

Effects on lung-cell physiology

Airway smooth muscle: An immunomodulatory cell

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Asthma is characterized in part by reversible airflow obstruction, hyperresponsiveness, and inflammation. Chronic obstructive pulmonary disease, which includes chronic bronchitis, emphysema, and possibly bronchiectasis, is defined as predominantly irreversible airflow obstruction associated with abnormal airway inflammation. Traditional concepts concerning airway inflammation have focused on trafficking leukocytes and on the effects of inflammatory mediators, cytokines, and chemokines secreted by these cells. Airway smooth muscle, the major effector cell responsible for bronchomotor tone, has been viewed as a target tissue responding to neurohumoral control and inflammatory mediators. New evidence, however, suggests that airway smooth muscle may secrete cytokines and chemokines and express cellular adhesion molecules that are important in modulating submucosal airway inflammation. Other new evidence suggests that β -adrenergic agents may inhibit some but not all of the inflammatory responses. In certain circumstances, increasing levels of cyclic adenosine monophosphate in the cytosol of airway smooth muscle promote the secretion of other cytokines or chemokines. The cellular and molecular mechanisms that regulate the immunomodulatory functions of airway smooth muscle may offer new and important therapeutic targets in treating these common lung diseases. (*J Allergy Clin Immunol* 2002;110:S269-74.)

Key words: Airway remodeling, smooth muscle hypertrophy, asthma, chronic obstructive pulmonary disease

Airway diseases such as asthma, chronic obstructive pulmonary disease, and obliterative bronchiolitis are characterized by airway inflammation. Studies show that airway inflammation, as measured by trafficking leukocytes that secrete inflammatory mediators, modulates airflow by promoting vascular permeability and stimulating airway smooth muscle (ASM) cell contraction. ASM, a critically important effector cell modulating bronchomotor tone, responds to inflammatory mediators by contracting, shortening, and increasing luminal resistance. New evidence, however, suggests that effector cells in the submucosa, including ASM, may serve an immunomodulatory

Abbreviations used

ASM:	Airway smooth muscle
cAMP:	Cyclic AMP
[cAMP] _i :	Intracellular cyclic AMP
CREB:	Cyclic AMP response element binding protein
ICAM-1:	Intercellular adhesion molecule-1
NF- κ B:	Nuclear factor κ B
PGE ₂ :	Prostaglandin E ₂
RANTES:	Chemokine regulated on activation, normal T cell expressed and secreted
VCAM-1:	Vascular cell adhesion molecule-1

role by expressing cellular adhesion molecules and by secreting chemokines and cytokines (Fig 1). The cellular signaling processes that promote the secretion of such molecules by ASM and fibroblasts appear to be resistant to corticosteroid inhibition. Alternatively, increasing levels of cyclic AMP (cAMP) in such effector cells appear to inhibit some but not all synthetic responses.

This review examines current evidence suggesting that ASM stimulated with cytokines can secrete proinflammatory molecules. It also discusses the downstream signaling pathways that promote synthetic function, as well as the role of cAMP and cAMP-dependent protein kinase in modulating these responses.

ASM GROWTH AND β -AGONISTS

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

The β_2 -agonists activate the β_2 -adrenergic receptor/ G_s /adenylyl cyclase pathway to elevate cAMP in ASM cells. Because of their cAMP-elevating ability, albuterol, fenoterol, and salmeterol have been shown to inhibit mitogen-induced proliferation of human ASM cells.¹⁻³ β_2 -Agonists and other cAMP-elevating agents are thought to arrest the G1 phase of the cell cycle by posttranscriptionally inhibiting cyclin D1 protein levels by action on a proteasome-dependent degradation pathway.⁴ Another mechanism was suggested by Musa et al,⁵ who examined the effects of forskolin, an activator of adenylyl cyclase, on DNA synthesis, cyclin D1 gene expression, cAMP response element binding protein (CREB) phosphorylation, and DNA binding in bovine ASM. This study

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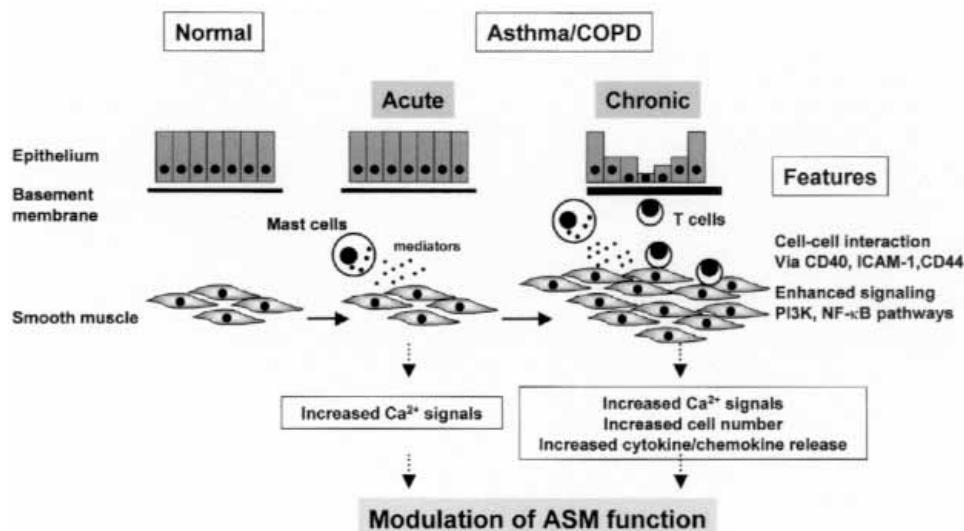


FIG 1. Factors affecting ASM function in the acute and chronic states of asthma or chronic obstructive pulmonary disease (COPD). During the acute inflammation, a variety of mediators, such as cytokines, can modulate ASM contractile function by enhancing calcium signaling to agonists. These mediators regulate the recruitment and activation of eosinophils and T lymphocytes in the airway mucosa, a characteristic histopathologic feature of the chronic disease. The persistence of airway inflammation through the production of cytokines and chemokines by both inflammatory and structural cells has the potential to stimulate ASM proliferation directly or as a result of interaction between T cells and ASM mediated by cell surface expression of various cellular adhesion molecule proteins, such as ICAM-1, CD40, and CD44. Enhanced activation of phosphatidylinositol 3-kinase (PI3K) or NF-κB in ASM may also stimulate mitogenic and synthetic functions.

showed that by increasing cAMP in ASM cells, forskolin suppressed cyclin D1 gene expression through phosphorylation and transactivation of CREB, suggesting that the effect of cAMP on cyclin D1 gene expression is exerted by way of *cis*-repression of cyclin D1 promoter.

In human ASM, the corticosteroids dexamethasone and fluticasone propionate have been shown to arrest ASM cells in the G1 phase.⁶ In that study 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 extracellular signal-regulated kinase cascade.⁶ Dexamethasone has different inhibitory effects on receptor tyrosine kinase-mediated ASM cell growth and G-protein-coupled receptor-mediated ASM cell growth, suggesting that steroid effects on ASM mitogenesis are complex. Further elucidation of the signaling and transcriptional targets for the inhibition of cell cycle progression by corticosteroids and β_2 -agonists may indicate how these therapies could be used optimally, possibly in combination, to modulate airway wall remodeling in patients with asthma.

ASM AS A TARGET FOR ANTI-INFLAMMATORY THERAPY

Because of the new evidence that ASM cells secrete and express immunomodulatory proteins, investigators are now studying the cellular and molecular processes

that regulate ASM synthetic function and are examining the roles of dexamethasone and β -agonists in modulating cytokine-induced synthetic responses.

Effects of intracellular cAMP-mobilizing agents on cytokine-induced synthetic responses

In asthma, β -agonist bronchodilators increase intracellular cAMP ($[cAMP]_i$) and stimulate cAMP-dependent protein kinase in ASM. In a similar manner, prostaglandin E₂ (PGE₂), which is produced in large quantities at sites of inflammation, increases $[cAMP]_i$ in human ASM cells and is a potent and effective bronchodilator.⁷ New evidence suggests that $[cAMP]_i$ -mobilizing agents in ASM cells also modulate cytokine-induced synthetic function (Fig 2).⁸ In TNF- α -stimulated ASM cells, expressions of both eotaxin and RANTES are effectively inhibited by isoproterenol, PGE₂, dibutyl $[cAMP]_i$, or the phosphodiesterase inhibitors rolipram and cilomast.⁹⁻¹¹ TNF- α -induced interleukin (IL)-8 secretion has also been shown to be inhibited by the combination of $[cAMP]_i$ -mobilizing agents and corticosteroids.¹² Similarly, sphingosine-1-phosphate, which activates a G_s-protein-coupled receptor and increases $[cAMP]_i$, abrogates TNF- α -induced RANTES secretion in ASM cells.¹³

In contrast to the effects of $[cAMP]_i$ on chemokine secretion, pharmacologic agents that increase $[cAMP]_i$

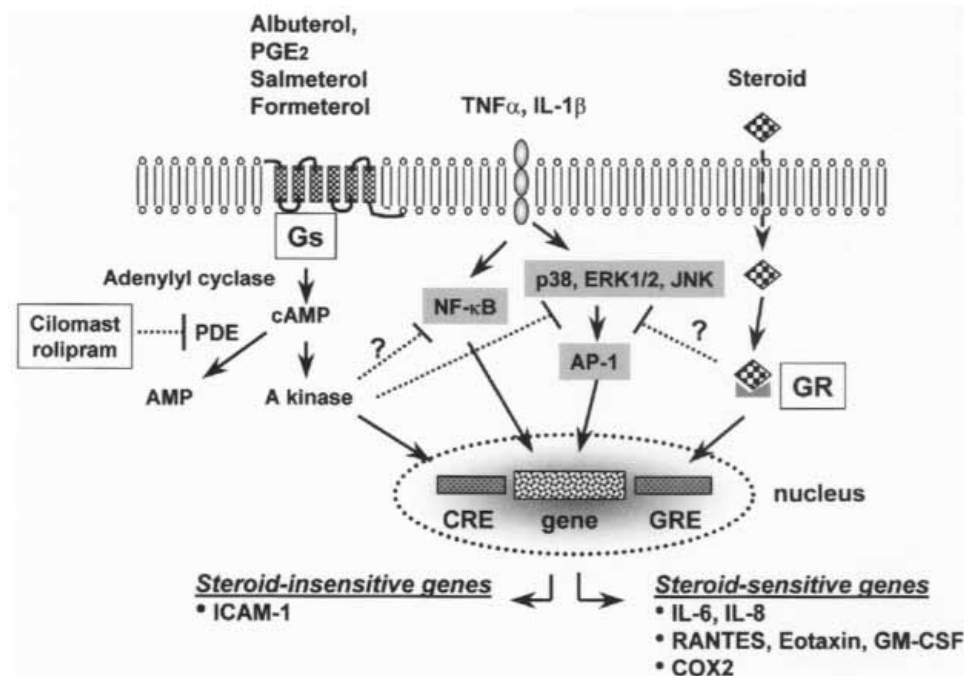


FIG 2. Putative signaling pathways that regulate cytokine-induced synthetic responses in ASM cells. The [cAMP]_i-mobilizing agents inhibit cytokine-induced chemokine and cellular adhesion molecule expression. IL-6 secretion, however, is augmented by agents that increase [cAMP]_i. Cytokine-induced activation of NF-κB in human ASM is corticosteroid-insensitive. PGE₂, Prostaglandin E₂; ERK, extracellular signal-regulated kinase; JNK, Jun-N-terminal kinase; PDE, phosphodiesterase; AP-1, activator protein-1; GR, glucocorticoid receptor; A kinase, cAMP-dependent protein kinase; CRE, cAMP response element; GRE, glucocorticoid response element; COX2, cyclooxygenase 2. (From Lazaar AL, Panettieri RA Jr. Airway smooth muscle as an immunomodulatory cell: a new target for pharmacotherapy? *Curr Opin Pharmacol* 2001;1:259-64. With permission.)

markedly stimulate secretion of IL-6 in human ASM cells.⁹ This stimulation appears to be due to effects on basal IL-6 promoter activity.¹⁴ Whether the secreted IL-6 modulates ASM cell function in an autocrine manner or alters leukocyte function in the submucosa remains unknown. Studies show that overexpression of IL-6 decreases acetylcholine responsiveness in transgenic mice,¹⁵ however, so the role of IL-6 in asthma may be that of an anti-inflammatory signal. More recently, it has been shown that cAMP limits secretion of GM-CSF by ASM cells. Cyclooxygenase inhibitors have been shown to reduce PGE₂ and enhance cytokine-induced secretion of GM-CSF,^{16,17} whereas phosphodiesterase type IV inhibitors have been found in vitro to reduce GM-CSF secretion and in an animal model to reduce antigen-induced airway hyperresponsiveness.^{17,18} Taken together, current evidence suggests that some but not all proinflammatory functions in ASM cells are inhibited by [cAMP]_i-mobilizing agents.

Reports conflict, however, concerning the effects of increased [cAMP]_i on lymphocyte adhesion and migration through cytokine-activated endothelial cells.^{19,20} The controversy regarding the role of [cAMP]_i in modulating cellular adhesion probably reflects differences in the cytokines used to stimulate endothelial cells or the temporal differences in the addition of the agonists used

to increase [cAMP]_i. Far less is known concerning [cAMP]_i effects on adhesion of leukocytes to smooth muscle. In human ASM cells, activation of [cAMP]_i-dependent pathways inhibits in part TNF-α-mediated induction of both intercellular adhesion molecule-1 (ICAM-1) expression and vascular cellular adhesion molecule-1 (VCAM-1) expression, as well as inhibiting adhesion of activated T cells to ASM cells. The basal expressions of ICAM-1 and VCAM-1, as well as the binding of activated T cells to unstimulated ASM, were resistant to increases in [cAMP]_i.²¹ This study shows that cytokine-induced expression of cellular adhesion molecules and T-cell adhesion to ASM cells are modulated by changes in [cAMP]_i.

Corticosteroids alter cytokine-induced synthetic responses

One of the most effective medications to date for controlling airway inflammation in asthma has been inhaled corticosteroids. Although steroids have been used for this purpose for more than 25 years, the precise mechanisms by which they improve lung function in asthma remain unclear. Current evidence suggests that chemokine and cytokine secretions induced by inflammatory mediators are inhibited by dexamethasone in human ASM cells. Cytokine-induced secretions of RANTES,^{9,22,23} mono-

cyte chemoattractant protein,²³ eotaxin,¹⁰ GM-CSF,²⁴ and IL-6²⁵ are abrogated by corticosteroids. In most of these studies, corticosteroid and [cAMP]_i-mobilizing agents were shown to act additively to inhibit chemokine and cytokine secretion. Recent data suggest that the effects of dexamethasone on RANTES expression are mediated through inhibition of the transcription factor activator protein-1.¹⁴

Nuclear factor κ B (NF- κ B) is another transcription factor that mediates chronic inflammatory responses in asthma, as well as in rheumatoid arthritis, psoriasis, and inflammatory bowel disease.²⁶ Notably, dexamethasone has been found to have no effect on TNF- α -induced or IL-1 β -induced NF- κ B activation in human ASM cells.²⁷ Furthermore, cytokine-induced ICAM-1 expression in ASM cells, which is completely dependent on NF- κ B activation, has been shown to be unaffected by dexamethasone, with IL-6 secretion affected only partially.¹⁴ In contrast, IL-1 β -induced cyclooxygenase2 expression has been found to be abrogated.²⁷⁻²⁹ Collectively these data support the notion that anti-inflammatory effects of steroids in asthma may not be due solely to modulation of cytokine-induced NF- κ B activation and are probably regulated by pathways such as activator protein-1 or other transcriptional factors.

CONCLUSIONS

ASM, an important effector cell type modulating bronchomotor tone, is now emerging as a potential immunomodulatory cell type. Although current evidence suggests that ASM cells are not antigen-presenting cells, ASM clearly secretes proinflammatory cytokines and chemokines. The recognition that increases in cAMP can modulate synthetic responses in ASM may offer new insight into the role of cAMP-mobilizing agents in modulating airway inflammation. Further, the recognition that increasing cAMP can also promote IL-6 secretion may suggest a role for IL-6 in mediating injury repair responses. Unfortunately, current evidence for ASM as an immunomodulatory cell is based on *in vitro* studies; further investigation *in vivo* is therefore warranted. Overall, the recognition of ASM as not only a contractile cell but also an immunomodulatory cell may offer new insights into characterizing the airway inflammation that occurs in diseases such as asthma, chronic bronchitis, and obliterative bronchiolitis.

DISCUSSION SESSION

Question: Have you looked at the consequences of the up-regulation of these adhesion molecules? Have you co-cultured, for example, with T cells or with eosinophils to determine whether that would up-regulate the secretory process?

Dr Panettieri: We have not done this with eosinophils. With regard to T cells, if the cellular adhesion molecule expression on the ASM is up-regulated, it facilitates T-cell binding. The binding of the T cell to the ASM stimulates mitogenesis in ASM. The DNA signaling pathway is not dependent on soluble mediators. For example, if we just use the supernatants of activated T cells, we do

not see proliferation. We think that it is the engagement of cellular adhesion molecules that is permissive for the proliferative response.

Question: Some have criticized this smooth muscle cell culture on the basis that the cytokine production seen in this culture system may not be representative of what occurs *in vivo*. Do you have any data on this?

Dr Panettieri: We and others examined this. RANTES expression in human ASM after segmental allergen challenges robustly increased in ASM in patients with asthma. This was not seen in normal cells. We are also pursuing studies looking at the IL-6 response. Others in the United Kingdom have clearly shown that RANTES is up-regulated in muscle in asthma. As we do more biopsy studies with patients with well-characterized asthma from whom we can get reproducible quantities of muscle to study, we are probably going to find that these responses track closely with the *in vitro* circumstances.

Question: Is the stimulation observed dependent on cell content or polarity?

Dr Panettieri: I think that an organ culture system or rebuilding the airway is precisely where we are going. We are using murine models in which we can individually knock out genes in the epithelium and the muscle, put it back, and rebuild the airway that way. Using slices of organ tissue is extremely complex. There are diffusional problems, and it is difficult to interpret some of the responses. The ASM will actually proliferate within about 18 hours or enter DNA synthesis at 18 hours. So, if one were to use thick organ culture, it would not be possible to demonstrate quickly whether cycling of the cells was occurring. Regarding polarity, our cells are adherent, so we cannot easily get underneath them to test. However, if we put them in a modified Boyden chamber, we could compare the situation above and below. I do not know of a polarity with regard to muscle secretion of proteins. It is an interesting and provocative concept.

Question: Is there an asthma pathologic condition showing widespread invasion of muscle with leukocytes?

Dr Panettieri: We do not have a myositis in asthma, but we do have individual migratory myofibroblasts, which are simply errant ASM cells that come into close contact with T cells. When we look at RANTES secretion in muscle biopsy specimens from patients with asthma, we find that the muscle is intensely staining, immunoreactive for RANTES. So this cell type that we thought was simply contractile actually produces a great deal of RANTES.

Comment: I really wonder whether migration of smooth muscle is the mechanism by which myofibroblasts appear in the submucosa. Clearly, transforming growth factor β action causes cultured fibroblasts that don't look anything to start with like myocytes to end up looking like myocytes by inducing the low-level expression of muscle markers. Fibroblasts will contract without those muscle markers anyway. So I am uncertain why we think that a cell that is so far away from the rest of the muscle cells originated in the muscle, especially when in atherosclerosis circulating stem cells undoubtedly contribute to some of the modulated previously contractile smooth muscle cells. That speaks against migration in the very system that prompts us to think that this happens in the airway.

Dr Panettieri: The point is well taken. I cannot tell you the stem cell from which this comes. Are these myofibroblasts that proliferate in the submucosa, or are these migratory muscle cells from the outside of the bundles that migrate out? I believe that there is a heterogeneity to the ASM. We need better tools to study this. We observed that agents that increase cAMP can have effects on migratory function in a physiologically relevant human ASM cell. The direct relevance of this to the *in vivo* circumstance remains up for discussion.

Question: Have you looked at the next step beyond cyclic nucleotide levels to see whether there is an increase in kinase

activity? Do you have a sense of where the phosphorylation targets are in the smooth muscle cells?

Dr Panettieri: We have looked at all those issues, with mixed success. Because if we're looking at a growth response, there is a window of opportunity there of 4 and 5 hours. So what is the biomarker of cAMP protein kinase A activation activity that would stretch that span that is relevant to look at growth that occurs in 36 hours? An agonist produces contraction within minutes, but the outcome related to growth factor-induced proliferation is quite complex. I think that we can show specific phosphorylation events. The direct relevance to the proliferative signal is difficult to understand. We are still looking for a tool that would tell us that cAMP-dependent protein kinase A activation blocks this signal. There are problems with using protein kinase activation inhibitors, especially in long-term incubation experiments. We have done single-cell microinjection with the regulatory subunit of cAMP kinase and can block the growth by directly injecting catalytic subunits, suggesting that at least protein kinase A activation appears to have the growth inhibitory signal.

Question: Regarding the chemotactic and proliferative effects, what would be the corollaries of these in a disease model? How would you show whether the agents you discussed play a chemotactic or a proliferative role in vivo?

Dr Panettieri: Well, I think that one of the most direct ways would be to count myofibroblasts in biopsy specimens or in histologic samples in allergen-induced rodent models. Evidence has suggested that there are more myofibroblasts in the submucosa in both human beings and rodents. Is this a proliferative or a migratory signal? To explore this, one could co-stain at that time with a specific marker looking for activation of growth. If this is not observed and an increase in the number of cells is seen, it could be a migration effect.

Comment: In some instances, phosphodiesterase inhibitors also affect adenosine receptors on the surface. Whether they affect the A1 or A2 receptors, one could picture effects on cAMP levels. This may explain in part what is seen.

Dr Panettieri: This might be the case with theophylline, but not for some of the other compounds that do not bind to adenosine receptors.

Question: In your experiments with the lymphocytes in co-culture, were these cells HLA-matched, and would this make a difference?

Dr Panettieri: No, these cells were not HLA-matched. We do not think that it matters. This was a relatively acute incubation and binding. ASM under no circumstances presents antigen.

Question: Is there a difference in ASM in terms of a contractile and a secretory phenotype, and can these cells change from one to the other?

Dr Panettieri: Early data clearly show a synthetic and a contractile phenotype. I believe that there is no terminally differentiated ASM cell. It is a phenotype that is modulated. I think that cells are plastic, and that this plasticity can occur under different circumstances. I am impressed in our biopsy studies that muscles that have tremendous amounts of actin and myosin are capable of secreting RANTES and IL-6. So a contractile phenotype cell can also secrete. My concern is with pigeonholing cells as either contractile or secretory. There is probably a continuum. The IL-6 secretion by ASM in response to β -agonists was surprising to us. We believe that this is actually an injury-repair response, suggesting that if the muscle does not secrete the IL-6 in response to cAMP, this could actually be detrimental.

Question: In your experiments, have you shown directional migration? Are the effects seen due to chemokinesis? Have you looked for chemokine receptors on these cells, and will they migrate in a directed fashion in response to chemokine?

Dr Panettieri: I think that your point is well taken. In our experi-

ments, agonist is placed in the bottom of the well, and the muscle is on the top. We then count the cells that have gone through the filter, and we count the number of nuclei there. The maximal response is at 4 hours. This is chemotaxis and not simply chemokinesis.

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