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Review Article

Epithelial–mesenchymal interactions in the pathogenesis of asthma

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Abstract

Asthma is regarded as an inflammatory disorder of the conducting airways characterized by a mast cell, eosinophil and T lymphocyte inflammatory response that is responsive to anti-inflammatory therapy, such as corticosteroids. In more severe and chronic disease, corticosteroids become less effective. As in other chronic inflammatory diseases, the tissue in which the cellular and mediator processes occur plays a major role in maintaining the response and creating a basis for disease persistence. Herein, we describe evidence that the airway epithelium interacting with the underlying mesenchymal cells recapitulates branching morphogenesis, as observed in the developing lung, to create airway wall remodeling. The reciprocal signaling between the susceptible epithelium and responsive mesenchyme (epithelial mesenchymal trophic unit) offers a new paradigm for asthma and creates new opportunities for developing therapeutics based on reversing the 'chronic wound' phenotype of asthmatic airways.

Key words: asthma, bronchial hyperresponsiveness, epidermal growth factor, epithelium, inflammation, mesenchyme, remodeling.

Introduction

A recent National Institutes of Health (NIH) workshop and a European Respiratory Society (ERS) Task Force both concluded that more work is needed to understand mechanisms of severe and chronic asthma.[1,2] This disease significantly affects quality of life and is an important socioeconomic burden. Recognizing that, in isolation, T-helper 2 (Th-2)-mediated inflammation is insufficient to cause asthma, we suggest that the asthmatic state results from a combination of the increased susceptibility of the bronchial epithelium to injury and prolonged tissue repair involving an imbalance in responses to heregulin receptor ligands, such as epithelium growth factor (EGF), tyrosine kinase receptor ligands, such as the transforming growth factor (TGF)-β and Th-2 T cell cytokines. These alter cell–cell communication between the epithelium and the underlying attenuated fibroblast sheath, causing activation of the epithelial mesenchymal trophic unit (EMTU), thereby propagating and amplifying remodeling of the airways and sustaining chronic inflammation, which are linked to both asthma chronicity and the reduced efficacy of corticosteroids.

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Asthma: A dynamic disorder of inflammation and remodeling

Asthma is an inflammatory disorder of the airways involving T cells, mast cells and eosinophils characteristic of a Th-2 response. However, inflammation alone does not explain many of the features of this chronic and relapsing disease. While atopy is an important risk factor for asthma, in the general population it only accounts for 40% of the attributable risk of having this disease.[3] Eosinophils have been assumed to play a central role in disease pathogenesis; however, studies with an anti-interleukin (IL)-5 blocking monoclonal antibody[4] and recombinant human IL-12[5] have failed to reveal efficacy, despite markedly reducing circulating and airway

eosinophil numbers. Thus, while being associated with asthma, atopy and airway eosinophilia would not seem to be critical requirements for disease expression. Genetic studies have also demonstrated that atopy and bronchial hyperresponsiveness (BHR) have different patterns of inheritance.[6] These findings imply that locally operating factors play an important role in predisposing individuals to asthma and provide an explanation for epidemiologic evidence that identifies pollutant exposure,[7] diet[8] and respiratory virus infection,[9] which all increase oxidant stress in the airways, as important disease risk factors.

Morphometry has revealed that thickening of asthmatic airways accounts for a large component of BHR and excessive airway narrowing observed in established disease. In moderate–severe disease, these structural changes, along with BHR, are poorly responsive to corticosteroids.[10] This failure of corticosteroids is reflected in the findings of our recent European Network For the Understanding Of Severe Asthma (ENFUMOSA) study, which has revealed that these patients exhibit a greatly impaired quality of life,[11] a component of fixed airflow obstruction and have clear evidence of airway wall remodeling.[12] In such patients, we have found evidence of persistent matrix turnover with higher levels of cleaved tenascin C, matrix metalloproteinase-2 and collagen VI, indicating active tissue remodeling.[13] Airway remodeling in adult asthma also provides an explanation for the accelerated decline in lung function observed over time.[13] The recent Childhood Asthma Management Program (CAMP) study in 5–11-year-old children has shown that the initial beneficial effect of an inhaled corticosteroid on the post-bronchodilator improvement in airway function observed during the first year of treatment was lost over the following 3 years.[14] This is best explained by airway remodeling that is insensitive to corticosteroids. A recent biopsy study has identified tissue remodeling as an early and consistent component of childhood asthma with fibroblast proliferation and collagen deposition in the subepithelial lamina reticularis being of greater diagnostic significance than tissue eosinophilia.[15] Although remodeling has been considered to be secondary to long-standing inflammation, biopsy studies in young children have shown tissue restructuring up to 4 years before the onset of symptoms,[16] indicating processes that begin early in the development of asthma and occur in parallel with, or may be obligatory for, the establishment of persistent inflammation.

Epithelial susceptibility to injury and the repair phenotype in asthma

The normal bronchial epithelium is a stratified structure consisting of a columnar layer supported by basal cells to serve as a physical and chemical barrier to the external environment. In asthma, the epithelium shows evidence of activation linked to structural damage and goblet cell metaplasia. Epithelial stress is seen in the form of widespread activation of the transcription factors nuclear factor-κB,[17,18] activator proteins (e.g. AP-1)[19] and signal transducer and activation of transcription (STAT)-1,[20] and by the increased expression of heat shock proteins[21] and the cyclin-dependent kinase inhibitor $p21^{waf}$.[22] The altered epithelium also becomes an important source of autacoid mediators, chemokines and growth factors[23] that sustain ongoing inflammation. To explain the disordered morphology and extent of activation, we have investigated whether the asthmatic epithelium is more susceptible to injury and/or has an altered response to damage.

Epithelial susceptibility

Epithelial disruption is characteristically increased in the asthmatic bronchial epithelium. It has been proposed that this damage is artefactual;[24] however, our findings of enhanced expression of the epidermal growth factor receptor (EGFR, HER-1, c-erbB1)[25] and the epithelial isoform of CD44[26] indicate that injury has occurred *in vivo*. We have found that EGFR and CD44 expression in asthma increases with disease severity and is evident throughout the epithelium, suggesting that damage is widespread.[25,26] Significantly, EGFR overexpression is insensitive to the action of corticosteroids and is positively correlated with the thickness of the lamina reticularis,[25] linking epithelial injury to underlying remodeling. The extent of epithelial shedding in asthma is not observed in other inflammatory diseases, such as chronic obstructive pulmonary disease (COPD), where the epithelium becomes multilayered due to squamous metaplasia while the underlying lamina reticularis remains normal. While these differences may reflect the quality of inflammation, airway eosinophilia is observed in the absence of asthma[27] and neutrophils may dominate inflammation in severe asthma, as in COPD.[12]

We have shown increased epithelial expression of Fas and Fas ligand in patients who have died with asthma. In bronchial biopsies, there is markedly increased immunostaining of asthmatic columnar (but notably not basal)

epithelial cells for p85, the caspase-3 cleavage product of poly (ADP-ribose) polymerase, indicating that epithelial apoptosis is increased in this disease.[28] While such observational studies are able to identify differences between asthmatic and normal subjects, they are unable to differentiate whether the changes are a cause or consequence of inflammation. To address this, we have established primary cultures using bronchial epithelial cells brushed from the airways of normal and asthmatic subjects in order to compare responses under identical conditions *in vitro*. Although no difference in the rate of proliferation of these cultures under optimal growth conditions has been found, when rendered quiescent by growth factor depletion those from asthmatic airways exhibit a significantly greater sensitivity to oxidant-induced apoptosis in the face of a normal apoptotic response to the DNA and RNA synthesis inhibitor actinomycin D.[28] This susceptibility to oxidants is unlikely to be a secondary effect of airway inflammation in being preserved through several generations *in vitro*. Because epidemiologic studies[27] and limited investigations in primates[29] have identified multiple interacting risk factors for asthma, including inhalant pollutants and diets low in anti-oxidants, we propose that the effect of environmental oxidants on a susceptible epithelium provides a plausible triggering mechanism for the induction of epithelial activation and damage in asthma. Once initiated, the resulting inflammatory cell influx causes secondary damage through the production of endogenous reactive oxygen, resulting in chronic tissue injury and persistent inflammation. Consistent with this proposal, we have shown recently that the epithelial expression of the neutrophil chemoattractants IL-8 and macrophage inflammatory protein (MIP)-1α is increased in severe asthma and that their appearance correlates with increased EGFR expression as a marker of epithelial damage. Further *in vitro* studies have revealed that oxidant or EGF treatment of primary bronchial epithelial cells enhances MIP-1α or IL-8 release, respectively.[30] Thus, the susceptibility of the epithelial barrier to the action of different components of the inhaled environment may play a key role in determining the asthmatic phenotype.

Growth arrest and epithelial repair

Our *in vitro* studies point to a central role for activation of the EGFR in the restoration of the bronchial epithelium following injury because: (i) EGF is a mitogen for bronchial epithelial cells;[22] (ii) mechanical damage induces rapid phosphorylation of the EGFR irrespective of the presence of exogenous ligand;[25] and (iii) wound closure is enhanced by EGF but not by the unrelated ligand keratinocyte growth factor (KGF, FGF-7).[25] Recognizing that EGF is a potent mitogen, the increase in epithelial EGFR in asthma is paradoxical because it is not matched by increased proliferation to replace columnar cells that have been shed[22,31] and, in this way, contrasts with the hyperproliferative state of the epithelium seen in COPD. Although studies with primary cultures of normal and asthmatic bronchial epithelial cells have shown similar proliferation rates when maintained in medium supplemented with exogenous EGF,[28] we have found a potential mechanism for reduced EGFR-mediated proliferation *in vivo* because the cyclin-dependent kinase inhibitor p21^{waf} is overexpressed in basal, as well as columnar, epithelial cells in bronchial biopsies from patients with severe asthma.[22] In response to injury, p21^{waf} acts as a checkpoint at the G1 to S-phase transition of the cell cycle, causing growth arrest to enable DNA repair to be completed before progression into S-phase or, where damage is irreparable, to direct exit from the cell cycle and activate apoptosis. In this way, the decisions to survive or to enter into apoptosis are irrefutably linked. The EGFR ligands (EGF, TGF-α, amphiregulin, heparin-binding epidermal growth factor-like growth factor (HB-EGF), epiregulin and betacellulin) are pivotal determinants of epithelial cell fate through their ability to act as survival factors[32] that protect against pro-apoptotic stimuli and as mitogens that signal cell cycle progression. Although we have not yet characterized the ability of EGF to protect against oxidant-induced apoptosis *in vitro*, studies in animal models have shown that EGF can protect against smoke-induced tracheal injury in sheep.[33]

Because expression of p21^{waf} is strongly induced by oxidant stress,[22] our finding[28] that asthmatic bronchial epithelial cells are more susceptible to oxidant injury provides one explanation for the high expression of p21^{waf} in the asthmatic epithelium. However, p21^{waf} is also induced by the antiproliferative growth factors TGF-β1 and TGF-β2, the levels of which are elevated in asthma, in COPD[34] and in response to epithelial injury *in vitro*.[25,35] Because mitogen-activated protein kinase (MAPK) activation by mitogens such as EGF antagonizes TGF-β signaling,[36] the overall fate of the epithelium will reflect integration of survival and proliferation signals provided by EGF-like growth factors and the counterbalancing effect pro-apoptotic and antiproliferative signals caused by injury and members of the TGF-β

family. Although expression of EGF, TGF-α and HB-EGF[37] is unchanged in asthmatic bronchial epithelium relative to normal controls, our unpublished studies in COPD indicate markedly increased TGF-α production. Thus, in COPD, increased activation of the EGFR by EGF and analogous ligands protects against cigarette smoke-induced injury and overrides the antiproliferative effect of TGF-β, whereas in asthma an insufficiency of these ligands provides a unifying mechanism for increased epithelial susceptibility and impaired repair. This is supported by our finding of a marked decrease in epithelial immunostaining for phosphotyrosine (a global marker of tyrosine kinase activation) in the bronchial epithelium in mild asthma[38] and the reported lack of MAPK activation.[39] In contrast, in corticosteroid-refractory asthma, tyrosine kinase activity is markedly increased in relation to disease severity or treatment.[38] Although we have not yet identified the phosphorylated proteins within the asthmatic epithelium, they are more likely to be linked to stress-induced growth arrest than to proliferation because, in the bronchial epithelium of severe asthmatic subjects, $p21^{waf}$ is elevated whereas proliferating cell nuclear antigen (PCNA) remains low.[22]

In asthma, we have also discovered that there is no increase in epithelial expression of the structurally related heregulin receptors HER-2 (c-erbB2) or HER-3 (c-erbB3),[37] whereas in COPD both these receptors are over-expressed in parallel with the EGFR (ST Holgate *et al.*, unpubl. obs., 2002). As EGFR : EGFR homodimers are only weak activators of the MAPK pathway,[40] this will further contribute to the inability of the asthmatic epithelium to counter antiproliferative signals provided by TGF-β. In contrast, EGFR : HER-2 heterodimers are potent signal transducers[41] and will augment epithelial proliferation in COPD, whereas c-erbB3-containing heterodimers are able to promote cell survival due to the presence of multiple binding sites for phosphatidylinositol 3-kinase on this receptor.[42] Based on these findings, we hypothesize that the duration of epithelial repair is prolonged in asthma due to an imbalance between proliferation and survival signals involving the EGFR/HER family and antiproliferative signals involving TGF-β.

The epithelial–mesenchymal trophic unit

Epithelial–mesenchymal signaling

High-resolution computed tomography, post-mortem and biopsy studies in chronic asthma have all revealed airway wall thickening. This involves deposition of interstitial collagens in the lamina reticularis,[43] matrix deposition in the submucosa, smooth muscle hyperplasia and microvascular and neuronal proliferation. Thickening of the lamina reticularis is diagnostic of this disease and, on the basis of measurements made in human airways[44] and in a guinea pig model of chronic antigen exposure,[45,46] it appears to reflect events linked to thickening of the entire airway wall.[47] In 1990, we described a layer of subepithelial mesenchymal cells with features of myofibroblasts, the number of which was increased in asthma in proportion to the thickness of the reticular collagen layer.[48] These cells correspond to the attenuated fibroblast sheath described by Evans *et al.*[49] lying adjacent to the lamina reticularis and forming a network similar to hepatic stellate cells which, when activated by liver damage, are the key effector cells responsible for fibrosis.[50] Because the bronchial epithelium is in intimate contact with the attenuated fibroblast sheath, 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.[51] We have already demonstrated that injury to epithelial monolayers *in vitro* results in increased release of fibroproliferative and fibrogenic growth factors including fibroblast growth factor (FGF)-2, insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF), endothelin (ET)-1 and latent and active TGF-β2.[35,52] We have also found that TGF-β, FGF-2 and ET-1 are increased in asthma,[53–55] with TGF-β and FGF-2 being encrypted in the extracellular matrix, as shown by their colocalization with the proteoglycans decorin and heparan sulfate, respectively.[56] To further establish the relationship between EGFR signaling in the repair and remodeling processes, we have used an EGFR-selective tyrosine kinase inhibitor that suppresses epithelial repair *in vitro* with a resultant increase in release of TGF-β2 by the damaged epithelial cells.[25] This points to parallel pathways operating in repairing epithelial cells, some of which direct efficient restitution and are regulated by the EGFR, whereas others control profibrogenic growth factor production independent of the EGFR. In 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.

Using a coculture model, we have obtained direct evidence that epithelial injury causes myofibroblast activation. Thus, polyarginine (a surrogate for eosinophil

basic proteins) or mechanical damage to confluent monolayers of bronchial epithelial cells grown on a collagen gel seeded with human myofibroblasts leads to enhanced proliferation and increased collagen gene expression due to the combined effects of FGF-2, IGF-1, PDGF-BB, TGF-β and ET-1.[35] Furthermore, in mild–moderate asthma, inhaled corticosteroids reduce airway inflammation and levels of IGF-1 and FGF-2, but with minimal improvement in BHR and no effect on collagen deposition in the lamina reticularis or on TGF-β levels.[57] Because we have shown that the corticosteroids reduced submucosal eosinophils by 80%, the persistently high TGF-β in bronchoalveolar lavage fluid (BALF) most likely derives from the injured and repairing epithelium and associated matrix turnover rather than from eosinophils. Because both epithelial EGFR expression[25] and TGF-β production are refractory to corticosteroids, the combined effects of these signaling pathways on the EMTU could provide mechanisms for tissue remodeling and explain the incomplete resolution of lung function with inhaled corticosteroids observed in chronic asthma.

Communication between the epithelium and the subepithelial fibroblast sheath is reminiscent of the processes that drive physiological remodeling of the airways during embryogenesis, where the epithelium and mesenchyme act as a 'trophic unit' to regulate airway growth and branching.[58] Consequently, we propose that the EMTU is reactivated in asthma to drive pathologic remodeling of the airways.[51] In subjects with asymptomatic BHR, longitudinal studies have shown that those who progress to asthma show parallel changes in inflammation and remodeling.[59] Thickening of the lamina reticularis in bronchial biopsies from young children is also present several years before asthma becomes clinically manifest.[16] During lung development, epithelial and mesenchymal growth is regulated, in part, by the balance of EGF and TGF-β signaling, as we suggest occurs in chronic asthma. In susceptible individuals, we propose that environmental factors interact with the EMTU in early life to initiate structural changes in the airways that may account for the decrease in lung function observed in young children who are susceptible to early wheezing[60] and for the loss of corticosteroid responsiveness on baseline lung function observed in the CAMP study.[14] This is supported by very recent studies in non-human primates, where intermittent exposure to ozone in the presence of allergen creates a phenotype resembling chronic asthma.[29] Therefore, bronchial epithelial susceptibility seems to either precede or occur in parallel with factors predisposing to Th-2-mediated inflammation and is an absolute requirement to establish the microenvironment for inflammation to become persistent in the airways and for remodeling to occur.

Propagation of remodeling responses by the EMTU

To study the mesenchymal cells that are involved in the remodeling responses in asthma, we have established protocols for their outgrowth from bronchial biopsies. These vimentin-positive fibroblasts can be grown readily from asthmatic mucosal biopsies and differ from those from normal airways in adopting stem cell characteristics, by proliferating rapidly in the absence of exogenous growth factors and by their ability to overcome contact inhibition in the presence of TGF-β1 or TGF-β2, enabling higher cell densities to be achieved.[61] In this regard, TGF-β treatment *in vitro* also causes submucosal fibroblasts to adopt a myofibroblast phenotype, as evidenced by induction of α-smooth muscle actin (SMA), acquisition of a contractile phenotype and synthesis of interstitial (repair) collagens. Although the relationship between myofibroblast activation and the underlying smooth muscle mass in asthma has yet to be studied, following allergen exposure we have found an increase in BALF of the smooth muscle and vascular mitogen, ET-1[55] and that TGF-β treatment of asthmatic mucosal fibroblasts *in vitro* causes them to release both ET-1 and another vascular mitogen, namely vascular endothelial growth factor (VEGF).[61] Thus, in addition to increased matrix deposition, activation of myofibroblasts contributes to smooth muscle and microvascular proliferation, which are both characteristic features of the remodeled asthmatic airway.[62]

In a rabbit model of partial outflow obstruction in the bladder, TGF-β causes serosal thickening due to accumulation of myofibroblasts, which, over time, change phenotype into smooth muscle cells.[63] In developing capillaries, endothelial cells and smooth muscle cells also share a common stem cell progenitor with VEGF and PDGF acting as the key determinants of cell fate.[64] Our own studies of normal and asthmatic airway (myo) fibroblasts reveal that these cells exhibit phenotypic plasticity, have some early features of smooth muscle (e.g. expression of the SM-22 protein) and can be further differentiated by TGF-β1 and TGF-β2 to express heavy chain myosin (HCM) and α-SMA.[61,65] This suggests that the cells have properties of both myofibroblasts and smooth

muscle cells and are probably derived from a common (? stem cell) progenitor. Thus, mediators released by the repairing bronchial epithelium provide a mechanism for myofibroblast activation to propagate and amplify airway remodeling. Such a mechanism would fit well with the recent findings on airway smooth muscle cultured from asthmatic airways.[62]

Interaction between IL-4 and IL-13 and the EMTU

Th-2 type inflammation of the airways is a characteristic feature of asthma, irrespective of atopy. In view of transgenic mice studies that have suggested that expression of an IL-13 (but not IL-4) transgene in the bronchial epithelium leads to submucosal remodeling,[66,67] we have investigated the role of IL-4 and IL-13 in asthmatic epithelial cell and fibroblast function. The expression of the high-affinity IL-13Rα2 chain is restricted to airway epithelial cells and fibroblasts and is coexpressed with IL-13Rα1 and IL-4Rα.[68] Both IL-4 and IL-13 signal via the transcription factor STAT-6,[69] the expression of which we have shown to be prominent in the bronchial epithelium and further increased in severe asthma.[70] While we have shown that IL-13 is able to induce myofibroblast transformation, it is two orders of magnitude less potent than TGF-β and is equipotent with IL-4 in this effect.[61] Because parallel experiments revealed that IL-13 causes a corticosteroid-insensitive increase in release of TGF-β2 from bronchial epithelial cells, it seems likely that IL-13-mediated submucosal remodeling is initiated largely through the bronchial epithelium.[61] A recent report from Lee *et al.*[70] supports this conclusion because both fibrosis and smooth muscle hyperplasia in the airways of mice expressing a bronchial epithelial-specific IL-13 transgene are TGF-β dependent. However, in human epithelial cells, IL-4 is as effective as IL-13 in promoting TGF-β release,[71] raising the possibility of an important species difference in epithelial IL-4 and IL-13 receptor expression.

While the remodeling effects of IL-4 and IL-13 can be attributed to epithelial activation, these cytokines also have direct pro-inflammatory effects on both epithelial cells and fibroblasts. Cultures of bronchial epithelial cells respond to IL-4 and IL-13 with increased STAT-6 phosphorylation accompanied by enhanced granulocyte–macrophage colony stimulating factor and IL-8 production, which is further augmented by enzymatically active extracts of house dust mite (*Dermatophagoides pteronyssinus*).[71,72] We have also found enhanced release of eotaxin from asthmatic fibroblasts[61] that may help explain the accumulation of eosinophils beneath the lamina reticularis in asthma. Thus, by interacting with the EMTU, IL-4 and IL-13 contribute to chronic inflammation and airway remodeling.

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