

Molecular mechanisms in allergy and clinical immunology

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Airway smooth muscle: A modulator of airway remodeling in asthma

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Asthma is a disease characterized, in part, by airway hyperresponsiveness and inflammation. Although asthma typically induces reversible airway obstruction, in some patients with asthma, airflow obstruction can become irreversible. Such obstruction might be a consequence of persistent structural changes in the airway wall caused by the frequent stimulation of airway smooth muscle (ASM) by contractile agonists, inflammatory mediators, and growth factors. Traditional concepts concerning airway inflammation have focused on trafficking leukocytes and on the effects of inflammatory mediators, cytokines, and chemokines secreted by these cells. Recent studies suggest that ASM cells might modulate airway remodeling by secreting cytokines, growth factors, or matrix proteins and by expressing cell adhesion molecules and other potential costimulatory molecules. These ASM cell functions might directly or indirectly modulate submucosal airway inflammation and promote airway remodeling. (*J Allergy Clin Immunol* 2005;116:488-95.)

Key words: Asthma, signal transduction, smooth muscle, proliferation, synthetic function

Chronic severe asthma is characterized by inflammation of the airways and growth of airway smooth muscle (ASM) cells. In patients with asthma, cytokines and growth factors promote myocyte proliferation and deposition of extracellular matrix (ECM) that, in part, constitute the process of airway remodeling. Pathologically,

Abbreviations used

ADAM: A disintegrinase and metalloproteinase
ASM: Airway smooth muscle
ECM: Extracellular matrix
ERK: Extracellular signal-regulated kinase
GPCR: G protein-coupled receptor
LTD₄: Leukotriene D₄
MCP: Monocyte chemotactic protein
MMP: Matrix metalloproteinase
PDGF: Platelet-derived growth factor
PI3K: Phosphatidylinositol 3-kinase
RTK: Receptor tyrosine kinase
STAT: Signal transducer and activator of transcription
VEGF: Vascular endothelial growth factor

angiogenesis and microvascular remodeling are prominent features of chronic severe asthma, changes that are likely mediated by multiple factors. We will discuss the potential for ASM to influence the remodeling process through its ability to synthesize and secrete inflammatory mediators and ECM, as well as express adhesion molecules and other immunomodulatory proteins.

INCREASED SMOOTH MUSCLE MASS

Myocyte hyperplasia

ASM mitogens act through different receptor-operated mechanisms. Although growth factors induce ASM cell mitogenesis by activating receptors with intrinsic protein receptor tyrosine kinase (RTK) activity, contractile agonists released from inflammatory cells mediate their effects through activation of G protein-coupled receptors (GPCRs). Importantly, synergy can occur between RTK and GPCRs to promote human ASM mitogenesis.¹

The mechanisms regulating smooth muscle proliferation have been the subject of numerous reviews.² Phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase (ERK) activation appear to be the dominant signal transduction pathways for RTK-, GPCR-, or cytokine-stimulated growth of ASM cells (Fig 1). PI3K phosphorylates membrane phosphoinositides, which function as second messengers and activate downstream

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effector molecules to regulate cell-cycle protein expression and thus modulate cell-cycle traversal.³ Activation of PI3K is critical for ASM cell-cycle progression,⁴⁻⁶ although other signaling events are also necessary to promote maximal ASM growth responses.⁷ For example, inhibition of ERK activity attenuates mitogen-induced DNA synthesis in ASM cells, suggesting that activation of ERK is also required for proliferation.^{8,9}

D-type cyclins (cyclins D1, D2, and D3) are key regulators of G₁ progression in mammalian cells, and consequently, cyclin D1 has been the most widely studied cyclin in ASM biology. In human ASM corticosteroids reduce mitogen-stimulated increases in cyclin D1 protein.¹⁰ Corticosteroid binding to the glucocorticoid receptor results in activation of the transcription factor C/EBP α and subsequent increased expression of the cyclin-dependent kinase inhibitor p21^{WAF/Cip1}, thus providing a potential mechanism for the antiproliferative effect of glucocorticoids on ASM cell growth.¹¹⁻¹³ A recent study suggests that ASM cells derived from patients with asthma lack expression of C/EBP α and are therefore resistant to the effects of glucocorticoids on proliferation.¹⁴ Although cyclin D1 expression might be necessary for cell growth, it is not sufficient. For example, IFN- γ inhibits ASM growth without modulating growth factor-induced upregulation of cyclin D1.¹⁵

Reactive oxygen species might also regulate ASM function. ASM cells express several, but not all, components of the phagocyte nicotinamide adenine dinucleotide phosphate oxidase, and reactive oxygen species can modulate mitogen-induced ASM cell proliferation.¹⁶⁻¹⁹ Additionally, signal transducer and activator of transcription (STAT) 3 has been shown to play an important role in platelet-derived growth factor (PDGF)-induced proliferation of human ASM cells. Activation of JAK2 and STAT3 by PDGF appears to be redox dependent and affects the proliferative response to mitogen independent of ERK activation.²⁰ Interestingly, IFN- γ and IFN- β activate STAT1/2, JAK1, and Tyk2 in ASM; these cytokines, however, are potent inhibitors of mitogen-induced proliferation, in part through upregulation of the interferon-inducible gene *IFI 16*.^{15,21}

Myocyte hypertrophy

There is renewed attention to the role of increased myocyte size or hypertrophy as a mechanism of increased ASM cell mass in asthma. Some mediators, such as IL-1 β , IL-6, TGF- β , angiotensin II, and cardiotrophin I, induce cellular hypertrophy *in vitro*, although the mechanisms remain unclear.²²⁻²⁴ In a primate model of asthma, the airways of monkeys sensitized to house dust mite antigen display an increase in smooth muscle mass, as well as smooth muscle bundle size.²⁵ Morphologic studies confirm the presence of ASM cell hypertrophy in some, but not all, asthmatic patients.²⁶ Increased cell size appears to negatively correlate with postbronchodilator FEV₁ and distinguishes between severe persistent asthma and milder disease.²⁷ In contrast, Woodruff et al²⁸ obtained bronchial biopsy specimens from patients with mild-to-moderate

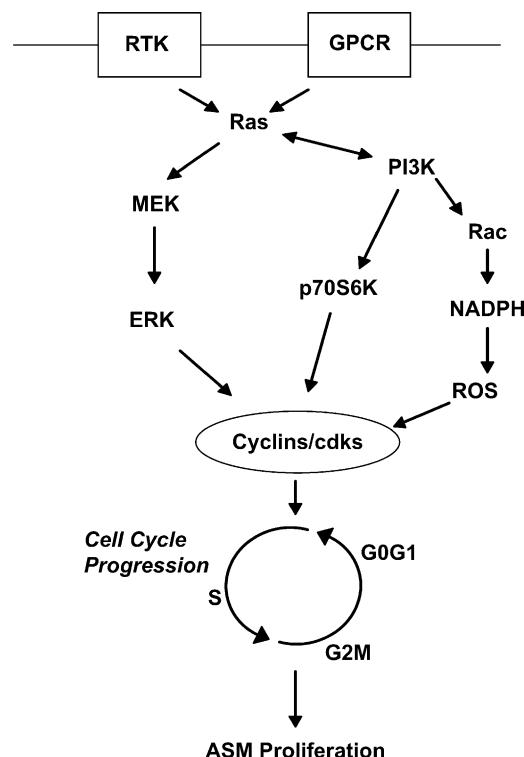


FIG 1. Overview of ASM cell mitogenesis. In this simplified diagram of the intracellular pathways regulating ASM cell mitogenesis, Ras serves as a point of convergence for growth factor RTK and GPCR signaling. Ras activates ERK and PI3K, leading to activation of cyclins and cyclin-dependent kinases (CDKs). Activation of cyclin D1 ultimately leads to cell-cycle progression and cell proliferation. MEK, mitogen-activated protein kinase/ERK; NADPH, nicotinamide adenine dinucleotide phosphate; p70S6K, Ribosomal S6 kinase; ROS, reactive oxygen species.

asthma and studied ASM cell gene expression and cell morphology. These investigators found no evidence for ASM cell hypertrophy, although they did confirm the presence of ASM hyperplasia. More work is needed in this particular area of ASM cell biology.

Smooth muscle migration

Hyperplasia and hypertrophy are important processes regulating increased smooth muscle mass in asthma. Analogous to vascular smooth muscle migration in atherosclerosis, however, ASM cell migration also likely promotes airway remodeling in chronic asthma. Evidence suggests that proliferating smooth muscle cells migrate along chemotactic gradients. During chronic inflammation, myocyte migration would be promoted by exposure of cells to a variety of cytokines and growth factors, as well as to an altered ECM.

Structurally, myofibroblasts display a phenotype intermediate between fibroblasts and smooth muscle cells, express α -smooth muscle actin, and have the ability to secrete ECM proteins. In addition, myofibroblasts secrete chemokines and prolong eosinophil survival.²⁹ Although myofibroblasts are found in the lamina reticularis, the

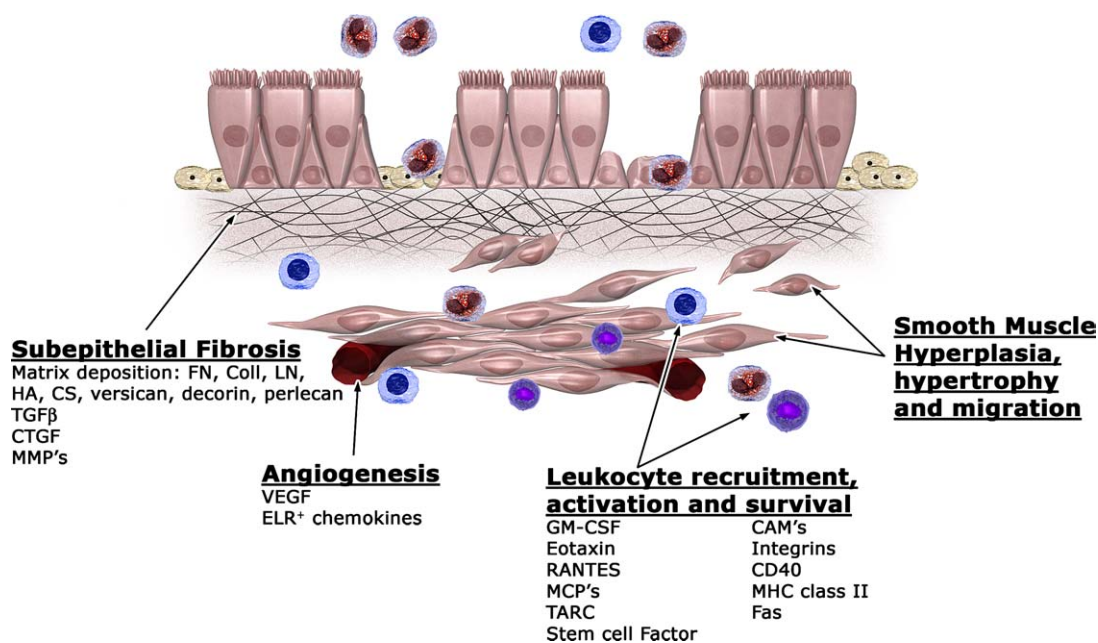


FIG 2. ASM-derived factors that promote airway remodeling, including matrix deposition, bronchial neo-vascularization, and leukocyte recruitment. Alterations in the ECM also affect ASM cell proliferation and migration. *FN*, Fibronectin; *Coll*, collagen; *LN*, laminin; *HA*, hyaluronan; *CS*, chondroitin sulfate; *CTGF*, connective tissue growth factor; *TARC*, thymus and activation-regulated chemokine; *CAM*, cell adhesion molecule.

origin of these cells remains unknown. Possibly the cells exist in small numbers within the lamina reticularis and proliferate locally after stimulation with inflammatory mediators or growth factors. Alternately, smooth muscle cells, under the influence of similar stimuli, migrate from the periphery of the smooth muscle bundle into the sub-mucosa. A final intriguing possibility suggests that cells are recruited from a circulating pool of fibrocytes, as has recently been described.³⁰ In this study CD34 $^{+}$ cells expressing procollagen I and α -smooth muscle actin were increased in the bronchial mucosa after allergen challenge in patients with asthma. Using a murine model of chronic allergic airway inflammation, the investigators demonstrated that these cells were recruited from a circulating population of CD34 $^{+}$ fibrocytes.³⁰

The potential for mesenchymal cells, such as myofibroblasts, to migrate into the airway wall is well established in patients with asthma.^{31,32} Interestingly, bronchoalveolar lavage-derived fibroblasts from patients with mild asthma have enhanced migratory capacity compared with tissue fibroblasts isolated from bronchial biopsy specimens.³³ *In vitro*, many studies support the capacity of ASM cells to migrate. Increased chemotaxis of ASM cells has been demonstrated to PDGF, TGF- β , basic fibroblast growth factor, and IL-1 β . Urokinase has been shown to increase chemotaxis in some, but not all, studies.^{34,35} Cysteinyl leukotrienes alone promote chemokinesis only; however, in combination with PDGF, increased chemotaxis is observed over that seen with PDGF alone.³⁶ Interestingly, vascular endothelial growth factor (VEGF), a potent inducer of vascular

smooth muscle migration, has no effect on ASM cell chemotaxis.^{37,38} Similarly, not all mitogens are promigratory; recent data demonstrate that only PDGF, and not epidermal growth factor or thrombin, induces migration in ASM cells.³⁹ The ECM also regulates the migratory phenotype, but its role in ASM migration has not been extensively characterized. Parameswaran et al⁴⁰ recently demonstrated that ASM cells migrate more on collagen III and V and fibronectin compared with on collagen I, elastin, or laminin. Corticosteroids have been shown to inhibit ASM cell migration *in vitro*. Phosphodiesterase 4 (PDE4) inhibitors and β_2 -agonists have a more modest effect, but this can be enhanced by low concentrations of corticosteroids.³⁷

INFLAMMATORY CELL INFILTRATE

Many chemokines, which act to recruit and activate leukocytes, are found in the bronchoalveolar lavage fluid and lung tissue of patients with asthma. ASM cells have been shown to secrete RANTES, eotaxin, IL-8, monocyte chemotactic protein (MCP) 1, MCP-2, MCP-3, thymus and activation-regulated chemokine, and GM-CSF in response to TNF- α and IL-1 β (Fig 2).⁴¹ Interestingly, polymorphisms in the IL-4 receptor on ASM might influence chemokine release.⁴² ASM cells might play a role in promoting both the recruitment and survival of eosinophils through secretion of GM-CSF and IL-5.⁴³⁻⁴⁵ ASM infiltration by mast cells also has been demonstrated in patients with asthma.⁴⁶ In addition to eotaxin, ASM

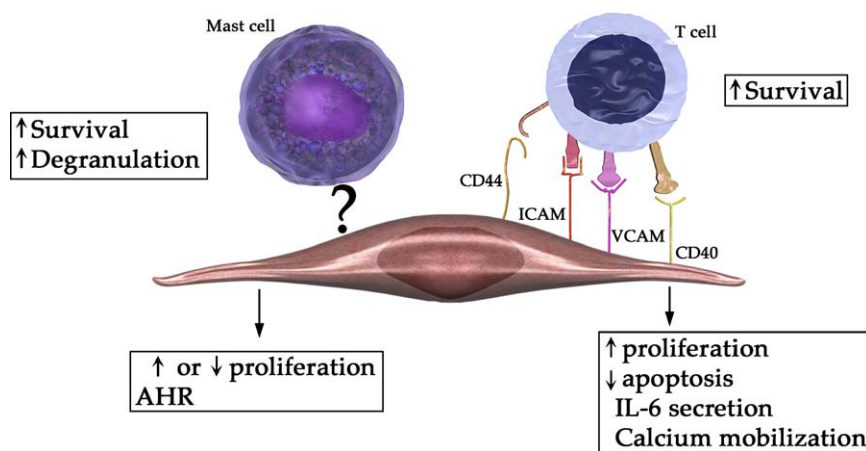


FIG 3. Cross-talk between ASM and inflammatory cells in asthma. T-cell adhesion through integrins and CD44 enhances ASM cell proliferation, agonist-induced calcium mobilization, and cytokine secretion. Mast cell-derived products enhance airway hyperresponsiveness (AHR) and modulate ASM proliferation. In turn, adhesion to ASM promotes T-cell and mast cell survival, as well as mast cell degranulation. The ? symbol highlights that the receptors mediating mast cell adhesion to ASM have not yet been defined.

cells secrete stem cell factor, which acts to recruit and retain mast cells within the smooth muscle layer.⁴⁷

In addition to secreting chemokines that recruit leukocytes into the airways, ASM might promote leukocyte retention and activation through the expression of cell adhesion molecules. ASM cells express intercellular adhesion molecule 1 and VCAM-1, which are inducible by inflammatory mediators and constitutively express CD44, the hyaluronan receptor.⁴⁸ ASM cells also express variable levels of integrin subunits, with the α_v , α_5 , and β_1 subunits predominating.⁴⁹ The ability of ASM-associated cell adhesion molecules to directly activate T cells remains controversial. Although ASM cells express MHC class II and CD40 after stimulation with IFN- γ ^{50,51} and coculture of T cells with ASM appears to upregulate T-cell expression of CD25,⁵² the physiologic relevance of these findings remains unknown because ASM cells are unable to present antigen to CD4 T cells.^{50,53} Recent data suggest, however, that coculture with ASM decreases apoptosis of activated T cells.⁵³ Similarly, coculture of mast cells and ASM enhances mast cell degranulation.⁵⁴ This effect requires cell-cell contact, although the receptors mediating mast cell adhesion to ASM have not yet been identified.⁵⁵

In contrast, coculture of activated T cells and ASM clearly induces smooth muscle cell DNA synthesis.^{48,53} Adhesion of activated T cells also appears to alter smooth muscle cell contractility,⁵² potentially because of the autocrine secretion of IL-5 and IL-1 β .⁴⁵ Others have shown that ASM cells cultured with activated T cells show increased calcium mobilization responses to leukotriene D₄ (LTD₄) and serotonin,⁵⁶ whereas release of lipid mediators and enzymes by activated mast cells likely promotes airway hyperresponsiveness and either inhibits (chymase) or promotes (tryptase) ASM cell proliferation.⁵⁷⁻⁵⁹ These studies highlight the finding that direct interactions between leukocytes and smooth muscle cells contribute to the modulation of the local milieu, resulting

in smooth muscle cell activation and growth and possibly leukocyte activation and survival (Fig 3).

SUBEPITHELIAL FIBROSIS

ECM proteins are critical for maintaining the structure and function of the airways. ASM cells, through production of ECM components, as well as matrix-modifying enzymes, might contribute to airway remodeling. Alterations in the ECM, in turn, modify the growth and synthetic function of ASM cells. The composition of the ECM is tightly controlled and involves a dynamic process of matrix deposition and degradation. In inflammatory processes, such as asthma, this balance is disturbed, resulting not only in an abnormal amount of matrix deposition but also in an altered composition of matrix components.

ASM cells secrete a wide variety of matrix proteins, including fibronectin, collagen, hyaluronan, laminin, and versican (Fig 2). TGF- β is secreted from ASM cells and, in turn, induces smooth muscle cell synthesis of hyaluronan and collagen.^{23,60,61} The mechanism of this effect likely involves, in part, induction of connective tissue growth factor by TGF- β .⁶² Recent studies have demonstrated that ASM cells also secrete VEGF, a potent endothelial mitogen that increases fibronectin expression by ASM.^{38,63} Finally, LTD₄ and epidermal growth factor increase expression of versican and fibronectin in ASM cells.⁶⁴

In asthma there is an increase in hyaluronan, fibronectin, tenascin, versican, laminin, and collagen types I, III, and V.⁴¹ Recently, human ASM cells were shown to secrete ECM proteins in response to asthmatic sera,⁶⁵ identifying ASM as a cellular source for ECM deposition in airways and implicating a novel mechanism by which ASM cells might modulate autocrine proliferative responses. However, treatment of cells with corticosteroids

TABLE I. Key concepts

1. ASM is the primary regulator of bronchomotor tone in asthma.
2. ASM cells actively synthesize proinflammatory mediators and ECM proteins that might promote airway remodeling and loss of lung function in asthma.
3. The function of genes, such as ADAM-33, in the pathogenesis of asthma has not yet been determined.
4. Genetic polymorphisms, as in the IL-4 receptor, might influence ASM cell synthetic function.
5. ASM cells derived from patients with asthma appear to differ in their synthetic function and response to the inhibitory effects of glucocorticoids.

TABLE II. Therapeutic implications

1. Corticosteroids effectively inhibit ASM cell proliferation, migration, and inflammatory mediator production but are less effective at decreasing synthesis of matrix proteins.
2. Alterations in the ECM seen in asthma might decrease smooth muscle responsiveness to inhaled β_2 -agonists and glucocorticoids.

has no effect on the production of matrix proteins by ASM cells.⁶⁵ In addition, ASM cells derived from asthmatic, but not healthy, subjects secrete significantly more perlecan, collagen I, and chondroitin sulfate and less laminin $\alpha 1$ and collagen IV compared with nonasthmatic ASM cells.⁶⁶

The increased matrix deposition seen in asthma is likely caused by increased secretion by mesenchymal cells, as well as an imbalance between matrix-degrading enzymes and inhibitors of these proteases. One class of proteins that has been intensively studied is the matrix metalloproteinase (MMP) family. MMP-1 expression is increased in the smooth muscle cells of patients with asthma, and studies have shown that LTD₄ increases expression of MMP-1, which acts to degrade insulin-like growth factor binding protein, a growth inhibitor.⁶⁷ Progelatinase A (MMP-2) is constitutively released by ASM cells but remains inactive because of high levels of tissue inhibitor of metalloproteinases 2 on the cell membrane,⁶⁸ although thrombin has been shown to activate MMP-2.⁶⁹ In contrast, tissue inhibitor of metalloproteinases 1 is secreted in large amounts into the conditioned media of ASM cells.⁶⁸ Membrane type 1 MMP is also found on ASM^{68,69}; this proteinase can activate MMP-2 and has been shown to cleave CD44 from the cell surface and promote cell migration.⁷⁰ ASM cells also express pro-MMP-3 and active MMP-3.⁶⁹ TNF- α induces the release of MMP-9, which can degrade matrix but also plays a critical role in cleaving latent TGF- β to its active form.⁷¹ The a disintegrinase and metalloproteinase (ADAM) family is a subfamily of metalloproteinases located on the cell surface. Polymorphisms in the ADAM-33 gene have been associated with airway hyperresponsiveness and asthma.⁷² ADAM-33 is expressed by ASM cells and lung fibroblasts,⁷² although there does not appear to be a significant difference in ADAM-33 protein in subjects with asthma compared with control subjects.⁷³

The composition of the ECM also influences ASM cell function. For example, PDGF-induced proliferation is enhanced by monomeric collagen I, fibronectin, and vitronectin, whereas other ECM components, such as fibrillar collagen I, collagen III, and tenascin-C, have no effect.^{74,75} Others have shown that ECM proteins secreted

by asthmatic ASM differ from nonasthmatic ASM. Cells plated on the ECM derived from asthmatic ASM displayed greater rates of proliferation; this is true for both nonasthmatic and asthmatic ASM cells.⁶⁶ In addition to altering ASM cell proliferation, ECM composition also affects ASM cell survival. Fibronectin, laminin, and collagens I and IV have been identified as important antiapoptotic elements, and elastin promotes ASM apoptosis.⁴⁹ Finally, in a study examining the effect of ECM composition on β_2 -adrenoceptor signaling in human ASM cells, the investigators demonstrated that fibronectin increased, whereas collagen V and laminin decreased, accumulation of the second messenger cyclic AMP when compared with collagens I or IV.⁷⁶ This suggests that altered ECM might affect responses to bronchodilator therapy in patients with asthma.

BRONCHIAL NEOVASCULARIZATION

Angiogenesis and microvascular remodeling are features of chronic severe asthma. These changes are likely mediated by multiple factors, and new clinical data support an important role for VEGF as a mediator of airway remodeling. Bronchial biopsy specimens taken from subjects with asthma reveal an increase in airway vascularity and expression of VEGF and VEGF receptors in the bronchial mucosa,⁷⁷ and increased levels of VEGF are found in the induced sputum of patients with asthma.⁷⁸ VEGF also increases vascular permeability,⁷⁹ thereby contributing to airway wall edema in asthma. Indeed, the number of cells expressing VEGF correlates with measurements of airway hyperreactivity,⁷⁷ and VEGF receptor inhibitors block both airway inflammation and airway hyperresponsiveness in an animal model of asthma.⁸⁰ More recently, lung-specific overexpression of VEGF has been shown to enhance T_H2 inflammation, myocyte hyperplasia, and airway hyperresponsiveness.⁸¹ Several cytokines or growth factors, such as TGF- β , IL-1 β , and PDGF, induce VEGF protein expression in ASM.³⁸ Recent reports demonstrate that prostaglandin E₂, bradykinin, and T_H2-type cytokines also increase smooth muscle cell expression of VEGF.^{63,82}

Members of the CXC family of chemokines also play a role in angiogenesis. These molecules have 4 highly conserved cysteine residues; the first and second cysteines are separated by a variable amino acid, thus the name CXC. Another conserved 3-amino-acid motif (Glu-Leu-Arg; "ELR" motif; ELR⁺), which immediately precedes the first cysteine, distinguishes those CXC members with angiogenic capability.⁸³ ASM cells secrete several CXC ELR⁺ chemokines, including CXCL8 (IL-8), CXCL5 (ENA-78), and CXCL1, CXCL2, and CXCL3 (GRO- α , GRO- β , and GRO- γ , respectively).^{84,85} Interestingly, ASM cells also secrete angiostatic ELR⁻ chemokines, such as CXCL10 (IP-10) and, to a lesser extent, Mig (CXCL9) and I-TAC (CXCL11).⁸⁶

CONCLUSIONS

ASM, the most important tissue regulating bronchomotor tone, plays a pivotal role in promoting and mediating airway remodeling (Table I). The physiologic consequences of airway remodeling, however, remain unclear. Undoubtedly, ASM *in vitro* could promote airway inflammation through the secretion of cytokines and chemokines and profoundly alter its phenotype in response to growth factors. The fundamental mechanisms that promote ASM cell growth, migration, and secretion of chemokines and cytokines differ from those of other cell types and might have therapeutic implications (Table II). Therefore the specificity of these responses might serve to aid in the identification of unique targets for therapeutic intervention. By far, the evidence supporting ASM *in vitro* as an immune modulatory cell must carry forward *in vivo* before the relevance of such effects are realized.

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