

Synthetic responses in airway smooth muscle

Peter H. Howarth, DM,^a Alan J. Knox, MD,^b Yassine Amrani, PhD,^c Omar Tliba, PhD,^c
Reynold A. Panettieri, Jr, MD,^c and Malcolm Johnson, PhD^d *Southampton, Nottingham, and
Greenford, Middlesex, United Kingdom, and Philadelphia, Pa*

Human airway smooth muscle (ASM) has several properties and functions that contribute to asthma pathogenesis, and increasing attention is being paid to its synthetic capabilities. ASM can promote the formation of the interstitial extracellular matrix, and in this respect, ASM from asthmatic subjects compared with normal subjects responds differently, both qualitatively and quantitatively. Thus, ASM cells are important regulating cells that potentially contribute to the known alterations within the extracellular matrix in asthma. In addition, through integrin-directed signaling, extracellular matrix components can alter the proliferative, survival, and cytoskeletal synthetic function of ASM cells. ASM also functions as a rich source of biologically active chemokines and cytokines that are capable of perpetuating airway inflammation in asthma and chronic obstructive pulmonary disease by promoting recruitment, activation, and trafficking of inflammatory cells in the airway milieu. Emerging evidence shows that airway remodeling may also be a result of the autocrine action of secreted inflammatory mediators, including T_H2 cytokines, growth factors, and COX-2–dependent prostanooids. Finally, ASM cells contain both β_2 -adrenergic receptors and glucocorticoid receptors and may represent a key target for β_2 -adrenergic receptor agonist/corticosteroid interactions. Combinations of long-acting β_2 -agonists and corticosteroids appear to have additive and/or synergistic effects in inhibiting inflammatory mediator release and the

migration and proliferation of ASM cells. (*J Allergy Clin Immunol* 2004;114:S32-50.)

Key words: Asthma, chronic obstructive pulmonary disease, airway smooth muscle, synthetic function, inflammation, airway remodeling, interstitial extracellular matrix, β_2 -adrenergic receptor agonists, corticosteroids

The traditional view of human airway smooth muscle (ASM) in asthma is that it is a passive partner in airway inflammation, contracting in response to proinflammatory mediators and neurotransmitters and relaxing in response to endogenous and exogenous bronchodilators.¹ Therefore, early studies of ASM function in asthma tended to concentrate on the contractile aspects to explain airway hyperresponsiveness, the characteristic of asthma in which the airways show an exaggerated response to a large number of different stimuli acting through disparate pathways.^{1,2} It has become apparent that this paradigm was a rather simplistic view and that ASM has several other important properties of relevance to obstructive lung diseases.

As techniques for reliable culture of ASM have been developed, studies have shown that ASM can express a synthetic function. This article presents evidence that ASM contributes to the formation of the interstitial extracellular matrix and that, in turn, components of the extracellular matrix influence ASM function and pharmacologic responsiveness. Also discussed is the role of ASM as a biological factory, serving as a rich source of many cytokines, chemokines, bronchoprotective factors, adhesion molecules, and growth factors that may modulate chronic airway inflammation and remodeling in asthma³ (Fig 1). The cytokines, often produced in large quantities, are capable of recruiting several inflammatory cells into the airway milieu. This article shows that, in addition, cytokines may exert direct paracrine or autocrine effects by inducing expression of inflammatory genes in airway cells, including ASM cells.

Airway smooth muscle cells contain both β_2 -adrenergic receptors (β_2 ARs) and glucocorticoid receptors (GRs) and are a potential target for interactions between β_2 AR agonists and corticosteroids. Individually, each of these 2 classes of drugs inhibits inflammatory mediator release from ASM cells, suppressing expression of cytokines, chemokines, growth factors, and adhesion molecules. Recent findings reviewed here suggest that combinations of long-acting β_2 -agonists (LABAs) and corticosteroids have additive and/or synergistic effects in inhibiting inflammatory mediator release as well as in inhibiting the migration and proliferation of ASM cells.

From ^aRespiratory Cell and Molecular Biology, Southampton General Hospital; ^bthe Division of Respiratory Medicine, City Hospital, Nottingham; ^cthe Pulmonary, Allergy and Critical Care Division, University of Pennsylvania Medical Center; and ^dGlaxoSmithKline Research and Development, Greenford, Middlesex.

Disclosure of potential conflict of interest: A. J. Knox has consultant arrangements with GlaxoSmithKline and Astra Zeneca; he receives grants/research support from GlaxoSmithKline. Y. Amrani receives grants/research support from NIH and Centocor; he is employed by the University of Pennsylvania. R. A. Panettieri has consultant arrangements with Merck, GlaxoSmithKline, Schering, and Epigenesis; he receives grants/research support from Merck, GlaxoSmithKline, and Centocor; is employed by the University of Pennsylvania; and is on the Speakers' Bureau of Merck, GlaxoSmithKline, Schering, and Epigenesis. M. Johnson is employed by GlaxoSmithKline Research and Development. P. H. Howarth and O. Tliba have no conflict of interest to disclose.

Supported by the Medical Research Council, the Wellcome Trust, the National Asthma Campaign, and Glaxo Smith Kline (Dr Knox); National Institutes of Health grants 2R01-HL55301 and 1P50-HL67663 (Dr Panettieri); and American Lung Association grant RG-062-N (Dr Amrani). Dr Amrani is a Parker B. Francis Fellow in Pulmonary Research.

Received for publication April 16, 2004; revised April 20, 2004; accepted for publication April 20, 2004.

Reprint requests: Peter H. Howarth, DM, Respiratory Cell and Molecular Biology, Mailpoint 810, Level D, Centre Block, Southampton General Hospital, Tremona Road, Southampton, Hampshire, SO16 6YD, United Kingdom. E-mail: phh1@soton.ac.uk.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2004.04.041

Abbreviations used

AP-1: Activator protein 1
ASM: Airway smooth muscle
β_2 AR: β_2 -Adrenergic receptor
cAMP: Cyclic adenosine monophosphate
C/EBP: CCAAT/enhancer-binding protein
COPD: Chronic obstructive pulmonary disease
CRE: Cyclic adenosine monophosphate–responsive element
CTGF: Connective tissue growth factor
ECM: Interstitial extracellular matrix
EGF: Epidermal growth factor
ERK: Extracellular signal-regulated kinase
FP: Fluticasone propionate
GPCR: G protein–coupled receptor
GR: Glucocorticoid receptor
GRE: Glucocorticoid response element
IC ₅₀ : Median inhibitory concentration
ICAM-1: Intercellular adhesion molecule 1
ICS: Inhaled corticosteroid
JAK: Janus-activated kinase
JNK: c-Jun NH ₂ -terminal kinase
LABA: Long-acting β_2 -adrenergic receptor agonist
LTD ₄ : Leukotriene D ₄
MAPK: Mitogen-activated protein kinase
MCP: Monocyte chemoattractant protein
MMP: Matrix metalloproteinase
NF- κ B: Nuclear factor κ B
PDGF: Platelet-derived growth factor
PGE ₂ : Prostaglandin E ₂
PKA: Protein kinase A
STAT: Signal transducer and activator of transcription
TARC: Thymus- and activation-regulated chemokine
TIMP: Tissue inhibitor of metalloproteinases
TNFR: TNF receptor homologue
VEGF: Vascular endothelial growth factor

EXTRACELLULAR MATRIX AND ASM IN ASTHMA

The term *extracellular matrix* encompasses all that is not cellular within tissue and includes both basement membrane connective tissue and interstitial connective tissue (Fig 2). The interstitial extracellular matrix (ECM) surrounds ASM, and there are bidirectional interactions: human ASM has synthetic capabilities of modulating ECM, and the composition of the ECM can influence ASM responses to stimulation, affecting its proliferative, migratory, and synthetic capabilities (Fig 3). Exposure of human ASM in culture to 10% serum from an asthmatic individual has been found to lead to greater generation of fibronectin, laminin γ , and the proteoglycans perlecan and chondroitin sulfate by these cells compared with exposure to 10% serum from a healthy individual.⁴ This synthetic response is not inhibited by corticosteroids. ASM was also found with serum stimulation to generate elastin, laminin β_1 , laminin β_2 , laminin γ_1 , thrombospondin, collagen I, collagen III, collagen IV, collagen V, and decorin.⁴ The factors in the serum responsible for this stimulation are

undefined, but because airway inflammation is associated with enhanced vascular permeability, it is probable that the plasma exudation delivers such stimuli to the tissue under these circumstances. The demonstration that human ASM is able to generate such a wide range of ECM proteins helps explain the observation that the interstitial deposition of collagens, elastin, laminin, hyaluron, versican, tenascin, and fibronectin is prominent around ASM cells.

TGF- β is a growth factor of relevance to these changes in that its known biological activities include stimulation of the synthesis of collagens I, collagen III, collagen V, fibronectin, tenascin, vitronectin, and several proteoglycans while downregulating synthesis of matrix metalloproteinase (MMP) and upregulating synthesis of tissue inhibitors of metalloproteinases (TIMP). Bovine ASM cells have been shown to release TGF- β_1 and TGF- β_2 in association with the generation of plasmin by these cells.⁵ At least in part, in this study, the release of TGF- β was a result of cleavage of TGF- β from its latency binding protein tethered to the ASM cell surface.⁵ This release was shown to be linked to synthesis of collagen I. Thus, the presence of plasmin within the serum may be one of the explanations for the stimulatory effect of serum on ECM synthesis by human ASM. *In vitro* studies with human ASM have shown that TGF- β (10 ng/mL) is able to upregulate gene expression for collagen I, collagen IV, fibronectin, elastin, and biglycan, while downregulating gene expression for decorin, a proteoglycan that regulates TGF- β activity.⁶ More recently, it has been shown that TGF- β regulates the expression of connective tissue growth factor (CTGF) gene and protein expression by human ASM⁷ and that this response is greater in ASM cells cultured from samples of the airways of asthmatic subjects than from those cultured from non-asthmatic airways. This difference amounted to approximately a 3-fold increased response at the mRNA level and an 18-fold increase in protein production for the same level of stimulation (TGF- β 1 ng/mL). No effect on CTGF was seen with stimulation of human ASM by platelet-derived growth factor (PDGF). CTGF has been shown in fibroblast culture to stimulate not only collagen synthesis but also other ECM proteins, such as fibronectin, and the induction of CTGF by TGF- β may be one of the mechanisms for ECM synthesis by human ASM.

Conflicting data exist on the ability of leukotriene D₄ (LTD₄) to stimulate ECM secretion by human ASM. One study reported that LTD₄ administered alone or in combination with TGF- β had no effect in either stimulating or augmenting gene expression for collagen I, collagen IV, fibronectin, elastin, or biglycan.⁶ LTD₄ did, however, augment the proliferative response to epidermal growth factor (EGF) and was able to induce a contractile response in these human cultured ASM cells. No gene expression for the proteoglycan versican was evident in ASM in this study, either at rest or after TGF- β stimulation. A more recent study investigated the effect of EGF in conjunction with LTD₄ on ECM synthesis, evaluating gene expression in human ASM for the proteoglycans versican, biglycan, decorin, and perlecan as well as gene expression for

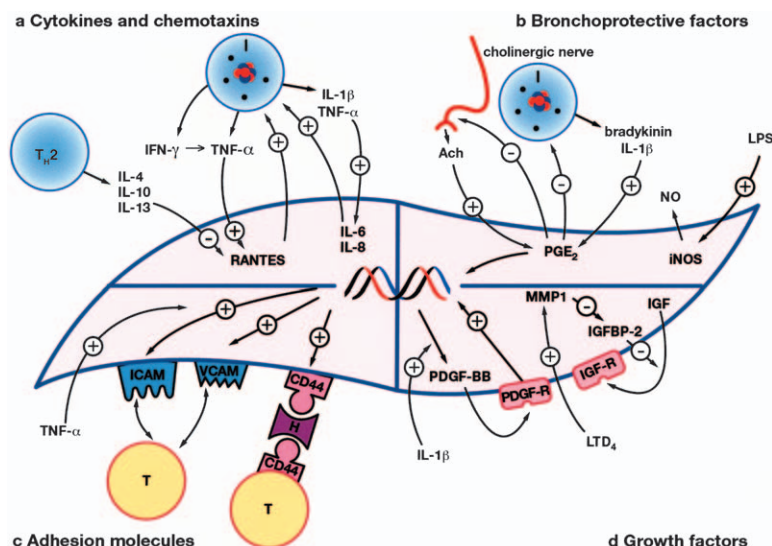


FIG 1. Human ASM produces cytokines, chemokines, inflammatory mediators, matrix-modifying enzymes, growth factors, and angiogenic factors. VCAM, Vascular cell adhesion molecule. Reprinted from *Trends in Pharmacological Sciences*, Vol 18, Johnson SR, Knox AJ, Synthetic Functions of Airway Smooth Muscle in Asthma, Pages 288-92, Copyright 1997, with permission from Elsevier.

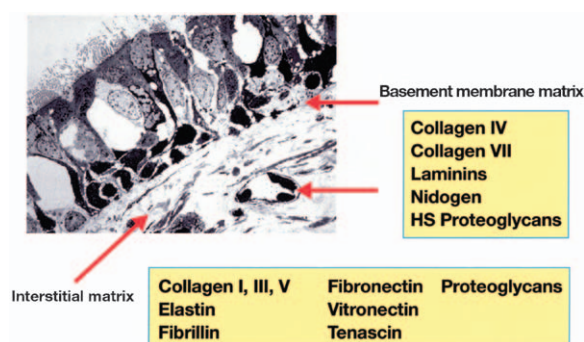


FIG 2. Extracellular matrix of the airways. HS, Heparan sulphate.

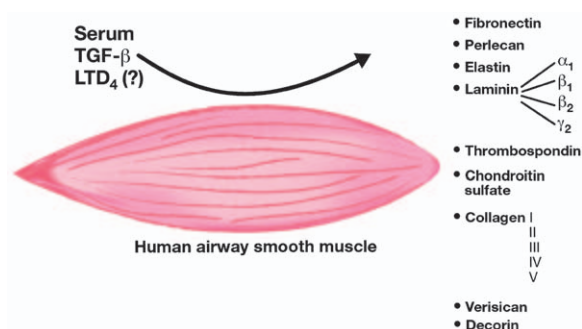


FIG 3. Synthetic capabilities of human ASM of relevance to the ECM.

collagen I and fibronectin.⁸ In ASM cells, decorin and collagen I transcripts accounted for the largest portion of the mRNA, with little or no versican mRNA evident in growth-associated ASM. Three days after EGF and LTD₄ stimulation, gene expression for versican V₀ increased 1.9-fold and for fibronectin increased 1.3-fold. No change was identified in versican V₁ splice variant, perlecan, or biglycan, and gene expression for decorin and collagen I decreased.⁸ Analysis of protein synthesis showed that EGF and LTD₄ preferentially increased production of V₀ relative to V₁ and that this alteration in ratio was partially prevented by montelukast pretreatment. This effect of montelukast is not dissimilar to the finding that the augmentation of EGF-induced ASM proliferation by LTD₄ is only partially inhibited by a cysteinyl leukotriene 1 receptor antagonist, suggestive of the additional involvement of a leukotriene receptor distinct from that associated with LTD₄-induced bronchoconstriction.⁶ No experiments with EGF alone or LTD₄ alone were reported,

so the relative importance of the LTD₄ stimulus to these responses is difficult to evaluate fully.

In addition to promoting ECM deposition, ASM may be capable of affecting its degradation. Human ASM cells have been shown to release progelatinase A (precursor for MMP-2) and, in association with TNF-α stimulation, to generate gelatinase B (MMP-9).⁹ Human umbilical vein endothelial cells can convert progelatinase A into its active form, a process that involves the precursor complexing with TIMP-2 and membrane-type MMP. The free membrane-type MMP that is available after TIMP-2 neutralization is free to effect this activation. However, this conversion does not seem to occur in cultured human ASM.⁹ The difference from that identified in human umbilical vein endothelial cell culture is considered to be caused by an excess of TIMP-2 on the ASM cell membrane, precluding any free membrane-type MMP activity. The autocrine production of MMP-2 by human ASM has also been identified as a regulatory step in the

ASM proliferative response.¹⁰ The local generation of TNF- α during airway inflammation in asthma, in particular from mast cells within human ASM,¹¹ would thus lead to an increase in MMP-9 from ASM. This may have differential effects in that although MMP-9 can degrade collagens IV, V, and X as well as laminin, MMP-9 has also been linked to the release of growth factors bound to proteoglycans within the ECM, such as fibroblast growth factor 2 and TGF- β , thereby allowing them to exert their bioactivity. In addition to its other activities, TGF- β is recognized to support attachment of ASM cells to the ECM, including fibronectin,¹² glycosaminoglycans,^{12,13} microfibrillar proteins,¹⁴ and collagens.^{15,16} This binding is integrin-dependent, involving $\alpha_7\beta_1$.¹³ The close association of ASM with these ECM components allows integrin-directed signaling from the ECM to ASM.

Influence of ECM on ASM function

The ECM surrounds cells within the airway wall, and integrin-directed signaling from the ECM components influences the responsiveness of the cells to differing stimuli.^{17,18} The influence of ECM components on human ASM proliferative and phenotypic status has been evaluated. On the basis that changes in the ECM composition in asthma favor increased deposition of collagen I and fibronectin, in contrast with a more prominent presence of laminin within the normal airway, Hirst et al¹⁹ evaluated the influence of these ECM components on the proliferative and phenotypic status of mitogen-stimulated cultured human ASM cells. They studied the ASM response to PDGF-BB and α -thrombin when the cells were cultured on plates precoated with 1 to 100 $\mu\text{g}/\text{mg}$ fibronectin, collagen I, or laminin and compared the findings with those from cells cultured either on undiluted matrigel (50 $\mu\text{g}/\text{cm}^2$) or directly onto plastic. These studies identified important differences relating to the differing culture environments. Both fibronectin and collagen I increased the mitogen-induced proliferative response in comparison with ASM grown on plastic, whereas both laminin and matrigel reduced the proliferative response. Immunohistochemical staining of these cells showed that the differing matrix components also influenced the organization of cytoskeletal contractile proteins. ASM grown for 5 days on plastic and unstimulated expressed smooth muscle myosin heavy chain, calponin, and smooth muscle α -actin myofilament immunoreactivity. Positive nuclear staining for Ki67, a proliferation index marker, was present in <5% of cells. After stimulation with PDGF-BB 5 $\mu\text{g}/\text{mL}$, Ki67 was expressed by >60% of cells, whereas the extent of immunoreactivity for the contractile proteins was significantly reduced. This reduction in contractile protein expression after stimulation was not evident in cells grown on either laminin or matrigel, and, consistent with the effects of these plate coatings on proliferation, there was significantly less Ki67 expression. By contrast, cells grown on either fibronectin or collagen I remained in the proliferative phenotype, with significantly greater Ki67 expression, but failed to demonstrate coordinated expression of contractile marker

proteins. The phenotype of ASM was thus critically influenced by signaling from the ECM matrix.

In an extension of this work, Freyer et al²⁰ investigated the influence of ECM proteins on human ASM survival and explored the relevance of integrin-directed signaling to this cell survival. The extracellular domains recognize short peptide sequences, termed *RGD sequences* because they have the composition Arg-Gly-Asp, that are present on some ECM proteins, such as fibronectin, vitronectin, nidogen, tenascin, and laminin. The interaction between the extracellular domain of the integrin receptor and the ECM RGD sequence leads to alterations in the intracellular domains that influence cell events such as differentiation, survival, migration, and cell adhesion. Freyer et al²⁰ identified a low level of apoptosis in cultured airway myofibroblasts, except when cells were grown on elastin, which does not bind cells via integrins. No difference in apoptotic rate was evident among cells cultured on plastic, collagen I, collagen IV, collagen V, fibronectin, vitronectin, or laminin, suggesting that they all provided antiapoptotic signaling. This hypothesis was confirmed by coincubation with cycloheximide, which inhibited cell protein synthesis but had no effect on ASM apoptosis. To identify the integrins involved in the signal survival transduction, blocking studies with anti-integrin antibodies were undertaken. These studies revealed that α_5 was an integral subunit in all responses, and β_1 was involved in survival signaling from collagen IV, collagen V, laminin, and fibronectin. The α_5 and β_1 integrin subunits were found to be universally expressed on cultured airway myofibroblasts, as was α_V , although blocking α_V had no effect on cell apoptosis.²⁰ Consistent with the findings of Hirst et al,¹⁹ the myofibroblast morphology was influenced by the differing ECM components. Cells cultured on either collagen IV or fibronectin appeared to exhibit a normal growth pattern, cytoskeletal formation, and spreading, whereas cells grown on collagen I, collagen V, or laminin had defective spreading, and cells cultured on collagen V also had defective cell-cell contact formation and impaired cytoskeletal formation. Those cultured on collagen I exhibited a dense cytoskeletal network. These studies reveal the influence of the ECM on ASM function and highlight the importance of ECM-integrin signaling in these regulatory actions. It is also apparent that changes in the ECM in asthma are likely to influence ASM behavior.

CHEMOKINE AND CYTOKINE PRODUCTION BY ASM

When an inflammatory process is initiated in an organ such as the lung, low-molecular-weight cytokines (chemokines) are produced that are responsible for recruiting inflammatory cells along diffusion gradients (Fig 4). Chemokines, which can be divided into several categories on the basis of their molecular structure,²¹ exhibit a degree of selectivity for distinct inflammatory cell populations. For example, eotaxin, RANTES, and

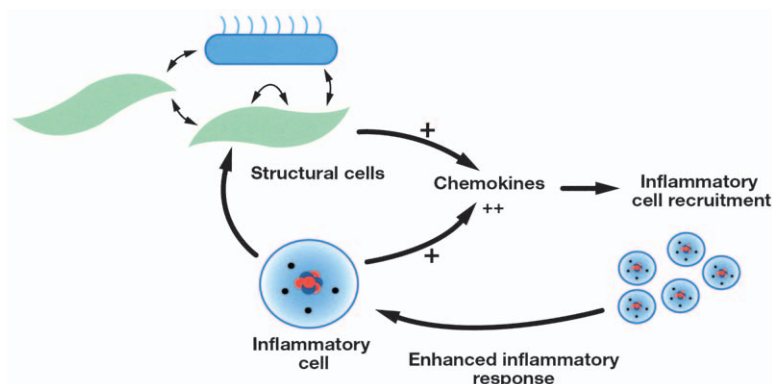


FIG 4. Chemokines produced by human ASM cells can attract several inflammatory cells into the airway milieu and amplify the signals generated by the inflammatory cells themselves.

IL-5 have been primarily considered to recruit eosinophils, although both eotaxin and RANTES have actions on other cell types; IL-8 potently recruits neutrophils; monocyte chemoattractant proteins (MCPs) recruit monocytes; thymus- and activation-regulated chemokine (TARC) recruits lymphocytes; and stem cell factor recruits mast cells. ASM has been shown to produce several chemokines, often in large quantities, that are capable of recruiting several inflammatory cells into the airway milieu. Chemokines produced by ASM cells and indeed by other structural cells, such as epithelium, fibroblasts, and myofibroblasts, amplify the signals generated by inflammatory cells themselves to produce a powerful chemokine stimulus that drives the inflammatory response. Chemokines can be generated by T_H2 cytokines such as IL-4 and IL-13, proinflammatory cytokines such as IL-1 β or TNF- α , or mediators that act on 7-transmembrane G protein-coupled receptors (GPCR) such as bradykinin,²²⁻²⁴ thrombin,²⁵ platelet activating factor,²⁶ and adenosine.²⁷ These agents appear to generate production of distinct profiles of chemokines through several common and disparate pathways.

Eotaxin

Eotaxin was first identified from bronchoalveolar lavage fluid of guinea pigs and was subsequently characterized as an important eosinophil chemoattractant in human asthma. ASM appears to be a particularly rich source of eotaxin, production of which is increased by proinflammatory cytokines, such as TNF- α and IL-1 β ,²⁸⁻³¹ and T_H2 cytokines, such as IL-4 and IL-13.^{32,33} A marked increase in eotaxin immunoreactivity in ASM was seen in an immunohistochemical study of the airways from patients with asthma,³¹ which clearly shows that the synthetic functions of ASM cells are not just an artefact of cell culture but are characteristic of human asthma *in vivo*. GPCR ligands such as bradykinin do not increase eotaxin production by ASM cells (Knox et al, unpublished data).

RANTES

RANTES is a CC chemokine and potent chemoattractant for monocytes, memory T cells, and eosinophils. RANTES is released by proinflammatory cytokine (TNF- α and IL-1 β) stimulation of ASM cells.³⁴⁻³⁷ Platelet activating factor can also increase RANTES release by ASM cells.²⁶

MCPs

Monocyte chemoattractant proteins are a family of chemokines that show structural similarities to eotaxin and are selective in the main for monocytes and lymphocytes. Increased levels are found in the airways in asthma. MCPs exist in 3 forms, designated MCP-1 to MCP-3. TNF- α and IL-1 β release all 3 isoforms when given in combination, but IL-1 β seems to be the more potent when given alone.^{32,38,39}

IL-8

IL-8, first isolated from monocytes as a neutrophil chemoattractant, is an 8.5-kd CXC chemokine that plays a major role in the initiation and maintenance of inflammatory responses. IL-8 also functions as an angiogenic factor and may contribute to angiogenesis, a prominent feature of the histopathology of several inflammatory diseases such as asthma, rheumatoid disease, and inflammatory bowel disease. IL-8 is an important neutrophil chemoattractant in chronic obstructive pulmonary disease (COPD) and may also have a role in neutrophil recruitment during exacerbations of asthma.

Proinflammatory cytokines such as TNF- α and IL-1 β are potent at releasing IL-8 from ASM cells,³⁹⁻⁴¹ whereas unlike with the eosinophil chemoattractant eotaxin, T_H2 cytokines such as IL-4 and IL-13 are ineffective. Studies from Pang and Knox⁴² have shown that bradykinin can also increase IL-8 release. Bradykinin, an important proinflammatory mediator in several inflammatory diseases, including asthma, acts on GPCRs. Interestingly, although some of the biological effects of bradykinin occur directly, many of its effects are mediated by

complex autocrine or paracrine loops involving intermediate factors such as prostaglandins.

TARC

Thymus- and activation-regulated chemokine is a highly specific ligand for the CCR4 receptor that is expressed on T_H2 but not T_H1 lymphocytes. TARC is upregulated in murine models of asthma, and antibodies against TARC attenuate airway eosinophilia and hyperresponsiveness in these models. TARC is also increased in bronchoalveolar lavage fluid and sputum after allergen challenge in asthmatic patients. It has recently been shown that TARC is produced by ASM cells, but only when IL-4 and IL-13 are given in combination with TNF- α ,⁴³ suggesting synergistic interactions in their signaling pathways.

Stem cell factor

Interest has begun to focus on the interactions between mast cells and ASM cells with the finding in a recent immunohistochemical study that the presence of mast cells in the ASM layer differentiated between patients with eosinophilic bronchitis—a condition in which cough is the prominent clinical manifestation—and asthma, which is characterized by wheezing.¹¹ Furthermore, studies from sensitized ASM *in vitro* show that localization of mast cells in the ASM layer is an important determinant of the resulting contractile response.⁴⁴ Although several chemokines are chemoattractant, for mast cells, including eotaxin and IFN- γ inducible protein of 10 KDa (IP-10), perhaps the most important is stem cell factor. Ongoing studies by Hamad et al⁴⁵ have shown that TGF- β can induce release of stem cell factor from ASM cells, but that other agents, such as IL-4, IL-13, IL-1 β , TNF- α , and bradykinin, are largely ineffective.

GM-CSF

GM-CSF stimulates the maturation, activation, and survival of several inflammatory cells, particularly eosinophils, and is an important cytokine in asthma and other allergic diseases. GM-CSF is produced by ASM cells when stimulated by a combination of IL-1 β and TNF- α ,^{37,46,47} suggesting that these agents not only stimulate ASM cells to produce chemokines such as eotaxin and RANTES, which attract eosinophils into the airways in asthma, but also produce factors that activate them and prolong their effective life in the airways. Thrombin also increases GM-CSF release.²⁵

IL-6

IL-6 is a pleiotropic cytokine that has both pro-inflammatory and anti-inflammatory properties in asthma. It is released by ASM cells in response to TNF- α and bradykinin.^{22,36,48-51}

Signaling pathways

Several complex signaling cascades are involved in regulating the various chemokines and cytokines pro-

duced by ASM cells. Regulation can occur at both transcriptional and posttranscriptional levels, although the former has been studied more in these cells.

Transcription factors. Various motifs within the 5'-flanking regions of the various chemokine gene promoters have the potential to bind several important transcription factors in a cell-dependent and stimulus-dependent manner. These have been studied extensively in other cell systems, but fewer data are available from studies in ASM cells. Several techniques can be used to study the transcriptional regulation of these genes, including transient transfection strategies with wild-type and mutated promoter constructs, transcription factor translocation studies, electrophoretic mobility shift assays, and chromatin immunoprecipitation assays.

T_H2 cytokines such as IL-4 and IL-13 act mainly through signal transducer and activator of transcription (STAT) pathways.⁵² Activation of STAT6 seems to be responsible for eotaxin release by these agents. In contrast, TNF- α activates eotaxin via nuclear factor κ B (NF- κ B; Knox, Unpublished data). NF- κ B is responsible for IL-6 expression in response to TNF- α , whereas nuclear factor for activated T cells and activator protein 1 (AP-1) mediate TNF- α -induced RANTES expression.⁴⁹

IL-1 β uses NF- κ B, CCAAT/enhancer-binding protein (C/EBP), and cyclic adenosine monophosphate (cAMP)-responsive element (CRE) to transduce its signals.^{24,53} The GPCR ligand bradykinin can activate several transcription factors in ASM cells.²²⁻²⁴ Transient transfection strategies with mutated promoter constructs and electrophoretic mobility shift assays have shown that NF- κ B, AP-1, and nuclear factor for IL-6 are all involved in IL-8 induction by bradykinin.²³ In contrast, bradykinin induced COX-2 via the CRE with no involvement of NF- κ B or nuclear factor for IL-6.²⁴ Complex autocrine loops involving endogenous prostaglandin E₂ (PGE₂) are responsible for some of these effects but not others. For example, bradykinin-induced activation of AP-1 and NF- κ B is prostanoid-dependent, because it is mimicked by exogenous PGE₂ and inhibited by indomethacin. In contrast, activation of NF- κ B occurs mainly via prostanoid-independent pathways.²³ Similarly, endogenous prostanoids partly mediate COX-2 induction in response to bradykinin in both ASM²⁴ and pulmonary artery smooth muscle cells via the CRE, suggesting that cAMP-linked prostaglandin EP₂ or EP₄ receptors are involved.⁵⁴ Activation of IL-6 by bradykinin appears to involve prostanoid-dependent activation of AP-1.²²

Kinase cascades. Mitogen-activated protein kinases (MAPKs) are a family of serine/threonine kinases that transduce extracellular signals to the nucleus.⁵⁵ Three groups of MAPKs that differ in their substrate specificity are recognized in mammalian cells: the extracellular signal-related kinases (ERKs), c-Jun NH₂-terminal kinases (JNKs), and p38 MAPKs. Once activated, MAPKs phosphorylate several intracellular substrates including transcription factors to switch on inflammatory genes such as chemokine and cytokine genes. Eotaxin, RANTES, and GM-CSF production in response to IL-1 β is inhibited by

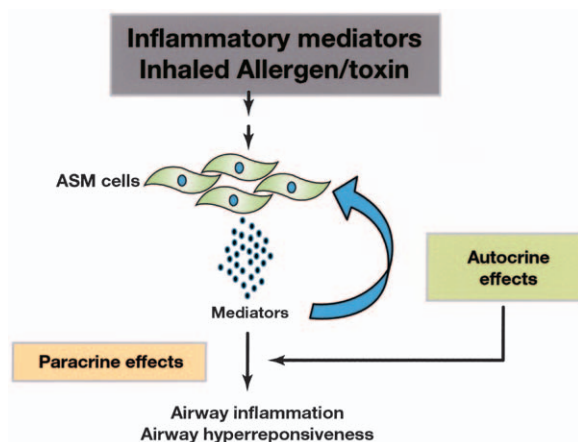


FIG 5. Autocrine and paracrine effects of ASM-derived mediators in asthma.

ERK inhibition.⁵⁶ Eotaxin production in response to IL-4 and IL-13 is also inhibited by ERK inhibitors.³³ In contrast, p38 MAPK inhibition hinders eotaxin but enhances GM-CSF production in response to IL-1 β .⁵⁶ RANTES production in response to platelet activating factor is inhibited by both p38 MAPKs and ERKs.²⁶ A recent study with the JNK inhibitor SP600125 has implicated JNKs in the production of RANTES, GM-CSF, and IL-8 in response to IL-1 β and TNF- α in ASM cells.³⁷

Inhibitory pathways. Transcription factors and the signaling kinases that activate them represent major intracellular targets for inhibition of inflammatory responses in asthma and COPD. Several agents have been shown to downregulate components of these pathways, particularly the corticosteroids and β_2 -agonists. Several other agents and pathways are worthy of mention. Carbon monoxide, which is produced endogenously in the airways via heme oxygenase, acts via cyclic guanosine monophosphate, which may be another important signaling pathway to target, because ASM cells contain abundant soluble and particulate guanylyl cyclases.⁵⁷ Carbon monoxide has been shown to inhibit IL-1 β -induced GM-CSF production.⁴⁷ The antioxidant N-acetyl cysteine has been shown to reduce MCP-1 and eotaxin production by inhibiting p38 MAPKs.⁵⁸ Phosphodiesterase inhibitors, particularly those selective for types III and IV, the cAMP-selective isoforms, also inhibit production of several chemokines. For example, TNF- α -induced production of eotaxin is inhibited by rolipram.²⁸

AUTOCRINE REGULATION OF ASM FUNCTION

As described, various T-cell-derived and mast cell-derived cytokines present in high levels in asthmatic airways, such as IL-4, IL-5, IL-13, and TNF- α , are believed to orchestrate both acute and chronic inflammatory responses by altering myocyte function.^{59,60} Although airway remodeling may be influenced by cytokines acting

indirectly by promoting the recruitment, activation, and trafficking of leukocytes, evidence now suggests that cytokines may also exert paracrine or autocrine effects by inducing expression of inflammatory genes in resident airway cells such as epithelium, fibroblasts, and ASM.^{61,62} Still, few reports have defined the precise signaling pathways by which cytokines, alone or in combination, regulate synthetic responses in ASM cells. Further, whether ASM-derived cytokines are physiologically active remains unknown. One previous report showed that conditioned media from IL-1 β -treated ASM cells increase eosinophil survival, an effect that was completely abrogated by blocking antibodies against GM-CSF.⁶³ Thus, ASM-derived GM-CSF mediates survival-enhancing effects on eosinophils induced by IL-1 β . New evidence shows that ASM-derived inflammatory mediators are biologically active in an autocrine manner (Fig 5).

Cytokine receptors are expressed on ASM cells

A variety of T_H1-type and T_H2-type cytokines, including TNF- α , IL-1, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN- γ , induce several cellular responses in ASM cells,⁶⁴ presumably by activating cognate receptors. Some studies have attempted to characterize the cytokine receptor types expressed in cultured ASM cells, as well as their precise role in regulating cellular responses, by using various experimental approaches. Levels of mRNA expression for IL-2, IL-12, and IFN- γ receptors were detected in rabbit ASM cells and were increased on exposure to asthmatic serum.⁶⁵ Others used flow-cytometric analysis to show the expression of α -chain of IL-4 receptor, but not γ -chain of IL-2 receptor, and functionally the activation of these receptors was characterized by using neutralizing antibodies to block IL-4-induced and IL-13-induced STAT6 phosphorylation and secretion of eotaxin in bronchial smooth muscle cells.⁶⁶ Laporte et al,⁶⁷ by using reverse transcriptase polymerase chain reaction techniques, confirmed the expression of α -chain on ASM cells and showed that IL-13, but not IL-4, decreased ASM responsiveness to β -agonists, suggesting a role of α -chain of IL-4 receptor in cytokine-induced modulation of ASM contractile function. Consistent with this observation are findings by Grunstein et al,⁶⁸ who showed the involvement of α -chain of IL-4 receptor in the impairment of rabbit ASM responsiveness to both contractile and relaxant agonists after IgE sensitization. Immunostaining and Western blot analysis revealed a differential expression of TNF- α receptors on cultured ASM cells, with TNF receptor homologue (TNFR)—1 expressed evenly over the cell surface, whereas TNFR2 was located more on the edge of the cells.⁶⁹⁻⁷¹ More importantly, although TNFR2 was found to be functionally active in inducing gene expression, the use of agonistic antibodies showed that TNFR1 was the predominant receptor regulating TNF- α -induced expression of proinflammatory mediators such as intercellular adhesion molecule 1 (ICAM-1), IL-6, and RANTES.^{70,72} The *in vivo* relevance of TNF- α effects on cultured ASM cells is supported by

the fact that both receptors are also expressed in native tracheal tissues, as demonstrated by using immunocytochemistry.⁷² The immunocytochemical and flow cytometry techniques also revealed that all 3 isoforms of TGF- β receptors are expressed on bovine ASM cells.^{73,74}

Autocrine effect of ASM-derived prostanoids

As mentioned, ASM-derived prostanoids regulate ASM synthetic function in an autocrine fashion.^{42,75,76} Autocrine prostanoid products may also be involved in bradykinin-induced IL-6 expression.⁷⁷ These findings suggest that ASM-derived prostanoids regulate a variety of inflammatory genes in ASM cells in an autocrine manner. Additional studies have shown that both TNF- α and IL-1 β , a cytokine mixture that maximally increases COX-2 expression and prostanoid production, impaired ASM responsiveness to β_2 AR stimulation⁷⁸ or suppressed ASM cell proliferation⁷⁹ via PGE₂-dependent pathways. Secreted PGE₂ also seems to modulate the transcriptional regulation of the COX-2 gene, because flurbiprofen, a COX inhibitor, significantly reduced IL-1 β -induced COX-2 expression in human bronchial ASM cells.⁸⁰ Interestingly, TGF- β 1, which also stimulates COX-2 expression and PGE₂ production in ASM cells, regulates IL-8 expression via prostanoid-independent mechanisms,⁸¹ demonstrating that the role of endogenous prostanoids in the regulation of gene expression is stimulus-specific.

Autocrine effect of ASM-derived T_H2-type cytokines

Evidence suggests that T_H2 cells and their derived cytokines play a central role in chronic airway inflammation in asthma.⁸² Interesting studies using rabbit tracheal preparations show that under certain experimental conditions, including asthmatic serum sensitization or rhinovirus exposure, the ASM-derived T_H2 cytokines IL-13, IL-4, and IL-5 enhance ASM contractile responses to acetylcholine in a coordinate and autocrine fashion but also decrease the relaxant responses to isoproterenol.⁸³⁻⁸⁷ These findings support the current notion that inflammatory cytokines may promote the development of bronchial hyperresponsiveness by directly altering ASM responsiveness to agonists that activate GPCRs.^{78,88-91} The molecular mechanisms by which cytokines induce abnormal ASM contractile and relaxant responsiveness remain unknown. By using cultured ASM cells, investigators showed that cytokines such as TNF- α , IL-1 β , and IL-13 modulate GPCR-associated signaling pathways by increasing calcium responses and/or altering cAMP production.^{88,92} Together, these data suggest that GPCR signaling pathways may be a potential target for the autocrine action of ASM-derived cytokines.

Autocrine effect of ASM-derived TGF- β 1

Another candidate gene associated with asthma severity is TGF- β , which is involved in airway remodeling. TGF- β promotes the production of ECM proteins, such as collagens and fibronectin.⁹³ Levels of TGF- β , a cytokine that is constitutively expressed in healthy airways, are

significantly increased in eosinophils in patients with severe asthma compared with patients with mild asthma.^{94,95} TGF- β is also produced by other inflammatory cells, including macrophages and activated T lymphocytes, as well as airway resident cells, such as epithelial cells and ASM cells.^{96,97} Further investigation by using neutralizing antibodies demonstrated that, as mentioned, the synthesis of procollagen I by ASM cells is regulated by TGF- β in an autocrine fashion.⁵ In addition, secretion of TGF- β 1 has been shown to correlate with ASM hypertrophy.⁹⁶ The autocrine activity of TGF- β 1 is an important observation, because others demonstrated that TGF- β 1 regulates several cellular responses in ASM cells. Exogenous TGF- β 1 inhibits both EGF-induced and thrombin-induced ASM cell proliferation in human ASM cells⁹⁸ and increases cell size and cell number in bovine and human ASM cells.^{97,99} TGF- β 1 also impairs β_2 AR function in tracheal tissues¹⁰⁰ and augments LTD₄-induced cell proliferation in bronchial ASM cells via increased expression of cysteinyl leukotriene 1 receptor.¹⁰¹ All 3 TGF- β isoforms also stimulate the secretion of vascular endothelial growth factor (VEGF) in human ASM cells.¹⁰² It is critical, therefore, to characterize better the signaling pathways associated with TGF- β 1 receptor in ASM cells.

Autocrine effect of ASM-derived IFN- γ

Janus-activated kinases (JAKs) and STATs serve as the primary components of the IFN signaling cascade, although the JAK/STAT pathway is also activated by other cytokines such as IL-4, IL-5, IL-6, and IL-13.¹⁰³ JAK1 and Tyk2 are activated by IFN- α/β (type 1); JAK1 and JAK2 are activated by IFN- γ (type 2).¹⁰⁴ JAKs phosphorylate STAT proteins that then assemble in dimeric or oligomeric forms and translocate to the nucleus. STATs regulate gene expression by binding to DNA motifs called IFN- γ activation sites, recognized by STAT1 homodimers (also known as *IFN- γ activation factor*). These sites overlap with interferon-stimulated response elements, activated by STAT1-STAT2 heterodimers, also termed *interferon-stimulated gene factor-3* when complexed to p48 or IFN regulatory factor 9.^{105,106}

Notably, STAT1 and STAT1-dependent genes such as ICAM-1 and IFN regulatory factor 1 are also activated in the epithelium of asthmatic patients,¹⁰⁷ illustrating the potential role of JAK/STAT1 in the pathogenesis in allergic asthma. In ASM, the relevance of such JAK/STAT pathways is supported by indirect evidence showing that PDGF-induced ASM cell proliferation requires induction of cyclin D₁ expression via a mechanism that involves JAK2/STAT3-dependent pathways.¹⁰⁸ In addition, activation of STAT6-dependent signaling pathways is associated with gene expression by IL-13 and IL-4 in human ASM cells.^{66,67} More importantly, IFN- γ , alone or in a synergistic manner with other cytokines such as TNF- α and/or IL-1 β , also stimulates the expression of various proinflammatory genes, such as COX-2,^{109,110} IL-8,¹¹¹ RANTES,¹¹² GM-CSF,¹¹³ and ICAM-1.¹¹⁴ IFN- γ has also been shown to modulate ASM responsiveness to both contractile and relaxant GPCR agonists. IFN- γ

enhances LTD₄-induced contraction in human ASM cells¹¹⁵ and ASM sensitivity to isoproterenol in guinea pig ASM.¹¹⁶ In contrast, Hakonarson et al⁶⁵ showed that IFN- γ prevents asthmatic serum-induced alterations in ASM responsiveness to either acetylcholine or isoproterenol. Although the JAK/STAT1 pathway is usually the primary component of the IFN- γ signaling cascade, Tliba et al¹¹⁷ recently showed for the first time that TNF- α , via the autocrine activation of IFN- β receptor-associated JAK1 and Tyk2, stimulates STAT1-dependent and STAT2-dependent gene expression in human ASM cells. The group also showed that autocrine IFN- β differentially regulates TNF- α -induced inflammatory gene expression, suppressing IL-6 expression and promoting RANTES secretion, whereas ICAM-1 expression was not affected (Fig 6). Although functional crosstalk between TNF- α and IFN signaling molecules has been reported in other cell types (mostly hemopoietic cells), these findings were unique. In 3T3-L1 adipocytes, TNF- α induces the phosphorylation of STAT1 by directly interacting with both JAK1 and JAK2,¹¹⁸ whereas in Hela cells, STAT1 physically interacts with TNFR1 and the TNF receptor-associated death domain protein, but not TNF receptor-associated factor 2.¹¹⁹ TNF- α also induces STAT1 phosphorylation at serine 727 in macrophages.¹²⁰ These data clearly show that autocrine IFN- β is a novel signaling component by which TNF- α regulates ASM function in human ASM cells (Fig 7).

Autocrine regulation of chemokine secretion

Complex autocrine loops can regulate the secretion of several chemokines and cytokines, particularly the generation of lipid mediators such as prostanoids. Prostanoids are produced from membrane phospholipids in a multistep process. COX, the main rate-limiting enzyme, converts arachidonic acid into prostaglandin H₂, which is subsequently converted into prostanoids by specific synthases. Three isoforms of COX have been identified. COX-1 is a housekeeping gene producing physiological levels of prostanoids, whereas COX-2 is induced in inflammatory conditions. A further isoform, COX-3, is a splice variant of COX-1 whose function is less clear. PGE₂ is the main COX product produced by ASM cells. COX-2 can be induced in ASM cells, mainly by IL-1 β and bradykinin.^{76,109,110} The effect of IL-1 β is more sustained, and bradykinin also induces an early prostanoid release by mobilizing arachidonic acid via phospholipase A₂. There are several functional effects of COX-2 induction. COX products regulate the synthetic functions of ASM cells, and they potentiate VEGF production.⁷⁵ Of relevance to this article, however, are studies showing that production of prostanoids may act as a negative feedback mechanism, inhibiting the production of some chemokines and cytokines while conversely augmenting the production of others. Studies with broad-spectrum and COX-2-selective COX inhibitors have shown that these agents potentiate the production of GM-CSF in response to IL-1 β and TNF- α , suggesting that endogenous prostanoids exert a brake on GM-CSF production.³⁰

Consistent with this, exogenously applied PGE₂ inhibited GM-CSF production. PGE₂ also reduced the release of RANTES in response to IL-1 β and TNF- α , but only when endogenous PGE₂ was inhibited with indomethacin.³⁵ Endogenous prostanoids are partly responsible for IL-8 generation in response to bradykinin, because COX inhibitors inhibit bradykinin-induced IL-8 release, whereas PGE₂ stimulates release.^{23,42} This effect seems to be specific to bradykinin, however, and it is not observed when IL-1 β or TGF- α is used to stimulate IL-8 release. The differences may be a result of differences in additional signaling pathways that these agents use, or perhaps the timing of PGE₂ generation, which is earlier with bradykinin.

ASM: A TARGET FOR β_2 -AGONIST/CORTICOSTEROID INTERACTIONS

β_2 -Agonists and corticosteroids are widely used in the treatment of bronchial asthma and COPD. The combination of LABAs and inhaled corticosteroids (ICSs) results in improved lung function, better symptom control, and reduced exacerbations compared with LABAs or higher doses of ICSs alone.^{121,122} Several studies have shown that LABAs potentiate the anti-inflammatory actions of corticosteroids in either an additive or a synergistic manner.^{123,124} Recent evidence suggests that ASM cells may have both a contractile and a synthetic function in the pathophysiology of respiratory disease, and, given the fact that ASM cells contain both β_2 ARs and GRs, it is possible that they represent a key target for interactions between β_2 -agonists and corticosteroids.

Mechanism of action of β_2 -agonists

All β_2 -agonists exert their biological and therapeutic effects through cell surface β_2 ARs, which are members of the 7-transmembrane GPCR family. After ligand binding to the active site of the receptor, the α -component of the associated G_s-protein dissociates and activates adenylate cyclase,¹²⁵ leading to the production of intracellular cAMP and subsequent activation of protein kinase A (PKA), which then phosphorylates several intracellular regulatory proteins.¹²⁶

β_2 -Agonists may also influence gene transcription through a cAMP mechanism whereby there is increased translocation of the catalytic subunit of PKA to the nucleus and phosphorylation of CRE binding protein, enhancing its DNA-binding and transactivation effects.¹²⁷ More recently, it has been reported that activation of the β_2 AR can also lead to coupling to G_i, resulting in stimulation of the ERK and p38 MAPK pathways.¹²⁸

The mechanisms of action of the LABAs, salmeterol and formoterol, have been reported previously.^{129,130} Briefly, salmeterol partitions into the cell membrane and diffuses laterally to the β_2 AR. The side chain of the molecule then binds to a discrete, hydrophobic region of the fourth transmembrane domain, the exo-site. Binding to

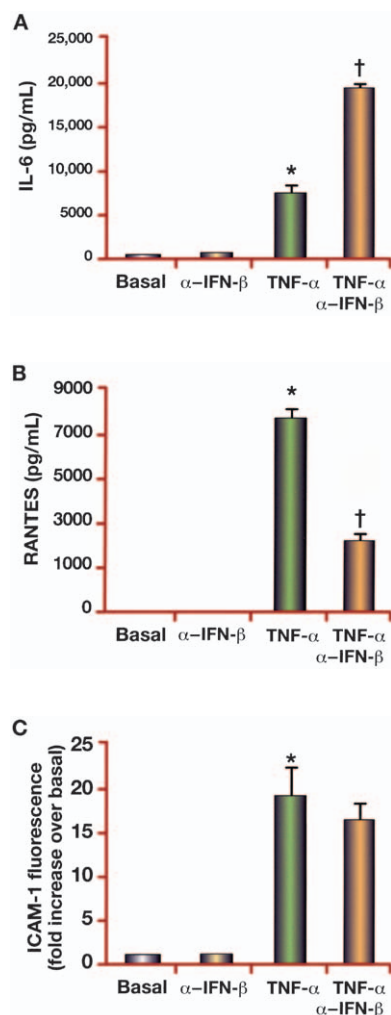


FIG 6. TNF- α , via the autocrine action of IFN- β , differentially regulates ICAM-1, IL-6, and RANTES expression. Cells were stimulated for 24 hours with TNF- α (10 ng/mL) in the presence or absence of neutralizing anti-IFN- β (5 mg/mL; 15 minutes). Secretion of IL-6 (**A**) and RANTES (**B**) or expression of ICAM-1 (**C**) levels was assessed as described in the report by Tliba et al.¹¹⁷ Values shown are means $\pm$ SEMs and are significantly different from those of controls. * P < .05 compared with basal levels. † P < .05 compared with cells treated with TNF- α alone. Adapted with permission from Tliba et al.¹¹⁷

the exo-site prevents salmeterol from dissociating from the receptor, and the saligenin head of the molecule is free to engage and disengage the active site by a Charnière (hinge) principle, leading to a long, concentration-independent duration of action.¹²⁹ Formoterol, which is moderately lipophilic, is taken up into the cell membrane as a depot. The drug then progressively leaches out to activate the β_2 AR, imparting a prolonged, concentration-dependent action.¹³⁰

Mechanisms of corticosteroid action

The target receptor for corticosteroids is the intracellular GR. Under resting conditions, the inactive GR is

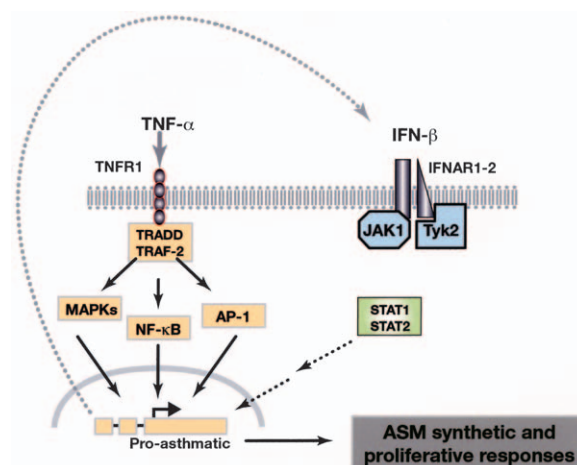


FIG 7. Proposed mechanisms for the regulation of ASM synthetic and proliferative responses by TNF- α in human ASM cells. TNF- α via TNFR1 induces both expression and secretion of IFN- β , which then acts in an autocrine manner on IFN type I receptors (IFNARs) to activate JAK1/Tyk2. Activation of IFN- β -associated signaling pathways (STAT1/STAT2) can cooperate with other signaling pathways (MAPK, NF- κ B, AP-1) to modulate differentially expression of proinflammatory genes and cell proliferation induced by TNF- α . TRADD, TNF receptor-associated death domain protein; TRAF, TNF receptor-associated factor.

largely located in the cytosol of the cell, associated with multichaperone proteins. The corticosteroid molecule first penetrates the cell membrane, then binds to the GR through the corticosteroid-binding domain.¹³¹ This induces a conformational change in the receptor protein, dissociation of the chaperone proteins, and the formation of an active corticosteroid-GR complex. The complex may then form a dimer and translocate from the cytosol to the nucleus of the cell, where it binds to specific DNA sequences (glucocorticoid response elements [GRE]) in the promoter region of target genes, leading to cofactor activation and either an increase or a decrease in gene transcription. This process is termed *transactivation*.¹³¹

Alternatively, the active corticosteroid-GR complex, as a monomer, can interact directly with intracellular transcription factors such as AP-1 or NF- κ B through a protein-protein interaction to attenuate transcription factor-mediated proinflammatory processes. This transrepression process involves recruitment of histone deacetylases and modulation of chromatin structure.¹³²

Interactions between corticosteroids and β_2 -agonists

Effect of corticosteroids on β_2 ARs. Corticosteroids can modulate β_2 ARs and their function by several mechanisms: protection against desensitization and the development of tolerance, increased efficiency of receptor coupling, and protection against inflammation-induced receptor downregulation and uncoupling.

Corticosteroids stimulate the transcription of β_2 ARs via binding to GREs in the 5'-noncoding promoter region of the β_2 AR gene. Dexamethasone was shown to increase

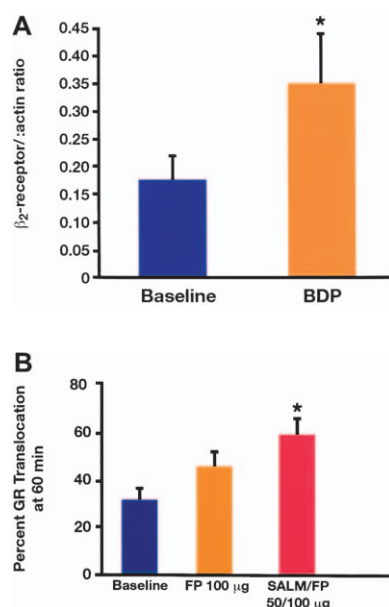


FIG 8. Effect of corticosteroids and LABAs on β_2 ARs and GRs *in vivo*. **(A)** Intranasal beclomethasone dipropionate (BDP; 100 μ g/d for 3 days) significantly ($*P < .04$) increased β_2 AR density (expressed as β_2 AR:actin ratio) on the nasal mucosa of mild asthmatic patients. Reprinted with permission from Baraniuk et al.¹³⁴ **(B)** The combination of salmeterol (SALM) and FP (50 μ g/100 μ g) significantly ($*P < .05$) increased GR nuclear translocation compared with FP (100 μ g) alone in sputum macrophages from patients with mild asthma 60 minutes after inhalation. Reprinted with permission from Usmani O, Maneechotesuwan K, Adcock I, Barnes PJ / 2002 / Glucocorticoid receptor activation following inhaled fluticasone and salmeterol / *American Journal of Respiratory and Critical Care Medicine* / Vol 165 / Page A616. Official Journal of the American Thoracic Society. ©American Thoracic Society.

β_2 AR mRNA (at 2 hours) and protein (at 24 hours) in human lung tissue *in vitro*.¹³³ This increase in transcription, which has also been shown in neutrophils and T cells, is both time-dependent and dose-dependent, consistent with the later induction of receptor binding activity. However, dexamethasone has not been found to alter the half-life of β_2 AR mRNA.¹³³

Baraniuk et al¹³⁴ reported that intranasal administration of beclomethasone dipropionate (100 μ g/d $\times$ 3 days) significantly increased the density of β_2 ARs on the nasal mucosa (Fig 8, A). Both systemic corticosteroids and ICSs reversed β_2 AR downregulation after exposure to high doses of short-acting β_2 -agonists.¹³⁵

The efficiency of coupling between the β_2 AR and G_s has also been reported to be modulated by corticosteroids.¹³⁶ As a result, β_2 AR-stimulated adenylate cyclase activity and cAMP accumulation increase after corticosteroid treatment. Animals that have been depleted of corticosteroids by adrenalectomy, in contrast, lose the ability to maintain the sensitivity of the β_2 AR-coupled adenylate cyclase system.

Other studies¹³⁷ have shown that by reducing the concentrations of proinflammatory cytokines such as IL-1 β and TGF- β_1 , corticosteroids regulate β_2 AR sensitivity and the coupling of β_2 ARs to adenylate cyclase. Such

effects may have clinical relevance in preventing the development of tolerance to β_2 -agonists in asthmatic patients and patients with COPD on chronic therapy.

Effect of LABAs on GRs. LABAs exert an effect on the GR by priming the GR for subsequent corticosteroid binding and by increasing the translocation of the GR from the cell cytosol to the nucleus. Modulation of the GR has been shown to be achieved by phosphorylation (activation) and dephosphorylation (inactivation). Priming of the GR by LABAs is achieved by MAPK-dependent phosphorylation.¹³⁸ After stimulation of the β_2 AR by salmeterol and dissociation of $G_{\alpha s}$, the residual $\beta\gamma$ -subunit of the G protein initiates an intracellular signaling cascade involving the nonreceptor tyrosine kinase C-Src and the G protein Ras, culminating in the stimulation of MAPK.¹³⁹ This has been demonstrated in human embryonic kidney cells transfected with a MAPK-luciferin/luciferase reporter construct and directly by isolation of phospho-MAPK.¹⁴⁰

Activation of this pathway by the LABA requires that the β_2 AR is phosphorylated, probably by PKA.¹³⁹ MAPK then phosphorylates the GR at several proline-directed serine residues in the N-terminal region of the receptor. Indeed, phospho-GR has been detected in cells containing both β_2 ARs and GR, when stimulated with salmeterol.¹⁴¹ It is possible that an increase in negative charge at the N-terminal domain of the receptor leads to a conformational change in the GR protein, leading to the priming event and rendering the receptor more sensitive to steroid-dependent activation.

Other studies have shown that translocation of the GR from the cell cytosol to the nucleus, a fundamental step in the anti-inflammatory activity of corticosteroids, is increased by the addition of a LABA. Eickelberg et al¹⁴² showed that treatment of human lung fibroblasts and smooth muscle cells with fluticasone propionate (FP) and salmeterol resulted in increased translocation of GR compared with FP alone. These effects have been confirmed with formoterol and budesonide.¹⁴³ Electrophoretic mobility shift assays confirmed that the LABA-activated nuclear GR actively bound to the GRE consensus sequence. The effects were β_2 AR-mediated and PKA-mediated, because receptor blockade with propranolol or the use of a PKA-inhibitor peptide abrogated GR activation.^{142,143}

These *in vitro* findings have now been confirmed *in vivo* in asthmatic patients treated with LABAs and corticosteroids. Usmani et al¹⁴⁴ showed that inhalation of salmeterol (50 μ g) and FP (100 μ g) increased the nuclear translocation of GR in sputum macrophages at 60 and 120 minutes significantly more than the same dose of FP alone and to the same extent as a 5-fold higher dose of the steroid (Fig 8, B). Whether these effects of salmeterol can be shown in the patient with COPD remains to be determined. In contrast, whereas budesonide (800 μ g) also significantly increased the translocation of the GR from the cytosol into the nuclei of peripheral blood leukocytes, budesonide in combination with formoterol (24 μ g) did not enhance this effect versus budesonide alone.¹⁴⁵

The interactions between corticosteroids and LABAs may be summarized as follows: corticosteroids increase β_2 AR synthesis, and LABAs prime GR for steroid-dependent activation and nuclear translocation. These mechanisms form a possible molecular and receptor basis for additive and synergistic interactions between the 2 classes of drugs in the treatment of asthma and COPD.

Effects of β_2 -agonists and corticosteroids in ASM

IL-8 release. β_2 -Agonists such as albuterol (salbutamol) and salmeterol (0.1–1 μ mol/L) caused a time-dependent increase in basal IL-8 accumulation that was significant after 4 and 8 hours, respectively.⁴⁰ The magnitude of IL-8 release by β_2 -agonists was smaller, however, than that after stimulation with TNF- α , suggesting that an increase in cAMP is a weak inducer of IL-8 generation by human ASM cells. Indeed, albuterol or salmeterol did not further increase the TNF- α response. In contrast, corticosteroids such as dexamethasone (1 μ mol/L) and FP (0.1 μ mol/L) resulted in a concentration-dependent inhibition (Fig 9), but did not abolish TNF- α -induced IL-8 release.⁴⁰ However, when dexamethasone or FP was used in combination with salmeterol, a further inhibition was observed that was significantly greater than with the corticosteroids alone (Fig 9). This represents a synergistic interaction between LABA and corticosteroids in ASM, which may have implications in asthma and COPD, in which the combined use of LABAs and ICSs significantly reduces exacerbations, conditions associated with IL-8-driven neutrophilia. The synergistic inhibition of IL-8 release was reversed by the β_2 -selective antagonist ICI 118551, confirming that the effect of salmeterol was mediated through the β_2 AR.⁴⁰ KT 5720, a potent and selective inhibitor of cAMP-dependent PKA, also significantly reduced the inhibitory effects of salmeterol and FP in combination, whereas the phosphodiesterase IV inhibitor rolipram further increased the level of IL-8 inhibition.⁴⁰ These observations indicate an involvement of the cAMP pathway in the synergy between salmeterol and corticosteroids in ASM cells.

The precise mechanism of the effects of cAMP on steroid-induced inhibition of IL-8 release is unknown. However, because there is no CRE on the IL-8 gene promoter, the effect is likely to be a secondary one on other transcriptional elements. In many cells, TNF- α -induced IL-8 release is mediated by NF- κ B and AP-1. Further studies are required to examine interactions between corticosteroids and β_2 -agonists on the transcriptional regulation of the IL-8 promoter to determine the mechanism of the synergistic effect in human ASM cells.

Eotaxin release. β_2 -Agonists had no effect on basal eotaxin but significantly inhibited TNF- α -induced release, with salmeterol 10-fold more potent than albuterol.²⁸ Pretreatment of cells with dexamethasone (0.1–10 μ mol/L) or FP (0.01–1 μ mol/L) inhibited responses to TNF- α in a concentration-dependent manner but did not abolish eotaxin release. When salmeterol was used in combination with dexamethasone or FP, TNF-

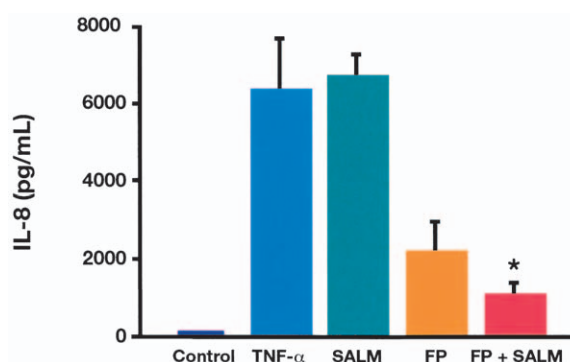


FIG 9. Combination of salmeterol (SALM) and FP (1 μ mol/L/0.1 μ mol/L) synergistically ($*P < .01$) inhibited TNF- α -induced IL-8 release from human ASM cells compared with SALM (1 μ mol/L) or FP (0.1 μ mol/L) alone. Reprinted with permission from Pang L, Knox AJ / 2000 / Synergistic inhibition by beta(2)-agonists and corticosteroids on tumor necrosis factor-alpha-induced interleukin-8 release from cultured human airway smooth-muscle cells / *American Journal of Respiratory Cell and Molecular Biology* / Vol 23 / Pages 79–85. Official Journal of the American Thoracic Society. ©American Thoracic Society.

α -induced eotaxin release was inhibited significantly more than with salmeterol alone.²⁸ These effects were reversed by ICI 118551 and were further increased by rolipram, implicating both the β_2 AR and the cAMP pathways.²⁸ However, studies using inhibitors of cAMP-dependent PKA and cyclic guanosine monophosphate-dependent protein kinase G suggested that neither PKA nor protein kinase G was involved in the enhanced inhibition of TNF- α -induced eotaxin release by the combination of LABAs and corticosteroids.²⁸

IL-6 and RANTES release. Recent studies have demonstrated that TNF- α stimulates the secretion of IL-6 and RANTES from ASM cells. The role of IL-6 may be that of an anti-inflammatory signal, modulating ASM in an autocrine manner. IL-6 release appears to be mediated by a NF- κ B-dependent pathway, whereas RANTES release is mediated by activation of AP-1.¹⁴⁶ The LABA formoterol increased TNF- α -induced IL-6 generation by approximately 6-fold, with a median effective concentration of 0.01 μ mol/L.¹⁴⁶ This was associated with binding to CRE and activation of the IL-6 promoter but did not involve AP-1 or NF- κ B.¹⁴⁶ The lower-efficacy LABA, salmeterol, at higher concentrations, induced only a 2-fold increase in IL-6 (Fig 10). In contrast, β_2 -agonists inhibited TNF- α -induced RANTES secretion in a concentration-dependent manner.¹⁴⁶ Formoterol was the most effective inhibitor, with a median inhibitory concentration (IC_{50}) of 1 μ mol/L. TNF- α -induced RANTES secretion was inhibited by approximately 70% at 10 μ mol/L. Iso- α -proterenol was 10-fold less potent than formoterol, but at 100 μ mol/L abrogated TNF- α -induced RANTES by approximately 80%. At a concentration of 10 μ mol/L, salmeterol and albuterol also inhibited RANTES secretion after TNF- α stimulation, by 46.2% and 31.0%, respectively.¹⁴⁶ β_2 -Agonists alone had no effect on RANTES secretion.¹⁴⁶ The rank order of effectiveness of β_2 -agonists

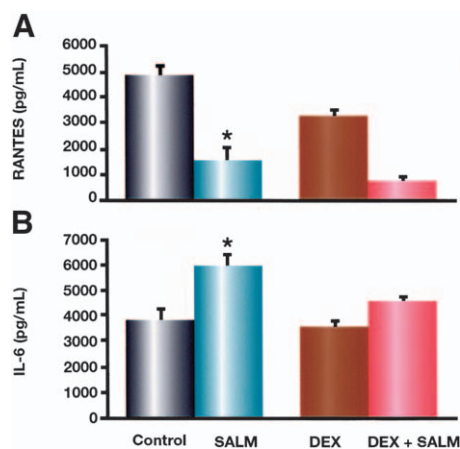


FIG 10. Salmeterol (SALM, 1 $\mu\text{mol/L}$) significantly ($*P < .05$) inhibited TNF- α -induced RANTES release (**A**) and significantly ($*P < .05$) increased IL-6 generation (**B**) by human ASM cells. Dexamethasone (0.1 nmol/L) also inhibited RANTES but had little effect on TNF- α -induced IL-6 release. The combination of SALM and dexamethasone (DEX) further enhanced RANTES inhibition over SALM or DEX alone, but it increased IL-6 release compared with DEX alone. Reprinted with permission from Ammit AJ, Lazaar AL, Irani C, O'Neill GM, Gordon ND, Amrani Y, et al / 2002 / Tumor necrosis factor-alpha-induced secretion of RANTES and interleukin-6 from human airway smooth muscle cells: modulation by glucocorticoids and beta-agonists / *American Journal of Respiratory Cell and Molecular Biology* / Vol 26 / Pages 465-74. Official Journal of the American Thoracic Society. ©American Thoracic Society.

in their ability to inhibit TNF- α -induced RANTES secretion is similar to their ability to induce secretion of IL-6 and increase cytosolic levels of cAMP in ASM. However, RANTES seems to be more sensitive than IL-6 to small changes in cAMP concentration, and it did not appear to be mediated via AP-1.

Corticosteroids have differential effects on the release of IL-6 and RANTES from ASM cells (Fig 10). Dexamethasone (0.001-1 $\mu\text{mol/L}$) completely abrogated TNF- α -induced RANTES secretion, with an IC_{50} of 1 nmol/L, but even at the highest concentration only partially inhibited IL-6.¹⁴⁶ The effect of corticosteroids on RANTES gene expression appears to be mediated via an AP-1-dependent pathway. In combination with salmeterol, the same degree of inhibition of TNF- α -induced RANTES release could be achieved with a 100-fold lower concentration of dexamethasone.¹⁴⁶ However, TNF- α -induced IL-6 secretion from ASM cells was significantly augmented by the combination of salmeterol and dexamethasone (Fig 10).

MCP release. As mentioned, stimulation of human ASM cells with a cytokine mixture (IL-1 β , TNF- α , IFN- γ) resulted in an increase in MCP release.³⁸ Dexamethasone (1 $\mu\text{mol/L}$) inhibited MCP-1 and MCP-2 release by approximately 50%. This may be one of the mechanisms whereby corticosteroids inhibit the influx of leukocytes into the airway and suppress inflammation in asthma. There are no reports of the effects of β_2 -agonists alone or in combination with corticosteroids on MCP release from ASM cells.

GM-CSF release. Albuterol (0.1-1 $\mu\text{mol/L}$) did not significantly alter the basal release of GM-CSF, but it attenuated cytokine-induced secretion with an IC_{50} of 16 nmol/L.¹⁴⁷ Pretreatment with ICI 118551 prevented the albuterol inhibition of IL-1 β -stimulated GM-CSF release, confirming an effect mediated by membrane β_2 ARs. The effects of albuterol were associated with a 15-fold increase in intracellular cAMP formation and were inhibited by H-89, an inhibitor of the PKA-dependent pathway.¹⁴⁷

IL-1 β -induced and TNF- α -induced GM-CSF release from human ASM cells was inhibited by approximately 85% with dexamethasone, although the corticosteroids did not affect basal secretion.⁴⁶ Combinations of LABAs and corticosteroids have been shown to have an additive inhibitory effect on GM-CSF release.¹⁴⁸

VEGF release. An interaction between β_2 -agonists and corticosteroids in ASM is also observed with other proinflammatory mediators, such as VEGF. Either salmeterol (0.1-0.5 $\mu\text{mol/L}$) or FP (0.01-0.1 nmol/L) alone inhibited TGF- β_1 -stimulated VEGF release from human ASM cells.¹⁴⁹ The corticosteroid was more potent, almost completely inhibiting VEGF secretion at 0.1 nmol/L. In addition, whereas FP was still effective in reducing VEGF release from IL-1 β -stimulated cells, the LABA was inactive. However, the combination of salmeterol and FP further inhibited IL-1 β -induced VEGF compared with the corticosteroids alone, suggesting a synergistic interaction.¹⁴⁹

ASM: Cell-cell interactions. Isoproterenol, at a concentration (100 $\mu\text{mol/L}$) previously shown to increase cAMP in human ASM cells, inhibited TNF- α -induced expression of ICAM-1 and vascular cell adhesion molecule 1 by approximately 60%.¹⁵⁰ This was associated with decreased adhesion of activated T cells to the ASM cells.¹⁵⁰ In contrast, dexamethasone only partially inhibited early (1-hour) ICAM-1 expression induced by either TNF- α or IL-1, but it had no effect on late (24-hour) expression. In cells transfected with a luciferase reporter, dexamethasone did not affect TNF- α -induced NF- κB -dependent transcription of ICAM-1.¹⁵⁰

Migration of smooth muscle cells induced by inflammatory mediators may contribute to airway remodeling and also involves cell-cell interactions. Basal levels of human ASM cell migration were decreased by salmeterol (1 $\mu\text{mol/L}$) by approximately 40%, whereas the LABA had little effect on PDGF-induced migration of the cells.¹⁵¹ Treatment of cells with dexamethasone or FP also attenuated basal, but not PDGF-stimulated, ASM cell migration. However, the combination of salmeterol and FP significantly inhibited the PDGF-migratory response (Fig 11, A), indicating a synergistic interaction between the LABA and corticosteroids.¹⁵¹

ASM cell proliferation. Inhibition of mitogen-induced proliferation of ASM cells by corticosteroids or β_2 -agonists has been previously reported.^{152,153} 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 through an action

on a proteasome-dependent degradation pathway.¹⁵³ Alternatively, suppressed cyclin D₁ gene expression may occur through phosphorylation and transactivation of CRE binding protein, suggesting that the effect of cAMP is exerted by way of *cis*-repression of cyclin D₁ promoter.¹⁴⁶ Salmeterol (30 to 100 nmol/L) has been shown to inhibit thrombin-induced proliferation of human ASM cells, and this was associated with a reduction in c-myc RNA expression.¹⁵⁴ There was no effect, however, on other mitogens, such as EGF.

In human ASM, dexamethasone and FP have also been shown to arrest ASM cells in the G1 phase.¹⁵² Corticosteroids reduced thrombin-stimulated increases in cyclin D₁ protein and messenger RNA levels, and they attenuated retinoblastoma protein phosphorylation through a pathway either downstream of or parallel to the ERK cascade. Dexamethasone, however, has different inhibitory effects on ASM cell growth mediated by receptor tyrosine kinase and ASM cell growth mediated by GPCR.¹⁵²

More recent studies have shown that corticosteroids inhibit proliferation via activation of C/EBP- α , which has a pivotal role in cell proliferation.^{155,156} In particular, after interaction with the corticosteroid-GR complex, C/EBP- α activates p21^{WAF1/Cip1}, a cyclin-dependent kinase inhibitor that exerts a brake on cell cycling.¹⁵⁶ Through this mechanism, dexamethasone strongly inhibited cell proliferation to 48% of control at 10⁻⁷ mol/L. This effect was abolished if C/EBP- α was depleted by cDNA antisense pretreatment. Similarly, salmeterol reduced cell proliferation in a dose-dependent manner, with a maximum of 74% of control at 10⁻⁶ mol/L, and it was also associated with increased expression of p21^{WAF1/Cip1}. Incubation with antisense against C/EBP- α mRNA reduced the LABA-induced p21^{WAF1/Cip1} expression. When the 2 drugs were applied in combination, effects on p21^{WAF1/Cip1} expression and on cell proliferation were synergistic.

Budesonide (10⁻⁸ to 10⁻¹⁰ mol/L) induced activation of p21^{WAF1/Cip1} in human ASM cells stimulated with fetal bovine serum.¹⁵⁷ By contrast, formoterol (10⁻¹⁰ to 10⁻⁸ mol/L) had no significant effect. However, the combination of formoterol and budesonide was shown to inhibit serum-induced proliferation in a concentration-dependent (10⁻¹² to 10⁻⁸ mol/L) manner. A synergistic inhibitory effect was observed when both drugs were used at $\leq 10^{-10}$ mol/L compared with each drug alone. The effect of the combination of formoterol (10⁻¹⁰ mol/L) and budesonide (10⁻¹⁰ mol/L) was associated with enhanced nuclear translocation of GR, which was apparent within 30 minutes and persisted for 6 hours. The combination also resulted in stronger GR-GRE binding and greater DNA binding of C/EBP- α than with either drug alone, and in enhanced activation of p21^{WAF1/Cip1}. The activation of p21^{WAF1/Cip1} by the combination of formoterol and budesonide was reversed by the GR antagonist RU486 and by the β -blocker propranolol, confirming an effect mediated by receptor-driven pathways (Fig 11, B). Similarly, antisense oligonucleotides against either C/EBP- α or p21^{WAF1/Cip1} abrogated the enhanced anti-

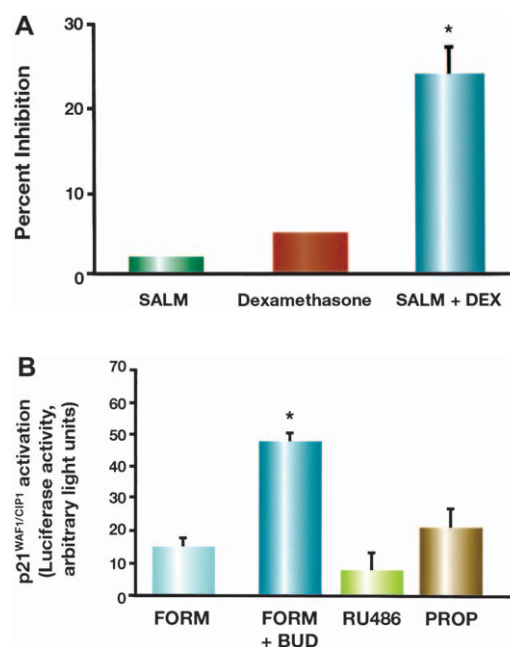


FIG 11. Effects of LABA/corticosteroid combinations on human ASM cell migration and proliferation. **(A)** The combination of salmeterol (SALM) and dexamethasone (DEX; 1 μ mol/L/10 μ mol/L) synergistically (* P < .05) inhibited PDGF-induced migration of human ASM cells compared with SALM (1 μ mol/L) or DEX (10 μ mol/L) alone. Data from Krymskaya et al.¹⁵¹ **(B)** The combination of formoterol (FORM) and budesonide (BUD; 0.1 nmol/L/0.1 nmol/L) significantly (* P < .01) increased p21^{WAF1/Cip1} activation (expressed as luciferase activity in arbitrary light units) in human ASM cells compared with FORM (0.1 nmol/L) alone. This effect was inhibited by the GR antagonist RU486 and by the β_2 AR antagonist propranolol (PROP). Adapted from Roth et al.¹⁵⁷

proliferative effects of the LABA/corticosteroid combination. These findings show that corticosteroids and LABAs both activate the GR and C/EBP- α and inhibit the proliferation of ASM via the action of p21^{WAF1/Cip1}.

SUMMARY AND IMPLICATIONS FOR FUTURE RESEARCH

Airway smooth muscle clearly can contribute to the formation of the ECM, and in this respect, ASM from asthmatic patients has both qualitative and quantitative differences in its response to stimuli. Thus, the ASM cells are important regulating cells potentially contributing to the known alterations within the ECM in asthma. In addition, through integrin-directed signaling, ECM components can change human ASM function, altering the proliferative, survival, and cytoskeletal synthetic function. The changes in the ECM in asthma, with enhanced collagen I and V deposition along with increased airway fibronectin, alter the behavior of human ASM, forming a proliferative phenotype with defective cytoskeletal organization. How this influences the contractile potential of the muscle remains unknown. Also incompletely defined is the influence of the change in the ECM

matrix—in particular, the changes related to ASM activation in asthma—on the contractile responsiveness of the ASM. It might be anticipated that enhanced deposition of collagen adjacent to the muscle increases the resistive load on the muscle and opposes the effects of ASM shortening. However, mechanical strain on bovine ASM has been shown to decrease the proliferative response,¹⁵⁸ and in view of the reciprocal findings identified, it may thus increase the synthesis of contractile proteins, enabling the ASM to overcome the effects of this resistive strain. Such changes would increase the contractile force of the ASM. In addition, human ASM-related changes in the ECM external to the ASM may uncouple ASM from the parenchyma and allow exaggerated airway narrowing.¹⁵⁹ Although endobronchial biopsies of large airways in asthma provide information relative to the airway wall from the epithelium to the ASM, they do not provide information relevant to the ASM-parenchyma/cartilage interface. Such information is obtainable only from analysis of postmortem or resected lung tissue.

The finding that ECM components influence not only human ASM function but also pharmacologic responsiveness raises different questions, particularly regarding mechanisms of resistance to steroid therapy and the nature of more severe asthma. Further exploration is required in these areas, and it will also be necessary to understand better the integrin-related signaling relevant to these and other facets of ECM-ASM signaling. The synthetic capabilities of ASM and its interaction with ECM suggest that these components of the airways cannot be considered in isolation when investigating the pathophysiology of asthma.

The production of chemokines by structural cells such as ASM cells may provide a mechanism whereby signals generated by inflammatory cells are amplified to produce an exaggerated inflammatory response. Agents capable of secreting chemokines activate distinct sets of kinases and transcription factors to mediate their effects. Inhibition of the pathways used by these cells in chemokine and cytokine production may provide novel therapeutic targets for the treatment of asthma and COPD.

It is clear that smooth muscle cells in other sites in the body exhibit functional heterogeneity, and it has been suggested that the same may be true in the airway. Studies from animal ASM cells that have been serum-deprived for prolonged periods show differentiation of these cells into 2 distinct populations: an elongated hypercontractile cell that stains more strongly for smooth muscle contractile proteins and a shorter, less contractile phenotype (reviewed in Hirst et al¹⁶⁰). It is unknown whether ASM cells in normal and asthmatic airways *in vivo* exhibit similar heterogeneity and whether the phenotype differs between normal and asthmatic airways, and these questions deserve further study. Interesting in this respect is a recent study comparing asthmatic with normal ASM cells that suggests that proliferation of asthmatic cells may be enhanced.¹⁶¹

Emerging evidence, mostly from reports using cultured ASM cells or isolated tracheal or bronchial preparations,

shows that a variety of stimuli indirectly modulate ASM synthetic and contractile functions via the autocrine action of secreted inflammatory mediators, including T_H2 cytokines, growth factors, and COX-2-dependent prostanoids. It is likely that a better understanding of the autocrine mechanisms regulating ASM cell function will have an effect on the development of new therapeutic drugs for the treatment of inflammatory airway diseases such as asthma. Additional studies are needed to determine the importance of the signaling crosstalk that may exist between stimuli and autocrine mediators.

Airway smooth muscle cells contain both β_2 ARs and GR and are a potential target for β_2 -agonist/corticosteroid interactions. With LABA/corticosteroid combinations, there is evidence of additive and/or synergistic inhibition of inflammatory mediator release and the migration and proliferation of ASM cells. These effects may have relevance to the clinical efficacy of LABA/ICS combination therapy in asthma and COPD.

Dr Knox thanks Dan Duthie for help with graphics.

REFERENCES

1. Thomson NC. Neurogenic and myogenic mechanisms of nonspecific bronchial hyperresponsiveness. *Eur J Respir Dis Suppl* 1983;128:206-12.
2. Holgate ST, Beasley R, Twentyman OP. The pathogenesis and significance of bronchial hyper-responsiveness in airways disease. *Clin Sci (Lond)* 1987;73:561-72.
3. Johnson SR, Knox AJ. Synthetic functions of airway smooth muscle in asthma. *Trends Pharmacol Sci* 1997;18:288-92.
4. Johnson PR, Black JL, Carlin S, Ge Q, Underwood PA. The production of extracellular matrix proteins by human passively sensitized airway smooth-muscle cells in culture: the effect of beclomethasone. *Am J Respir Crit Care Med* 2000;162:2145-51.
5. Coutts A, Chen G, Stephens N, Hirst S, Douglas D, Eichholtz T, et al. Release of biologically active TGF-beta from airway smooth muscle cells induces autocrine synthesis of collagen. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L999-1008.
6. Panettieri RA, Tan EM, Ciocka V, Luttmann MA, Leonard TB, Hay DW. Effects of LTD4 on human airway smooth muscle cell proliferation, matrix expression, and contraction in vitro: differential sensitivity to cysteinyl leukotriene receptor antagonists. *Am J Respir Cell Mol Biol* 1998;19:453-61.
7. Burgess JK, Johnson PR, Ge Q, Au WW, Poniris MH, McParland BE, et al. Expression of connective tissue growth factor in asthmatic airway smooth muscle cells. *Am J Respir Crit Care Med* 2003;167:71-7.
8. Potter-Perigo S, Baker C, Tsoi C, Braun KR, Isenath S, Altman GM, et al. Regulation of proteoglycan synthesis by leukotriene d4 and epidermal growth factor in bronchial smooth muscle cells. *Am J Respir Cell Mol Biol* 2004;30:101-8.
9. Foda HD, George S, Rollo E, Drews M, Conner C, Cao J, et al. Regulation of gelatinases in human airway smooth muscle cells: mechanism of progelatinase A activation. *Am J Physiol* 1999;277:L174-82.
10. Johnson S, Knox A. Autocrine production of matrix metalloproteinase-2 is required for human airway smooth muscle proliferation. *Am J Physiol* 1999;277:L1109-17.
11. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002;346:1699-705.
12. Billings PC, Whitbeck JC, Adams CS, Abrams WR, Cohen AJ, Engelsberg BN, et al. The transforming growth factor-beta-inducible matrix protein (beta)ig-h3 interacts with fibronectin. *J Biol Chem* 2002;277:28003-9.

13. Ferguson JW, Thoma BS, Mikesh MF, Kramer RH, Bennett KL, Purchio A, et al. The extracellular matrix protein betaG-H3 is expressed at myotendinous junctions and supports muscle cell adhesion. *Cell Tissue Res* 2003;313:93-105.
14. Gibson MA, Kumaratilake JS, Cleary EG. Immunohistochemical and ultrastructural localization of MP78/70 (betaig-h3) in extracellular matrix of developing and mature bovine tissues. *J Histochem Cytochem* 1997;45:1683-96.
15. Gibson MA, Hatzinikolas G, Kumaratilake JS, Sandberg LB, Nicholl JK, Sutherland GR, et al. Further characterization of proteins associated with elastic fiber microfibrils including the molecular cloning of MAGP-2 (MP25). *J Biol Chem* 1996;271:1096-103.
16. Hashimoto K, Noshiro M, Ohno S, Kawamoto T, Satakeda H, Akagawa Y, et al. Characterization of a cartilage-derived 66-kDa protein (RGD-CAP/beta ig-h3) that binds to collagen. *Biochim Biophys Acta* 1997;1355:303-14.
17. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;110:673-87.
18. Danen EH, Sonnenberg A. Integrins in regulation of tissue development and function. *J Pathol* 2003;200:471-80.
19. Hirst SJ, Twort CH, Lee TH. Differential effects of extracellular matrix proteins on human airway smooth muscle cell proliferation and phenotype. *Am J Respir Cell Mol Biol* 2000;23:335-44.
20. Freyer AM, Johnson SR, Hall IP. Effects of growth factors and extracellular matrix on survival of human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 2001;25:569-76.
21. Rizzo-Vasquez Y, Spina D. Role of cytokines and chemokines in bronchial hyperresponsiveness and airway inflammation. *Pharmacol Ther* 2002;94:185-211.
22. Huang CD, Tliba O, Panettieri RA Jr, Amrani Y. Bradykinin induces interleukin-6 production in human airway smooth muscle cells: modulation by Th2 cytokines and dexamethasone. *Am J Respir Cell Mol Biol* 2003;28:330-8.
23. Zhu YM, Bradbury DA, Pang L, Knox AJ. Transcriptional regulation of interleukin (IL)-8 by bradykinin in human airway smooth muscle cells involves prostanoid-dependent activation of AP-1 and nuclear factor (NF)-IL-6 and prostanoid-independent activation of NF-kappaB. *J Biol Chem* 2003;278:29366-75.
24. Nie M, Pang L, Inoue H, Knox AJ. Transcriptional regulation of cyclooxygenase 2 by bradykinin and interleukin-1beta in human airway smooth muscle cells: involvement of different promoter elements, transcription factors, and histone h4 acetylation. *Mol Cell Biol* 2003;23:9233-44.
25. Tran T, Stewart AG. Protease-activated receptor (PAR)-independent growth and pro-inflammatory actions of thrombin on human cultured airway smooth muscle. *Br J Pharmacol* 2003;138:865-75.
26. Maruoka S, Hashimoto S, Gon Y, Takeshita I, Horie T. PAF-induced RANTES production by human airway smooth muscle cells requires both p38 MAP kinase and Erk. *Am J Respir Crit Care Med* 2000;161:922-9.
27. Zhong H, Belardinelli L, Maa T, Feoktistov I, Biaggioni I, Zeng D. A2B adenosine receptors increase cytokine release by bronchial smooth muscle cells. *Am J Respir Cell Mol Biol* 2004;30:118-25.
28. Pang L, Knox AJ. Regulation of TNF-alpha-induced eotaxin release from cultured human airway smooth muscle cells by beta2-agonists and corticosteroids. *FASEB J* 2001;15:261-9.
29. Chung KF, Patel HJ, Fadlon EJ, Rousell J, Haddad EB, Jose PJ, et al. Induction of eotaxin expression and release from human airway smooth muscle cells by IL-1beta and TNFalpha: effects of IL-10 and corticosteroids. *Br J Pharmacol* 1999;127:1145-50.
30. Lazzeri N, Belvisi MG, Patel HJ, Yacoub MH, Fan Chung K, Mitchell JA. Effects of prostaglandin E2 and cAMP elevating drugs on GM-CSF release by cultured human airway smooth muscle cells: relevance to asthma therapy. *Am J Respir Cell Mol Biol* 2001;24:44-8.
31. Ghaffar O, Hamid Q, Renzi PM, Allakhverdi Z, Molet S, Hogg JC, et al. Constitutive and cytokine-stimulated expression of eotaxin by human airway smooth muscle cells. *Am J Respir Crit Care Med* 1999;159:1933-42.
32. Wuyts WA, Vanaudenaerde BM, Dupont LJ, Demedts MG, Verleden GM. Modulation by cAMP of IL-1beta-induced eotaxin and MCP-1 expression and release in human airway smooth muscle cells. *Eur Respir J* 2003;22:220-6.
33. Moore PE, Church TL, Chism DD, Panettieri RA Jr Shore SA. IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L847-53.
34. John M, Hirst SJ, Jose PJ, Robichaud A, Berkman N, Witt C, et al. Human airway smooth muscle cells express and release RANTES in response to T helper 1 cytokines: regulation by T helper 2 cytokines and corticosteroids. *J Immunol* 1997;158:1841-7.
35. Lazzeri N, Belvisi MG, Patel HJ, Chung KF, Yacoub MH, Mitchell JA. RANTES release by human airway smooth muscle: effects of prostaglandin E(2) and fenoterol. *Eur J Pharmacol* 2001;433:231-5.
36. Ammit AJ, Hoffman RK, Amrani Y, Lazaar AL, Hay DW, Torphy TJ, et al. Tumor necrosis factor-alpha-induced secretion of RANTES and interleukin-6 from human airway smooth-muscle cells: modulation by cyclic adenosine monophosphate. *Am J Respir Cell Mol Biol* 2000;23:794-802.
37. Oltmanns U, Issa R, Sukkar MB, John M, Chung KF. Role of c-jun N-terminal kinase in the induced release of GM-CSF, RANTES and IL-8 from human airway smooth muscle cells. *Br J Pharmacol* 2003;139:1228-34.
38. Pye JL, Dupont LJ, Menten P, Van Coillie E, Opendakker G, Van Damme J, et al. Expression of monocyte chemotactic protein (MCP)-1, MCP-2, and MCP-3 by human airway smooth-muscle cells: modulation by corticosteroids and T-helper 2 cytokines. *Am J Respir Cell Mol Biol* 1999;21:528-36.
39. Watson ML, Grix SP, Jordan NJ, Place GA, Dodd S, Leithead J, et al. Interleukin 8 and monocyte chemoattractant protein 1 production by cultured human airway smooth muscle cells. *Cytokine* 1998;10:346-52.
40. Pang L, Knox AJ. Synergistic inhibition by beta(2)-agonists and corticosteroids on tumor necrosis factor-alpha-induced interleukin-8 release from cultured human airway smooth-muscle cells. *Am J Respir Cell Mol Biol* 2000;23:79-85.
41. John M, Au BT, Jose PJ, Lim S, Saunders M, Barnes PJ, et al. Expression and release of interleukin-8 by human airway smooth muscle cells: inhibition by Th-2 cytokines and corticosteroids. *Am J Respir Cell Mol Biol* 1998;18:84-90.
42. Pang L, Knox AJ. Bradykinin stimulates IL-8 production in cultured human airway smooth muscle cells: role of cyclooxygenase products. *J Immunol* 1998;161:2509-15.
43. Faffe DS, Whitehead T, Moore PE, Baraldo S, Flynt L, Bourgeois K, et al. IL-13 and IL-4 promote TARC release in human airway smooth muscle cells: role of IL-4 receptor genotype. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L907-14.
44. Ammit AJ, Bekir SS, Johnson PR, Hughes JM, Armour CL, Black JL. Mast cell numbers are increased in the smooth muscle of human sensitized isolated bronchi. *Am J Respir Crit Care Med* 1997;155:1123-9.
45. Hamad AM, Pang LH, Knox AJ. TGF-beta stimulates stem cell factor production by human airways smooth muscle cells. *Am J Respir Crit Care Med* 2003;167:A690.
46. Saunders MA, Mitchell JA, Seldon PM, Yacoub MH, Barnes PJ, Gienbycz MA, et al. Release of granulocyte-macrophage colony stimulating factor by human cultured airway smooth muscle cells: suppression by dexamethasone. *Br J Pharmacol* 1997;120:545-6.
47. Song R, Ning W, Liu F, Ameredes BT, Calhoun WJ, Otterbein LE, et al. Regulation of IL-1beta -induced GM-CSF production in human airway smooth muscle cells by carbon monoxide. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L50-6.
48. Elias JA, Wu Y, Zheng T, Panettieri R. Cytokine- and virus-stimulated airway smooth muscle cells produce IL-11 and other IL-6-type cytokines. *Am J Physiol* 1997;273:L648-55.
49. Ammit AJ, Lazaar AL, Irani C, O'Neill GM, Gordon ND, Amrani Y, et al. Tumor necrosis factor-alpha-induced secretion of RANTES and interleukin-6 from human airway smooth muscle cells: modulation by glucocorticoids and beta-agonists. *Am J Respir Cell Mol Biol* 2002;26:465-74.
50. Amrani Y, Ammit AJ, Panettieri RA Jr. Tumor necrosis factor receptor (TNFR) 1 but not TNFR2, mediates tumor necrosis factor-alpha-induced interleukin-6 and RANTES in human airway smooth muscle cells: role of p38 and p42/44 mitogen-activated protein kinases. *Mol Pharmacol* 2001;60:646-55.

51. McKay S, Bromhaar MM, de Jongste JC, Hoogsteden HC, Saxena PR, Sharma HS. Pro-inflammatory cytokines induce c-fos expression followed by IL-6 release in human airway smooth muscle cells. *Mediators Inflamm* 2001;10:135-42.
52. Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene* 2002;285:1-24.
53. Wuyts WA, Vanaudenaerde BM, Dupont LJ, Demedts MG, Verleden GM. Involvement of p38 MAPK, JNK, p42/p44 ERK and NF-kappaB in IL-1beta-induced chemokine release in human airway smooth muscle cells. *Respir Med* 2003;97:811-7.
54. Bradbury DA, Newton R, Zhu YM, El-Haroun H, Corbett L, Knox AJ. Cyclooxygenase-2 induction by bradykinin in human pulmonary artery smooth muscle cells is mediated by the cyclic AMP response element through a novel autocrine loop involving endogenous prostaglandin E2, E-prostanoid 2 (EP2), and EP4 receptors. *J Biol Chem* 2003;278:49954-64.
55. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol* 2002;20:55-72.
56. Hallsworth MP, Moir LM, Lai D, Hirst SJ. Inhibitors of mitogen-activated protein kinases differentially regulate eosinophil-activating cytokine release from human airway smooth muscle. *Am J Respir Crit Care Med* 2001;164:688-97.
57. Hamad AM, Clayton A, Islam B, Knox AJ. Guanylyl cyclases, nitric oxide, natriuretic peptides, and airway smooth muscle function. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L973-83.
58. Wuyts WA, Vanaudenaerde BM, Dupont LJ, Demedts MG, Verleden GM. N-acetylcysteine reduces chemokine release via inhibition of p38 MAPK in human airway smooth muscle cells. *Eur Respir J* 2003;22:43-9.
59. Chung KF, Barnes PJ. Cytokines in asthma. *Thorax* 1999;54:825-57.
60. Renaud JC. New insights into the role of cytokines in asthma. *J Clin Pathol* 2001;54:577-89.
61. Holgate S, Davies D, Lackie P, Wilson S, Puddicombe S, Lordan J. Epithelial-mesenchymal interactions in the pathogenesis of asthma. *J Allergy Clin Immunol* 2000;105:193-204.
62. Amrani Y, Chen H, Panettieri RA Jr. Activation of tumor necrosis factor receptor 1 in airway smooth muscle: a potential pathway that modulates bronchial hyper-responsiveness in asthma? *Respir Res* 2000;1:49-53.
63. Hallsworth MP, Soh CP, Twort CH, Lee TH, Hirst SJ. Cultured human airway smooth muscle cells stimulated by interleukin-1beta enhance eosinophil survival. *Am J Respir Cell Mol Biol* 1998;19:910-9.
64. Chung K. Airway smooth muscle cells: contributing to and regulating airway mucosal inflammation? *Eur Respir J* 2000;15:961-8.
65. Hakonarson H, Maskeri N, Carter C, Grunstein MM. Regulation of TH1- and TH2-type cytokine expression and action in atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 1999;103:1077-87.
66. Hirst SJ, Hallsworth MP, Peng Q, Lee TH. Selective induction of eotaxin release by interleukin-13 or interleukin-4 in human airway smooth muscle cells is synergistic with interleukin-1beta and is mediated by the interleukin-4 receptor alpha-chain. *Am J Respir Crit Care Med* 2002;165:1161-71.
67. Laporte JC, Moore PE, Baraldo S, Jouvin MH, Church TL, Schwartzman IN, et al. Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. *Am J Respir Crit Care Med* 2001;164:141-8.
68. Grunstein MM, Hakonarson H, Leiter J, Chen M, Whelan R, Grunstein JS, et al. IL-13-dependent autocrine signaling mediates altered responsiveness of IgE-sensitized airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L520-8.
69. Amrani Y, Panettieri RJ, Frossard N, Bronner C. Activation of the TNF alpha-p55 receptor induces myocyte proliferation and modulates agonist-evoked calcium transients in cultured human tracheal smooth muscle cells. *Am J Respir Cell Mol Biol* 1996;15:55-63.
70. Amrani Y, Ammit AJ, Panettieri RA Jr. Tumor necrosis factor receptor (TNFR) 1, but not TNFR2, mediates tumor necrosis factor-alpha-induced interleukin-6 and RANTES in human airway smooth muscle cells: role of p38 and p42/44 mitogen-activated protein kinases. *Mol Pharmacol* 2001;60:646-55.
71. McFarlane S, Jupp O, Cobban H, Hunter I, Anderson H, Vandenabeele P, et al. Stimulation of stress-activated but not mitogen-activated protein kinases by tumor necrosis factor receptor subtypes in airway smooth muscle. *Biochem Pharmacol* 2001;61:749-59.
72. Amrani Y, Lazaar AL, Hoffman R, Amin K, Ousmer S, Panettieri RA Jr. Activation of p55 tumor necrosis factor-alpha receptor-1 coupled to tumor necrosis factor receptor-associated factor 2 stimulates intercellular adhesion molecule-1 expression by modulating a thapsigargin-sensitive pathway in human tracheal smooth muscle cells. *Mol Pharmacol* 2000;58:237-45.
73. Chen G, Grotendorst G, Eichholtz T, Khalil N. GM-CSF increases airway smooth muscle cell connective tissue expression by inducing TGF-beta receptors. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L548-56.
74. Chen G, Khalil N. In vitro wounding of airway smooth muscle cell monolayers increases expression of TGF-beta receptors. *Respir Physiol Neurobiol* 2002;132:341-6.
75. Knox AJ, Corbett L, Stocks J, Holland E, Zhu YM, Pang L. Human airway smooth muscle cells secrete vascular endothelial growth factor: up-regulation by bradykinin via a protein kinase C and prostanoid-dependent mechanism. *FASEB J* 2001;15:2480-8.
76. Pang L, Knox AJ. PGE2 release by bradykinin in human airway smooth muscle cells: involvement of cyclooxygenase-2 induction. *Am J Physiol* 1997;273:L1132-40.
77. Huang CD, Tliba O, Panettieri RA Jr, Amrani Y. Bradykinin induces interleukin-6 production in human airway smooth muscle cells: modulation by Th2 cytokines and dexamethasone. *Am J Respir Cell Mol Biol* 2003;28:330-8.
78. Shore SA, Moore PE. Effects of cytokines on contractile and dilator responses of airway smooth muscle. *Clin Exp Pharmacol Physiol* 2002;29:859-66.
79. Vlahos R, Stewart AG. Interleukin-1 {alpha} and tumor necrosis factor-{alpha} modulate airway smooth muscle DNA synthesis by induction of cyclo-oxygenase-2: inhibition by dexamethasone and fluticasone propionate. *Br J Pharmacol* 1999;126:1315-24.
80. Bonazzi A, Bolla M, Buccellati C, Hernandez A, Zarini S, Vigano T, et al. Effect of endogenous and exogenous prostaglandin E(2) on interleukin-1 beta-induced cyclooxygenase-2 expression in human airway smooth-muscle cells. *Am J Respir Crit Care Med* 2000;162:2272-7.
81. Fong CY, Pang L, Holland E, Knox AJ. TGF-beta1 stimulates IL-8 release, COX-2 expression, and PGE(2) release in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L201-7.
82. Stirling RG, Chung KF. New immunological approaches and cytokine targets in asthma and allergy. *Eur Respir J* 2000;16:1158-74.
83. Grunstein MM, Hakonarson H, Leiter J, Chen M, Whelan R, Grunstein JS, et al. Autocrine signaling by IL-10 mediates altered responsiveness of atopic sensitized airway smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L1130-7.
84. Grunstein MM, Hakonarson H, Hodinka RL, Maskeri N, Kim C, Chuang S. Mechanism of cooperative effects of rhinovirus and atopic sensitization on airway responsiveness. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L229-38.
85. Hakonarson H, Herrick DJ, Serrano PG, Grunstein MM. Autocrine role of interleukin 1beta in altered responsiveness of atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 1997;99:117-24.
86. Hakonarson H, Maskeri N, Carter C, Chuang S, Grunstein MM. Autocrine interaction between IL-5 and IL-1beta mediates altered responsiveness of atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 1999;104:657-67.
87. Hakonarson H, Grunstein MM. Autocrine regulation of airway smooth muscle responsiveness. *Respir Physiol Neurobiol* 2003;137:263-76.
88. Amrani Y, Panettieri RA Jr. Modulation of calcium homeostasis as a mechanism for altering smooth muscle responsiveness in asthma. *Curr Opin Allergy Clin Immunol* 2002;2:39-45.
89. Chen H, Tliba O, Van Besien CR, Panettieri RA Jr, Amrani Y. Selected contribution: TNF-{alpha} modulates murine tracheal rings responsiveness to G-protein-coupled receptor agonists and KCl. *J Appl Physiol* 2003;95:864-72.
90. Parris JR, Cobban HJ, Littlejohn AF, MacEwan DJ, Nixon GF. Tumor necrosis factor-alpha activates a calcium sensitization pathway in guinea-pig bronchial smooth muscle. *J Physiol* 1999;518:561-9.
91. Hunter I, Cobban HJ, Vandenabeele P, MacEwan DJ, Nixon GF. Tumor necrosis factor-alpha-induced activation of RhoA in airway smooth

- muscle cells: role in the Ca(2+) sensitization of myosin light chain(20) phosphorylation. *Mol Pharmacol* 2003;63:714-21.
92. Tliba O, Deshpande D, Van Besien C, Chen H, Kannan M, Panettieri RA, et al. IL-13 enhances agonist-evoked calcium signals and contractile responses in airway smooth muscle. *Br J Pharmacol* 2003; 140:1159-62.
93. Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and its role in asthma. *Pulm Pharmacol Ther* 2003;16:181-96.
94. Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, et al. Transforming growth factor beta 1 (TGF beta 1) gene expression by eosinophils in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1996;15:404-9.
95. Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17: 326-33.
96. McKay S, de Jongste JC, Saxena PR, Sharma HS. Angiotensin II induces hypertrophy of human airway smooth muscle cells: expression of transcription factors and transforming growth factor-beta1. *Am J Respir Cell Mol Biol* 1998;18:823-33.
97. Black PN, Young PG, Skinner SJ. Response of airway smooth muscle cells to TGF-beta 1: effects on growth and synthesis of glycosaminoglycans. *Am J Physiol* 1996;271:L910-7.
98. Cohen MD, Ciocca V, Panettieri RA Jr. TGF-beta 1 modulates human airway smooth-muscle cell proliferation induced by mitogens. *Am J Respir Cell Mol Biol* 1997;16:85-90.
99. Cohen P, Rajah R, Rosenbloom J, Herrick DJ. IGFBP-3 mediates TGF-beta1-induced cell growth in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L545-51.
100. Ishikawa T, Kume H, Kondo M, Ito Y, Yamaki K, Shimokata K. Inhibitory effects of interferon-gamma on the heterologous desensitization of beta-adrenoceptors by transforming growth factor-beta 1 in tracheal smooth muscle. *Clin Exp Allergy* 2003;33:808-15.
101. Espinosa K, Bosse Y, Stankova J, Rola-Pleszczynski M. CysLT1 receptor upregulation by TGF-beta and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD4. *J Allergy Clin Immunol* 2003;111:1032-40.
102. Wen F-Q, Liu X, Manda W, Terasaki Y, Kobayashi T, Abe S, et al. T(H)2 Cytokine-enhanced and TGF-[beta]-enhanced vascular endothelial growth factor production by cultured human airway smooth muscle cells is attenuated by IFN-[gamma] and corticosteroids. *J Allergy Clin Immunol* 2003;111:1307-18.
103. Liu KD, Gaffen SL, Goldsmith MA. JAK/STAT signaling by cytokine receptors. *Curr Opin Immunol* 1998;10:271-8.
104. Darnell JE Jr. STATs and gene regulation. *Science* 1997;277:1630-5.
105. Horvath CM. STAT proteins and transcriptional responses to extracellular signals. *Trends Biochem Sci* 2000;25:496-502.
106. Hoey T, Schindler U. STAT structure and function in signaling. *Curr Opin Genet Dev* 1998;8:582-7.
107. Sampath D, Castro M, Look D, Holtzman M. Constitutive activation of an epithelial signal transducer and activator of transcription (STAT) pathway in asthma. *J Clin Invest* 1999;103:1353-6.
108. Simon AR, Takahashi S, Severgnini M, Fanburg BL, Cochran BH. Role of the JAK-STAT pathway in PDGF-stimulated proliferation of human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2002; 282:L1296-304.
109. Belvisi MG, Saunders MA, Haddad el B, Hirst SJ, Yacoub MH, Barnes PJ, et al. Induction of cyclo-oxygenase-2 by cytokines in human cultured airway smooth muscle cells: novel inflammatory role of this cell type. *Br J Pharmacol* 1997;120:910-6.
110. Pang L, Knox AJ. Effect of interleukin-1 beta, tumour necrosis factor-alpha and interferon-gamma on the induction of cyclo-oxygenase-2 in cultured human airway smooth muscle cells. *Br J Pharmacol* 1997;121:579-87.
111. John M, Au BT, Jose PJ, Lim S, Saunders M, Barnes PJ, et al. Expression and release of interleukin-8 by human airway smooth muscle cells: inhibition by Th-2 cytokines and corticosteroids. *Am J Respir Cell Mol Biol* 1998;18:84-90.
112. John M, Hirst SJ, Jose PJ, Robichaud A, Berkman N, Witt C, et al. Human airway smooth muscle cells express and release RANTES in response to T helper 1 cytokines: regulation by T helper 2 cytokines and corticosteroids. *J Immunol* 1997;158:1841-7.
113. Saunders MA, Mitchell JA, Seldon PM, Yacoub MH, Barnes PJ, Gienbycz MA, et al. Release of granulocyte-macrophage colony stimulating factor by human cultured airway smooth muscle cells: suppression by dexamethasone. *Br J Pharmacol* 1997;120:545-6.
114. Lazaar AL, Albelda SM, Pilewski JM, Brennan B, Pure E, Panettieri RA Jr. T lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle cell DNA synthesis. *J Exp Med* 1994;180:807-16.
115. Amrani Y, Moore PE, Hoffman R, Shore SA, Panettieri RA Jr. Interferon-gamma modulates cysteinyl leukotriene receptor-1 expression and function in human airway myocytes. *Am J Respir Crit Care Med* 2001;164:2098-101.
116. Chen H, Munakata M, Amishima M, Ukita H, Masaki Y, Homma Y, et al. Gamma-interferon modifies guinea pig airway functions in vitro. *Eur Respir J* 1994;7:74-80.
117. Tliba O, Tliba S, Huang CD, Hoffman RK, DeLong P, Panettieri RA, et al. TNFalpha modulates airway smooth muscle function via the autocrine action of IFNbeta. *J Biol Chem* 2003;278:50615-23.
118. Guo D, Dunbar JD, Yang CH, Pfeffer LM, Donner DB. Induction of Jak/STAT signaling by activation of the type 1 TNF receptor. *J Immunol* 1998;160:2742-50.
119. Wang Y, Wu TR, Cai S, Welte T, Chin YE. Stat1 as a component of tumor necrosis factor alpha receptor 1-TRADD signaling complex to inhibit NF-kappa B activation. *Mol Cell Biol* 2000;20:4505-12.
120. Kovarik P, Stoiber D, Eysers PA, Menghini R, Neining A, Gaestel M, et al. Stress-induced phosphorylation of STAT1 at Ser727 requires p38 mitogen-activated protein kinase whereas IFN-gamma uses a different signaling pathway. *Proc Natl Acad Sci U S A* 1999;96:13956-61.
121. Condeelis JJ, Goldstein S, Kalberg C, Yancey S, Emmett A, Rickard K. The addition of salmeterol to fluticasone propionate versus increasing the dose of fluticasone propionate in patients with persistent asthma. Salmeterol Study Group. *Ann Allergy Asthma Immunol* 1999;82:383-9.
122. Szafranski W, Cukier A, Ramirez A, Menga G, Sansores R, Nahabedian S, et al. Efficacy and safety of budesonide/formoterol in the management of chronic obstructive pulmonary disease. *Eur Respir J* 2003;21:74-81.
123. Myo S, Zhu X, Myou S, Boetticher E, Meliton AY, Lambertino AT, et al. Additive inhibition of fluticasone propionate (FP) and salmeterol on beta(2)-integrin-mediated adhesion to human eosinophils. *Am J Respir Crit Care Med* 2003;167:A754.
124. Edwards MR, Johnson M, Johnston SL. Synergistic effects of salmeterol and fluticasone in human rhinovirus-induced pro-inflammatory cytokine production. *Am J Respir Crit Care Med* 2003;167:A399.
125. Johnson M. The beta-adrenoceptor. *Am J Respir Crit Care Med* 1998; 158:S146-53.
126. Johnson M, Coleman RA. Mechanisms of action of beta(2)-adrenoceptor agonists. In: Busse WW, Holgate ST, editors. *Asthma and rhinitis*. Boston: Blackwell Scientific Publications; 1995. p. 1278-95.
127. Montminy MR, Gonzalez GA, Yamamoto KK. Regulation of cAMP-inducible genes by CREB. *Trends Neurosci* 1990;13:184-8.
128. Daaka Y, Luttrell LM, Lefkowitz RJ. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* 1997;390:88-91.
129. Johnson M. Mechanisms of action of beta(2)-adrenoceptor agonists. In: Busse WW, Holgate ST, editors. *Asthma and rhinitis*. Oxford: Blackwell Scientific Publications; 2000. p. 1541-57.
130. Anderson GP. Formoterol: pharmacology, molecular basis of agonism, and mechanism of long duration of a highly potent and selective beta 2-adrenoceptor agonist bronchodilator. *Life Sci* 1993;52:2145-60.
131. Barnes PJ. Molecular mechanisms of steroid action in asthma. *J Allergy Clin Immunol* 1996;97:159-68.
132. Adcock IM, Ito K. Molecular mechanisms of corticosteroid actions. *Monaldi Arch Chest Dis* 2000;55:256-66.
133. Mak JC, Nishikawa M, Barnes PJ. Glucocorticosteroids increase beta 2-adrenergic receptor transcription in human lung. *Am J Physiol* 1995; 268:L41-6.
134. Baraniuk JN, Ali M, Brody D, Maniscalco J, Gaumond E, Fitzgerald T, et al. Glucocorticoids induce beta2-adrenergic receptor function in human nasal mucosa. *Am J Respir Crit Care Med* 1997;155:704-10.
135. Brodde OE, Howe U, Egerszegi S, Konietzko N, Michel MC. Effect of prednisolone and ketotifen on beta 2-adrenoceptors in asthmatic patients receiving beta 2-bronchodilators. *Eur J Clin Pharmacol* 1988;34:145-50.

136. Mak JC, Nishikawa M, Shirasaki H, Miyayasu K, Barnes PJ. Protective effects of a glucocorticoid on downregulation of pulmonary beta 2-adrenergic receptors in vivo. *J Clin Invest* 1995;96:99-106.
137. Koto H, Mak JC, Haddad EB, Xu WB, Salmon M, Barnes PJ, et al. Mechanisms of impaired beta-adrenoceptor-induced airway relaxation by interleukin-1beta in vivo in the rat. *J Clin Invest* 1996;98:1780-7.
138. Johnson M. Combination therapy for asthma: complementary effects of long-acting beta(2)-agonists and corticosteroids. *Curr Opin Allergy Clin Immunol* 2002;15:16-22.
139. Adcock IM, Maneechotesuwan K, Usmani O. Molecular interactions between glucocorticoids and long-acting beta2-agonists. *J Allergy Clin Immunol* 2002;110:S261-8.
140. Latif ML, Hill SJ. Salmeterol induction of mitogen-activated protein kinase (MAPK) in transfected HEK cells. *Br J Pharmacol* 1998;125:717-26.
141. Adcock I, Maneechotesuwan K, Usmani O. Effects of LABA on GR function: possible role of GR phosphorylation. *J Allergy Clin Immunol* 2002;110:S265-6.
142. Eickelberg O, Roth M, Lox R, Bruce V, Rudiger J, Johnson M, et al. Ligand-independent activation of the glucocorticoid receptor by beta2-adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. *J Biol Chem* 1999;274:1005-10.
143. Roth M, Rudiger J, Bihl M, Leufgen H, Cornelius B, Genacy M, et al. The beta-2-agonist formoterol activates the glucocorticoid receptor in vivo. *Eur Respir J* 2000;16:437S.
144. Usmani O, Maneechotesuwan K, Adcock I, Barnes PJ. Glucocorticoid receptor activation following inhaled fluticasone and salmeterol. *Am J Respir Crit Care Med* 2002;165:A616.
145. Leufgen H, Rudiger J, Herrmann M, Mayar I, Soler M, Perruchoud M, et al. Glucocorticoid receptor activation by steroids and long-acting beta(2)-agonists in vivo: a double blind crossover study. *Am J Respir Crit Care Med* 2002;165:A618.
146. Panettieri RA Jr. Airway smooth muscle: an immunomodulatory cell. *J Allergy Clin Immunol* 2002;110:S269-74.
147. Hallsworth MP, Twort CH, Lee TH, Hirst SJ. Beta(2)-adrenoceptor agonists inhibit release of eosinophil-activating cytokines from human airway smooth muscle cells. *Br J Pharmacol* 2001;132:729-41.
148. Korn SH, Jerre A, Brattsand R. Effects of formoterol and budesonide on GM-CSF and IL-8 secretion by triggered human bronchial epithelial cells. *Eur Respir J* 2001;17:1070-7.
149. Corbett L, Stocks J, Pang L, Knox AJ. TGF-beta increases VEGF release from cultured human airway smooth muscle cells: regulation by steroids and beta agonists. *Am J Respir Crit Care Med* 2002;165:A114.
150. Panettieri RA Jr, Lazaar AL, Pure E, Albelda SM. Activation of cAMP-dependent pathways in human airway smooth muscle cells inhibits TNF-alpha-induced ICAM-1 and VCAM-1 expression and T-lymphocyte adhesion. *J Immunol* 1995;154:2538-65.
151. Krymskaya VP, Goncharova EA, Panettieri RA. CAMP-mobilizing and anti-inflammatory agents in migration of human airway smooth muscle (HASM) cells. *Am J Respir Crit Care Med* 2002;165:A346.
152. Fernandes D, Guida E, Koutsoubos V, Harris T, Vadielloo P, Wilson JW, et al. Glucocorticoids inhibit proliferation, cyclin D1 expression, and retinoblastoma protein phosphorylation, but not activity of the extracellular-regulated kinases in human cultured airway smooth muscle. *Am J Respir Cell Mol Biol* 1999;21:77-88.
153. Stewart AG, Harris T, Fernandes DJ, Schachte LC, Koutsoubos V, Guida E, et al. Beta2-adrenergic receptor agonists and cAMP arrest human cultured airway smooth muscle cells in the G(1) phase of the cell cycle: role of proteasome degradation of cyclin D1. *Mol Pharmacol* 1999;56:1079-86.
154. Stewart AG, Tomlinson PR, Wilson JW. Beta 2-adrenoceptor agonist-mediated inhibition of human airway smooth muscle cell proliferation: importance of the duration of beta 2-adrenoceptor stimulation. *Br J Pharmacol* 1997;121:361-8.
155. Ramji DP, Foka P. CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochem J* 2002;365:561-75.
156. Rudiger J, Bihl M, Cornelius BC, Block HL, Johnson M, Perruchoud AP, et al. Addition of beta(2)-agonists to glucocorticoid treatment augments glucocorticoid action via transcription factor C/EBP. *Am J Respir Crit Care Med* 2000;161:A467.
157. Roth M, Johnson PR, Rudiger JJ, King GG, Ge Q, Burgess JK, et al. Interaction between glucocorticoids and beta2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. *Lancet* 2002;360:1293-9.
158. Bonacci JV, Harris T, Stewart AG. Impact of extracellular matrix and strain on proliferation of bovine airway smooth muscle. *Clin Exp Pharmacol Physiol* 2003;30:324-8.
159. Macklem PT. Theoretical basis of airway instability. Roger S. Mitchell Lecture. *Chest* 1995;107:87S-8S.
160. Hirst SJ, Walker TR, Chilvers ER. Phenotypic diversity and molecular mechanisms of airway smooth muscle proliferation in asthma. *Eur Respir J* 2000;16:159-77.
161. Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, et al. Airway smooth muscle cell proliferation is increased in asthma. *Am J Respir Crit Care Med* 2001;164:474-7.