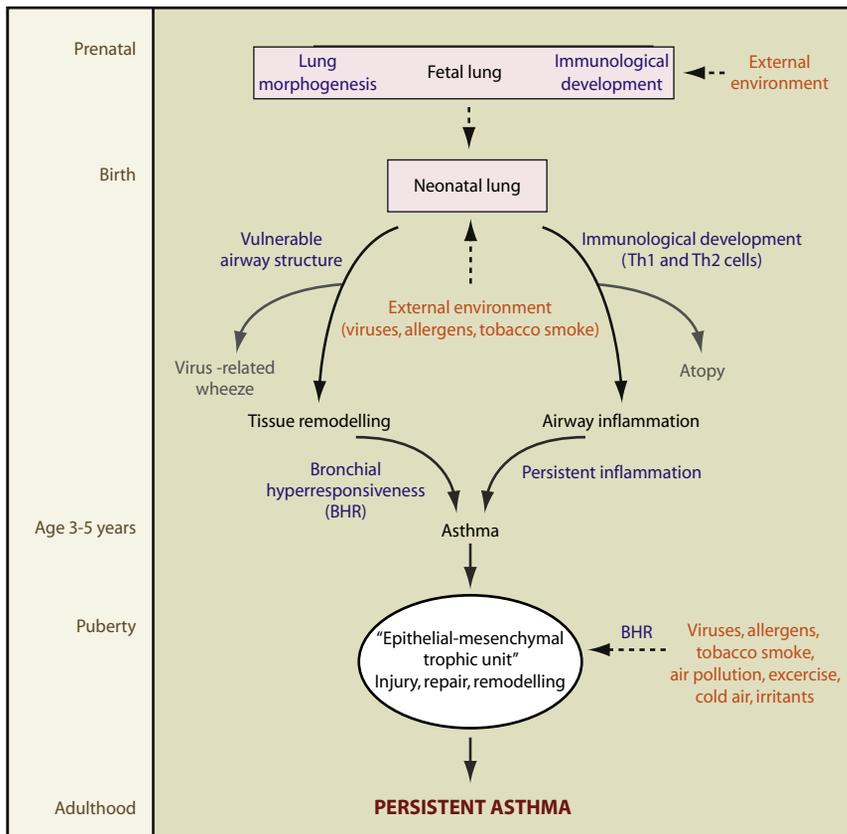
As discussed by Lambrecht and Hammad, airway DCs play a critical role in initiating and regulating early inflammatory events. In the first year of life, infants do not typically exhibit airway DCs in the absence of inflammation, but severe respiratory infection is a powerful stimulus for their appearance (Tschernig et al., 2001). Thus, it is possible that respiratory virus infection of the asthmatic epithelium causes airway DC maturation with a pref-

erential bias toward a Th2 response to the penetrating allergen. This proposal is supported by showing that, in response to TLR3 agonists, bronchial epithelial cells from asthmatic subjects make less IFN- $\beta$  and more TSLP than those from normal donors. Other epithelial defects that are intrinsic to the asthmatic epithelium are a reduction in antioxidant capability and impaired formation of tight junctions leading to reduced barrier function.

Taken together, a structurally and functionally defective airway epithelium underlies abnormal responses to respiratory viruses and other components of the inhaled environment (Figure 1). This would promote a microenvironment that facilitates allergic sensitization, supports different types of inflammation, and predisposes the airways to development of asthma during childhood.

### Airway Remodeling in Asthma

Bronchial biopsies from very young children with early life virus-associated wheezing reveal little abnormal pathology, but by the age of 3 years, epithelial injury and thickening of the *lamina reticularis* is evident, either in the absence or presence of Th2 type inflammation. While thickening of the *lamina reticularis* is diagnostic of asthma in children and adults, there is doubt over its importance to airway remodeling because it does not relate to asthma duration, although may increase with severity. Based upon its presence in asthma and following lung transplantation, deposition of new matrix in the *lamina reticularis* is indicative of chronic epithelial injury (Holgate, 2007b). The proliferation of airway smooth muscle, epithelial mucous metaplasia, increase in neural and vascular networks, and the deposition of matrix throughout the airway wall translates into airway hyper-responsiveness, fixed airflow obstruction, and a progress decline in lung function over time that characterizes chronic persistent asthma. In adult and childhood asthma, epithelial overexpression of epidermal growth factor receptor, reduced markers of cell proliferation (Ki67, PCNA), and increased nuclear translocation of the cell-cycle inhibitor P21<sup>waf</sup> are consistent with impaired epithelial repair responses. Most recently, airway epithelial cells cultured from atopic asthmatic, when compared to those from atopic or nonatopic normal children, show



**Figure 1. Schematic Representation of Factors that Interact in the Initiation and Persistence of Asthma**

The developing fetal lung expresses genes that predispose the airways to both structural and immunological changes that favor the development of asthma in early childhood. An interaction between vulnerable airways and multiple inhaled environmental insults, including allergens, environmental tobacco smoke, viruses, pollutants, and other chemicals, translate into airway inflammation and tissue remodeling. This involves increased susceptibility of the epithelium to injury, impaired repair, and activation of mesenchymal cells, leading to persistent inflammation, airway hyper-reactivity, and remodeling. These interactions are also influenced by gender and age as well as physical interactions generated by repeated bronchoconstriction. Environmental factors acting at different stages throughout the life course probably act together in generating the different asthma subtypes, susceptibility to treatment, and natural history. The hyper-reactive asthmatic airway manifests in variable airflow obstruction in response to a range of different insults such as allergens, exercise, cold air, and irritants.

greatly impaired wound repair responses following injury (Stevens et al., 2008). An additional important stimulus for profibrogenic growth factor release is epithelial distortion consequent upon repeated cycles of bronchoconstriction. The consequences of these events are engagement of the trophic activity of the EMTU to promote airway remodeling and increased airway smooth muscle (Figure 1).

### An Approach to Prevention and Treatment

The important role of the physical and functional barrier functions in atopic dermatitis (eczema), food allergy, and rhi-

nosinusitis is now being realized with defects in filaggrin, S100, and claudin proteins, respectively, indicating that this might be a generic feature for acquiring mucosal allergy. Based on this primary role of the airway epithelium in the group of disorders' causation and persistence, an approach to preventing and treating asthma could be to increase the resistance of the epithelium to the inhaled environment, perhaps in addition to suppressing the immune and inflammatory responses once they have become established.

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