Airway Remodeling in Asthma: Therapeutic Implications of Mechanisms

Asthma is currently recognized as a chronic inflammatory disorder of the airways that leads to tissue injury and subsequent structural changes collectively called airway remodeling. Transgenic modeling of inflammatory mediators allows for the discovery of unexpected effects, dissection of downstream signaling events, and clues to future therapies.

extracellular pathogens like parasites. However, the absence of either population leads to enhanced immunopathology, even in conditions classically thought to depend on the other cell type.

It is thought that inflammation alone may lead to some features of asthma, including reversible bronchospasm. However, as with many chronic inflammatory disorders, asthmatic airway inflammation is also believed to cause tissue injury and subsequent structural changes. These changes are referred to collectively as airway remodeling and include an increase in overall wall thickness, an increase in airway fibrosis and smooth muscle mass, abnormalities in composition of the extracellular matrix, and an increase in vascularity (37). These changes have attracted interest due to the increased realization that these changes may account for aspects of asthmatic physiology that are poorly addressed with current anti-inflammatory strategies (10).

Descriptive Analysis of Airway Remodeling

In fatal asthma, pathological analysis has shown that most elements of the airway wall (smooth muscle, non-smooth muscle connective tissue, mucus glands) are increased (FIGURE 1) (13, 26, 45, 47, 53). The changes (except for the increase in mucus glands) are found in airways of all sizes (82). The pathological changes in airways of patients with nonfatal asthma are much less pronounced, with changes seen predominantly in small airways (2–4 mm in diameter) (13, 53). Airway wall thickness as measured radiologically also correlates with disease severity and length of time with disease, with small airways again being the predominant abnormality in milder disease (4, 7, 33, 35, 49, 63, 72, 76).

An increase in airway smooth muscle mass is one of the best-known features of asthmatic remodeling (45). Like total wall thickness, smooth muscle thickness correlates with fatal vs. nonfatal asthma (13, 47, 53). The increase in smooth muscle mass is...
disproportionate to the increase in total airway wall thickness. The degree to which hyperplasia vs. hypertrophy contributes to this response continues to be controversial, but both probably contribute in different patient populations (5, 27, 44, 105). The degree to which this increased smooth muscle is abnormal is also controversial (5, 105). The degree to which this increased smooth muscle is abnormal is also controversial (5, 105). The degree to which this increased smooth muscle is abnormal is also controversial (5, 105).

An increase in fibrosis just below the large airway epithelium occurs as a prominent feature in asthma. This layer has been extensively studied due to its accessibility on endobronchial biopsy. The normal layer of collagen under the airway is ~5 μm thick, which increases to 7–23 μm in patients with asthma. Initially described as basement membrane thickening, it is now apparent that the true basement membrane (the lamina rara and densa as seen on electron microscopy, which contain laminin and collagen IV) is not grossly altered (80). There is, rather, thickening of the area just below the true basement membrane, the lamina reticularis, with deposition of interstitial collagens I, III, and V. Studies have reported correlations of subepithelial fibrosis as measured in larger cartilaginous airways with overall wall thickness in the same airways but not in small airways elsewhere in the same lung (46, 49). There are inconsistent results with respect to enhanced collagen deposition in the submucosa (area between the layer of dense subepithelial fibrosis and smooth muscle) (5, 19, 104). Abnormalities have also been noted in noncollagenous matrix, including elastin, proteoglycans, and cartilage (56).

The significance of this specific feature of asthmatic airway remodeling is unclear. Although measurements of airway distensibility correlate well with subepithelial fibrosis (99), other functional measurements (clinical illness scores, measures of pulmonary function and airway hyperresponsiveness) show variable correlations (5, 6, 16, 18, 40). Other groups have identified both severe asthmatics with no increase in subepithelial fibrosis and nonasthmatics with increased subepithelial fibrosis (14, 19, 101). Subepithelial fibrosis is actually a very early marker for the asthmatic phenotype in children and does not correlate with length of time with the disease nor necessarily with the severity of inflammation (8, 19, 23, 54, 78). It has therefore been suggested that subepithelial fibrosis represents disordered epithelial-mesenchymal signaling rather than a direct response to inflammatory injury (36). A tracheal explant model has shown that cigarette smoke can induce remodeling in the absence of inflammation, indicating that other pathways to fibrosis need to be considered (98). Myofibroblasts are specialized cells with features of both fibroblasts and myocytes. They have the synthetic machinery of fibroblasts used for synthesis of extracellular matrix but also have at least some components of the contractile apparatus of myocytes. They are well known to be increased in tissues undergoing repair, such as in wounds or in pulmonary interstitial fibrosis. In human asthma, the submucosa shows an increase in myofibroblasts that correlates with the thickness of the lamina reticularis but not with severity of disease (5, 9, 40). Since myofibroblasts are well-known sources of interstitial collagens, it is plausible that the myofibroblasts are the source of the subepithelial fibrosis. The origin and fate of these cells is somewhat obscure. Cells with this phenotype appear very quickly after antigen challenge, possibly implying a quiescent precursor cell that acquires myofibroblastic markers without necessarily dividing (32). Other data suggest that these cells may arise from circulating precursors (86).

Increased vascularity is a common feature of chronic inflammation. In humans and in a sheep model, an increase in blood flow was noted after antigen exposure at a time point corresponding to increased airway resistance (51, 64). In addition, increased vascular congestion, leading to wall thickening, has been suggested as the basis of exercise-induced asthma, the airway hyperresponsiveness seen in congestive heart failure and in normal subjects after a rapid infusion of intravenous fluids (11, 81). Using autopsy material, two groups have shown that there is evidence for increased vascular congestion as measured histologically (12, 53). New vessel growth as determined by a number of vascular profiles has also been demonstrated on biopsy material (62, 73). More severe asthmatics have a greater number of vessels than milder asthmatics (85, 97). There is a correlation of vascularity with airway hyperresponsiveness and change in lung function after bronchodilator treatment (42, 73). The vessels that are increased appear to be capillaries and venules and are concentrated just under the airway epithelium (74, 85). Abnormalities of various types of vessels in the airway mucosa have also been described (85).

One novel approach to this issue used a high-magnification bronchovideoscope that allows imaging of vessels in vivo, although it does not distinguish between new vessel growth and engorged vessels (90). Using this technique, increased vascularity was noted in both newly diagnosed patients and in patients with long-standing asthma. Paradoxically, but consistent with results measuring other aspects of airway remodeling, there is no correlation of degree of abnormality with length of time with disease. As in animal models, vascularity is denser in intercartilaginous areas than over cartilage (66).

Mechanisms of Airway Remodeling

A large number of mediators have been described
in airways of asthmatics that could theoretically be relevant to airway remodeling. In many cases, the presence of these mediators correlates with the severity of airway remodeling or some clinical feature. However, it is difficult to know the significance of these findings in the absence of a specific inhibitor of that mediator. So far, only one specific mediator, IL-5, has been targeted in humans. IL-5 was well established as potentially relevant to asthma on account of its powerful influence on eosinophil development and priming. Treatment of asthmatics with a humanized anti-IL-5 antibody confirmed this suspicion, because it caused a marked reduction in circulating and airway lumen eosinophils and a lesser reduction in airway tissue eosinophils. Furthermore, treatment with this antibody was able to reduce matrix proteins present beneath the epithelial basement membrane such as tenascin C and lumican (30). Whether this effect of anti-IL-5 on matrix turnover is eosinophil dependent is not known because airway fibroblasts and epithelial cells also possess IL-5 receptors. Unfortunately, despite these impressive biological effects, the antibody failed to produce any clinically significant effect on asthmatic physiology.

Animal models therefore remain a mainstay in this area. One approach to determining the role of these mediators is to express them in a transgenic fashion, either constitutively or inducibly. Because asthma is a chronic disease, this has the advantage of looking at long-term outcomes, which are particularly relevant to airway remodeling. Furthermore, by isolating the contributions of individual mediators, it is possible to examine the downstream pathway of these mediators, thereby more readily highlighting possible inhibitors of these pathways.

IL-13 had been shown to be critically important in acute models of allergic inflammation (103). It was originally discovered as an IL-4-like molecule with which it shares some receptor subunits. It has since become clear that IL-13 is more important in the effector phase and that IL-4 is more important in the initiation phase of Th2 inflammation. With the use of both conventional and inducible transgenic modeling, IL-13 was shown to be a potent inducer of an eosinophil-, macrophage-, and lymphocyte-rich inflammatory response, airway fibrosis, mucus metaplasia, and airway hyperresponsiveness (FIGURE 2) (107).

IL-13 mediates many of its effects through a signal transducer and activator of transcription (STAT)-6 signaling pathway, and human asthmatics have elevated levels of STAT-6 in airway epithelium (69). To assess the role of STAT-6 on IL-13-induced pathology, the IL-13-overexpressing mice were bred either with mice deficient in STAT-6 or mice that only express STAT-6 in airway epithelium. STAT-6 was required for virtually all effects of IL-13. When STAT-6 was only present on airway epithelium, the mice had no inflammatory infiltrate or airway fibrosis but still showed airway mucus metaplasia, airway obstruction, and airway hyperresponsiveness (52). This confirms that airway fibrosis was not essential to the airway’s hyperresponsiveness seen in that model.
found in exaggerated quantities in sputum, bronchoalveolar lavage (BAL) fluid, and biopsies from patients with asthma. TIMP-1 is also produced in an exaggerated fashion by asthmatic alveolar macrophages and is present in exaggerated quantities in the sputum and biopsies of patients with asthma. The ratio of MMP-9 and TIMP-1 in asthmatics is lower than in control patients and correlates with the degree of airway obstruction. (50).

In the IL-13-overexpressing mice, MMP-2, -9, -12, -13, and -14 are markedly upregulated (106). Perhaps not surprisingly given the large number of proteases produced in these mice, the IL-13-overexpressing mice develop emphysema with marked destruction of lung parenchyma. Inhibiting these enzymes, either through the use of protease inhibitors or by breeding to mice deficient in specific enzymes (MMP-9 or MMP-12), significantly decreases the emphysema and inflammation but not the mucus in these animals. Moreover, MMP-9 deficiency had no effect on MMP-2, -12, -13, and -14 induction, nor did it affect recovery of eosinophils, macrophages, or lymphocytes from BAL fluids. On the other hand, MMP-9 deficiency increased the recovery of neutrophils from BAL fluids, possibly due to enhanced levels of the neutrophil-tropic chemokines KC and MIP-2. MMP-12 deficiency diminished the induction of MMP-2, -9, -13, and -14 and decreased the recovery of leukocytes, eosinophils, and macrophages, but not lymphocytes or neutrophils, from BAL. MMP-9 and -12, therefore, play different roles in the generation of IL-13-induced inflammation, with IL-13 induction of MMPs-2, -9, -13, and -14 mediated at least partially by an MMP-12-dependent pathway.

Other Th2 cytokines, such as IL-9 and IL-10, appear to function at least partly via inducing IL-13 (57, 102). IL-10 has been considered in some cases a Th2 cytokine but has also been implicated in immunoregulation. Mice that overexpress IL-10 in the airway show a reduction in aspects of innate immunoregulation, including endotoxin-induced tumor necrosis factor production and neutrophil accumulation. However, IL-10 also causes mucus metaplasia, B cell- and T cell-rich inflammation, and airway fibrosis and augments the levels of mRNA encoding Gob-5, mucins, and IL-13. These responses are mediated by multiple mechanisms, with mucus metaplasia being dependent on an IL-13–IL-4Rα–STAT-6 pathway, whereas the inflammation and fibrosis were independent of that pathway.

The most heavily studied mediator of tissue fibrosis is transforming growth factor (TGF)-β. TGF-β1 is made by many cells within the lungs, such as epithelial cells, macrophages, eosinophils, lymphocytes, and fibroblasts. TGF-β1 can induce fibroblast and smooth muscle proliferation and enhances matrix production. TGF-β1 mRNA appears to be increased in moderate and severe asthmatics compared with normal subjects, and the expression of this cytokine is directly related to the degree of subepithelial fibrosis (68, 71). As determined by using in situ hybridization, eosinophils and fibroblasts are the principle cells synthesizing TGF-β1 in the airway, whereas alveolar macrophages release more TGF-β1 from asthmatics than from controls (39, 95, 96). Expression of downstream signaling molecules from TGF-β also shows an increase in asthma and a correlation with subepithelial fibrosis (70, 84). Consistent with this data, the IL-13 transgenic mice induced both total and active TGF-β1 expression via a plasmin- and MMP-9-dependent pathway (58). Macrophages were the major site of TGF-β1 production as assessed by immunohistochemistry and in situ hybridization. IL-13-induced fibrosis was significantly ameliorated by treatment with the TGF-β1 antagonist soluble TGFβRII-Fc. It is possible, therefore, that the main significance of MMP-9 overexpression in human asthma may be to activate TGF-β1.

A large number of factors are known that control airway smooth muscle cell proliferation in vitro (77). However, little is certain about their role in vivo. IL-11, a member of the IL-6-type cytokine family, shares a common receptor-signaling subunit with IL-6 and has been shown to induce airway hyperresponsiveness in mice when administered intratracheally (28). It is produced by a variety of lung stromal cells, including epithelial cells, smooth muscle cells, and fibroblasts, in response to a variety of stimuli, including asthma-related viruses, histamine, and eosinophil major basic protein (28, 29). Overexpression of IL-11 in the lungs caused an increase in peribronchial fibrosis and an increase in true airway smooth muscle mass and myofibroblasts (91). There is an increase in baseline airway resistance and marked airway hyperresponsiveness. The studies with the IL-11 mice led to analysis of human airways that showed that IL-11 is overexpressed in chronic human asthma but only in the most severely remodeled airways (67).

Increased vascularity may be due to any number of proangiogenic factors, especially vascular endothelial growth factor (VEGF). VEGF was originally described as vascular permeability factor on the basis of its ability to generate tissue edema. It has subsequently been appreciated to be a multifunctional angiogenic regulator that stimulates epithelial cell proliferation, blood vessel formation, and endothelial cell survival (20, 31). VEGF levels are increased in asthmatics, and levels correlate directly with disease activity and inversely with airway caliber and airway responsiveness (3, 38, 42, 60). VEGF has been postulated to contribute to

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asthmatic tissue edema via its vascular permeability factor effect (2, 92). IL-13 is known to cause an increase in VEGF production (24).

Mice that overexpress VEGF show a dramatic endothelial sprouting under the airway epithelium (59). The new vessels are larger than capillaries, with endothelial cells that are thin with occasional fenestrations, and are enveloped by pericyte processes and basement membranes. These lungs show a marked increase in vascular permeability, as shown by wet/dry ratios and Evans blue dye extravasations. Thus VEGF is a potent inducer of angiogenesis and edema in the murine airway and lung. Unexpectedly, airways also show evidence for remodeling, with an increase in smooth muscle mass around airways relatively early after transgene expression. This was followed later by an increase in collagen around airways. TGF-β1 was also increased at this latter time point. These airway and lung changes were accompanied by an increase in airway hyperresponsiveness.

VEGF also caused a marked increase in inflammation, with an increase in mononuclear cells, T and B cells, and eosinophils. There was an increase in the number and activation state of dendritic cells that correlated with ability to prime the mice via the airways, a feature not found in wild-type mice.

As expected from the IL-13-overexpressing mice, after allergen challenge, VEGF was found to be markedly increased in BAL fluids. In the lung, VEGF is localized to airway epithelial cells, mononuclear cells, and T cells. In vitro, polarized Th2 cells are much more potent producers of VEGF than Th1 cells. When a VEGF receptor antagonist was used in vivo, there was a dramatic decrease in BAL and tissue inflammation as well as airway hyperresponsiveness. There was also a decrease in IL-13 and IL-4 production.

These results suggest a positive feed-forward circuit in which allergen challenge increases VEGF while VEGF also increases allergic inflammation, dendritic cell activation, and airway remodeling. It is thought that viral infection early in life can predispose one to the development of asthma (87, 89). Because viral infection can also lead to increased VEGF (28), these results suggest a mechanism whereby this may occur (FIGURE 3). Finally, given the ability of VEGF to promote both inflammation and airway remodeling, this data supports a possible role for VEGF antagonists in therapy of asthma, especially in severe asthma, which is resistant to conventional therapy.

**Physiological Significance of Airway Remodeling**

Airway remodeling has been invoked to explain various aspects of asthma severity, including airway hyperresponsiveness and fixed airway obstruction (10). The cross-sectional studies comparing fatal vs. nonfatal asthma certainly support the general concept of a relationship between airway remodeling and asthma severity. Although experimental studies of allergic models show that airway hyperresponsiveness can be induced without airway remodeling (22) and individual cytokines are known that can increase airway smooth muscle responsiveness both in vitro and in vivo (1, 28), a murine model of airway remodeling suggests that airway dysfunction persists after resolution of inflammation, implicating the airway remodeling itself in airway dysfunction (61). Extensive mathematical modeling also suggests effects of airway remodeling on airway function similar to those seen clinically (37).

Fixed airway obstruction associated with progressive loss of lung function has now been demonstrated in several cohort studies of both adults and children (25, 55, 79, 94). Although the pathological basis for this phenomenon is not completely known, a recent biopsy study has examined patients with varying degrees of asthma severity, including fixed airway obstruction, and has shown a remarkable correlation of severity with measures of smooth muscle area, smooth muscle hypertrophy, and density of fibroblasts in the airways.
way wall (5). It is interesting that another group had not only not seen this effect, they also saw a correlation of subepithelial fibrosis with disease severity, which was specifically not found by the first group (18). It is possible that this represents disease heterogeneity.

A number of papers have addressed the issue of reversibility of airway remodeling. Early studies showed no effect of steroids on subepithelial fibrosis (48, 65, 80). More recent randomized studies have shown a significant reduction in subepithelial fibrosis after short-term or long-term therapy or after withdrawal from exposure to antigen (16, 41, 83, 93, 100). It has been suggested that prior treatment with steroids, delayed treatment, or low doses and short courses of steroids prevented an effect from being noted in the earlier studies. Steroids also decrease the number of (myo)fibroblasts in the submucosa and reduce airway vascular- ity (17, 41, 43, 73). The decrease in vessel number correlates with the decrease in airway hyperresponsive- siveness, reduction in inflammation, and change in subepithelial collagen (17, 43). One study suggests that addition of a β2-agonist to inhaled steroids may reduce vascularity further (74). These pathological studies have been used to support the suggestion from clinical trials that early treatment with inhaled steroids is required to prevent irreversible loss of lung function, at least in adults (34, 75, 88).

A few studies have addressed the issue of response of putative remodeling mediators to therapy. TGF-β appears resistant to steroid therapy, whether there is a reduction in measures of airway remodeling or not (15, 41). However, since remodeling presumably requires more than one mediator and because alternative pathways are likely, it is hard to interpret this kind of data.

There remain many areas of uncertainty in this field. Despite the suggestion that airway remodeling explains the lack of response to therapy of some patients, no study has specifically shown that those patients who either fail to respond to therapy or progress despite therapy do in fact show airway remodeling (or excess expression of some mediator of remodeling) that fails to respond or progresses despite a reduction in inflammation. No prospective study has examined the effects of therapy on remodeling (or excess expression of some mediator explaining the lack of response to therapy of some patients). If this hypothesis was true, it would ultimately be necessary to characterize the pathological basis of each patient’s physiology before determining which therapy would be most beneficial in reversing or preventing airway remodeling in that individual patient.

References


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