Molecular mechanisms in allergy and clinical immunology
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# Asthma therapy and airway remodeling

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**Asthma is characterized by variable degrees of chronic inflammation and structural alterations in the airways. The most prominent abnormalities include epithelial denudation, goblet cell metaplasia, subepithelial thickening, increased airway smooth muscle mass, bronchial gland enlargement, angiogenesis, and alterations in extracellular matrix components, involving large and small airways. Chronic inflammation is thought to initiate and perpetuate cycles of tissue injury and repair in asthma, although remodeling may also occur in parallel with inflammation. In the absence of definite evidence on how different remodeling features affect lung function in asthma, the working hypothesis should be that structural alterations can lead to the development of persistent airway hyperresponsiveness and fixed airway obstruction. It is still unanswered whether and when to begin treating patients with asthma to prevent or reverse deleterious remodeling, which components of remodeling to target, and how to monitor remodeling. Consequently, efforts are being made to understand better the effects of conventional anti-inflammatory therapies, such as glucocorticosteroids, on airway structural changes. Animal models, *in vitro* studies, and some clinical studies have advanced present knowledge on the cellular and molecular pathways involved in airway remodeling. This has encouraged the development of biologicals aimed to target various components of airway remodeling. Progress in this area requires the explicit linking of modern structure-function analysis with innovative biopharmaceutical approaches.**
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***Key words:*** *Asthma, therapy, remodeling, corticosteroids, leukotriene receptor antagonist, airway, basement membrane, smooth muscle, epithelium, extracellular matrix*

*Abbreviations used*
ASM: Airway smooth muscle
BAL: Bronchoalveolar lavage
BM: Basement membrane
CTGF: Connective tissue growth factor
ECM: Extracellular matrix
FP: Fluticasone propionate
MMP: Matrix metalloprotease
PDE: Phosphodiesterase
VEGF: Vascular endothelial growth factor

Asthma is a clinical diagnosis based on episodic symptoms and variable airways obstruction.[1] It has been generally recognized that the disease is also characterized by variable degrees of chronic inflammation and structural alterations in the airways.[2,3] The structural alterations, collectively called *airway remodeling*, encompass complex changes in composition, content, and organization of the various cellular and molecular constituents of the airway wall.[4] The most striking abnormalities are epithelial denudation, goblet cell metaplasia, subepithelial thickening, increased airway smooth muscle mass, bronchial gland enlargement, angiogenesis, and alterations in the extracellular matrix (ECM) components.[5] These alterations involve large and small airways and the surrounding peribronchiolar areas[3-6] (Fig 1).

Traditionally, it is thought that $T_H2$-mediated chronic inflammation triggers and perpetuates a vicious circle of tissue injury–tissue repair culminating in remodeling. Data on pediatric severe asthma showing the early presence of structural alterations in bronchial tissue raised the alternative hypothesis that inflammation and remodeling may occur in parallel, beginning at early stages of the disease.[7,8] During normal lung development, there is a cross-talk between the bronchial epithelium and the underlying mesenchymal cells (the bronchial epithelial mesenchymal trophic unit), which is fundamental for adequate branching morphogenesis.[9] Davies et al[4] proposed that abnormal injury occurring in genetically susceptible bronchial epithelium because of components of the inhaled

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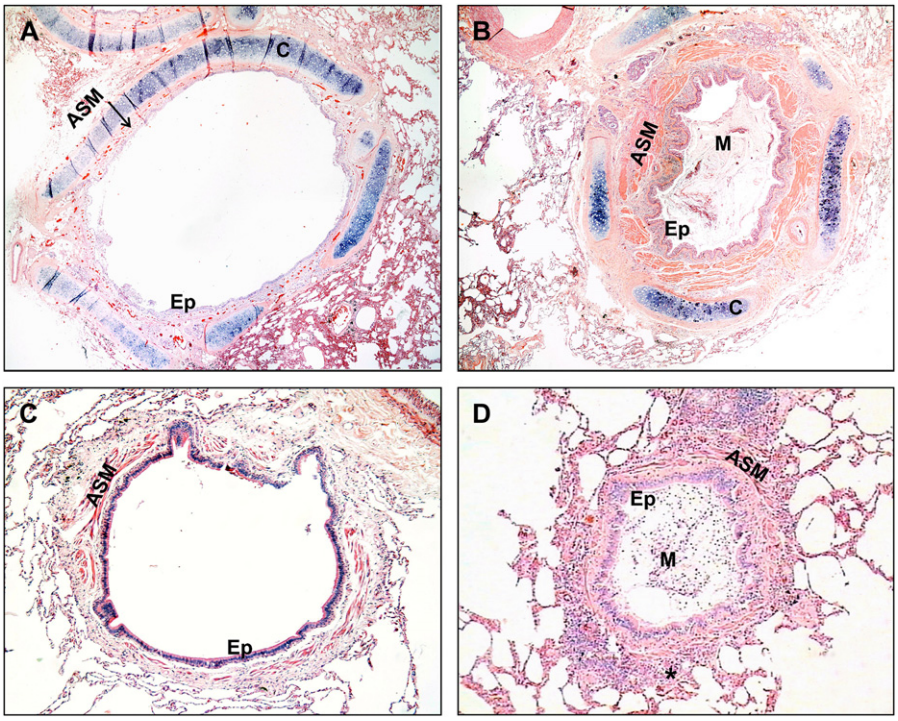



**FIG 1. A and C,** Normal airways. **B and D,** Airways from a patient with fatal asthma. Mucus plug within the airway lumen, epithelial folding and thickened ASM layer in *B.* Mucus plugging and increased ASM thickness in *D,* with spreading of the inflammation to the surrounding peribronchiolar alveoli.* Hematoxylin and eosin. *A and B,* ×25. *C and D,* ×100. *C,* Cartilage; *Ep,* epithelium; *M,* mucus.

environment could reactivate the epithelial mesenchymal trophic unit in asthma, driving smooth muscle proliferation and remodeling. Therefore, myofibroblasts, mesenchymal cells that have the capacity to secrete ECM proteins and growth factors, are believed to play a key role in the remodeling process.

The current working hypothesis is that airway remodeling could explain at least some aspects of disease severity in animal models, such as airway hyperresponsiveness[10] and fixed airway obstruction.[11] Southam et al[12] presented recent data on chronic allergen-sensitized mice, showing that a complex temporal interplay may exist between airway inflammation, structural changes, and the onset/maintenance of airway hyperresponsiveness. With regard to the human situation, we know that many patients exhibit persistent airway hyperresponsiveness, fixed airflow limitation, excessive airway narrowing, and/or enhanced airway closure[13] despite maximal therapy.[14] It is very likely that components of airway remodeling contribute to this, particularly in severe asthma. However, definite clinical evidence of a causal relationship is still missing. We do not know whether and how each component of airway remodeling, alone or in combination, affects lung function. Previous studies comparing fatal asthma and nonfatal asthma reported associations between increased airway thickness and asthma severity.[15] Biopsy studies in patients with different asthma severities also demonstrated that increased collagen content and airway smooth muscle (ASM) cell mass (among other remodeling features within the airway mucosa) were present in patients with severe asthma compared with patients with moderate and mild asthma.[15-19] Other studies, however, had more difficulties in finding differences in collagen content between patients with asthma of different severities,[20] or associations between basement membrane thickening and progressive decline in lung function.[21] Hence, the relevance of the different components of airways remodeling for the clinical severity and progression of asthma still remains to be established. As a consequence, the question is which components of airway remodeling should be treated.

Tissue turnover and restructuring is a physiological, homeostatic process.[22] This may help to preserve optimal functional properties of the airways.[23] Airway wall thickening and loss of alveolar attachments[24] in asthma are likely to enhance airway narrowing.[25] However, the airways in patients with asthma are stiffer than in normal subjects,[26,27] possibly because of fibrosis, mucosal folding, and/or smooth muscle stiffening,[28,29] which may actually counteract airway narrowing.[30] Therefore, before considering any interventions aimed to prevent or reverse structural changes of the airways in asthma, there should be good evidence that the targeted tissue elements contribute to impaired airway function.

This raises a large challenge to the field. How should airway structure be sampled and monitored? Endobronchial

biopsies are sampling a very superficial and selected area of the airways,[31,32] disregarding the small airways that are extremely difficult to assess.[33] Although high-resolution computed tomography scanning allows quantitative airway morphometry,[34] it cannot distinguish the histologic components of the airways. Hence, when targeting airway remodeling by therapeutic interventions, the outcome parameters should be carefully specified.

To assess the effects of different drugs on the cellular and molecular components of airway remodeling, animal experiments and *in vitro* models are indispensable despite their intrinsic limitations.[35] In such models, corticosteroids have been studied intensively, because these drugs are the most effective therapy for the control of airway inflammation in asthma.[36] However, less is known about how corticosteroids affect structural cells involved in remodeling, such as fibroblasts, myofibroblasts, ASM cells, and epithelial cells.[37] That is why the current review first focuses on data describing how corticosteroids and other currently available drugs affect each component of airway remodeling. Thereafter, we explore newly developed interventions[38] and their potential for influencing airway compartments involved in remodeling in asthma.

## EFFECTS OF CURRENTLY AVAILABLE ASTHMA DRUGS ON AIRWAY STRUCTURE

### Bronchial epithelium

Increased epithelial shedding caused by epithelial fragility is a feature frequently described in asthma,[39] leading to mucosal denudation and increased exposure of the mucosal nerve endings to irritant factors, enhanced penetration of allergens, and reduced mucociliary clearance (Fig 2). Although part of the observed shedding is probably artifactual because of bronchoscopy techniques,[40] the frequent sloughing of epithelial cells observed in bronchoalveolar lavage (BAL) fluid from patients with asthma suggests that some degree of epithelial shedding does occur in asthma.[31] Goblet cell metaplasia is another important feature of asthma that, together with the bronchial gland enlargement, leads to hypersecretion and airway obstruction.

There is controversy about the role of corticosteroids on epithelial damage in asthma. Some *in vitro* studies suggested that corticosteroids increase apoptosis of epithelial cells, which could further contribute to the chronic epithelial damage already present in this disease.[41] In contrast, glucocorticoids can inhibit cell death induced by cytokines.[42] In mechanically denudated guinea pig tracheal epithelium, budesonide did not interfere with an efficient restitution of the epithelium,[43] whereas dexamethasone prolonged the repair potential after repeated episodes of epithelial injury in mucociliated human bronchial epithelial cells.[44]

Defective mechanisms of epithelial repair are believed to occur in asthma. Persistent activation of the epithelial growth factor receptor with paradoxical increased expression of antiproliferative markers such as p21/waf have been described in adult and pediatric patients with severe asthma,[45,46] and both features seem to be unresponsive to steroid treatment. Hence, sustained epithelial activation associated with chronic inflammation is proposed to be the major trigger of the remodeling process.[4] Partial restoration of the epithelium has been observed after the use of corticosteroids in a retrospective biopsy study[47] showing that treating patients with asthma with inhaled corticosteroids for 10 years decreased inflammation and partially improved epithelial damage. However, this was not associated with improvement of bronchial hyperresponsiveness. When examining this prospectively, compared with bronchodilator treatment alone, 3 months of budesonide therapy in patients with asthma increased the number of ciliated cells.[48]

Goblet cell metaplasia and mucus hypersecretion are features of asthma, and airway mucus hypersecretion is recognized as an important contributor to morbidity and mortality in patients with lung diseases such as asthma and chronic obstructive pulmonary disease. Patients with asthma have altered mucin expression in the goblet cells, but the clinical or functional significance of this different composition is still unclear.[49] Corticosteroids and bronchodilators are not primarily targeted to act on goblet cell activity but seem to have direct and indirect suppressive effects on mucus production.[49] In addition, in animal models, corticosteroids appear to be effective in reducing goblet cell metaplasia.[50,51] The effects of ciclesonide and fluticasone propionate (FP) on reducing goblet cell metaplasia in a rat model of allergic inflammation were comparable.[50] Apparently, this also occurs in human asthma, because De Kluijver et al[52] observed a decrease in the number of goblet cells in patients with mild asthma after 2 weeks of inhaled corticosteroids.

Are there alternatives to corticosteroids available with regard to effects on bronchial epithelium? In mice, leukotriene receptor antagonists have a positive effect on reducing epithelial changes induced by allergic sensitization.[53,54] In addition, pranlukast and zafirlukast greatly suppressed ovalbumin-induced secretion in the guinea pig trachea, suggesting a role of these drugs in the treatment of airway diseases with a hypersecretory component.[55]

### Basement membrane components and thickness

Because of its constant accessibility in bronchial biopsies, the basement membrane has been studied extensively in asthma[6] (Fig 2). It has been considered ''the remodeling marker'' in several studies. It consists of the thickening of the subepithelial lamina reticularis that lies underneath the true basal lamina of the bronchial epithelium, sometimes referred to as *pseudo-thickening*. In patients without asthma, this structure measures approximately 5 to 6 μm, whereas it is consistently thickened in biopsies of patients with asthma with mean values of approximately 9 μm.[56] A study in symptomatic infants with reversible airway obstruction could not demonstrate basement membrane (BM) thickening,[57] whereas it was already present in older children with severe asthma,[7] suggesting that BM thickness develops early in the disease course. Further, an association between BM thickness and disease duration and

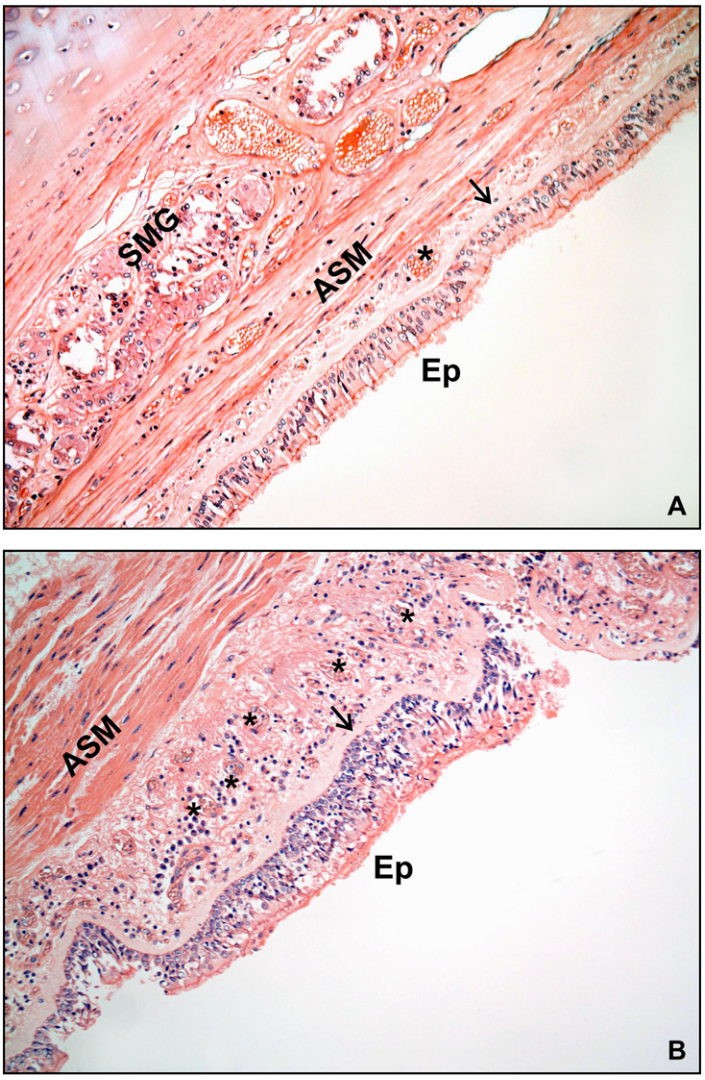



**FIG 2. A,** Normal bronchial mucosa. The epithelium is intact and composed of ciliated columnar cells. **B,** Bronchial mucosa from a patient with fatal asthma. There is epithelial damage and basement membrane thickening *(arrow)*. The lamina propria is thickened with inflammation and numerous capillaries.* The ASM layer is thickened. Hematoxylin and eosin. ×200. *Ep,* Epithelium; *SMG,* submucosal glands.

disease duration or severity have not been consistently demonstrated.[7]

Accumulation of collagen III, I, and IV and fibronectin at the BM level occurs in patients with asthma.[58] More recent ultrastructural studies reported that accumulation of (thinner) reticulin fibers and not (thicker) interstitial collagen fibers accounts for the thickened BM in asthma, suggesting that this phenomenon in asthma is different from the collagen deposition observed in interstitial lung diseases.[59] It must be stressed that in sensitized animals, the typical characteristic hyaline thickening of the BM as seen in human asthma is not observed. What has been called *subepithelial thickening* in these models would correspond to the entire lamina propria in human airways.

Several studies assessed the effects of asthma treatment in the reversibility of BM thickness. Differences in methodology, small samples, different corticosteroids and doses, and timing make results seemingly controversial and difficult to interpret. Some studies could not demonstrate reductions in BM thickness after the use of inhaled corticosteroids,[60,61] whereas others could.[62,63] The latter studies suggest that reducing BM thickness may require prolonged and high-dose inhaled steroid therapy. Reduction in collagen type III deposition, decrease of matrix metalloprotease (MMP)-9, and increase in tissue inhibitor of metalloprotease 1 levels could be involved in the mechanisms of the reversal of BM thickness.[64] It remains to be established whether such a decrease in BM thickness has any benefits on airway function.

Laminin is a glycoprotein found at the airway BM level, playing a major role in airway morphogenesis, particularly of the smooth muscle. Altraja et al[65] have previously

observed increased deposition of laminin chains at BM level in patients with chronic and occupational asthma. This could reflect an increased tissue turnover in the airway wall, possibly as a result of airway inflammation. Concomitant treatment with dexamethasone reduced laminin and receptor levels within the airways in an experimental model of asthma, but this was not accompanied by a reduction in airway hyperresponsiveness.[66] Furthermore, the expression of tenascin, one of the BM glycoproteins, was found to be decreased after the use of inhaled corticosteroids during the birch pollen season, but without any corresponding improvements in lung function.[67]

All these studies point toward the capability of corticosteroids to change the thickness and constitution of the bronchial reticular basement membrane in asthma, but unless its functional benefit has been demonstrated, this should not be considered a therapeutic target.

## Lamina propria

The lamina propria of the airways is composed of structural cells such as fibroblasts and myofibroblasts, vessels, ECM components, and inflammatory cells. In large airways, this wall layer accounts for a significant portion of the airway (Fig 2).

*Fibroblasts and myofibroblasts.* Fibroblasts and myofibroblasts play a crucial role in the mechanisms of altered airway structure in asthma because of their capacity to secrete growth factors and ECM elements.[68] It has been observed that the steroid triamcinolone acetonide and the combination of FP and salmeterol have the ability to downregulate fibroblast proliferation *in vitro*.[69,70] However, studies using fibroblasts from patients with asthma show that dexamethasone can increase fibroblast proliferation and stimulate G1-S phase transition.[71,72] This is an important observation, suggesting that corticosteroids may also promote elements of airway remodeling in asthma. FP displays anti-inflammatory effects on human lung fibroblasts during their myofibroblastic differentiation. At early stages of differentiation, FP inhibits the activation of Janus kinase/signal transducer and activator of transcription pathways induced by IL-13 in lung myofibroblasts.[73] FP also inhibits constitutive and TGF-β–induced expression of α–smooth muscle actin in fibroblasts, the main marker of myofibroblastic differentiation, both in very early and in mild differentiated myofibroblasts.[73] These *in vitro* results have been extended by *in vivo* studies. In a mouse model of chronic allergic sensitization, 3 months of systemic corticosteroids reduced myofibroblasts (defined as the cells that expressed α–smooth muscle actin and collagen I), TGF-β expression, and peribronchial fibrosis, but not ASM thickness.[74] However, to our knowledge, there are no clinical studies available on the effects of corticosteroids on fibroblast or myofibroblast activity and proliferation.

It is interesting to note that theophylline inhibits fibroblast proliferation and suppresses TGF-β–induced collagen I mRNA *in vitro*.[75] Furthermore, recent data suggest that the leukotriene receptor antagonist montelukast may lead to a decrease in airway wall myofibroblasts.[76] This indicates that it is worth examining other interventions than just corticosteroids in changing fibroblast or myofibroblast function in asthma.

*Extracellular matrix elements.* Collagens, elastin, proteoglycans, and glycoproteins compose the extracellular matrix of the airway wall and are mainly secreted by structural cells such as fibroblasts, myofibroblasts, and ASM cells.[68] Altered content and composition of extracellular matrix have been described in large and small airways of patients with asthma of different severities.[52,77-81]

Aerosolized corticosteroids have shown to be effective in preventing and reversing enhanced fibronectin deposition during concomitant repeated allergen exposure.[82,83] However, these effects appeared to be dependent on the timing[82,83] and dose of the inhaled corticosteroids.[84] Treatment with low doses or treatment after allergen exposure had no effect on fibronectin deposition, which suggests that adequate therapy during a particular time window may be required to prevent and suppress components of airway remodeling.

There are few data on the ECM composition in the entire lamina propria in patients with asthma with or without antiasthma treatment. Wilson and Li[77] have shown that the bronchial submucosal region of patients with asthma has more type III and type V collagen than controls, but these results were not reproduced by Chu et al[20] when examining patients with asthma of different severities and controls. Patients with moderate-severe asthma have increased deposition of type III, type I collagens, and the profibrotic cytokines IL-11, IL-17, and TGF-β compared with healthy controls and patients with mild asthma.[85] When patients with asthma were treated with 2 weeks of oral corticosteroids, the levels of IL-11 and IL-17, but not of TGF-β or collagens, were reduced.[85] These results again suggest that some components of airway remodeling may not be responsive to steroid therapy.

Proteoglycans are important ECM components of the airway wall and are involved in mechanics, water balance, regulation of inflammation, cell migration, and proliferation.[86] Previous studies have demonstrated differential deposition of its components within the airways.[52,79,81] The combination of budesonide and formoterol inhibits the serum-induced production of proteoglycans *in vitro*.[87] However, in asthmatic airways *in vivo*, we have observed that a 2-week course of inhaled corticosteroids resulted in increased density of the proteoglycans versican and biglycan, without associated changes in fibronectin or BM thickness.[52] This study shows that treatment with corticosteroids differentially affects ECM components in asthma, but again it is unclear whether this has functional relevance. Long-term longitudinal studies are surely needed to determine whether the steroid induced changes are beneficial or detrimental to lung function decline in asthma.

Elastin is a major component of the lung ECM, presumably having a pivotal role in airway patency and lung elastic recoil. Changes in elastin have been described in patients with asthma,[23,80,88] but to our knowledge, there are no intervention studies addressing elastin content in asthma.

## Bronchial vessels

Engorgement of bronchial vessels at the large and small airway levels is a feature of asthma, and morphometric studies have revealed increases in both the number of vessels and the vessel area.[89-92] Furthermore, increased microvascular permeability is observed in asthmatic airways.[93] These features could indirectly amplify the inflammatory response and contribute to enhanced airway thickness.[94]

Inhaled corticosteroids can decrease airway vascularity in the airways of patients with asthma, which is associated with a decrease in BM thickness, $FEV_1$, and airway responsiveness.[95] Different corticosteroids seem to have different potencies in the reduction of airway vasoconstriction, with FP and budesonide causing greater airway vasoconstriction than beclomethasone dipropionate (BDP).[96] Corticosteroids may also inhibit angiogenesis by regulating growth factors such as vascular endothelial growth factor (VEGF), an important regulator of angiogenesis. In patients with asthma treated with 800 μg/d beclomethasone, VEGF levels in induced sputum were decreased, and were associated with the degree of airway narrowing and vascular permeability.[97] Taken together, high doses of corticosteroids seem to be necessary to reduce structural changes in airway vessels that are accompanied by decreases in VEGF expression.[94] Feltis et al[98] recently extended these results, showing that reduction in subepithelial vascularity after 3 months of treatment with high-dose FP (750 μg twice daily) was associated with a concomitant reduction in VEGF-positive vessels, a reduction in angiogenic sprouts per vessel, and a decrease in VEGF receptors and in angiopoietin 1 in steroid-naive patients with asthma.

There are few data about the effects of other antiasthma drugs on vessel remodeling. $\beta_2$-Agonists can have an inhibitory effect on plasma exudation and vascular permeability.[99] Indeed, adding salmeterol to low-dose inhaled steroid treatment (200-400 μg/d BDP or 200-400 mg/d budesonide) results in decreased vessel density in the lamina propria of patients with asthma.[100] Leukotriene receptor antagonists may also have beneficial effects on vascular permeability and airway mucosa blood flow. In sensitized mice, the use of a leukotriene receptor antagonist led to a decrease in vascular permeability and a decrease in VEGF levels.[101] In human beings, 2 weeks of daily treatment with 10 mg montelukast or 400 μg FP appeared to have equivalent effects on reducing airway mucosal blood flow in patients with asthma.[102] So far, no human studies have addressed the effects on leukotriene receptor antagonists on the structural changes of the bronchial microvasculature.

## ASM cells

Increase in ASM mass, caused by cell hyperplasia, hypertrophy, and/or increased extracellular matrix deposition, is an important component of the structurally altered airway in asthma (Figs 1 and 2) and is presumed to be a major determinant of enhanced bronchoconstriction and airway hyperresponsiveness.[103] Increase in the ASM mass occurs along the entire airway tree, and it is the major contributor to the increased area of the inner airway wall in asthma.[103] Increases in ASM mass have been related to asthma severity,[15,18] whereas smooth muscle amount and changes in its plasticity are likely to be key players in the dynamics of airway narrowing.[29,104,105]

A shift toward a synthetic phenotype with increased proliferation rates of the ASM cells is believed to play a role in the mechanisms leading to the ASM thickening.[106] Several mediators in the asthmatic airways, such as contractile agonists, cytokines, growth factors, and ECM proteins, can induce ASM proliferation.[106] However, it must be emphasized that increased proliferation rates have not been confirmed in human beings *in vivo* yet. It is possible that ASM *in vivo* proliferation occurs very slowly, occurs only intermittently, or is already completed before or at the onset of asthma.[103]

The mechanisms by which corticosteroids could affect ASM proliferation are of great interest and are currently being clarified.[107] Corticosteroids not only modulate the secretion of chemokines and cytokines that are involved in ASM function and proliferation,[108] but also can exert a direct effect on ASM cells. *In vitro* experiments have shown that the corticosteroids dexamethasone and FP arrest human ASM cells in the G1 phase of the cell cycle and inhibit some but not all growth factor–induced proliferation of ASM cells.[109] Corticosteroids have the capacity to inhibit the action of some, but not all, mitogens involved in ASM proliferation. Corticosteroids inhibit the ones signaling via G protein coupled receptors, but less efficiently the ones signaling via receptor tyrosine kinase pathway.[110] A mechanism proposed to explain the absence of an effective antiproliferative action by corticosteroids on ASM cells of patients with asthma would be the absence of the CCAAT/enhancer binding protein α.[111] This protein forms a complex with the glucocorticoid receptor, leading to activation of the cell cycle inhibitor p21; its absence could explain the failure of glucocorticoids to inhibit ASM proliferation of asthmatic cell cultures *in vitro*.

It has been recently observed that corticosteroids have significant inhibitory effects on ASM contractile protein expression by reducing human ASM-actin protein abundance and incorporation into contractile filaments.[112] The authors suggest that these effects appear to be mediated via the attenuation of mRNA translation and enhancement of protein degradation.

Extracellular matrix components are known to affect ASM growth and its synthetic properties, and it has been hypothesized that altered composition of the ECM could contribute to ASM dysfunction in asthma.[113] It was recently demonstrated that culturing these cells on collagen type I prevented the antimitogenic actions of glucocorticoids, but not of $\beta_2$-adrenoceptor agonists. In contrast, glucocorticoids were efficient in regulating ASM production of GM-CSF, whereas $\beta_2$-adrenoceptor agonists were not.[114] These results suggest that combination therapy may have increased efficacy over glucocorticoids alone in controlling remodeling events.

When treating smooth muscle cells in culture after exposure to 10% serum from an individual with asthma with or without beclomethasone (0.01-100 nM), it appeared that there was a significant increase in the production of ECM components in the cells cultured with the asthmatic serum. However, beclomethasone did not reverse the increase in ECM protein.[115] Burgess et al[116] extended these results, showing that treating cells with a corticosteroid or long-acting $\beta_2$-agonist did not inhibit TGF-β–stimulated release of connective tissue growth factor (CTGF), collagen I, fibronectin, and versican. Moreover, corticosteroid alone increased the release of CTGF, collagen I and fibronectin. These findings raise the point that corticosteroids may not be effective in reducing ECM deposition and that they may even increase matrix production in certain circumstances, as demonstrated by De Kluijver et al[52] in bronchial biopsies.

To date, there are no longitudinal studies evaluating the effect of pharmacologic therapy on the structure of ASM in asthma. In a murine model of asthma, daily administration of intranasal FP for 3 months inhibited the thickening of the ASM layer, decreased TGF-β and its intracellular effectors pSmad2 and pSmad3, and increased pSmad7 levels.[117] However, even though 3 months of systemic usage of dexamethasone prevented many structural alterations in mice submitted to allergic sensitization, it did not decrease ASM thickness.[74] Hence, the route of administration may be one of the determinants of steroid effects on ASM. Interestingly, the combination of allergen avoidance and treatment with dexamethasone for 1 month was capable of reverting airway remodeling in chronically sensitized mice, including the thickened ASM layer.[118]

How about other available antiasthma drugs? β-Agonists cause increases in the levels of 3′,5′ cAMP in ASM cells, and besides their bronchodilator effect could also inhibit mitogen-induced proliferation on ASM cells.[119] Maintenance treatment with the long-acting anticholinergic, tiotropium bromide, appears to prevent ASM thickening and to decrease myosin expression and ASM contractility in a guinea pig model of chronic allergic sensitization.[120] In addition, montelukast reversed increased smooth muscle mass and subepithelial fibrosis in an animal model of chronic allergic sensitization,[53] although lower doses of leukotriene receptor antagonists were not able to decrease ASM mass in another experimental study.[54] Given the emerging importance of smooth muscle activity, proliferation, and plasticity in the pathogenesis of asthma,[121] these findings indicate that it is worth revisiting the effects of widely used asthma therapy on these cells in asthma.

The issue has been raised that none of the physiological functions of the normal ASM are essential to lung physiology.[122] Ablation of the ASM by thermoplasty has therefore been proposed as an alternative to treat/cure asthma.[123] Preliminary data indicate that bronchial thermoplasty reduces ASM[124] and increases airway compliance[125] without serious complications. The first uncontrolled study suggested that this intervention led to improvement in $FEV_1$ and in airway hyperresponsiveness.[126] However, a recent randomized, controlled trial in patients with moderate to severe asthma reported improvements in the rate of mild exacerbations, morning expiratory peak flow, and symptom scores, but no effect on $FEV_1$ and airway responsiveness after 1 year of follow-up.[127] Even though it cannot be excluded that bronchial thermoplasty in a nonblind design may have introduced some level of placebo effect on clinical outcome, the current results certainly warrant more detailed studies on histologic and functional outcomes.

## NEW DRUGS

### Cytokine modulators

*Anti–TNF-α.* Evidence suggests that the TNF-α axis is upregulated in patients with refractory asthma, as indicated by increased levels of TNF-α, its receptors and converting enzyme in blood monocytes, bronchoalveolar lavage, and bronchial biopsies.[128,129] Two (1 controlled, 1 uncontrolled) studies in patients with severe asthma revealed that the use for 10 to 12 weeks of etanercept, which binds specifically to both TNF-α and TNF-β, thereby preventing free cytokine binding to TNF receptors, resulted in decreases in asthma symptoms and bronchial hyperresponsiveness and an increase in lung function in absence of changes of cellular inflammation in induced sputum.[128,129] This might point toward possible changes in airway remodeling, perhaps in ASM structure, which need to be addressed. Infliximab is a recombinant human-murine chimeric mAb that binds and neutralizes the soluble TNF-α homotrimer and its membrane-bound precursor. This drug was tested in patients with moderate asthma (3 infusions in 6 weeks) and resulted in the decrease of moderate exacerbations and sputum cytokine levels, but no effect was noted in lung function parameters. The drug was well tolerated.[130] The use of 2 dual inhibitors of TNF-α–converting enzyme and matrix metalloproteinases (PKF242-484, PKF241-466) in murine models of acute airway inflammation showed significant reductions on lung inflammation.[131] If the adverse effects of this treatment remain to be medically acceptable, exploration of anti–TNF-α strategies in asthma require priority in the clinical research of severe asthma.

*Blocking $T_H2$ cytokines.* $T_H2$ cytokines play an important role in orchestrating the inflammatory pathways of allergic asthma. Attempts to develop drugs that block $T_H2$ cytokine action or inhibit their synthesis or release are therefore the subject of long-standing investigation.[132] A nonspecific inhibitor, suplatast tosilate, suppresses selectively the synthesis of IL-4, IL-5 *in vitro*[133] and is available as an asthma controller in Japan. Beneficial effects on inflammation, symptoms, and lung function have been reported in steroid-dependent patients with asthma after 8 weeks of use.[134] Part of this effect may be a result of a decrease in PAS/alcian blue–positive bronchial goblet cells, as was measured by MUC5AC staining in bronchial epithelium by Hoshino et al.[135]

**TABLE I.** Potential therapeutic targets with effect on airway structure in animal models

| Target | Drug | Species | Effect on inflammatory parameters | Effect on airway structure | Reference |
|---|---|---|---|---|---|
| CCR3 | Low-molecular-weight CCR3 antagonist | Mouse | Reduction in eosinophils, no changes in lymphocytes or macrophages | Prevention of goblet cell hyperplasia, subepithelial fibrosis, and accumulation of myofibroblasts | 152 |
| IL-13 | Neutralizing IL-13 mAb | Mouse | Reduction in eosinophils, no changes in inflammatory cells | Reduction in mucus production and bronchiolar collagen deposition | 153 |
| TGF-β | 1. Neutralizing anti–TGF-β antibody | 1. Mouse | 1. No effect on inflammation and $T_H2$ cytokine production | 1. Reduced peribronchiolar ECM deposition, ASM proliferation, and mucus production | 1. 154 |
| | 2. Inhibitor of TGF-β receptor I kinase (SD-208) | 2. Rat | 2. Decrease in eosinophils, CD2 T-cell counts, and Smad2/3 intracellular expression | 2. Decreased epithelial and ASM cell proliferation and goblet cell hyperplasia | 2. 155 |
| Tryptase | Tryptase inhibitor MOL 6131 | Mouse | Reduction in total cells, eosinophils, and in the release of IL-4 and IL-13 in BAL fluid, reduction in airway tissue eosinophilia | Reduction in goblet cell hyperplasia, mucus secretion, and peribronchial edema | 156 |
| Immunomodulation | Immunostimulatory sequences–oligodeoxy-nucleotides (CpG-DNA) | 1. Mouse | 1. Decrease in VEGF, MMP-9 in bronchial epithelium, TGF-β levels, and CD4 T lymphocytes | 1. Prevents collagen deposition and mucus production, reduction in angiogenesis, reversion of peribronchial collagen deposition. | 1. 157-160 |
| | | 2. Monkey | 2. Fewer eosinophils and interstitial mast cells (no differences in glands or ASM mast cells) | 2. Thinner basement membranes and fewer mucus cells | 2. 161 |
| Toll-like receptor 7/8 | Toll-like receptor 7/8 ligand S28463 | Rat | Reduction in airway inflammation and $T_H1/T_H2$ cytokine levels | Prevents goblet cell hyperplasia and ASM mass increase by reducing proliferation | 162 |

IL-4 levels are important in regulating several steps of allergic inflammation, such as IgE isotype switching, upregulation of vascular adhesion molecule 1, and $T_H2$ cell commitment.[136] It has been previously shown that IL-4–deficient mice exhibit reduced collagen airway deposition after chronic allergen exposure.[137] An inhaled recombinant human soluble IL-4 receptor (altrakincept) has been developed,[132] but there are no data on possible effects on structural cells available in asthma yet. IL-13 may be a more attractive target with regard to airway remodeling. It is detectable in bronchial mucosa and sputum[138] and has been implicated in profibrotic activity.[139] Anti–IL-13 strategies, such as IL-13–deficient mice[137] or IL-4 receptor (R)α antisense oligonucleotides,[140] demonstrated reduced airway collagen and reduced goblet cell metaplasia and mucus staining, respectively. Hence, IL-13 seems to be a target worth exploring in the prevention (or reversal) of airway remodeling.

IL-5 is a major regulator of eosinophilopoiesis, and treatment with the anti–IL-5 humanized monoclonal antibody (hMoAB) (mepolizumab) almost abolished circulating and sputum eosinophils[141] while reducing tissue and bone marrow eosinophils by about 50%.[142] Notably, such treatment resulted in a significant reduction in the expression of tenascin, lumican, and procollagen III in the bronchial mucosal reticular BM and in TGF-β–positive cells compared with placebo.[143] These data suggest a role for eosinophilic release of TGF-β in patients with asthma, which can have implications for the understanding of airway remodeling.

## Anti-IgE

IgE is a key mediator of the inflammatory allergic reactions such as asthma and rhinitis. Omalizumab is a humanized anti-IgE mAb that binds free circulating IgE, thereby preventing the interaction between IgE and its receptors on inflammatory cells. Clinical trials have shown that omalizumab has beneficial effects in patients with severe persistent asthma by reducing the risk of exacerbations and hospitalization and improving symptom control, lung function, and quality of life in patients.[144] At the bronchial level, 16 weeks of treatment with omalizumab decreased IgE, associated with a marked reduction in airway eosinophilia and in expression of the

high-affinity IgE receptor and the $T_H2$ cytokine IL-4.[145] In epithelial cells, anti-IgE can reduce TNF-α and TGF-β expression, which may point toward possible indirect influence on airway structure.[146]

### Metalloprotease inhibitors

Matrix metalloproteases are a family of enzymes consisting of 24 zinc-dependent endopeptidases capable of degrading ECM components, having a role in cell migration, angiogenesis, and tissue remodeling.[147,148] Expression of several MMPs has been associated with asthma. Increases in MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 have been described in sputum, BAL, or tissue from patients with asthma. MMP-9 BAL levels are significantly enhanced in patients with severe asthma and in patients experiencing acute severe attacks.[147] Targeting MMPs could be therefore an alternative to treat asthma. A broad spectrum synthetic inhibitor, marimastat, initially used in a clinical trial with patients with cancer, was tested in patients with mild asthma.[149] Compared with placebo, marimastat reduced bronchial hyperresponsiveness to inhaled allergen without significant changes in sputum inflammatory cells, exhaled nitric oxide, $FEV_1$, asthma symptoms, or albuterol use. In patients with cancer, serious musculoskeletal side effects were observed with this drug, and trials have stopped. Targeted therapy against specific MMPs will be probably necessary when treating lung diseases.

### Phosphodiesterase inhibitors

The phosphodiesterases (PDEs) control the degradation of cAMP and cGMP to their inactive 5′ monophosphates, having important roles in regulating signaling responses to intracellular gradients of cAMP and cGMP. In an animal model of chronic allergic sensitization, the PDE4 inhibitor roflumilast reduced airway inflammation, subepithelial collagen, and thickening of the airway epithelium, but not the accumulation of TGF-β.[51] The same PDE4 inhibitor was able to reduce CTGF and ECM deposition from ASM cells *in vitro*, whereas corticosteroids and long-acting $\beta_2$-agonists were not.[116] This is indicative of the potential of PDE4 inhibitors to influence airway structure, which requires further testing in human *in vivo* studies.

### Other drugs

Several new promising approaches and new targets for asthma are currently under investigation,[150,151] but in many of them, there are no human data yet, or the effects on airway structure have not been investigated. Table I shows some new therapeutic targets which, in animals, have been shown to have effects on airway structure. Some of these approaches will surely deserve human proof of concept studies.

## CONCLUSION

Airway remodeling has become a key concept in today's asthma research. However, the level of uncertainties grows with the level of excitement. It appears to be an adequate working hypothesis that the observed changes in airway structure in asthma can have untoward effects on airway function, and thus should have therapeutic implications.

Progress in this area depends on linking disciplines: on explicit integration of innovative biopharmaceutics with modern structure-function analysis.[163,164] Where can we find this in a single laboratory? Public and private institutes should collaborate to promote such synthesis. During the next 5 years, we must develop balanced answers to the following questions:

- At which stage is airway remodeling impairing lung function?
- What are the key components of airway structure that determine fixed airway obstruction and excessive airway narrowing?
- How are these components differentially regulated?
- How can such regulatory mechanisms selectively be manipulated?
- Can this adequately prevent and reverse the detrimental components of airway remodeling in asthma?

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