

Molecular mechanisms in allergy and clinical immunology

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Immune mechanisms of smooth muscle hyperreactivity in asthma

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Asthma is characterized by bronchial hyperresponsiveness to a variety of bronchospasmogenic stimuli. To study the pathophysiologic mechanisms underlying the increased sensitivity and degree of maximal airway narrowing, various *in vivo* and *in vitro* models have been developed with methods of active and passive sensitization. These studies indicated a major role for alterations in the smooth muscle itself rather than neural dysfunction or airway inflammation as the underlying cause for the development of bronchial hyperresponsiveness. During the last years smooth muscle cells were found to exhibit not only the "classical" contractile phenotype but also a proliferative-synthetic phenotype, which is capable of producing proinflammatory cytokines, chemotaxins, and growth factors. Allergic sensitization can alter both contractile and secretory functions, thereby indicating that the smooth muscle cell could contribute directly to the persistence of airway inflammation in asthma. A better understanding of the changes within the smooth muscle cells and of the mechanisms that lead to their induction could contribute to the development of novel therapeutic approaches for the treatment of asthma. (*J Allergy Clin Immunol* 2000;105:673-82.)

Key words: Immune mechanisms, bronchial smooth muscle, hyperreactivity, asthma, sensitization, IgE, cytokines, airway inflammation

Since the early 1920s histologic studies have reported a marked increase in the amount of smooth muscle in airways from asthmatic subjects,¹ and this abnormality was thought to be the pathophysiologic basis of bronchial hyperresponsiveness. The importance of smooth muscle mass for the degree of airway responsiveness was later demonstrated by a study of Lambert et al² showing that for a given maximal contractile stimulus greater muscle thickness allows the development of greater force and thus enhanced narrowing of the airway lumen.

In this classical view, smooth muscle cells have been looked at as tissue that responds to exogenous stimulation

Abbreviations used

[Ca ⁺⁺] _i :	Intracellular calcium concentration
EFS:	Electrical field stimulation
ICAM-1:	Intercellular adhesion molecule-1
LTC ₄ :	Leukotriene C ₄
MBP:	Major basic protein
MLCK:	Myosin light chain kinase
PC ₂₀ FEV ₁ :	Provocative concentration of an inhaled stimulus that causes a 20% reduction in FEV ₁
pEC ₅₀ :	The negative logarithm of the concentration of a stimulus that causes 50% of the maximal response
T _H :	T helper cell
VCAM:	Vascular adhesion molecule

with contraction or relaxation, thereby regulating airway caliber. However, more recently it has been recognized that smooth muscle cells not only exhibit a contractile but also a noncontractile, synthetic-proliferative phenotype (Fig 1).³⁻⁶ Freshly isolated airway smooth muscle cells in culture are able to change phenotype, from the typically contractile to a noncontractile, while expressing a different pattern of proteins.⁴ This change in phenotype appears to depend on culture conditions (ie, the presence of fetal serum in the culture medium). Although smooth muscle cells under these conditions proliferate and are capable of producing growth factors, proinflammatory cytokines, and chemokines,⁶ removal of the serum from the medium reinduces the differentiation into contractile myocytes.⁵ Because the increase in bronchial smooth muscle mass in asthma is due to cell hypertrophy and to hyperplasia,⁷ the potential relevance of phenotype plasticity and its possible relationship to altered smooth muscle function in disease states has been suggested.³

The observations that smooth muscle exhibits not only contractile but also secretory functions⁶ (Table I) lead away from the classical view of smooth muscle as a mere recipient of exogenous (contractile) stimuli and toward its possible role as a immunomodulatory cell and hence an active contributor to airway inflammation and dysfunction; smooth muscle itself might be capable of initiating and maintaining airway inflammation. Furthermore, allergic sensitization of airways and the exposure to certain cytokines elicit significant functional changes,

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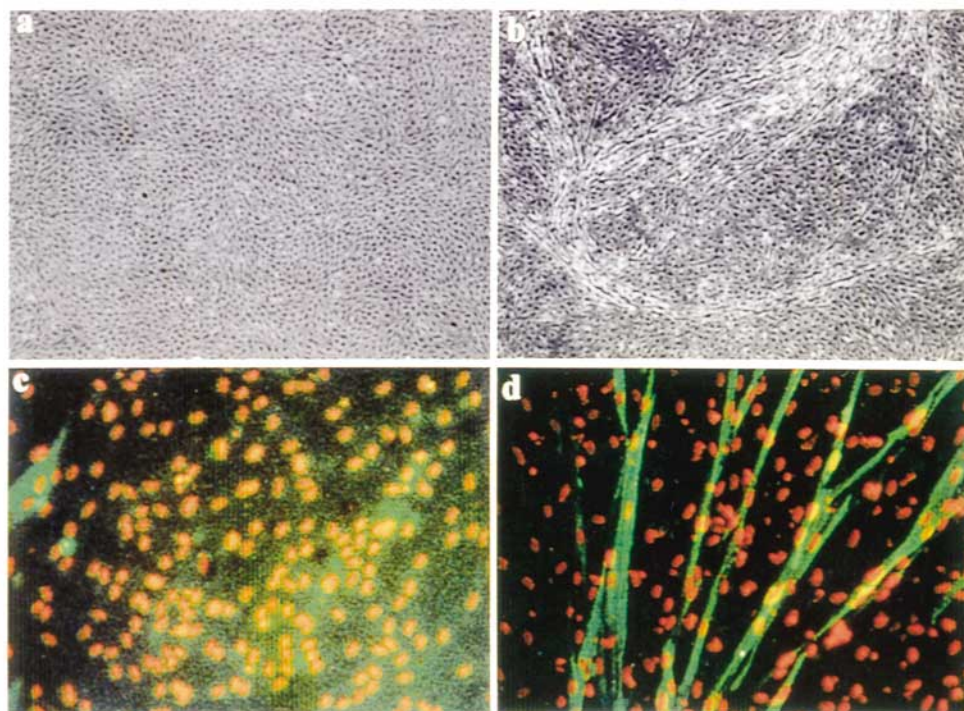


FIG 1. Phenotype plasticity. Smooth muscle cells in culture. **a** and **b**, Phase-contrast micrographs. (Original magnification $\times 40$.) **c** and **d**, Fluorescence immunocytochemical-staining smooth muscle myosin heavy-chain antibody (green). Nuclei were stained with propidium iodide (red). (Original magnification $\times 100$.) Passage 1 canine tracheal myocytes in primary culture demonstrating typical appearance of **(a, c)** confluent, synthetic-proliferative and **(b, d)** 11-day serum-deprived, elongated, contractile smooth muscle phenotypes. (Photographs adapted from Halayko AJ, Camoretti-Mercado B, Forsythe SM, Vieira JE, Mitchell JA, Wylam ME, et al. Divergent differentiation paths in airway smooth muscle culture: induction of functionally contractile myocytes. *Am J Physiol* 1999;276:L197-206.)

TABLE I. Stimuli of smooth muscle function

Contractile stimuli	Synthetic-proliferative stimuli
Acetylcholine/carbachol	Acetylcholine
Methacholine	Cysteinyl leukotrienes
Histamine	Bradykinin
Cysteinyl leukotrienes	Sensitizing serum
Neurokinin A	TNF- α , INF- γ
	IL-1 β , IL-4, IL-10, IL-13

resulting in altered force-velocity characteristics during smooth muscle contraction^{8,9} as well as altered expression of adhesion molecules¹⁰ and cytokines.¹¹

This article will review why airway smooth muscle dysfunction is believed to play an important role in the pathophysiologic mechanisms of bronchial hyperresponsiveness in asthma; it will discuss the immune mechanisms, which are likely to cause alterations in smooth muscle function and the subsequent changes within the smooth muscle itself.

EPIDEMIOLOGY AND CLINIC

The definition of asthma includes a dysfunction in the control of airway caliber that appears to be clinically well

characterized; however, the underlying pathomechanisms are far from being completely understood. Because airway hyperresponsiveness can be induced in nonasthmatic individuals by the transfer of serum from patients with asthma,¹² epidemiologic studies have tried to identify clinical parameters that might be associated with bronchial hyperresponsiveness and therefore reflect mechanisms that are important for the development of asthma. It was recognized that the prevalence of asthma¹³ and the degree of bronchial hyperresponsiveness^{14,15} were associated with increased total serum IgE levels. Furthermore, in subjects without known atopy current smoking was found to be related to airway hyperresponsiveness¹⁶ as well as elevated IgE levels.¹⁷⁻¹⁹ In accordance with these epidemiologic findings, we were able to show that isolated airways obtained from patients with high total serum IgE are hyperreactive to histamine under in vitro conditions in comparison with airways from patients with low serum IgE (Fig 2).²⁰ Interestingly, almost all patients with high IgE levels were current smokers without known history of atopy or asthma. Because in vivo as well as in vitro enhanced serum IgE levels were associated with bronchial hyperreactivity independently of atopy, IgE appears to be a serum marker reflecting not only airway immune responses but also smooth muscle hyperreactivity.²¹

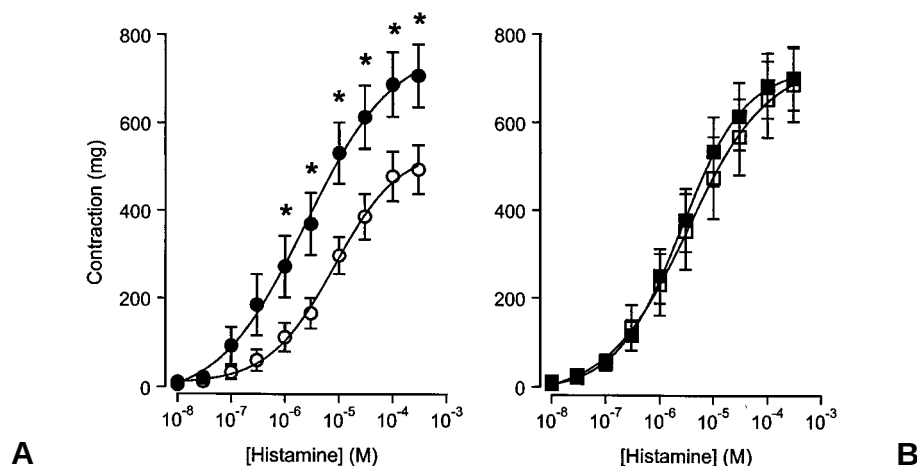


FIG 2. Contractile concentration-effect curves to histamine of human bronchi from donors with total serum IgE (a) <10U/mL (circles) and (b) >200U/mL (squares) in nonsensitized (open) and passively sensitized (closed) preparations. Contractions were expressed as milligram changes in tension. Data are the mean $\pm$ SEM of 10 experiments in the low-IgE and of 9 experiments in the high-IgE group. Asterisk, $P < .05$, regarding contractile responses to individual concentrations of histamine. (Data adapted from Schmidt D, Watson N, Rühlmann E, Magnussen H, Rabe KF. Serum IgE levels predict human airway reactivity in vitro. Clin Exp Allergy 2000;30:233-41.²⁰)

Bronchial responsiveness is reflected by increased sensitivity and a maximal degree of airway narrowing owing to a variety of bronchospasmogenic stimuli such as allergen, methacholine and histamine,²² leukotrienes,²³ neurokinins,²⁴ adenosine, and exercise challenges.²⁵ Considering the increase in smooth muscle mass in airways of asthmatic patients as described histologically,⁷ its possible functional role,² and the different pathways through which exogenous stimuli exhibit a more severe bronchoconstriction, it is likely that an alteration of the end organ (ie, the smooth muscle cell itself) is involved in the pathophysiologic mechanisms of clinical bronchial hyperresponsiveness.

However, the importance of the contribution of bronchial smooth muscle in asthma was temporarily questioned by studies that aimed to investigate the relationship between in vivo and in vitro airway reactivity. Studies failed to demonstrate a correlation between the degree of nonspecific responsiveness in vivo, as determined by the provocation concentration of an inhaled stimulus that causes a 20% reduction in the FEV₁ (PC₂₀FEV₁) of histamine and the sensitivity of isolated airway preparations to histamine in vitro, expressed as the negative logarithm of the histamine concentration that caused 50% of the maximal response (pEC₅₀).²⁶⁻³²

The failure of several studies to establish a relationship between in vivo and in vitro airway reactivity, which would have supported the role of smooth muscle in asthma, was mainly based on the lack of an individual correlation between PC₂₀FEV₁ and pEC₅₀. However, the PC₂₀FEV₁ reflects the sensitivity of smooth muscle contraction measured under almost auxotonic conditions in vivo, whereas in standard experimental setups the pEC₅₀ is based on measure-

ments under isometric conditions in vitro. Additionally, PC₂₀ measurements have been developed as a simple clinical tool to attest and relate a suspected diagnosis (ie, asthma) to a physiologic abnormality. In contrast to in vitro experiments, these in vivo measurements involve only the beginning part of a full dose-response curve. Therefore it appears not to be justified to refuse the possibility of a smooth muscle disorder from the lack of correlation between PC₂₀ and in vitro findings. Furthermore, it might be argued that PC₂₀ measurements, although clinically feasible, are not adequate to reflect the molecular abnormalities underlying airway hyperresponsiveness.

In contrast, when serum IgE levels (as mentioned before)²⁰ or even the clinical diagnosis of asthma was chosen as a reference, a relationship between in vivo and in vitro airway reactivity appeared. De Jongste et al³¹ assessed maximal contractile responses of isolated airways in vitro and observed enhanced contractile responses to histamine, methacholine, and leukotriene C₄ (LTC₄) in airway preparations from a patient with asthma, in comparison with airways from nonasthmatic subjects. Antonissen et al³³ demonstrated that isolated airway preparations obtained from allergen-sensitized dogs, in which sensitization was confirmed by elevated serum IgE levels and the development of bronchial hyperresponsiveness in vivo, exhibited significantly greater smooth muscle shortening velocity and capacity compared with airways from nonsensitized control animals (Fig 3). The authors interpreted their findings as indication that bronchial hyperresponsiveness predominantly results from alterations in smooth muscle function rather than from an imbalance in the autonomic nervous system or inflammatory mechanisms.

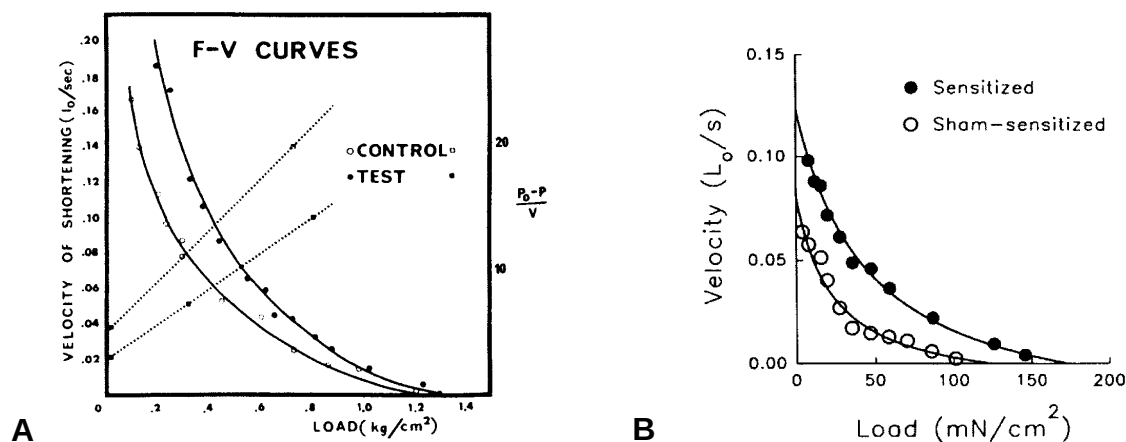


FIG 3. **A** Typical force-velocity (*F-V*) curves elicited in a paired (littermate) experiment in a canine model of asthma. Isolated airways of dogs, which had been sensitized *in vivo* to ovalbumin (test) or its adjuvant aluminum hydroxide (control) were investigated under isometric and isotonic conditions *ex vitro*. Linearized transforms prove relationships are hyperbolic; a goodness-of-fit test yielded a correlation coefficient of $r^2 = 0.95$ for both lines. Note the increased velocity of shortening of sensitized tracheal smooth muscle, without a significant difference in opening pressure (P_0). (Data adapted from Antonissen LA, Mitchell RW, Kroeger EA, Kepron W, Tse KS, Stephens NL. Mechanical alterations of airway smooth muscle in a canine asthmatic model. *J Appl Physiol* 1979;46:681-7.33) **B** Representative force-velocity curves of smooth muscle from a passively sensitized and paired sham-sensitized human bronchial ring. Sensitized tissue demonstrated significantly greater maximal velocity of shortening (V_{max} , y-axis intercept) and greater velocities of shortening at all afterloads compared with a paired, sham-sensitized bronchial ring. For these preparations V_{max} was 0.1236 L_0/s for sensitized and 0.0803 L_0/s for the control. Mean maximal isometric force development (P_0 , x-axis intercept) was not different for 8 pairs of human bronchial rings studied. (Data adapted from Mitchell RW, Rühlmann E, Magnussen H, Leff AR, Rabe KF. Passive sensitization of human bronchi augments smooth muscle shortening velocity and capacity. *Am J Physiol* 1994;267:218-22.8)

PASSIVE SENSITIZATION OF AIRWAY SMOOTH MUSCLE

In the early 1920s it was recognized by Prausnitz and Küstner¹² that allergen sensitivity can be induced in a nonallergic individual through the transfer of serum from an allergic subject. In experiments performed on themselves, they demonstrated that intradermal injection of serum from Küstner, who was allergic to fish, into Prausnitz's skin led to the appearance of a wheal-and-flare reaction when fish antigen was injected at the same spot 24 hours later. This was the first demonstration in humans that a substance (reagin) present in the serum could transfer skin sensitivity to specific allergen. The Prausnitz-Küstner test remained the classic method to determine skin-sensitizing antibodies for many years until it was realized that other diseases, such as hepatitis, could also be transferred.

Subsequent studies showed, in analogy, that on one side the exposure to serum from asthmatic patients caused an increase in mediator release in isolated cells³⁴⁻³⁶ and on the other initiated an allergen-induced bronchoconstriction in isolated human airways.³⁷ The incubation with sensitizing serum not only induced a specific response to allergen but also increased responsiveness to a variety of nonspecific stimuli.³⁸⁻⁴¹ This approach, which is now known as passive sensitization, has revealed that factors present in serum from atopic subjects are likely to be major determinants of bronchial hyperreactivity.⁴²

In accordance with the study in dogs performed by Antonissen et al,³³ passive sensitization of isolated human airways also increases smooth muscle shortening velocity and capacity in response to electrical field stimulation (EFS) (Fig 3)⁸ and, moreover, augments myogenic responses.⁹ These findings indicate that passive sensitization induces an increase in airway reactivity through changes in airway smooth muscle function, which underlines the importance of this model for the investigation of bronchial hyperresponsiveness in asthma. It is noteworthy that almost all isolated airways obtained from patients with normal serum IgE levels can be passively sensitized. This does not preclude that otherwise genetically determined and environmental factors modulate airway reactivity, but it underlines the essential role of the smooth muscle itself for the induction of bronchial hyperresponsiveness.

SENSITIZATION-INDUCED CHANGES IN SMOOTH MUSCLE CELLS

As mentioned previously, there is strong evidence that dysfunction of the smooth muscle itself contributes to bronchial hyperresponsiveness.⁴³ Because smooth muscle contraction is dependent on an increase in intracellular calcium concentration ($[Ca^{++}]_i$) and the subsequent activation of myosin light chain kinase (MLCK), it has been suggested that an alteration in this pathway could contribute to smooth muscle hyperreactivity. Indeed,

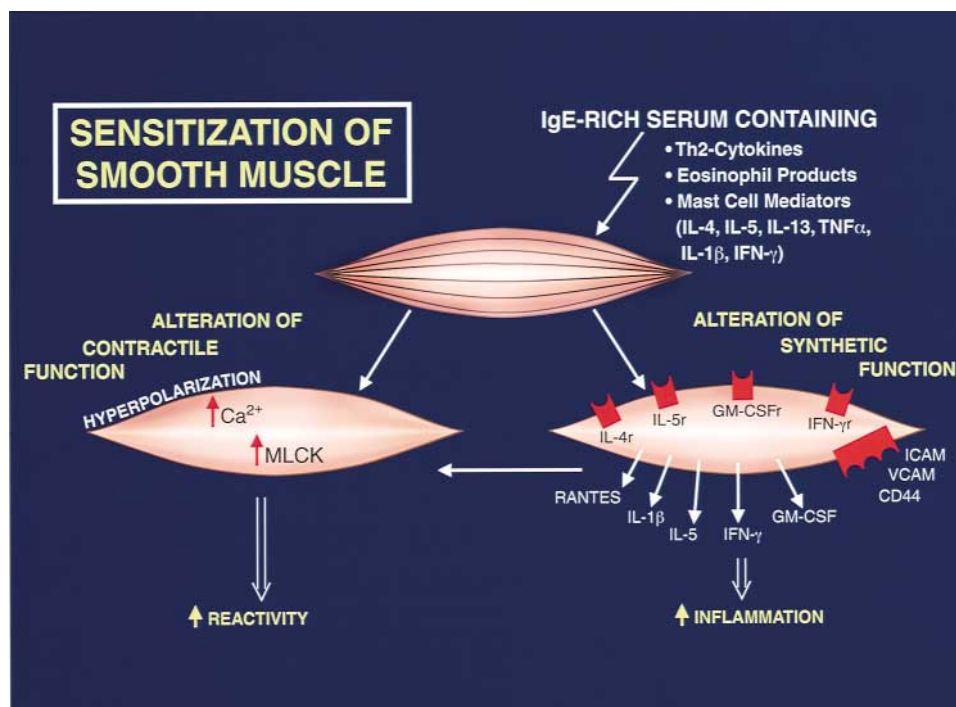


FIG 4. Sensitization-induced changes in smooth muscle function. The incubation of smooth muscle with IgE-rich serum or the exposure to certain inflammatory cytokines causes changes in smooth muscle contractile and synthetic function. Alteration in contractile function is reflected by an increase in smooth muscle shortening velocity and capacity as well as increased reactivity to bronchospasmogenic stimuli. These changes are believed to occur through an increase of [Ca $^{2+}$]_i concentrations, enhanced MLCK activity, or changes in the cell membrane potential. Moreover, sensitization might alter smooth muscle synthetic function through increased production of inflammatory cytokines, chemotaxins, and expression of cytokine receptors and adhesion molecules, all of which can contribute to the changes in smooth muscle reactivity and initiate or maintain airway inflammation. ICAM, Intercellular adhesion molecule; VCAM, vascular cell adhesion molecule.

Jiang et al⁴⁴ found an increased activity of MLCK in airways from sensitized dogs. The data suggested that the increase in MLCK activity resulted from an greater MLCK content in sensitized tissues, but it was indicated by the authors that an increase in MLCK activity could also result from an increased [Ca $^{2+}$]_i.

A possible role of altered calcium signaling in the pathophysiologic mechanisms of abnormal smooth muscle function in asthma has been suggested by clinical studies investigating the effects of drugs that interact with calcium channels. These drugs were shown to reverse already established airway hyperreactivity.⁴⁵ Perpina et al⁴⁶ suggested that the increase in airway contraction by sensitization resulted from an enhanced calcium entry or [Ca $^{2+}$]_i release in response to bronchospasmogenic stimuli. These changes in calcium signaling of sensitized airways could result from increased electrical activity as measured in airways of patients with asthma⁴⁷ or from sensitization-induced hyperpolarization of the smooth muscle cell membrane as shown by Souhrada and Souhrada.⁴⁸ Because in the latter study heating of the sensitizing serum prevented the change in resting membrane potential, it was concluded that temperature-sensitive components can lead to dysfunction of ion channels or pumps through binding

and subsequent hyperpolarization of the cell membrane.⁴⁸

In addition to sensitization by serum, incubation with cytokines such as TNF- α and IL-1 β is capable of modulating calcium signaling in airway smooth muscle in response to contractile agonists; however, these cytokines do not alter calcium signaling in smooth muscle cells under resting conditions.⁴⁹

Furthermore, it has to be taken into consideration that sensitization or the exposure to certain cytokines modifies not only contractile but also secretory functions of smooth muscle cells (Table II). After sensitization, smooth muscle cells can up-regulate their endogenous expression of T helper (T_H) type 1- and T_H2-like cytokine production as well as their receptors.¹¹ In addition, stimulation with IL-1, transforming growth factor- β 1, and experimental viral infection can lead to increased production of IL-6 and IL-11 by smooth muscle cells⁵⁰. Possibly the increased autocrine secretion of IL-1 β after passive sensitization is directly involved in the increased contractile capability of sensitized airway preparations.⁵¹ This view on smooth muscle cells as active players and potential immunomodulators opens up new prospects and might significantly improve our understanding of sensitization-induced changes in air-

TABLE II. Alteration of smooth muscle function

Sensitizing agent		Changes in contractile function	Changes within smooth muscle cells/synthetic function
IgE-rich serum	In vivo	⇒Allergen responses ¹²	
		↓PC ₂₀ FEV ₁ ^{23,24}	
	Ex vivo/in vitro	↑Maximal airway narrowing ²²	
		⇒Allergen responses ⁵²	↑[Ca ⁺⁺] _i ⁴⁶
		↑Maximal contraction ³⁸⁻⁴²	↑MLCK ⁴⁴
IL-4, IL-5 IL-5, GM-CSF	In vivo	↑Sensitivity ³⁸⁻⁴²	IL-1β, ⁵¹ IL-2/IL-2 receptor, IL-4 receptor, IL-5
		↑Shortening velocity to EFS ⁸	IL-5 receptor, IL-12, IFN-γ/IFN-γ receptor
	Ex vivo/in vitro	↑Shortening capacity to EFS ⁸	GM-CSF/GM-CSF receptor, ¹¹ RANTES ⁶
		↓PC ₂₀ FEV ₁ ^{2,73}	
		↑Maximal contraction ¹¹	
TNF-α IFN-γ, IL-1β	Ex vivo/in vitro	↑Sensitivity ¹¹	
		↑Maximal contraction to EFS ⁷⁷	ICAM-1, VCAM, CD44 ¹⁰
		↑Maximal contraction ¹¹	IL-6, IL-11, ⁵⁰ IL- ⁸⁶

way reactivity and concomitant airway inflammation in asthma (Fig 4).

ROLE OF IgE

On the basis of epidemiologic and experimental studies it has been suggested that serum IgE levels play an important role in the pathogenesis of smooth muscle hyperreactivity. Bronchial hyperresponsiveness was shown to be associated with serum IgE levels¹⁴ and to be transferable by IgE-rich serum from asthmatic to nonasthmatic subjects.¹² Incubation with serum from atopic individuals containing high levels of IgE was shown to induce hyperreactivity in isolated airway preparations⁵² and IgE was believed to cause abnormal smooth muscle contractile function through binding to the smooth muscle cell membrane and subsequent hyperpolarization.⁴⁸

Our own results show that the induction of allergen responses by passive sensitization is IgE mediated and highly specific for a selected allergen. Isolated airways that have been passively sensitized with serum containing high total IgE and high specific IgE antibodies against house dust mite exhibit contractile responses to house dust mite but not to other antigens.⁵² The presence of anti-IgE antibodies during passive sensitization, which reduces free IgE to undetectable levels⁵³ or, alternatively, the removal of serum IgE by immunomagnetic extraction of IgE complexes⁴² prevented allergen-induced contractile responses. Results were different regarding the increase in nonspecific responsiveness by passive sensitization because neither the presence of anti-IgE antibodies during sensitization⁵³ nor the removal of IgE from the sensitizing serum⁴² altered the induction of nonspecific hyperresponsiveness to histamine.

Taken together, these experiments indicate that only the induction of allergen responses depends on IgE, whereas the increase in histamine responsiveness is independent of IgE. Therefore a still unknown factor present in the sensitizing serum, which is closely associated with IgE, but not IgE itself, appears to be responsible for the induction of nonspecific smooth muscle hyperreactivity.

The experimentally based hypothesis that treatment of allergic asthma with anti-IgE antibodies should exhibit greater effects on IgE-mediated allergen responses as on hyperresponsiveness to nonspecific stimuli has been supported by recent clinical studies. These studies showed that treatment with the anti-IgE antibody rhuMAB-E25, which recognizes the specific Fc portion of circulating IgE that binds to the high-affinity IgE receptor FcεRI, reduced free serum IgE by about 90% and significantly decreased bronchoconstriction induced by allergen inhalation.^{54,55} Although Fahy et al⁵⁴ found no significant reduction of nonspecific responsiveness to methacholine, Boulet et al⁵⁵ observed a small but significant effect on PC₂₀FEV₁ of about 0.9 doubling doses. However, it is unclear whether in the latter study the small reduction of methacholine hyperresponsiveness was a direct effect of E25 or an indirect effect through reduction of airway inflammation, as a consequence of decreased mediator release from mast cells.

These promising results and the discrepancy between the effects of anti-IgE therapy on allergen-induced bronchoconstriction nonspecific bronchial responsiveness are in accordance with the in vitro data that predict the greater IgE dependence of allergen responses. Current studies aim to identify the serum factor(s) responsible for the induction of the nonspecific bronchial hyperresponsiveness. These factors were shown to be inactivated by heat,⁴⁸ are likely to be produced through similar mechanisms as IgE, or able to increase Ig switching from IgG to IgE⁵⁶ and might be enhanced by smoking. Because IL-4 exhibits the above-mentioned characteristics,⁵⁷⁻⁶¹ the factor is thought to be IL-4 itself or, more generally, to be found among the set of components that are associated with T_H2-type cytokine production.

IgE RECEPTORS

Furthermore, IgE receptors might play a role in the development of hyperreactivity; through direct interaction with IgE it has also been suggested that these recep-

tors might act as adhesion molecules and as “cytokines.” Two distinct receptors for IgE are known: the high-affinity receptor FcεRI and the low-affinity receptor FcεRII (CD23). Although the high-affinity receptor has its predominant role for the release of allergic mediators from inflammatory cells, the low-affinity receptor FcεRII and its soluble forms are not only capable of binding IgE but also exhibit functions that do not involve interactions with IgE, such as cell-cell interaction and cytokine-like activities.⁶²

Interestingly, the FcεRII receptor, in addition to its location on a variety of immune cells, has also been found on rabbit smooth muscle cells.⁶³ It was suggested that sensitization might enhance the expression of the low-affinity receptor and therefore be involved in the functional changes that are reflected in increased smooth muscle reactivity.⁶³ However, the precise role of this receptor and its increased expression on isolated human smooth muscle cells in the development of functional changes induced by sensitization remains to be demonstrated.

CELLS AND CYTOKINES

The degree of bronchial hyperresponsiveness is not only associated with elevated IgE levels but also with an increase in the number of inflammatory cell types within the airways.^{64,65} This is underlined by the findings of Kumagai et al,⁶⁶ who demonstrated in a murine model of asthma that the inhibition of inflammatory cell transmigration from the vascular bed into the alveolar space prevented allergen-induced airway inflammation and the development of nonspecific hyperreactivity to methacholine.

One of the major nonconstituent cell populations (cell populations that do not contribute to the normal architecture of the airways) that are found within the airway mucosa are T lymphocytes.⁶⁷ T cell-mediated immune responses are believed to be important contributors to airway hyperreactivity in asthmatic patients.⁶⁸ This is supported by the findings of de Sanctis et al⁶⁹ demonstrating that the transfer of T cells from a hyperresponsive mouse strain into a hyporeactive strain induces nonspecific airway hyperreactivity. This effect was thought to result from an enhanced susceptibility of the T cells to activating stimuli or a difference in T-cell subpopulations.

The characterization of lymphocyte populations in asthmatic and nonasthmatic individuals demonstrated differences in T-cell subtypes. In biopsy specimens and bronchoalveolar lavage fluid from patients with asthma, significantly higher numbers of T_H2-type cells were seen compared with control subjects, whereas there was no difference in the number of T_H1-type cells.^{65,70,71} T_H2-like cytokines such as IL-4 and IL-5 favor the production of IgE and the growth and activation of eosinophils and mast cells, but, noteworthy, they also enhance airway hyperresponsiveness *in vivo*⁷² as well as *in vitro*.¹¹

Both IL-4 and IL-5 inhalation cause increased bronchial responsiveness, airway eosinophilia,^{72,73} and an increase in the number of activated eosinophils^{64,74,75} as well as the levels of their products such as major basic

protein (MBP)⁷⁶ and TNF-α⁷⁷ that are associated with altered airway function. However, these observations did not clarify whether the changes in airway reactivity was due to direct effects of IL-4 and IL-5 or whether it was mediated through the infiltration by activated eosinophils. However, IL-13, which shares both a receptor component and signaling pathways with IL-4, was found to be necessary and sufficient for the expression of the pathophysiologic characteristics of asthma independently from IgE and eosinophils.⁷⁸

Hakonarson et al¹¹ demonstrated that preincubation with IL-5 or GM-CSF significantly enhanced responses to acetylcholine in isolated airways from rabbits, whereas the increase in responsiveness induced by passive sensitization was prevented by the addition of IL-2 to the serum. The same study showed that passive sensitization increased the expression of all 3 cytokines and their receptors on smooth muscle cells in culture. This underlines the possible relevance for smooth muscle secretory function in the regulation of airway reactivity and suggests an apparently direct effect of, for example, IL-5 on smooth muscle.

A preliminary report from Leckie et al⁷⁹ claims that a single intravenous application of the anti-IL5 antibody SB240563 caused a significant reduction in blood and sputum eosinophils for several weeks but did not modify late-phase responses to allergen in patients with asthma. These findings support the notion that IL-5 plays an important role in eosinophil recruitment but might question the relevance of eosinophils as well as IL-5 for the pathogenesis of bronchial hyperresponsiveness in asthma. However, it may be argued that the treatment period was too short to observe effects owing to the removal of IL-5 on alterations in smooth muscle reactivity that had persisted for years before. Furthermore, T_H2 type cytokines other than IL-5 or their combined effects might be the major determinants for smooth muscle hyperreactivity.

CONCLUDING REMARKS

The pathophysiologic basis of asthma and airway hyperresponsiveness can be thought of as a combination of altered bronchial smooth muscle function and airway inflammation. The relationship between both aspects is reflected by the association of bronchial hyperreactivity and asthma with serum IgE levels^{13,14} and markers of airway inflammation.^{68,80} Looking at the interaction between smooth muscle function and inflammation, it has to be considered that smooth muscle cells themselves are capable of producing proinflammatory cytokines, chemotaxins, and growth factors.^{6,11} However, it is not clear to which extent initiation of the disease inflammatory processes cause alterations of bronchial smooth muscle and, conversely, to which extent alterations in smooth muscle support or even initiate airway inflammation.

The mechanisms of airway sensitization appear to be linked to stimulation of the production of cytokines and chemokines such as IL-1β, IL-5, and RANTES by smooth muscle cells. IL-1β can enhance proliferation of the smooth muscle through increased secretion of growth

factors and expression of their receptors, whereas RANTES and IL-5 can attract and activate a variety of inflammatory cells.⁶ The release of TNF- α from these cells increases expression of adhesion molecules on smooth muscle cells, thereby enabling the interaction with T cells. Conversely, T-cell cytokines such as IL-4, IL-13, and GM-CSF are also able to attract and activate inflammatory cells.

Because greater smooth muscle mass and mediators released from smooth muscle cells may contribute to airway hyperreactivity, it might be questioned whether airway inflammation, as often believed, plays the key role in bronchial hyperresponsiveness. Possibly sensitization-induced alterations of smooth muscle function alone are sufficient. Environmental factors such as air pollutants,^{81,82} upper respiratory tract infections,^{83,84} occupational exposure to sensitizing agents,⁸⁵ or allergen exposure⁸⁶ can enhance bronchial responsiveness, at least temporarily. Although the effects of repeated exposure to these factors are not known, it has been demonstrated that they can initiate airway inflammation. It is easily conceived that chronic airway inflammation could contribute to the development of persistent bronchial hyperresponsiveness and facilitate the shift from nonsymptomatic bronchial hyperresponsiveness to symptomatic asthma.

Nevertheless, sensitization-induced changes in smooth muscle reactivity play a pivotal role in bronchial hyperresponsiveness as demonstrated by clinical studies in patients with asthma and by experimental models using methods of active or passive sensitization. Although a number of factors such as various cytokines and IgE have been shown to alter airway smooth muscle function, so far no single mediator has been identified that would mimic all effects of allergic sensitization.

Therefore the development of innovative preventive strategies for the treatment of bronchial asthma should possibly be targeted at the smooth muscle, in addition to the beaten track of airway inflammation.

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