

The immunopharmacology of mild asthma

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If cell numbers, activation state, or mediators, for example, can be correlated with some clinical measure of disease severity, a major effector role in the disease may be postulated. Mast cells, along with eosinophils and lymphocytes, are present in increased numbers in the airways of patients with asthma. Mast cell mediators are also increased in persons with allergies, with the concentrations of histamine, tryptase, and prostaglandin D₂ being proportional to the degree of airway obstruction and bronchial hyperresponsiveness. Increased numbers of activated mast cells and eosinophils (but not T cells or macrophages) were also found in bronchoalveolar lavage fluid in children. The mast cell is also known to release a range of cytokines (e.g., tumor necrosis factor- α and IL-4) that have various important functions, including upregulation of the endothelial adhesion molecules that are responsible for eosinophil recruitment from the microvascular circulation into the airways and subsequent activation. Mast cell staining for secreted IL-4 was found to be proportional to the infiltration of eosinophils and lower airway symptoms in patients with seasonal asthma, which is compatible with the concept that mast cells alone can sustain a continuing allergic inflammatory response. The mast cell proteases chymase and tryptase are also important for eosinophil recruitment and activation and for increasing mucus secretion and microvascular permeability. The evidence that the human mast cell is capable of releasing proteases and cytokines that have the capacity to initiate and maintain a chronic inflammatory response provides a mechanism whereby the clinical efficacy of nedocromil sodium in patients with chronic mild to moderate asthma can be explained. (J Allergy Clin Immunol 1996;98:S7-16.)

Key words: Allergic inflammation, cytokines, mast cell, mild asthma

During the past decade we have seen major developments in the perception of asthma as a disease of airway inflammation rather than purely in terms of disordered smooth muscle function. A major driver of this conceptual change originated when it became possible to obtain airway samples from patients with disease of varying severity through the application of fiberoptic bronchoscopy.¹ Early studies focused on mild disease and, to our surprise, revealed extensive evidence of ongoing airway inflammation, sometimes in the absence of symptoms and objective evidence of airway dysfunction.² Analysis of cells and mediators in bronchoalveolar lavage (BAL) fluid and the application of immunohistochemistry and electronmicroscopy to mucosal biopsy specimens confirmed what had long been suspected, namely, that the mast cell and the eosinophil were the major effec-

Abbreviations used

AMP:	Adenosine 5'-monophosphate
BAL:	Bronchoalveolar lavage
BHR:	Bronchial hyperresponsiveness
EAR:	Early asthmatic response
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
ICAM-1:	Intercellular adhesion molecule-1
LAR:	Late asthmatic response
NF κ B:	Nuclear factor κ B
PG:	Prostaglandin
SCF:	Stem cell factor
TNF- α :	Tumor necrosis factor- α

tor cells of the inflammatory response, irrespective of whether the disease had an allergic or a nonallergic basis.^{3,4} Many investigators, catalyzed by these initial observations, have undertaken a systematic analysis of the inflammatory components of asthma and have attempted to correlate their cellular and mediator findings with clinical and physiologic indices of disease activity and severity. The application of RNA-based methodology

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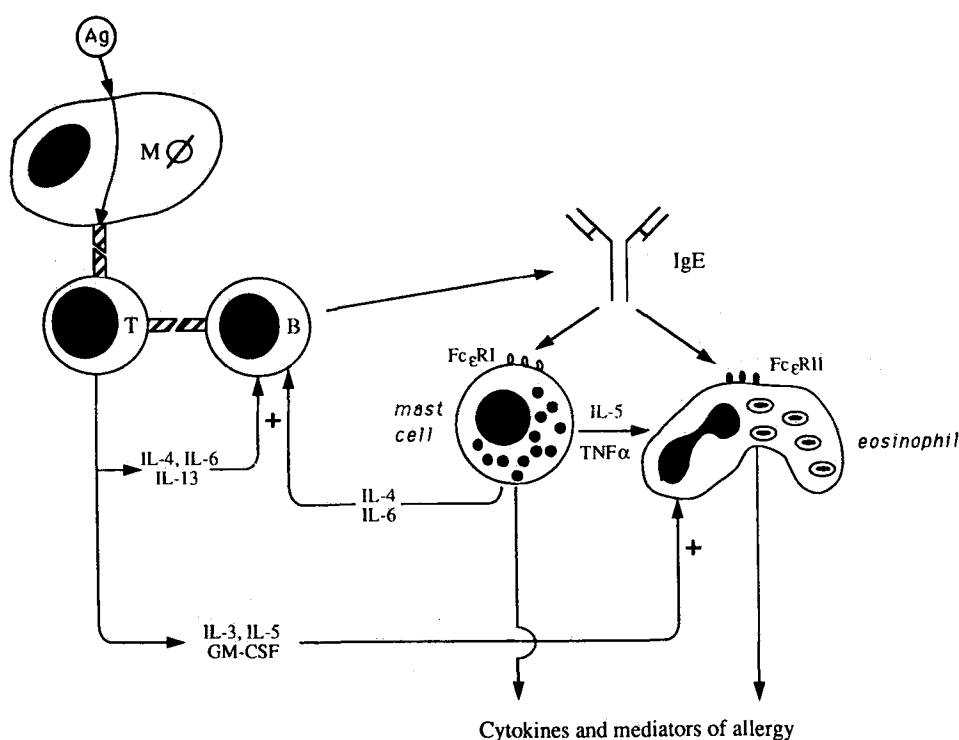


FIG. 1. Diagrammatic representation of cytokine networks involved in the regulation of allergic inflammation.

and improved methods of mediator detection have provided convincing evidence that, at least in the allergic form of asthma, initial sensitization and subsequent expansion of the mast cell and eosinophil populations result from the release of specific cytokines encoded on chromosome 5q.^{5,6} These include IL-3, IL-4, IL-5, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-13, the recently described homologue of IL-4.⁷ Figure 1 summarizes the biologic effects of these cytokines on mast cell, basophil, eosinophil, and B-lymphocyte function and also emphasizes the important role that IL-4 plays in directing T-cell differentiation toward those that produce cytokines of the IL-4 gene cluster.

In sensitized subjects, provocation of the airways with inhaled allergen produces early and late phases of airway obstruction in parallel with a progressive acquisition of bronchial hyperresponsiveness (BHR) to nonspecific stimuli. A combination of direct measurement of mediators or their metabolites, the use of pharmacologic agents that either inhibit the generation of a specific mediator or antagonize its receptor-mediated pharmacologic effects, and the use of inhibitors of cell activation such as cromolyn sodium and nedocromil sodium have together provided con-

vincing evidence that the early asthmatic response (EAR) results from the IgE-dependent activation of airway mast cells with the release of specific autacoid mediators, of which histamine, prostaglandin (PG) D₂, and leukotriene C₄ are probably the most important.⁸ However, it is the late asthmatic response (LAR) that has most recently attracted interest, mainly because it has been thought of as a paradigm for the inflammatory response in clinical asthma. Whereas BAL undertaken 24 hours after allergen provocation has shown eosinophil influx in atopic patients with mild asthma, at this point late-phase airway obstruction has usually recovered, although the airways remain hyperresponsive.⁹ If BAL fluid is examined 4 to 18 hours after local instillation of allergen into sensitized airways, during the onset of the LAR, increased numbers of neutrophils and eosinophils are recovered.^{10,11} As early as 15 minutes after challenge there is retention of T lymphocytes by the airways,¹² which persists for up to 6 hours¹⁰ and is accompanied by a reduction in the number of T cells recovered by lavage that specifically express the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1).¹⁰ By 18 to 24 hours, the lavage T-cell response returns to

normal, although there is a preferential increase in CD4+ cells expressing T_{H2}-type cytokines.^{5, 13, 14} The BAL neutrophilia at 4 to 6 hours seems to be largely secondary to epithelial cell activation and release of the neutrophil chemoattractant IL-8.¹⁵

When examining bronchial biopsy specimens obtained from the airways 4 to 6 hours after exposure to either allergen or saline solution, we were impressed by the marked increase in submucosal eosinophils and neutrophils (identified respectively by their granular content of eosinophil cationic protein and neutrophil elastase) and also the migration of mast cells (identified through their granular content of tryptase) toward the bronchial epithelium.¹⁶ The retention of neutrophils and eosinophils within the submucosa, together with evidence for release of their granular contents into the airway wall, indicated that not only are these cells selectively recruited into the airways with allergen, but they are also activated. In contrast to findings in BAL, at the 4 to 6 hour point an increase occurred in the number of infiltrating T cells that were equally divided between the CD4+ and CD8+ subpopulations; however, evidence for T-cell activation was minimal.¹⁶

In further studies, the BAL and biopsy findings of patients with mild asthma were investigated 24 hours after local instillation of allergen or saline solution into the airways. The BAL eosinophilia persisted but, in addition, there was now evidence of T-cell activation, increased IL-5 transcription, and cytokine product release.^{11, 13, 14} In bronchial biopsy specimens taken 24 hours after instillation of allergen, the tissue eosinophilia and neutrophilia had largely resolved, although there remained a small but significant increase in T cells exhibiting enhanced expression of interleukin-2 receptor (CD25).

On the basis of these studies it appears that, whereas T-cell activation is a late feature of allergen provocation, the secretion of cytokines from T cells could not account for the cellular events of the late-phase response because their release would first require increased transcription before any product could be generated. In vitro studies have shown that it takes 4 to 6 hours for this to reach a peak. It is this disparity that led us to explore possible alternative mechanisms to explain the allergen-provoked leukocyte influx.

The introduction of allergen onto sensitized airways led, within 4 to 6 hours, to the increased expression of two cell adhesion molecules on the microvascular endothelium, E-selectin and ICAM-1.¹⁶ Enhanced expression of these adhesion molecules was associated with an increase in tissue

infiltration of leukocytes bearing complementary ligands, supporting the view that leukocyte-endothelial interactions are crucial to the pathogenesis of the LAR. In in vitro systems, E-selectin and ICAM-1 are upregulated by specific cytokines, namely tumor necrosis factor- α (TNF- α), IL-1 β , and interferon- γ .¹⁷ By interacting with specific receptors on the surface of endothelial cells, these cytokines increase the generation of reactive oxygen intermediates with subsequent activation of a specific cytoplasmic protease, which cleaves a small peptide from the nuclear recognition site of the heterodimeric transcription factor, nuclear factor κ B (NF κ B). Subsequent dissociation of the inhibitor I κ B and the liberation of free heterodimer (P50/P65) enables NF κ B to traverse the nuclear membrane.¹⁸ Within the nucleus, NF κ B binds to a specific decanucleotide consensus sequence in the promoter regions for the genes of E-selectin, ICAM-1, and vascular cell adhesion molecule-1, resulting in increased transcription. NF κ B is also involved in the endothelial secretion of IL-8 and TNF- α . Thus, central to the allergen-induced recruitment of leukocytes into the airways is their entrapment within the microvasculature followed by their transendothelial migration and activation. Because we have shown that these processes are well established 4 to 6 hours after allergen exposure, it was important to look for a cellular source of cytokine that could be rapidly released and become bioavailable.

THE MAST CELL AS A KEY EFFECTOR CELL OF THE LATE-PHASE ALLERGIC RESPONSE

It has been known for some time that rodent mast cells, when suitably stimulated, could generate a range of cytokines,^{19, 20} but until recently it was not known whether this also occurred in human mast cells. The application of immunohistochemistry to bronchial and nasal mucosal biopsy specimens from patients with allergic asthma or rhinitis revealed strong immunostaining of mast cells (identified through their content of tryptase) for a range of cytokines, specifically TNF- α , IL-4, IL-5, and IL-6.²¹⁻²³ Immunoelectron microscopy further demonstrated that these cytokines were located within the mast cell secretory granules and, after IgE-dependent activation, could be released along with the other preformed mediators.^{21, 24} Thus, as described in the mouse cutaneous allergen late-phase model of Gordon and Galli²⁰ and Galli,²⁵ TNF- α derived from mast cells seems to be a particularly important candidate for initiating the increased expression of E-selectin and ICAM-1

and subsequent leukocyte recruitment. Vascular cell adhesion molecule-1 is an endothelial adhesion molecule that has been particularly linked to the selective recruitment of eosinophils by interacting with the integrin ligand very late activation protein-4 (VLA-4). It is upregulated 24 hours after allergen exposure²⁶ and requires IL-4 specifically to enhance and stabilize its expression by TNF- α . We believe that these findings are important because they demonstrate that human mast cells have the capacity to release potentially large quantities of cytokines relevant to leukocyte recruitment and, as in the mouse, could account for almost the entire late-phase inflammatory response.

Although the late-phase airway obstruction has usually recovered within 8 to 10 hours after allergen provocation, BHR may persist for up to 3 to 5 days and sometimes longer. We believe that the mast cell also plays an important role in orchestrating this aspect of the allergic tissue response through increased cytokine transcription and the generation of newly formed products. With use of mast cells dispersed from human lung and skin and purified to more than 98% by a combination of differential centrifugation and immunomagnetic selection, and taking advantage of the existence of stem cell factor (SCF) receptor (c-kit) on mast cells,²⁷ we have been able to show that, in the presence of SCF, ligation of cell-bound IgE results in a marked increase in transcription of TNF- α , IL-4, and IL-5, which persists for up to 24 hours, 12 hours, and 72 hours, respectively, after a single immunologic stimulus and is paralleled by release of cytokine product²⁸ (Fig. 2). The amount of IL-5 generated by human mast cells is large, up to 2500 pg/10⁶ cells. In the case of IL-4, its tight binding to the glycosaminoglycan side chains of heparin²⁹ and its rapid degradation by mast cell proteases³⁰ make it difficult to detect by standard immunoassays, although, with use of an IL-4 T-cell line, biologically active IL-4 can be demonstrated on the surface of mast cells.²¹ Both IL-4 and IL-5 transcription are highly IgE dependent, whereas TNF- α is more dependent on SCF, although gene expression is enhanced by IgE-dependent cell signaling. Thus the continued release of cytokines from activated mast cells, which have the potential to respond to a specific allergen, provides convincing evidence for incriminating this cell as a major source of cytokines capable of supporting the allergen-specific LAR.

Application of reverse transcriptase polymerase chain reaction to highly purified mast cells has also revealed their capacity to generate IL-3, IL-10, IL-13, GM-CSF, and SCF itself. The immunohis-

tochemical identification of SCF within the secretory granules of a proportion of human mast cells suggests that there may be important autocrine loops in allergic diseases, as has also been described for the eosinophil.

Human mast cells have been phenotyped according to their content of neutral proteases, with those at mucosal surfaces containing predominantly tryptase (MC_T cells), whereas the mast cells more associated with connective tissue elements contain larger amounts of tryptase and, in addition, chymase, carboxypeptidase A, and cathepsin G. In the airway of the patient with asthma, IL-5, IL-6, and TNF- α appear to partition with MC_T cells, whereas IL-4 is found in the granules of both mast cell populations.³¹ The proteases themselves also have important mediator functions. Tryptase is a potent chemoattractant and secretagogue for human eosinophils,³² stimulates ICAM-1 expression and proliferation of and IL-8 secretion by bronchial epithelial cells,³³ and shares with the mast cell autacoids the capacity to increase microvascular permeability³⁴ (Fig. 3). Chymase is a potent mucus secretagogue,³⁵ degrades certain cytokines including IL-4,³⁰ and, like tryptase, can increase microvascular permeability.³⁴ These functions are relevant to many of the features of chronic airway inflammation in asthma and provide a further basis for incriminating the mast cell in chronic and acute airway events.

PHARMACOLOGIC REGULATION OF MAST CELL MEDIATOR RELEASE

It has long been recognized that cromolyn sodium and nedocromil sodium inhibit mast cell mediator release. This mechanism is thought to explain the inhibitory effect of these drugs on the allergen-induced EAR and bronchoconstriction provoked by exercise, isocapnic hyperventilation, inhaled hypertonic saline solution, and adenosine 5'-monophosphate (AMP).³⁶ Provocation of sensitized nasal airways with allergen results in an increase in TNF- α recovered from the mucosa by lavage.³⁷ Nedocromil sodium has also been shown to inhibit TNF- α release and transcription from activated rodent mast cells.³⁸ Given that human mast cells are an important source of preformed and newly generated cytokines, the suppressive action of cromolyn sodium and nedocromil sodium on the LAR and acquired BHR after allergen provocation can be accounted for by inhibition of mast cell cytokine release. In support of such a mechanism, Calhoun et al.³⁹ have shown an inhibitory effect of nedocromil sodium on the allergen-induced increase in BAL histamine during the EAR and suppression of eosinophils at 24 hours.

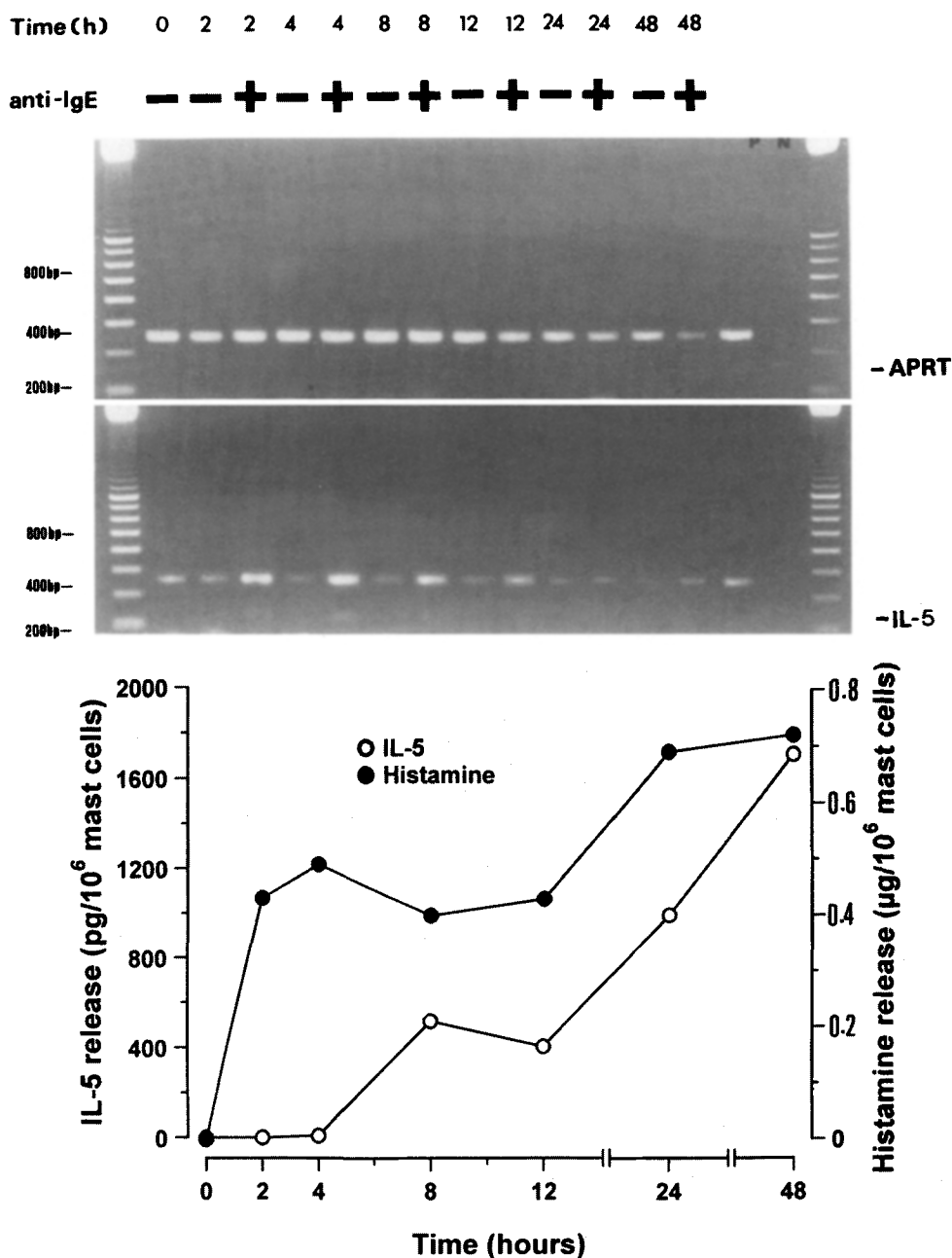


FIG. 2. Transcription of IL-5 by highly purified (>98%) human lung mast cells in the presence (+) and absence (–) of antihuman IgE. The time course of the PCR products (*upper panel*) are compared with the time course of histamine and IL-5 product release. Mast cells were preincubated with a low concentration of stem cell factor (10 ng/ml).

THE MAST CELL IN THE EVOLUTION OF CHRONIC AIRWAY INFLAMMATION IN ASTHMA

Even in the absence of provocation, BAL fluids from patients with allergic asthma demonstrate increased concentrations of mast cell mediators,

including histamine, PGD₂, and tryptase in proportion to the degree of baseline airway obstruction and BHR. Clinical experience with cromolyn sodium and, more recently, with nedocromil sodium has led to the view that these drugs are more active in treating asthma associated with respiratory al-

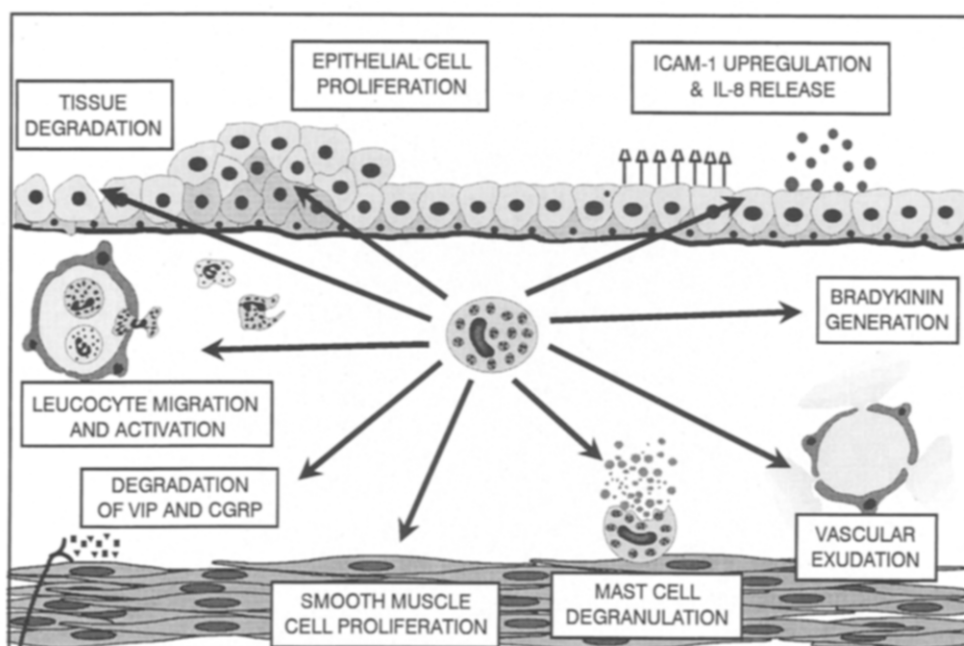


FIG. 3. Diagrammatic representations of targets for the action of mast cell tryptase pertinent to the airway inflammation of asthma.

lergy and in younger patients. In patients with allergic asthma, BAL has consistently demonstrated an increased recovery of mast cells from the airway lumen in proportion to the mast cell content of the respiratory epithelium (but not the submucosa). In its milder form, it seems that it is the mast cells at the very surface of the airways that are responsible for a significant component of the clinical expression of the disease, including the response to allergen, exercise, and other mast cell-dependent bronchoprovocants. In patients with seasonal asthma, we have shown an increased immunostaining of mast cells with the monoclonal antibody 3H4, which recognizes the secreted form of IL-4^{22, 23} in proportion to lower airway symptoms and eosinophil infiltration, suggesting that mast cells alone can sustain a continuing allergic inflammatory response.⁴⁰ The proven efficacy of cromolyn sodium and nedocromil sodium in patients with seasonal asthma adds to the view that these drugs have an important modulatory effect on mast cells because they are not known to inhibit T-cell function.

In 1983 we demonstrated that adenosine is a potent bronchoconstrictor in patients with asthma but not in healthy subjects.⁴¹ Bronchoconstriction provoked by adenosine and AMP was observed in patients with both allergic and nonallergic asthma and could be powerfully inhibited by histamine H₁

antagonists,^{42, 43} cromolyn sodium, and nedocromil sodium, the latter drug being approximately 10 times more potent than the former.^{44, 45} Most recently, we have shown that AMP-induced bronchoconstriction is associated with an increase in the plasma concentrations of histamine⁴⁶ and elevated levels of histamine, PGD₂, and tryptase recovered from the airways by BAL.⁴⁷ In vitro studies have confirmed the stimulatory effects of adenosine on isolated human mast cells through an adenosine A₃ or A_{2b} receptor subtype, which is linked to G proteins that stimulate adenylate cyclase and the phosphatidyl inositol pathway.⁴⁸

Both in children and in adults, the therapeutic response of asthmatic airways to inhaled corticosteroids is more closely matched by a reduction in airway responsiveness to AMP than it is to methacholine.⁴⁹ In addition, Avital et al.⁵⁰ has recently demonstrated that AMP hyperresponsiveness in children with asthma correlates better with the clinical expression of their disease when compared with the more traditional tests of BHR—: histamine and methacholine. Finally, with use of a novel cannula technique to obtain BAL fluid from the airways of children with asthma, Heaney et al.⁵¹ have demonstrated increased numbers of activated mast cells and eosinophils but not T cells or macrophages. In a comparable group of children with intermittent virus-related wheezing, the

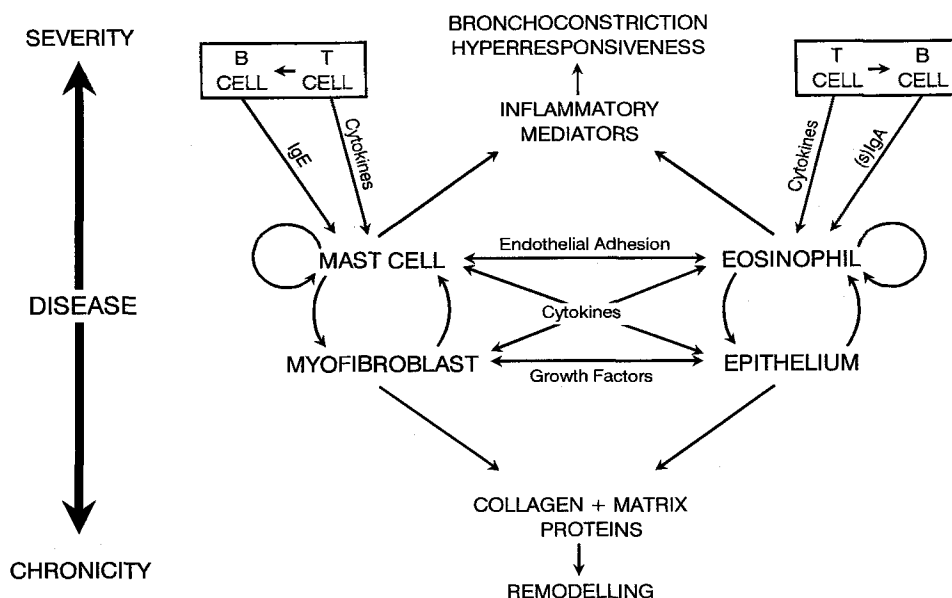


FIG. 4. Interactions between immunologic, inflammatory, and remodeling processes in determining the severity and chronicity of asthma.

prominent inflammatory cell was the neutrophil rather than the mast cell or eosinophil. Taken together, these findings support the view that the human mast cell is an important contributor to the continuing airway inflammatory response of persons with mild asthma and could explain the therapeutic efficacy of cromolyn sodium and nedocromil sodium. Similarly, by inhibiting the production of mast cell growth factors, the clinical efficacy of topical corticosteroids could also operate in part by replacing the mast cell population in the airways.⁵²

In a collaborative study with Vrugt et al.⁵³ at the Dutch Asthma Centre in Davos, the airways of patients with severe corticosteroid-dependent asthma have been shown to exhibit increased T-cell infiltration and activation when assessed by both BAL and mucosal biopsy. The extent of T-cell involvement in these patients with steroid-dependent asthma appeared not to be related to the degree of eosinophil infiltration. In a Southampton-based study, the number of eosinophils in the mucosa was even less than that observed in patients with mild disease. It is possible, however, that the eosinophil infiltrate, rather than being close to the surface of the airways (and accessible to lavage and mucosal biopsy), is present to a greater degree outside the airway smooth muscle. This certainly appears to be true in patients who have died of severe asthma and may be an important factor in determining BHR in severe chronic

disease.^{54,55} The presence of eosinophils and activated T cells outside the airway smooth muscle would also limit their accessibility to inhaled corticosteroids and might explain why patients with severe chronic disease are more dependent on oral corticosteroid medication. Despite receiving large doses of corticosteroids orally (30 to 60 mg daily), the subjects with asthma receiving treatment in the Davos Centre who had severe "allergic" disease exhibited enhanced mRNA expression for IL-5 and IL-13 in mucosal biopsy specimens assessed by reverse transcriptase polymerase chain reaction. Thus it would appear that similar cytokines that have the inflammatory response in mild to moderate disease also are important in maintaining the airway inflammatory response in patients with more severe asthma, although the cellular compartment expressing this favors the T cell rather than the mast cell.

Our recent studies have further suggested that, in patients with chronic severe disease, cytokine production by the bronchial epithelium and subepithelial myofibroblasts is an important factor in maintaining the inflammatory response.^{56,57} With use of a mucosal explant system from nasal polyps, we have shown that in the presence of specific allergen there is enhanced production of IL-8 and GM-CSF, two cytokines that are capable of prolonging eosinophil survival by inhibiting apoptosis, thereby enhancing their capacity to serve as mediator secreting cells.⁵⁸ Through the release of trans-

forming growth factor- β ,⁵⁹ basic fibroblast growth factor,⁶⁰ and endothelin,^{61, 62} the airway epithelium also drives a proliferative fibrotic response that could contribute to the airway wall remodeling characteristic of severe and chronic asthma.⁶³ Involvement of the formed elements of the airway, in both the inflammatory and the tissue remodeling responses, provides an additional mechanism whereby the airways in patients with severe asthma become less responsive to corticosteroids and exhibit a progressive decline in baseline pulmonary function.

CONCLUDING COMMENTS: IMPLICATIONS FOR THE DRUG MANAGEMENT OF ASTHMA

The newfound evidence that the human mast cell is capable of releasing proteases and cytokines that have the capacity to initiate and maintain a chronic inflammatory response provides a mechanism whereby the clinical efficacy of nedocromil sodium and related drugs in chronic mild to moderate asthma can be explained. Moreover, viewing the transition of asthma from mild intermittent disease associated with allergen exposure through to chronic airway inflammation and, finally, to fixed airway obstruction provides a basis for the different drug needs of this disease during its natural history, with the inflammatory response progressively evolving from being mast cell-dependent to a greater involvement of T cells and, finally, to constitutive elements of the airway wall (Fig. 4). Superimposed on this background are the environmental factors that result in acute variations in the disease phenotype. Included among these are variations in allergen exposure, resulting in enhanced eosinophil recruitment through mast cell- and T-cell-dependent mechanisms, and respiratory tract viruses, including human rhinoviruses that interact with the lower airway resulting in increased chemokine secretion accompanied by T-cell activation and eosinophil recruitment.⁶⁴ Because the airway response to inhaled allergens and viruses will depend on the basal level of airway inflammation, it seems appropriate within guidelines of asthma therapy to target regular treatment at reducing the underlying level of airway inflammation, thereby diminishing the opportunity for acute relapses. In choosing the most appropriate anti-inflammatory drugs it is also prudent to weigh the therapeutic advantages against possible systemic and local side effects, particularly in patients at the milder end of the disease spectrum.

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