Epithelial–Mesenchymal Communication in the Pathogenesis of Chronic Asthma

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Although Th-2–mediated inflammation is a key therapeutic target in asthma, its relationship to altered structure and functions of the airways is largely unknown. In addition to inflammation, asthma is a disorder involving the airway epithelium that is more vulnerable to environmental injury and responds to this by impaired healing. This establishes a chronic wound scenario that is capable of sustaining chronic inflammation as well as remodeling. This response occurs as a consequence of activation of the epithelial–mesenchymal unit, involving reciprocal activities of growth factors belonging to the fibroblast growth factor, epidermal growth factor, and transforming growth factor-β families. The observation that structural changes in the airways in children at or before the onset of asthma occurs irrespective of inflammation might suggest that premodeling is required before Th-2 inflammatory responses can be sustained. Once established, altered function of constitutive airway cells, including fibroblasts, smooth muscle, nerves, and the epithelium, provides an abnormal microenvironment in which to generate a separate set of signals that underpin the acute/subacute inflammation characteristic of asthma exacerbations, triggered by viruses, pollutants, and allergens.

Keywords: asthma; airway remodeling; epithelial–mesenchymal trophic unit

As the late Professor Ann Woodcock repeatedly emphasized during her distinguished career, asthma is a disorder of conducting airways that contract too much and too easily. Bronchial hyperresponsiveness (BHR) has been recognized as a prominent feature of asthma, although it has been reported in a wide range of airway disorders, including chronic obstructive pulmonary disease, sarcoidosis, and heart failure. Although broadly BHR correlates with asthma severity, a cause and effect relationship is far from clear. Thus, in longitudinal studies in which repeated measurements of BHR have been made and correlated with other physiologic indices and clinical manifestations of asthma, the relationship between BHR and clinical outcome measures in asthma is relatively weak (1, 2). The reason for this is that BHR, as measured in the laboratory with bronchial provocation tests using histamine or methacholine, relates only loosely to environmental stimuli that cause airway obstruction in naturally occurring asthma such as air pollutants, cold air, exercise, allergens, and infections (3). Indeed, each of these stimuli invokes airway narrowing indirectly through the release of mediators from a variety of effector cells, including neurones. In addition, there are stimuli such as respiratory virus infections that can cause severe exacerbations of asthma, even in subjects with mild BHR (4).

THE INFLAMMATORY COMPONENTS OF ASTHMA

The recognition that asthma is an inflammatory disorder of the airways in which acute and subacute inflammatory responses are superimposed on a chronic underlying inflammation has provided a strong basis for intervening in this disease with anti-inflammatory drugs, especially inhaled corticosteroids. In mild to moderate asthma, this drug class is highly effective at suppressing BHR in parallel with resolution of inflammation, suggesting that their predominant mode of action is on the inflammatory component of asthma. Research over the last 3 decades has provided overwhelming evidence for an important role for mast cells, eosinophils, and basophils orchestrated by antigen-presenting cells via T and B lymphocytes. The importance of these pathways, at least in a component of allergic asthma, is supported by the efficacy of target-specific interventions such as leukotriene receptor antagonists and anti-human IgE monoclonal antibodies.

Lessons on the cellular and mediator mechanisms of asthma have been gained through the use of pharmacologic agents. The clear efficacy of inhaled corticosteroids in asthma has been suggested to result from the effect of these drugs in suppressing the production of Th2 cytokines, which underpin mast cell eosinophil and basophil responses (5). Data in animal models and limited studies in humans have identified interleukin (IL)-5 as being particularly relevant to asthma on account of its powerful influence on eosinophil development and priming. To target this cytokine, humanized monoclonal antibodies have been developed and subjected to clinical trial in chronic asthma. One blocking monoclonal antibody, mepolizumab, directed to IL-5, although being highly efficacious in reducing eosinophils in the circulation and airway lumen, had no effect on the early or late asthmatic response to allergen challenge (6) nor when administered on three occasions at 4-week intervals on clinical or physiologic indices of asthma (C. Compton, personal communication). Despite producing a marked circulating and airway lumen eosinopenea in asthma, mepolizumab has been shown to reduce the airway submucosal content of eosinophils by only 55% (7) and appeared to have no effect on the subtype distribution or activation status of circulating T cells (8). That this was sufficient to influence molecular events in the asthmatic airway has been shown in an extension of this study demonstrating a reduction in matrix proteins present beneath the epithelial basement membrane such as tenascin C and lumican (7). Whether this effect of anti–IL-5 on matrix turnover is eosinophil dependent is not known because airway fibroblasts and epithelial cells are also known to possess IL-5 receptors.

These findings with anti–IL-5 are in clear contrast to a bronchial biopsy study undertaken before and after 12 weeks of treatment with the anti-human IgE monoclonal antibody (omalizumab), which produced an 80% reduction in airway mucosal tissue eosinophil content paralleled by almost a total loss of $Fc_{\varepsilon}R1$ staining cells (including mast cells, basophils, and dendritic cells) as well as a reduction in $CD4^+$, $CD8^+$ T cells, and $CD20^+$

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B cells (9). In several large clinical trials in allergic asthma, omalizumab has been shown to be efficacious both as an add-on therapy to inhaled corticosteroids and allowing corticosteroid reduction. One explanation for the discrepancy between these two forms of targeted therapy to achieve efficacy is the need for antiasthmatic therapeutics to intervene on more than one immune and inflammatory cell type or that a major inhibitory affect on tissue eosinophils is required rather than the 50% reduction observed with mepolizumab (7). One possibility is a combination of blocking strategies for IL-5 in combination with antichemokines, especially targeted toward the eotaxins and their receptor CCR3. Studies indicate that the beneficial effects of corticosteroids that were once though to be largely due to their effect in depleting airways of eosinophils exert their actions on multiple cells to generate efficacy in asthma.

CHRONIC ASTHMA: MORE THAN AN INFLAMMATORY DISEASE

The persistence of airway dysfunction in the presence of high-dose inhaled corticosteroids presents an important clinical problem, which, in part, has been resolved by the addition of a long-acting inhaled β2-adrenoceptor agonist (10, 11). Thus, whereas the inhaled corticosteroid suppresses inflammation, the long-acting β2 agonists will relax underlying airway smooth muscle. The synergy between these two therapeutics suggests that there are components of chronic asthma, particularly in patients with more severe disease, that are not amenable to corticosteroid suppression (12). This leads us to the conclusion that there may be two parallel events necessary for asthma to become chronic and persistent: (*1*) a susceptible airway linked to altered structure and function ("remodeling") and (*2*) a microenvironment capable of sustaining a chronic inflammatory response. One example of the latter is the strong correlation between the mast cell (but not eosinophil) content of airway smooth muscle that correlate strongly with variable airflow obstruction and BHR (13), whereas eosinophils present in the airway lumen and in induced sputum correlate strongly with asthma exacerbations (14). Asthmatic airway smooth muscle is more capable of sustaining a connective tissue-like (tryptase- and chymase-positive) mast cell population when compared with normal airway smooth muscle, probably due to the enhanced release of mast cell growth factors. In chronic asthma, recent observations suggest that abnormal tissue injury and repair provide the appropriate microenvironment for persistence of airway inflammation and remodeling in asthma (15).

Using the caspase 3 cleavage product of poly-ADP ribose polymerase "p85" as a marker for early apoptosis, immunostaining of bronchial biopsies has revealed patches of columnar cells accompanied by clumps of $p85^{+}$ epithelial cells in lavage fluid that were not found in biopsies from individuals without asthma (16). These studies, along with increased fas and fas ligand expression in severe asthma (17) and evidence of DNA fragmentation (18), suggest that in this disease, the airway epithelium is more susceptible to injury than is the normal epithelium, and as a consequence, clusters of epithelial cells enter into premature program cell death. That this is an intrinsic abnormality of the asthmatic epithelium has been further shown using cultures of airway epithelial cells obtained by bronchial brushing (16). When these cells are grown to confluence at passages 2 to 3 and then exposed to an oxident stimulus such as H_2O_2, they exhibit a threefold increase in the apoptosis marker anexin 5 (which identifies disruption to the plasma membrane and exposure of phosphatidylserine) when compared with epithelial cells cultured from normal airways. Further studies by Bayram and colleagues (19) reveal that asthmatic epithelial cells exhibit increased permeability to carbon^{14}-labeled bovine serum albumen movement across the epithelium *in vitro* when exposed to 10- to 100-ppb ozone. The mechanisms responsible for this increase susceptibility to oxidant injury are not known, but it could be due to the low expression of antioxidant protective mechanisms.

CHRONIC ASTHMA: A DISORDER OF ALTERED RESPONSE TO INJURY

When epithelial surfaces are injured, the normal response is for them to upregulate receptors, specifically members of the epidermal growth factor receptor (EGFR) family that drive proliferation and repair. Asthmatic biopsies demonstrate a marked increase in the expression of the EGF receptor (C erb B1, Her 1), whose expression increases in proportion to disease severity and chronicity irrespective of whether patients are taking inhaled corticosteroids (20, 21). EGFR immunostaining in severe asthma is throughout the repairing epithelium and also on the luminal surface, a feature not observed in the normal epithelium. *In vitro*, damage to the airway epithelium in culture results in the secretion of an array of EGFR ligands (including EGF, transforming growth factor [TGF]-α, and amphiregulin), which dimerize EGFRs, resulting in activation of their tyrosine kinase and cell proliferation through mitogen-activated protein kinase pathways (20). However, contrary to expectations, the high level of EGFR expression in the asthmatic epithelium is not paralleled by an increase in markers of cell proliferation such as proliferating cell nuclear antigen, even in patients with severe asthma taking high doses of inhaled and/or corticosteroids (22). In mild asthma, there is also marked cytoplasmic staining of columnar cells for $p21^{waf}$, indicating an attempt to protect the surface epithelial cells from premature program cell death. However, in severe disease, $p21^{waf}$ is found throughout the epithelium and specifically at a nuclear location, where it impairs proliferation by arresting cells at the G1 phase of the cell cycle (23). These findings lead us to the view that in chronic asthma the airway epithelium is impaired in its ability to restitute itself after injury and, as a consequence, enters into a chronic wound response.

High-resolution computed tomography and postmortem and biopsy studies in chronic asthma have all revealed airway wall thickening (24). This involves deposition of interstitial collagens in the lamina reticularis, matrix deposition in the submucosal, smooth muscle hyperplasia, and microvascular and neuronal proliferation. Thickening of the lamina reticularis is diagnostic of asthma (25), and on the basis of measurements made in human airways, it appears to reflect thickening of the entire airway wall, including the airway smooth muscle (26). In 1990, we described a layer of subepithelial mesenchymal cells with features of myofibroblasts whose number was increased in asthma in proportion to the thickness of the reticular collagen layer (27). These cells corresponded to the attenuated fibroblast sheath described by Evans and colleagues (28) lying adjacent to the lamina reticularis and forming a network capable of activation from signals provided by the overlying epithelial cells (Figure 1). These two cellular layers are in a key position to coordinate responses to challenges from the inhaled environment into the deeper layers of the submucosa (15, 16). Most recently, Benayoun and colleagues (29) have shown that in airway biopsies in carefully phenotyped patients, fibroblast accumulation and smooth muscle hypertrophy in proximal airways are selective determinants of severe persistent asthma. The importance of mesenchymal cells in translating environmental signals via the airway epithelium has recently been emphasized.

EPITHELIAL–MESENCHYMAL COMMUNICATION

Injury or distortion (30, 31) of epithelial monolayers *in vitro* results in increased release of fibroproliferative and fibrogenic

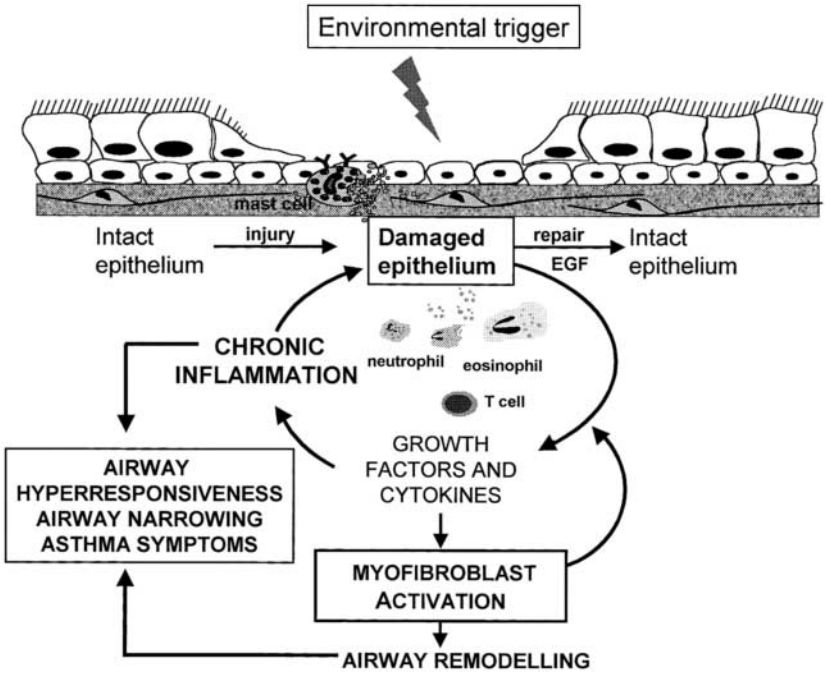

Figure 1. Activation of the epithelial–mesenchymal trophic unit (EMTU) in chronic asthma. This involves communication between the damaged and stressed epithelium and underlying fibroblast sheath to cause mesenchymal cell proliferation and leading to remodeling. Th2 cytokines generated as part of the inflammatory response such as interleukin (IL)-4 and IL-13 interact with the epithelial–mesenchymal trophic unit to augment remodeling. EGF = epidermal growth factor.

growth factors, including fibroblast growth factor-2, insulin-like growth factor-1, platelet-derived growth factor, endothelin-1, and both latent and active TGF-β2. The ability of asthmatic epithelium to generate these growth factors is increased when compared with normal epithelium (32). They are present in increased concentrations at the airway surface and become encrypted in the extracellular matrix, as shown by their colocalizations with proteoglycans (33). The relationship between EGFR signaling and the repair and remodeling processes has been further defined using an EGFR-selective tyrosine kinase inhibitor (tyrphostin, AG1478), which when present at the time of epithelial injury *in vitro* results in impaired repair paralleled by an increase in the release of both active and latent TGF-β2 (20). This points to parallel pathways operating in repairing epithelial cells, some of which direct efficient restitution and are regulated by EGFR, whereas others control profibrogenic growth factor production independent of EGFR. In chronic asthma, we suggest that impaired epithelial proliferation causes the bronchial epithelium to spend longer in a repair phenotype, resulting in increased secretion of profibrogenic growth factors. It is likely that the profibrogenic growth factors, including TGF-β, originate both from the damaged epithelium and inflammatory cells such as eosinophils and mast cells, and in this way, chronic inflammation continues to augment a remodeling response (7, 34).

Communication between the epithelium and the underlying epithelial fibroblast sheet is reminiscent of the process that drives branching morphogenesis in the fetus where the epithelium and mesenchyme act as a trophic unit (35). Thus, in chronic asthma, we propose that the epithelial mesenchymal trophic unit, so crucial to lung development in the fetus, becomes reactivated to drive pathologic remodeling. TGF-β and the related bone morphogenic proteins that are secreted by the epithelium and inflammatory cells are powerful stimuli for differentiating airway mesenchymal cells into myofibroblasts (36, 37), which have both contractile and secretory functions. Most recently, we have further shown that this differentiation step is mediated through the autocrine secretion of connective tissue growth factor. A characteristic feature of myofibroblasts is the alignment of both α-actin and heavy-chain myosin filaments in bundles, which after appropriate stimulation are capable of contracting. Contraction of myofibroblasts is of critical importance in the closure of chronic wounds. The relationship between myofibroblasts and airway smooth muscle cells has yet to be determined, but one possibility is that the airway fibroblast provides a continued source of precursor cells to generate new smooth muscle, which in many respects has characteristics of myofibroblasts (i.e., contract, secrete cytokines and chemokines and matrix molecules) (13, 15). In a rabbit model of partial outflow obstruction in the bladder, TGF-β causes wall thickening because of accumulation of myofibroblasts, which change into smooth muscle cells over time (38). Even in patients with mild asthma, after allergen provocation, myofibroblast have been observed to migrate from the epithelium toward smooth muscle (or fibromyoblasts from the smooth muscle to the epithelium), suggesting a dynamic interrelationship between these two airway compartments (39).

In addition to driving differentiation of airway mesenchymal cells to a contractile phenotype, TGF-β enhances the capacity of these cells to release a wide variety of additional growth factors and cytokines (40–42). Some of these such as connective tissue growth factor contribute to airway wall thickening by inducing the secretion of instititial collagens and proteoglycans. TGF-β2 also increases the release of vascular endothelial growth factor and endothelin. An additional property of fibroblasts cultured from asthmatic airways is their ability to proliferate in the absence of exogenous growth factors (43). This can be observed *in vitro* between 36 and 72 hours and can be blocked using an antibody against the EGFR, supporting the view that asthmatic mesenchymal cells have enhanced capacity to generate EGFR ligands such as TGF-α and amphiregulin (40, 43). Enhanced proliferative responses have also been reported for asthmatic airway smooth muscle, which also has an enhanced capacity to generate connective tissue growth factor.

INTERACTION BETWEEN IL-4 AND IL-13 AND THE EPITHELIAL–MESENCHYMAL TROPHIC UNIT

In view of transgenic mice studies that have suggested that expression of an IL-13 transgene in the bronchial epithelium leads

to submucosal remodeling (44), we have investigated the role of IL-4 and IL-13 in asthmatic epithelial and fibroblast function. Both IL-4 and IL-13 signal via signal transducer and activator of transcription 6, whose expression is prominent in the bronchial epithelium of asthmatic airways (45). Although others and we have shown that IL-13 is able to induce myofibroblast transformation (46) and rescue the cells from apoptosis (47), this Th2 cytokine is two orders of magnitude less potent than TGF-β. In human epithelial cells, IL-4 is as effective as IL-13 in promoting TGF-β release, which is inhibited by dominant-negative signal transducer and activator of transcription 6 (48). Epithelial cells respond to IL-4 and IL-13 with increased granulocyte–macrophage colony-stimulating factor and IL-8 production, which is further augmented by enzymatically active extracts of allergens. In the case of asthmatic fibroblasts, IL-4 and IL-13 enhance the production of cytokines such as eotaxin, regulated upon activation, normal T cell expressed and secreted, and granulocyte–macrophage colony-stimulating factor, which might help explain the accumulation of eosinophils beneath the lamina reticularis in asthma. Thus, by interacting with epithelial mesenchymal trophic unit, both IL-4 and IL-13 contribute to chronic inflammation and airway remodeling (Figure 1).

Airway mesenchymal cells (including smooth muscle) have the capacity to generate a range of proinflammatory cytokines and chemokines, which could help support chronic airway inflammation (41, 42). This mechanism helps explain why in asthma (but interestingly not in eosinophilic bronchitis) there is a disease-related increase in the colonization of airway smooth muscle by connective tissue–like mast cells (13, 49). The secretory behavior of asthmatic mesenchymal cells may provide an explanation why airway mastocytosis persists and is associated with BHR and variable airflow obstruction. Another asthma-specific feature is the ability of the Th2 cytokines IL-4 and IL-13 to increase preferentially the production of TGF-α from epithelial cells cultured from asthmatic airways *in vitro* (50). TGF-α, acting via the EGFR, is a powerful stimulus for epithelial goblet cell metaplasia. This helps explain why epithelial cells that are cultured *in vitro* in the presence of IL-4 or IL-13 and then differentiated at an air liquid interface become heavily colonized by goblet cells in preference to ciliated columnar cells.

AIRWAY WALL REMODELING OR PREMODELING AS THE BASIS OF ASTHMA

BHR is now understood as a major feature of childhood asthma and differentiates this airway disorder from intermittent virus-induced wheezing (51, 51). In subjects with asymptomatic BHR, longitudinal studies have shown that those who progress to asthma exhibit parallel changes in inflammation and remodeling. Recent bronchial biopsy studies in children have shown that thickening of the lamina reticularis correlates poorly with eosinophilic inflammation (52, 53). Our own studies on 4- to 11-year-old children have shown not only thickening of the lamina reticularis in children with asthma but also strong correlations between this and increased epithelial EGFR and reduced $p21^{waf}$ expression (54), as found in adult asthma (20, 21, 23). Because these changes appear to be present in both moderate and severe childhood asthma and be independent of eosinophil numbers, it is possible that disordered epithelial–mesenchymal signaling is a key feature in the origin of human asthma. It is known that during branching morphogenesis, activation of EGFRs increases the airway expression of metalloprotease enzymes to increase extracellular matrix degradation and at the same time proliferate the epithelium. Conversely, increased TGF-β expression reduces metalloprotease expression and extracellular matrix degradation and provides an inhibitory signal to the epithelium for proliferation. Intermittent EGF and TGF-β dominance produces alternating linear and branching of the developing airways (55). A disorder in this process may account for the continued premodeling of the airways of children destined to develop asthma. Thus, in addition to gene–environmental interactions necessary to generate a persisting Th2-mediated inflammatory response, for chronic asthma to develop, it is likely that altered epithelium mesenchymal signaling is required because of some fundamental abnormality in the functioning of the epithelial mesenchymal trophic unit. Such a mechanism might explain why the early introduction of inhaled corticosteroids when asthma is first recognized has powerful effects on those aspects of the disease linked to airway inflammation but has relatively little effect on the declining lung function consequent on an altered structure of the airway wall measured as the postbronchodilator FEV_1 (56–58). It also provides a good explanation for the wide variety of environmental factors that cause exacerbations through perturbations of a susceptible epithelium (Figure 1). Taken together, these studies raise the important point that the cellular and molecular mechanisms of chronic and severe asthma may be different from that of mild disease, with the former requiring early recruitment of epithelial–mesenchymal signaling, whereas the latter is more dependent on Th2 inflammatory pathways alone. This might help explain part of the corticosteroid refractoriness observed in chronic severe disease.

A HISTORICAL PERSPECTIVE

In 1838, John Forbes entered this as a footnote to his translation of Laennec's third edition on the effect of asthma: "Asthma…in every other case a more correct pathology in the disease will put us in the way of a more rational practice. Instead of wasting our efforts in attempting to ward off paroxysms of a purely spasmodic nature by measures directed to the central nervous system, our attention will be directed to the real disease, the structural alteration and preternatural sensibility of the bronchial membrane." Whether this preternatural sensibility of the epithelium has a genetic or acquired basis is yet to be determined, but by recognizing this as a key component of asthma, novel therapies could be developed that protect the epithelium against injury and restore the epithelial mesenchymal trophic unit to normal functioning.

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