

Asthma persistence versus progression: Does airway smooth muscle function predict irreversible airflow obstruction?

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ABSTRACT

Asthma, a disease characterized by airway inflammation and reversible bronchoconstriction, manifests a variety of phenotypes, and in some, but not all, asthma leads to a persistent and/or progressive loss of lung function. Conceptually, asthma persistence is identified by a stable decrement in lung function over time, and asthma progression can be identified by a gradual loss in pulmonary function associated with increasing symptoms. Whether these phenotypes are determined by distinct cellular and molecular mechanisms or whether unique genotypes define asthma persistence versus progression remains unknown. Recent evidence suggests that airway remodeling in some is associated with asthma persistence and/or progression, although the direct link of airway remodeling to these phenotypes remains tenuous. The development of in vitro cell models using nontransformed human airway smooth muscle (ASM) cells has been a useful approach in defining corticosteroid sensitivity and bronchodilator response. New evidence suggests some, but not all, immunomodulatory function of ASM is modulated by corticosteroid treatment. Additionally, the combinations of interferon and TNF- α render ASM insensitive to corticosteroids. The further development of cellular and ex vivo systems that can predict corticosteroid sensitivity and bronchodilator unresponsiveness may be useful in identifying new biomarkers and therapeutic approaches to address asthma persistence and progression.

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Key words: Airway remodeling, allergy, bronchoconstriction, bronchodilation, corticosteroids, corticosteroid insensitivity, cytokine, guidelines, inflammation, resistance

Asthma, a common chronic disease characterized by airway inflammation and bronchoconstriction, manifests as a variety of phenotypes in which its progression is difficult to predict. The precise mechanisms that induce variability in the clinical course of asthma remain unclear. Current guidelines in the management of asthma suggest that disease severity is defined by decrements in pulmonary function as measured by forced expiratory volume in 1 second (FEV₁) and by the use of high-dose corticosteroids in the management of symptoms. In some patients with asthma,

the disease induces a stable loss in pulmonary function and an occurrence of symptoms termed persistence, and in others, over time there is a gradual loss in pulmonary function with increasing symptoms, *viz.*, progression. Whether these phenotypes are determined by distinct cellular and molecular mechanisms or whether unique genotypes define asthma persistence versus progression remains unknown. This article will address the clinical concepts of asthma persistence and progression. Additionally, the role of airway smooth muscle (ASM) in contributing to these phenotypes will be reviewed.

DISEASE PERSISTENCE VERSUS PROGRESSION

Although clinicians recognize the difference between asthma persistence and progression, there remains no case definition or uniformity on the parameters that should be measured or evaluated to define such processes. In adults, the rate of decrease in lung function over time (FEV₁) is greater in those with asthma than in healthy subjects.^{1–3} Additionally, the rate of decrease among smokers is more profound in those with asthma than those without asthma.² Interestingly, the decline in FEV₁ is heterogeneous; not all patients experience

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disease progression, and asthma progression is difficult to predict for any given patient. Epidemiologic studies that tracked asthma severity over time indicate that asthma symptoms resolve in some pediatric and adult patients, remain stable in others, and go into remission and then recur in adulthood for some.^{4,5} Specific risk factors for the persistence of asthma into adulthood include house-dust mite allergy, smoking exposure, airway hyperresponsiveness in childhood, and female gender, whereas risk factors for asthma relapse include dust-mite allergy, airway hyperresponsiveness, and early age onset of asthma.⁵ Alternatively, risk factors for progressive loss of lung function have been described by Ulrik and colleagues⁶ and include severe symptoms and use of anti-inflammatory therapy while atopy and early onset of asthma did not predict progression.

Mechanisms that may predict progression or persistence of asthma may include airway inflammation, airway remodeling, or a combination of both processes. Airway remodeling manifests as a complex constellation of findings in the airway that include goblet cell and smooth muscle hypertrophy–hyperplasia, subepithelial fibrosis, and angiogenesis.^{7,8} For patients with irreversible airflow obstruction, inflammation might be necessary but perhaps not entirely sufficient to induce an irreversible component, some of which may be caused by the alterations in the structure of the bronchial wall.^{9,10} Conceptually, irreversibility may be a consequence of structural or myogenic changes rather than the direct consequence of inflammation. Factors associated with persistent airflow obstruction (<50% predicted versus >80% predicted postbronchodilator FEV₁) in patients with severe asthma include prolonged disease duration, peripheral blood eosinophilia, and bronchial wall thickening on high-resolution computerized tomography scanning.¹¹ Despite evidence suggesting these factors are important in predicting progression, the predictive value of any one component was not helpful in the early identification of irreversible airflow obstruction. Some investigators postulate that airway remodeling that develops potentially over years could evoke an irreversible airway obstruction phenotype.

Although most patients with asthma have some degree of airway remodeling, there has been no direct association or correlation between the physiologically relevant changes as defined primarily by objective measures of airway obstruction and any aspect of remodeling other than angiogenesis.¹² The number of bronchial wall blood vessels correlates with the severity of asthma¹³; however, neither the degree nor the thickening of the reticular basement membrane nor the increase in ASM mass was convincingly associated with the duration or the severity of asthma.

Given the clinical relevance of asthma persistence versus progression, a useful *working* definition for these phenotypes may include relative corticosteroid insensitivity and a lack of bronchodilator responsiveness. The identification of patients whose postbronchodilator response does not normalize pulmonary function or those who manifest substantial airway hyperresponsiveness despite normal baseline pulmonary function may identify an at-risk cohort for disease progression or persistence. Additionally, the lack of a complete response to either inhaled or oral corticosteroids may also have diagnostic and prognostic value in determining the susceptibility of individuals to asthma persistence versus progression. Current National Institutes of Health Expert Panel Guidelines define clinical response to therapy as achieving total control that includes elimination of symptoms and normalization of lung function.¹⁴ The precise mechanisms that induce corticosteroid insensitivity and bronchodilator unresponsiveness are likely complex and myriad; however, recent studies using cultured human ASM provide insight into new therapeutic targets and/or biomarkers of disease progression and persistence.^{15,16}

ASM FUNCTION: A MODEL TO SHOW CORTICOSTEROID INSENSITIVITY AND BRONCHODILATOR UNRESPONSIVENESS

Although asthma typically induces reversible airway obstruction, in some patients airflow obstruction can become fixed. Such obstruction may be a consequence of persistent structural changes in the airway wall caused by the frequent stimulation of ASM by contractile agonists, inflammatory mediators, and growth factors. Importantly, the bronchoconstriction evoked by smooth muscle shortening promotes airway obstruction and constitutes the hallmark of asthma and chronic obstructive pulmonary disease. ASM functions as the primary effector cell that regulates bronchomotor tone. Evidence now suggests that ASM may undergo hypertrophy and/or hyperplasia and modulate inflammatory responses by secreting chemokines and cytokines, as shown in Fig. 1.

Although asthma typically induces reversible airflow obstruction, in some patients, airflow obstruction becomes irreversible. The airway remodeling that occurs in asthma has been attributed in part to increased smooth muscle mass. Although increased ASM mass is, in part, caused by ASM cell proliferation, the mechanisms that regulate ASM cell growth remain unclear. Many studies have characterized the stimulation of ASM growth in response to mitogenic agents such as polypeptide growth factors, inflammatory mediators, and cytokines. The observation that contractile agonists induce ASM cell proliferation may be a critical link between the chronic stimulation of muscle contrac-

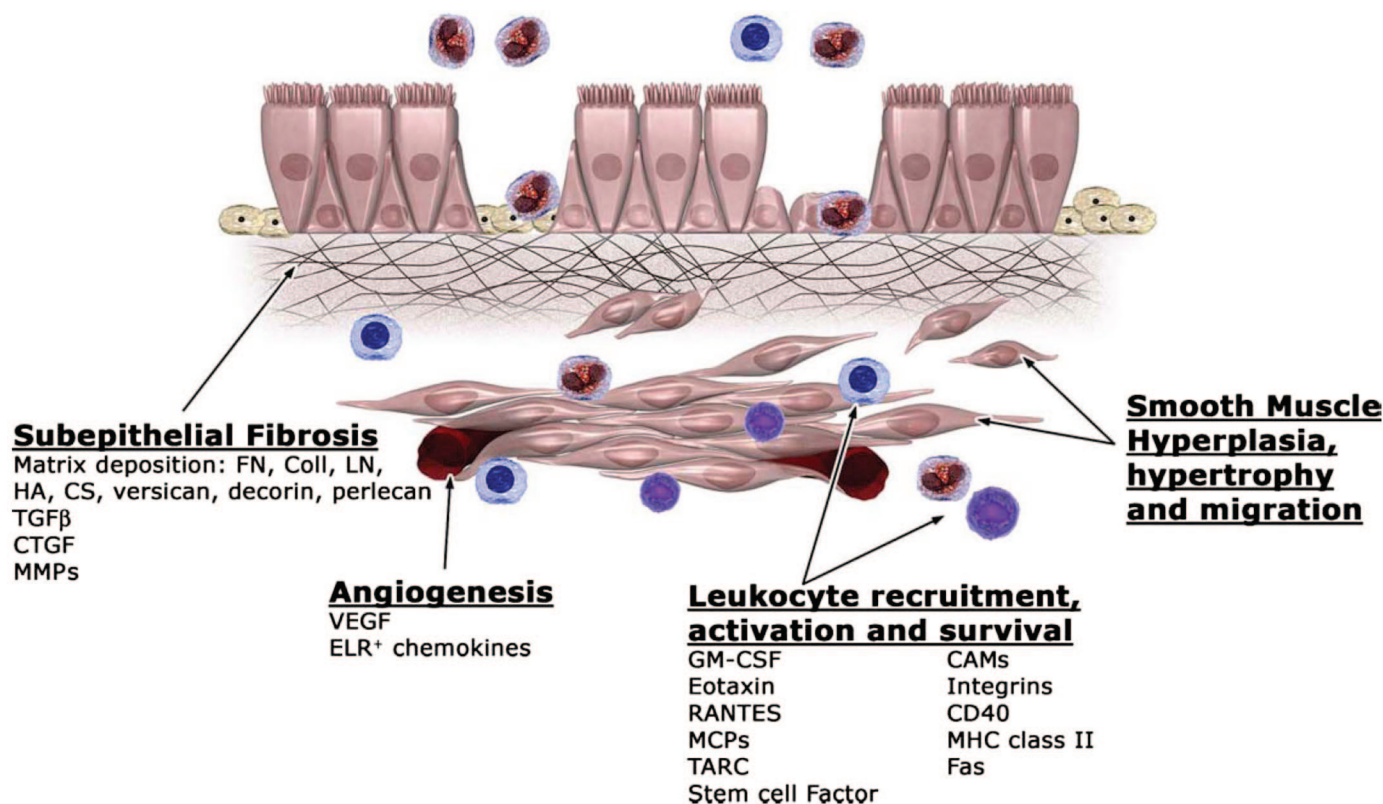


Figure 1. Airway smooth muscle (ASM)-derived factors that promote airway remodeling, including matrix deposition, bronchial neovascularization, and leukocyte recruitment. Alterations in the extracellular matrix also affect ASM cell proliferation and migration. FN, fibronectin; Coll, collagen; LN, laminin; HA, hyaluronan; CS, chondroitin sulfate; CTGF, connective tissue growth factor; TARC, thymus and activation-regulated chemokine; CAM, cell adhesion molecule (used with permission from Lazaar AL, and Panettieri RA Jr. Airway smooth muscle: A modulator of airway remodeling. *J Allergy Clin Immunol* 116:488–495, 2005).

tion and myocyte proliferation.¹⁷ Although the specific cellular and molecular mechanisms by which agonists induce cell proliferation remain to be elucidated, similarities exist between signal transduction processes activated by these agents and those of known growth factors, which can also stimulate smooth muscle contraction. The identification of the critical regulatory sites that mediate ASM cell proliferation may improve our understanding of the mechanisms that regulate airway inflammation and possibly provide new therapeutic approaches to alter airway remodeling seen in patients with chronic airflow obstruction.

The most widely used therapeutic agents for the control of asthma symptoms are the corticosteroids and the β_2 -adrenergic agonists. Inhaled corticosteroids inhibit inflammatory cell activation, whereas β_2 -agonists are effective bronchodilators. In addition, these agents are potent inhibitors of ASM cell proliferation.

The β_2 -agonists activate the β_2 -adrenergic receptor/Gs/adenylyl cyclase pathway to elevate cAMP in ASM cells. Because of their cAMP-elevating ability, albuterol, fenoterol, and salmeterol have been shown to inhibit mitogen-induced proliferation of human ASM cells.^{18–20} The β_2 -agonists and other cAMP-elevating

agents are thought to arrest the G1 phase of the cell cycle by posttranscriptionally inhibiting cyclin D1 protein levels by action on a proteasome-dependent degradation pathway.²¹ Another mechanism was suggested by Musa *et al.*²² who examined the effects of forskolin, an activator of adenylyl cyclase, on DNA synthesis, cyclin D1 gene expression, cAMP response element binding protein phosphorylation, and DNA binding in bovine ASM. This study showed that by increasing cAMP in ASM cells, forskolin suppressed cyclin D1 gene expression through phosphorylation and transactivation of cAMP response element binding protein, suggesting that the effect of cAMP on cyclin D1 gene expression is exerted by way of *cis*-repression of cyclin D1 promoter.

In human ASM, the corticosteroids dexamethasone and fluticasone propionate appear to arrest ASM cells in the G1 phase.²³ In that study, corticosteroids reduced thrombin-stimulated increases in cyclin D1 protein and mRNA levels, and they attenuated retinoblastoma protein phosphorylation through a pathway either downstream of or parallel to the extracellular signal-regulated kinase cascade.²³ Dexamethasone has different inhibitory effects on receptor tyrosine kinase-

mediated ASM cell growth and G protein-coupled receptor-mediated ASM cell growth, suggesting that corticosteroid effects on ASM mitogenesis are complex. Recently, *in vitro* studies showed that the antiproliferative effects of the corticosteroids were impaired by contact of ASM with collagen but not on laminin,²⁴ suggesting that in the collagen-rich microenvironment of the inflamed and fibrotic asthmatic airway, ASM may contribute to the lack of corticosteroid responsiveness in patients with asthma. *In vitro* human bronchial epithelial cells stimulated by allergens and matrix that release cytokines that are near smooth muscle cells are affected more by anti-IgE compared with corticosteroids after asthma.²⁵

ASM: AN IMMUNOMODULATORY CELL?

The variety of cell types that reside in or infiltrate the inflamed submucosa present the potential for many important cell-cell interactions. Eosinophils, macrophages, and, particularly, lymphocytes, are postulated as also being critical in the initiation and perpetuation of the asthmatic response. One mechanism by which immunocytes exert their effects is the production of proinflammatory mediators that may act directly or indirectly on ASM. The presence of several cytokine mRNAs has been detected within the airways of asthmatic subjects.^{26,27} A study of 18 patients with allergic asthma was performed to investigate the *in vitro* effects of montelukast on cysteinyl leukotrienes and IL-5 production and expression by peripheral blood mononuclear cells. In seven of nine patients tested, IL-5 mRNA was induced by both ragweed and dust-mite antigen, and montelukast at 10 mM completely blocked IL-5 mRNA expression. Therefore, montelukast may be anti-inflammatory by inhibiting IL-5 mRNA expression and reducing cysteinyl leukotriene secretion by peripheral blood mononuclear cells from asthmatic patients.²⁸ A second potential mechanism may involve direct contact of immunocytes with airway myocytes. Although many cell-cell interactions likely contribute to airway hyperresponsiveness in asthma, there is evidence to support that T cells and ASM cells can directly interact *via* cell adhesion molecules (CAMs).²⁹ The capacity for ASM cells to respond to numerous cytokines has revealed the extensive immunoregulatory potential of these cells. In response to cytokines such as IL-1 β , TNF- α , and interferon (IFN) γ , ASM cells can be induced to express a host of CAMs that allow interactions between the ASM and inflammatory cells that infiltrate the airways. Further advances in understanding the immunoregulatory potential of ASM have come with the discovery that cytokines also up-regulate the expression of multiple toll-like receptors in ASM cells.³⁰ These latter receptors are pattern-recognition receptors that mediate innate and adaptive immune and inflammatory responses to microbial infection, tissue injury, or inflammation.

Effects of Corticosteroids on Cytokine-Induced Synthetic Responses

One of the most effective medications to date for controlling airway inflammation in asthma has been inhaled corticosteroids. Although corticosteroids have been used for this purpose for >25 years, the precise mechanisms by which they improve lung function in asthma remain unclear. Most anti-inflammatory effects of corticosteroids are mediated *via* the glucocorticoid receptor (GR) α -isoform (GR α), which suppresses expression of inflammatory genes through mechanisms known as transactivation or transrepression.³¹ As a result of alternative splicing mechanisms, another GR isoform, *viz.*, GR β , has been described.³² Current evidence suggests that chemokine and cytokine secretions induced by inflammatory mediators are inhibited by dexamethasone in human ASM cells. Cytokine-induced secretions of RANTES,^{33–35} monocyte chemoattractant protein,³⁵ eotaxin,³⁶ granulocyte macrophage colony-stimulating factor,³⁷ and IL-6³⁸ are abrogated by corticosteroids. In most of these studies, corticosteroid and [cAMP]_i-mobilizing agents were shown to act additively to inhibit chemokine and cytokine secretion. Data suggest that the effects of dexamethasone on RANTES expression are mediated through inhibition of the transcription factor activator protein 1.³⁹ Nuclear factor κ B (NF- κ B) is another transcription factor that mediates chronic inflammatory responses in asthma, as well as in rheumatoid arthritis, psoriasis, and inflammatory bowel disease.⁴⁰ Notably, dexamethasone has been found to have no effect on TNF- α -induced or IL-1 β -induced NF- κ B activation in human ASM cells.⁴¹ Furthermore, cytokine-induced intracellular adhesion molecule (ICAM) 1 expression in ASM cells, which is completely dependent on NF- κ B activation, has been shown to be unaffected by dexamethasone, with IL-6 secretion affected only partially.³⁹ In contrast, IL-1 β -induced cyclooxygenase 2 expression has been found to be abrogated.^{41–43} Collectively, these studies support the notion that anti-inflammatory effects of corticosteroids in asthma may not be caused by solely modulation of cytokine-induced NF- κ B activation and are probably regulated by pathways such as activator protein 1 or other transcriptional factors.

Multiple Cytokine Stimulation on ASM Modulates Corticosteroid Responsiveness

We and others recently showed that treatment of ASM cells with a combination of IFNs with TNF- α impairs the ability of corticosteroids to inhibit the expression of various proinflammatory genes such as CD38, RANTES, and ICAM-1 by a mechanism involving the up-regulation of GR β isoform.⁴⁴ Interestingly, corticosteroids augment IFN- γ /TNF- α -induced fractalkine and toll-like receptor 2 expression in ASM^{45,46};

whether this involves similar mechanisms involved in the attenuation of corticosteroid activity by IFN- γ /TNF- α remains to be established. Although the pathological role of the GR β isoform is not well understood, previous reports indicate a strong correlation between corticosteroid resistance in individuals with asthma and the expression levels of GR β .⁴⁷ More importantly, increased GR β in the airways has been detected in patients who died of asthma.⁴⁸ Indeed, by its ability to act as a dominant-negative inhibitor of steroid action in other cell types,⁴⁹ GR β has been associated with corticosteroid resistance in different inflammatory diseases,⁵⁰ GR β overexpression in ASM cells also prevents the capacity for corticosteroids to (1) induce transactivation activity and (2) inhibit cytokine-induced proinflammatory gene expression.⁴⁴

Short-term treatment of ASM cells with IFNs and TNF- α inhibits, in a GR β -independent manner, the capacity for corticosteroids to induce transactivation partially through the cellular accumulation of interferon regulatory factor-1 (IRF-1).⁵¹ IRF-1 is an early response gene involved in diverse transcriptional regulatory processes,⁵² and a strong association was found between IRF-1 polymorphism and childhood atopic asthma.⁵³ Early corticosteroid insensitivity seen after short incubation with IFNs and TNF- α could be reproduced by enhancing IRF-1 cellular levels using constitutively active IRF-1, which dose-dependently inhibited glucocorticoid response element-dependent gene transcription.⁵¹ Consistently, reducing IRF-1 cellular levels using siRNA approach in TNF/IFN-treated ASM cells significantly restored corticosteroid transactivation activities. These findings show for the first time that IRF-1 is a novel alternative GR β -independent mechanism mediating corticosteroid insensitivity induced by TNF- α and IFN. The fact that different studies showed that the expression of IRF-1 was increased after viral infections,⁵⁴ combined with the suppressive effect of IRF-1 on corticosteroid signaling in ASM cells,⁵¹ may, in part, explain the corticosteroid insensitivity seen in patients with asthma after viral exacerbations.⁵⁵

CONCLUSION

Asthma, an extraordinarily common lung disease, is characterized by airway inflammation and reversible airflow obstruction. In some patients, a loss of lung function persists for years while in others, there is a progressive loss of lung function. Despite the clinical identification of patients who exhibit asthma persistence and progression, the cellular and molecular mechanisms that induce these phenotypes remain unclear. Recent *ex vivo* and *in vitro* systems using physiologically relevant structural cells may be important models for the identification of biomarkers and thera-

peutic approaches to prevent irreversible airflow obstruction. Evidence now suggests that combinations of cytokines with TNF- α and IFN may induce a corticosteroid insensitivity in structural cells that may have important therapeutic consequences for asthma progression and persistence.

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