

Inflammatory cells in asthma: Mechanisms and implications for therapy

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Recent clinical studies have brought asthma's complex inflammatory processes into clearer focus, and understanding them can help to delineate therapeutic implications. Asthma is a chronic airway inflammatory disease characterized by the infiltration of airway T cells, CD⁺ (T helper) cells, mast cells, basophils, macrophages, and eosinophils. The cysteinyl leukotrienes also are important mediators in asthma and modulators of cytokine function, and they have been implicated in the pathophysiology of asthma through multiple mechanisms. Although the role of eosinophils in asthma and their contribution to bronchial hyperresponsiveness are still debated, it is widely accepted that their numbers and activation status are increased. Eosinophils may be targets for various pharmacologic activities of leukotriene receptor antagonists through their ability to downregulate a number of events that may be key to the effector function of these cells. (*J Allergy Clin Immunol* 2003;111:S5-17.)

Key words: Asthma pathogenesis, T cells, mast cells, basophils, macrophages, eosinophils, cytokines, cysteinyl leukotrienes

A sensitized individual's initial response to allergen is dominated by products associated with mast cell activation, particularly histamine, prostaglandin D₂ (PGD₂), leukotriene C₄ (LTC₄), and tryptase. Within hours of the response, inflammatory cells are recruited from the circulation, including T cells, neutrophils, eosinophils, basophils, and monocytes. Understanding the complex mechanisms of asthma's inflammatory processes can help to delineate therapeutic implications, and recent clinical studies have highlighted mechanisms of this inflammatory process. For example, the effect of anti-IgE therapy on airway response to allergen bronchoprovocation has underlined the critical role of mast cells and basophils, which express the high-affinity IgE receptor. Studies with the leukotriene receptor antagonists (LTRAs) have demonstrated that cysteinyl leukotrienes (CysLTs) are important for most early and late physiologic responses to allergen bronchoprovocation and that CysLTs play a central role in the allergic airway and the recruitment of inflammatory cells.

Abbreviations used

5-LO:	5-lipoxygenase
AA:	Arachidonic acid
BAL:	Bronchoalveolar lavage
CysLT:	Cysteinyl leukotriene
LTC ₄ :	Leukotriene C ₄
LTD ₄ :	Leukotriene D ₄
LTE ₄ :	Leukotriene E ₄
LTRA:	Leukotriene receptor antagonist
MBP:	Major basic protein
PGD ₂ :	Prostaglandin D ₂
SAC:	Segmental allergen challenge

This article reviews cellular mechanisms that are part of asthma's inflammatory processes and details recent clinical studies that shed light on those processes, providing a clearer understanding of specific roles played by therapeutic agents, particularly the leukotriene modifiers.

INFLAMMATORY CELLS IN ASTHMA

Asthma is a chronic airway inflammatory disease characterized by infiltration of the airway T cells. In both normal and asthmatic airway mucosa, the prominent cells are the T lymphocytes, which are activated in response to antigen stimulation, or during acute asthma exacerbations, and produce high levels of cytokines. They are subdivided into two broad subsets according to their surface cell markers and distinct functions: the CD4⁺ (T helper) and the CD8⁺ (T cytotoxic) cells. CD4⁺ cells are further subdivided into T_{H1} and T_{H2} cells, depending on the type of cytokines that they produce. Another subtype of CD4⁺ cells has been identified, the T_{H3} cells, which produce high levels of transforming growth factor β and various amounts of IL-4 and IL-10.¹ The T_{H3} cells are associated with oral tolerance and are suggested to be regulatory T helper cells.¹⁻⁵ Other cells involved in the pathogenesis of asthma include mast cells, basophils, macrophages, and eosinophils. The interactions among all these cells and their products perpetuate the inflammatory response.

CYTOKINES

The initial indication for cytokine involvement in the pathogenesis of asthma came from studies performed in the early 1990s showing that atopic asthma was associated with local T_{H2} cytokine expression. IL-3, IL-4, IL-5,

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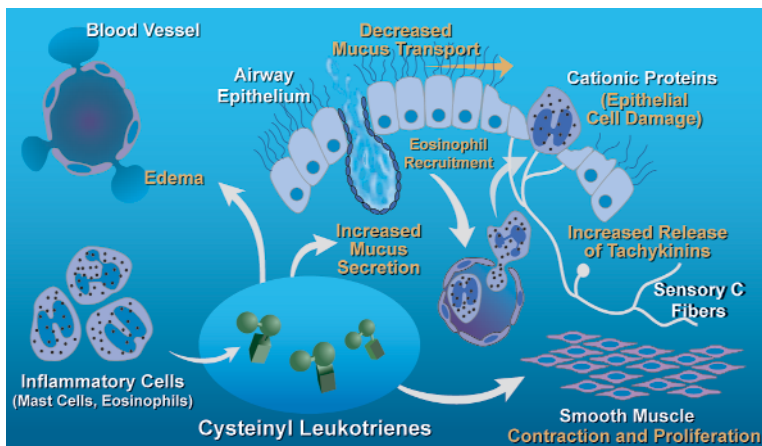


FIG 1. Potential sites and effects of cysteinyl leukotrienes relevant to asthma pathophysiology. Adapted from Hay DW, Torphy TJ, Undem BJ. Cysteinyl leukotrienes in asthma: old mediators up to new tricks. *Trends Pharmacol Sci* 1995;16:304-9.

and GM-CSF were upregulated in asthmatic patients relative to control subjects. These cytokines were significantly upregulated after antigen challenge, and their receptors were identified locally on the surface of inflammatory cells.⁶ Studies have confirmed the existence of the prominent T_H2 -type cytokine profile not only in asthma but also in allergic rhinitis and atopic dermatitis.

Many of these cytokines have been found in human beings and have been shown to be associated with pathologic changes of asthma.⁶ For example, IL-13 is associated not only with IgE synthesis and chemoattraction of eosinophils but also with mucus hypersecretion, fibroblast activation, and the regulation of airway smooth muscle function.⁷ Another T_H2 cytokine, IL-9, is upregulated preferentially and is associated with airway hyperresponsiveness, mucus hypersecretion, eosinophil function, IgE regulation, and the upregulation of calcium-activated chloride channel.⁸⁻¹⁰

The list of chemokines associated with asthma has expanded and includes eotaxin, monocyte chemoattractant protein 4, and RANTES. Although transforming growth factor β has long been considered the major profibrotic cytokine associated with subepithelial fibrosis and production of extracellular matrix proteins, other cytokines such as IL-11 and IL-17 have also demonstrated profibrotic activity in association with severe asthma.¹¹⁻¹³

LEUKOTRIENES

Although not cytokines, the CysLTs have emerged as important mediators in asthma and as modulators of cytokine function. Leukotrienes are lipid mediators resulting from the catabolism of the arachidonic acid (AA) released from the cell membrane by phospholipase A_2 after cell activation. After its release, AA is metabolized either by the cyclooxygenase pathway, generating prostaglandins and thromboxanes, or by the 5-lipoxy-

genase (5-LO) pathway, which in association with 5-LO-activating protein as a helper protein produces the leukotrienes: leukotriene B_4 , LTC₄, leukotriene D_4 (LTD₄), and leukotriene E_4 (LTE₄), with the last three forming the CysLT group. LTC₄ is metabolized enzymatically to LTD₄ and subsequently to LTE₄, which is excreted in the urine. The CysLTs are produced in eosinophils, monocytes, macrophages, mast cells, basophils, and, to a lesser extent, endothelial cells and T lymphocytes.¹⁴

Increased production of CysLTs has been detected in bronchoalveolar lavage (BAL)^{15,16} and urine¹⁷ samples from patients with asthma, especially after allergen challenge¹⁸ or during an acute asthma attack.¹⁹ In allergic airway inflammation, the expressions of 5-LO and 5-LO-activating protein enzymes are increased; their mRNA is present in endothelial and inflammatory cells after allergen challenge in mice.²⁰ Furthermore, an overexpression of LTC₄ synthase has been demonstrated in bronchial biopsy specimens from asthmatic patients.²¹⁻²³

The CysLTs also have been implicated in the pathophysiology of asthma by way of multiple mechanisms, including mucus hypersecretion, increased microvascular permeability, ciliary activity impairment, inflammatory cell recruitment, edema, and neuronal dysfunction (Fig 1).²⁴⁻²⁹ The CysLTs also induce eosinophil recruitment into the airways of guinea pigs *in vitro*³⁰ as well as in patients with asthma *in vivo*.^{31,32} Most important, these molecules increase airway hyperresponsiveness and cause smooth muscle hypertrophy in both healthy subjects and asthmatic patients.^{32,33}

MAST CELLS

Mast cells make up a small proportion of cells recovered by BAL, but within the airway tissue as many as 20% of inflammatory cells are mast cells.^{34,35} In BAL specimens, normal mast cell numbers range from 0.02%

to 0.48%.^{36,37} Normal mast cell numbers^{36,38-41} or increases of 2- to 6-fold, have been reported in patients with atopic^{34,37,42-47} as well as nonatopic asthma.⁴⁸ On the airway surface and in the submucosa, mast cells are mostly the mucosal type, containing tryptase in secretory granules designated MC_T , as opposed to the tissue-type mast cell containing both tryptase and chymase, designated MC_{TC} .

Present on the surface of and within the airway, mast cells are well positioned to respond to a provocative stimulus. Normally, they are the only resident cells in the airway that can interact with allergen by way of the IgE bound to the high-affinity receptor FcεRI. On allergen challenge of the airways, the mast cells respond within minutes, releasing both preformed mediators such as histamine and tryptase and newly synthesized products such as PGD₂ and LTC₄.^{16,38,40,49-52} Clearly, the immediate response to allergen challenge is dominated by products that are found with the mast cell. These products are potent bronchoconstrictors and may induce alterations in vascular permeability. Mast cell numbers in the bronchial mucosa also may increase after the late-phase response to allergen challenge.⁵³ In addition to allergen, such other stimuli as exercise, aspirin, and chemicals may invoke mast cell degranulation, leading to bronchoconstriction and vascular changes.

Ongoing mast cell degranulation has been shown to be present in chronic asthma, as evidenced by increased levels of the mast cell mediators histamine, PGD₂, and tryptase,^{34,36,39,41,54,55} although BAL histamine levels may be elevated in persons with allergic rhinitis without asthma.^{36,45} In vitro both spontaneous and IgE-mediated release of histamine has been enhanced in BAL mast cells of asthmatic versus nonasthmatic persons,⁴⁶ and spontaneous histamine release has been increased in patients with symptomatic versus asymptomatic asthma.⁴¹

Mast cells may further participate in asthma's inflammatory changes through the elaboration of cytokines. In response to IgE-dependent stimuli, mouse mast cell lines have been shown to produce a profile of cytokines, including IL-3, IL-4, IL-5, and IL-6, similar to the T_H2 profile produced by T lymphocytes. Human lung mast cells have been shown to release IL-4,⁵⁶ IL-5,⁵⁷ and IL-13⁵⁸ in vitro, and mucosal biopsy specimens from asthmatic persons have revealed positive staining by immunohistochemical means for IL-4, IL-5, IL-6, and TNF-α in mast cells.⁵⁹ In IgE-mediated reactions, mast cells are most likely an important immediate source of TNF-α. Unlike other sources of TNF-α, such as macrophages, resting mast cells contain preformed stores available for immediate release.⁶⁰ Further localization of cytokines to mast cell subsets reveals preferential IL-4 expression by MC_T mast cells, with predominantly IL-5 and IL-6 expression by the MC_{TC} subset.⁶¹

BASOPHILS

Basophils possess high levels of the FcεRI receptor and are capable of an immediate response to allergen.

Although basophils are not present in healthy airways, they are present in the airways of asthmatic persons under a variety of circumstances. Basophils have been reported in the sputum of patients with symptomatic asthma,⁶² and recent studies have demonstrated basophil infiltration of airways in cases of fatal asthma^{63,64} and in bronchial biopsy specimens from patients with asthma.^{65,66} During the late response to allergen challenge, large numbers of basophils have appeared in BAL specimens after segmental allergen challenge (SAC)^{38,67} and have been noted in airway tissue after inhalation bronchoprovocation.^{66,68} Like mast cells, basophils release histamine on activation; unlike mast cells, however, they do not produce PGD₂. The major product of AA metabolism in the basophil appears to be LTC₄. On a per cell basis, basophils produce as much LTC₄ as do mast cells and much more than do eosinophils. Recently, basophils have also been found to be a rich source of IL-4 and IL-13, demonstrating both spontaneous release and response to IgE-mediated stimuli.^{69,70} In fact, basophil production of these cytokines rivals that reported for T-cell clones. Mixed lymphocyte populations produce only 10% to 20% as much IL-4 as do basophils.⁷¹

MACROPHAGES

Macrophages are the predominant cell recovered by BAL in both nonasthmatic and asthmatic persons. Although most macrophages are recovered from alveoli, small volume lavage or lavage of isolated airway segments⁷² supports macrophage predominance in conducting airways as well as alveoli. Thus, macrophages are well positioned to respond to and regulate inflammation along the airway. Although the prominence of macrophages along the airway surface and their diverse functions strongly implicate macrophages as playing a role in asthma, it is unclear whether that role is one of promoting or preventing inflammatory responses. On the one hand, macrophages can perform accessory cell functions by presenting antigen and providing secondary signals (eg, IL-1) required for the differentiation and proliferation of specific lymphocyte responses. These functions may play a role in sensitizing the airway to respond to further exposures. On the other hand, in some systems, alveolar macrophages have been found to be poor antigen-presenting cells, and in the large proportions of macrophages to lymphocytes (5:1 to 10:1) found on the airway surface, macrophages most likely suppress lymphocyte responses.⁷³ Thus, the role of the resident macrophage in initiating immune responses remains unclear. Adding to this complexity are the findings that blood monocytes are better antigen-presenting cells than are macrophages and may be recruited to inflammation sites. In addition, dendritic cells are present in the airways and appear to be much more potent antigen-presenting cells than are macrophages.⁷⁴

Nevertheless, airway macrophages may participate in airway inflammation through multiple mechanisms. Alveolar macrophages express the low-affinity receptor

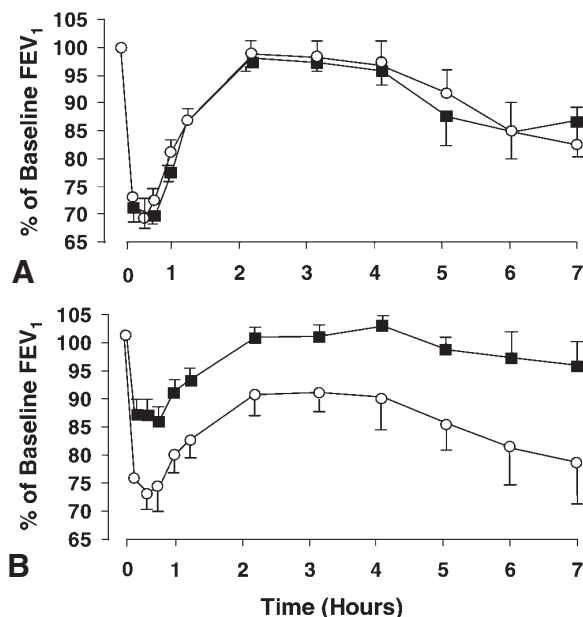


FIG 2. Effect of anti-IgE therapy on allergen bronchoprovocation. FEV₁ is shown as a percentage of baseline in the first 7 hours after allergen challenge. The early phase is during hour 1, and the late phase is from hours 2 to 7. The responses to placebo (**A**) and rhuMAB-E25 (**B**) are depicted at baseline (*open circles*) and at the end of 9 weeks of treatment (*closed squares*). RhuMAB-E25 significantly reduced allergen-induced bronchoconstriction during both early- and late-phase responses to allergen bronchoprovocation. From Fahy JV, Fleming HE, Wong HH, Liu JT, Su JO, Reimann J, et al. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1997;155:1828-34.

for IgE FcεR2, and expression appears to be increased in asthmatic persons relative to healthy subjects.⁷⁶ Macrophage release of lysosomal enzymes in response to SAC has been demonstrated *in vivo*.⁷⁷ *In vitro* studies have revealed that alveolar macrophages can respond to antigen through IgE to release leukotriene B₄, LTC₄, PGD₂, superoxide anion, and lysosomal enzymes.⁷⁸⁻⁸¹ Macrophages also produce other inflammatory mediators, such as platelet-activating factor, prostaglandin F_{2α}, and thromboxane.^{82,83} These mediators may play important roles in producing bronchoconstriction or in causing inflammatory changes, including cell recruitment and altered vascular permeability.

Pro-inflammatory cytokines produced by macrophages include IL-1, TNF-α, IL-6, and GM-CSF, which may induce endothelial cell activation, cellular recruitment, and prolonged eosinophil survival. Interleukin-6 and TNF-α may be released by IgE-dependent stimulation.⁸⁴ Macrophages also elaborate histamine-releasing factors that appear to act on the basophil and mast cell by way of binding to surface IgE.⁸⁵⁻⁸⁷ Thus, macrophages may play a role in perpetuating mast cell activation in asthma and late-phase responses independently of repeated exposures to specific allergen.

CLINICAL STUDIES SUPPORTING THE ROLE OF MAST CELLS AND BASOPHILS IN ASTHMA

Anti-IgE

In the pathogenesis of allergic disease, IgE plays a central role. Mast cells and basophils are the primary cells that bear the high-affinity IgE receptor, and a critical role for these cells is supported by the effect of anti-IgE therapy on the airway response to allergen bronchoprovocation. Omalizumab is a humanized murine monoclonal antibody (rhuMAB-E25) that binds to the same portion of IgE as the high-affinity IgE receptor. Because the anti-IgE antibody and the cell surface receptor compete for the same site (FcεR, binding domain) on IgE, the anti-IgE antibody can only bind free IgE and will not cause mediator release by cross-linking IgE on the cell surface. Results from 2 clinical studies have demonstrated that treatment with omalizumab inhibits the early- and late-phase responses to allergen challenge. In one study, shown in Fig 2, treatment with omalizumab reduced free serum IgE by nearly 90%, increased the dose of allergen required for an early response, and inhibited the maximum early and late changes in FEV₁ by about 40% and 60%, respectively.⁸⁸ In a separate study, omalizumab was given intravenously at an initial dose of 2 mg/kg, followed by 1 mg/kg at 1 week and every 2 weeks thereafter for a total of 10 weeks.⁸⁹ Free serum IgE was reduced by 89%, and the dose of inhaled allergen causing a 15% fall in FEV₁ was significantly increased by 2.3 to 2.7, doubling concentrations on days 20, 55, and 77. Methacholine reactivity was also significantly decreased by day 76.

Antihistamine and antileukotriene therapy

The concept that histamine and leukotrienes are responsible for most of the early and late physiologic responses to allergen bronchoprovocation is supported by a study in which identical bronchoprovocations were performed after 1 week of pretreatment with the LTRA zafirlukast (80 mg twice daily), the antihistamine loratadine (10 mg twice daily), or both in combination.⁹⁰ As shown in Fig 3, the results clearly demonstrated the inhibition of both the early and late responses with each agent alone. Zafirlukast was more effective than loratadine in the early ($P < .05$) but not the late response. Combination therapy inhibited the early response by 75% and the late response by 74% and was more effective than either drug alone during the late response ($P < .05$). These results clearly support a role for mast cell-derived and basophil-derived mediators in both the early and late bronchoconstrictive responses to allergen.

On the basis of the effects of multiple antileukotriene therapies on both the early- and late-phase responses, the central role of leukotrienes in this allergic airway reaction is firmly established. Whereas antileukotriene therapy may affect cell recruitment airway edema, and during the late-phase response blocking smooth muscle contrac-

tion, may be a major mechanism of antileukotriene therapy. In a recent study, increasing doses of the β -agonist terbutaline were administered intravenously 7 hours after allergen bronchoprovocation, when FEV₁ was 57% of baseline. At the end of infusion at 45 minutes, FEV₁ had returned to 100% of baseline and 84% of the maximal attainable value.⁹¹ The reversal of late-phase airway obstruction by a β -agonist supports the role of smooth contraction in this response to allergen challenge, in which leukotrienes (and histamine) appear to play major roles in the contractile responses.

SAC

The cellular, mediator, and cytokine responses underlying the physiologic response to allergen exposure have been examined with the SAC model, in which allergen is instilled directly into an airway segment during bronchoscopy and events occurring within minutes, hours, or days can be examined, generally by BAL. This model also has been used to examine the effects of prednisone and leukotriene modifier therapy on inflammatory changes. Cellular effects of these asthma medications on BAL cells after SAC are summarized in Table I.

The initial response to allergen in the sensitized individual is clearly dominated by products associated with mast cell activation, particularly histamine, PGD₂, LTC₄, and tryptase.^{38,51,52,97} These products are released within minutes and appear in the absence of cellular changes. Lipid products not produced by mast cells are also present, however, which implies the activation of other resident cells within the lung by the initial events. Within hours, immune and inflammatory cells are recruited from the circulation. This recruitment involves virtually all cell types including granulocytes (neutrophils, eosinophils, and basophils) and mononuclear cells (monocytes and lymphocytes); however, a remarkable selectivity of cells characterizes the allergic response, specifically eosinophils, basophils, and helper T cells. The T cells are further characterized as memory T cells and express a cytokine profile (eg, IL-4, IL-5) consistent with T_H2 cells.⁹¹ These cells have in common the expression on their surface of the adhesion molecule very late antigen 4, which binds to vascular cell adhesion molecule 1 on endothelial cells, suggesting a shared pathway for recruitment. IL-4 and IL-13 are T_H2 cytokines that specifically induce vascular cell adhesion molecule 1 expression.^{97,98} Whether there is a sequential recruitment of granulocytes followed by a mononuclear infiltration is not clear, but within 24 hours, all cell types are present. Furthermore, the mononuclear cell population shifts from one dominated by mature alveolar macrophages to one characterized by monocytes and monocytoïd cells expressing blood dendritic cell-specific antigens⁹⁹ (Liu MC, personal unpublished data).

The effects of leukotriene-modifying medications on inflammation induced by SAC have demonstrated inhibition of multiple cell types recruited during SAC. An examination of pretreatment with the 5-LO inhibitor

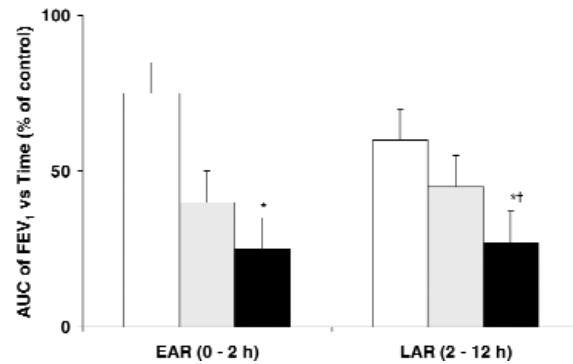


FIG 3. Effect of antihistamine and antileukotriene therapy on allergen bronchoprovocation. Results demonstrate inhibition of both early (EAR) and late (LAR) responses to all treatments and supported a role for mast cell-derived and basophil-derived mediators in both the early and late bronchoconstrictive responses to allergen. The early- and late-airway responses after the different treatments are expressed as area under the curve in percentage of the response during the control bronchoprovocation performed in the absence of drugs. All treatments caused significant reductions of both phases ($P < .05$). Bar heights represent mean; error bars represent SE. Asterisk denotes significant difference between zafirlukast plus loratadine (black bars) and loratadine alone (white bars); dagger denotes significant difference between zafirlukast plus loratadine and zafirlukast alone (shaded bars). From Roquet A, Dahlen B, Kumlin M, Ihre E, Anstren G, Binks S, et al. Combined antagonism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. *Am J Respir Crit Care Med* 1997;155:1856-63.

zileuton (600 mg four times daily) for 8 days demonstrated a significant increase in eosinophils in BAL at 24 hours during placebo treatment but no significant increase in eosinophils during active treatment. A study that examined a 7-day pretreatment with the LTRA zafirlukast (20 mg twice daily) demonstrated significant decreases in lymphocytes and alcian blue-positive cells in BAL at 48 hours.⁹³ TNF in BAL was also inhibited. Finally, researchers examined pretreatment for 6 weeks with zileuton (600 mg four times daily versus placebo) in a parallel design protocol.⁹⁴ On the basis of the initial response to SAC, the group could be divided into low and high leukotriene producers according to leukotriene levels in BAL at 24 hours after SAC. High leukotriene producers had greater eosinophil, total protein, and cytokine (TNF- α , IL-5, IL-6) levels than did low leukotriene producers. Eosinophil influx was significantly inhibited only in the group producing high levels of leukotrienes. Whereas the changes in airway obstruction inhibited by antileukotriene therapy probably represent effects on smooth muscle function, the results of SAC studies support an additional role of leukotrienes in the recruitment of inflammatory cells. This is further supported by studies with inhaled leukotrienes demonstrating both eosinophil and metachromatic cell (probably basophil) recruitment. Increased numbers of eosinophils and neutrophils appeared in the airway mucosa 4 hours after inhalation challenge with LTE₄. Numbers of eosinophils were 10-fold greater than those of neutrophils.¹⁰⁰ A

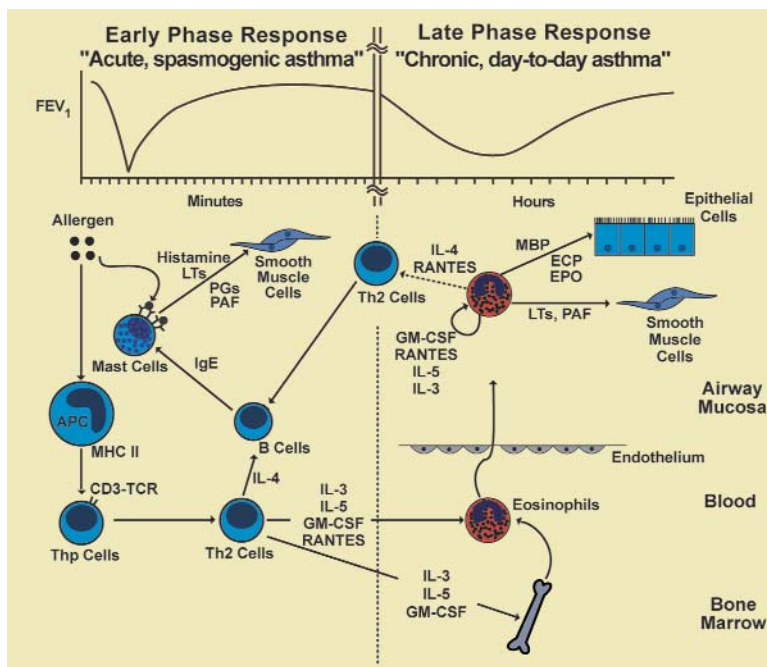


FIG 4. A scheme of putative immune and inflammatory events associated with the pathophysiology of asthma, with emphasis on early- versus late-phase asthmatic responses. Sites of potential anti-inflammatory action of LTRAs are shown by *large arrows*. ECP, Eosinophil cationic protein; EPO, eosinophil peroxidase; PG, prostaglandin; PAF, platelet activating factor; APC, antigen presenting cell; TCR, T-cell receptor.

TABLE I. Effects of prednisone and leukotriene-modifying agents on BAL cells after SAC

Medication	Cellular effect	Reference
Zileuton	No significant increase in eosinophils after SAC	Kane et al, ⁹² 1996
Zafirlukast	Inhibition of lymphocytes and basophils	Calhoun et al, ⁹³ 1998
Zileuton	Inhibition of eosinophils in high leukotriene producers	Hasday et al, ⁹⁴ 2000
Prednisone	Inhibition of eosinophils, basophils, and lymphocyte subsets	Dworski et al, ⁹⁵ 1994; Liu et al, ⁹⁶ 2001

recent study also demonstrated increased sputum eosinophils, as well as increased airway eosinophils and metachromatic cells (probably basophils), after inhalation of LTE₄.¹⁰¹

The effect of systemic steroid therapy with prednisone has also been examined in this model. Pretreatment with prednisone had no effect on the release of mast cell-derived mediators in the initial response,^{92,93} but subsequent events were inhibited.⁹³ In particular, significant decreases in cell recruitment of eosinophils, basophils, and T-cell subsets (CD4, CD45RA, and CD45RO cells) but not neutrophils occurred. Increases in airway permeability, kinins, the adhesion molecule E-selectin, and cytokine (IL-4, IL-5, IL-2, and transforming growth factor α) gene expression or protein induced by SAC were also inhibited. Increases in GM-CSF were not inhibited. Clearly, steroid therapy suppressed multiple components of the allergic airway response.

EOSINOPHILS

Activated T cells and eosinophils are important pathophysiological elements in asthma (Fig 4). The numbers of

these cells have been correlated broadly with disease severity.¹⁰² Mucosal damage in chronic asthma has been shown to be associated with cytotoxic and pro-inflammatory mediator release from activated eosinophils.^{103,104} These products include reactive oxygen species and cytotoxic granule and vesicular proteins: major basic protein (MBP), eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin, as well as cytokines and chemokines together with phospholipid-derived, pharmacologically active mediators. Cytokines released from T_H2-type cells, particularly IL-3, IL-5, and GM-CSF, are thought to regulate eosinophil priming, activation, and survival.^{105,106}

CysLTs

Eosinophils are a rich source of CysLTs¹⁰³ that are derived from native AA by the action of phospholipase A₂.¹⁰⁷ Human eosinophils synthesize and release relatively large concentrations of LTC₄ (as much as 70 ng/10⁶ cells) after stimulation with the calcium ionophore A23187.¹⁰⁸ In general, eosinophils obtained from asthmatic subjects appear to produce more LTC₄

than do those from healthy control donors.^{109,110} Experimentally, coculture of eosinophils with endothelial cells¹¹¹ or exogenous addition of cytokines (eg, IL-3, IL-5, GM-CSF, and TNF) has resulted in the upregulation of ionophore-induced release of LTC₄.^{106,111,112}

Leukotriene C₄ metabolism produces LTD₄ and LTE₄, which are rapidly degraded in the body; small amounts of LTE₄ can be measured in the urine.¹¹³ In human beings the 5-LO pathway is expressed only in myeloid cells, including mast cells, basophils, neutrophils, eosinophils, and alveolar macrophages.¹¹⁴ The CysLTs appear to have selective eosinophilotactic activity^{27,100} and promote transmigration of these cells through endothelial barriers.¹¹⁵

There are two receptors for CysLTs on smooth muscle cells, CysLT₁ and CysLT₂. The CysLT₁ receptor is the regulator for bronchial smooth muscle contraction and thus is directly relevant to asthma treatment.¹¹⁶ On the other hand, CysLT₂ appears to be involved mainly in pulmonary vein contraction.¹¹⁷ In addition to LTD₄, both LTC₄ and LTE₄ bind to CysLT₁, although LTE₄ exhibits a greatly reduced binding capacity.¹¹⁶ Evidence at the level of mRNA expression has suggested that eosinophils may have the capacity to synthesize CysLT₁ receptors.¹¹⁵ Recent studies have also suggested that eosinophils may express CysLTs, which may have implications for the capacity of these cells to respond to LTRAs in the setting of allergic and airway inflammation.¹¹⁸

LEUKOTRIENE MODIFIERS

The contribution of the CysLTs in bronchoconstriction has been inferred from recent developments in the field of leukotriene-modifying therapies.^{33,119,120} LTD₄ receptor antagonists (montelukast, zafirlukast, and pranlukast) inhibit the biologic activities of LTD₄ and the other members of the CysLT family by competing for their receptors on smooth muscle cells.¹²¹ Clinical trials of LTRAs, including montelukast and zafirlukast,¹²²⁻¹²⁶ have significantly controlled asthma symptoms in a substantial proportion of patients. Although the role of eosinophils in asthma and their contribution to bronchial hyperresponsiveness are still debated,¹²⁷ it is widely accepted that in asthma the number and activation status of eosinophils are increased and that eosinophil granule-derived products cause mucosal injury that may promote irreversible changes (tissue remodeling). The actions of LTRAs go beyond their potent bronchodilatory effects, particularly those that appear to downregulate various eosinophil effector parameters. Both montelukast and zafirlukast decrease peripheral blood and airway eosinophil numbers.^{93,128} In cellular infiltrate obtained from induced sputum, reductions in the number of sputum eosinophils were significantly less after treatment with montelukast.¹²⁹ These findings provide further support to the notion that CysLTs have eosinophilotactic properties.^{27,100,128}

In a rat model of allergic airway responsiveness after allergen challenge, eosinophil (MBP positive) and IL-5 mRNA-positive cell numbers were significantly reduced

in both BAL and lung tissue from rats that received montelukast compared with untreated animals.¹³⁰ In contrast, there are negligible data on the activation status of eosinophils in human airway tissue in LTRA-treated versus untreated subjects. Such data would expand current understanding and better define the anti-inflammatory properties of LTRAs in vivo. It is becoming clear that although LTRAs such as montelukast and zafirlukast dampen the eosinophilic response, the precise pathways underlying such an effect remain to be established. There is a need for a better identification of the spatial and temporal targeting of the effects of LTRAs during the life cycle of the eosinophil in inflammation, from eosinopoiesis to its demise in a given inflammatory site.

The first potential site for the effect of LTRAs is in early T-cell events associated with the development of the late-phase response. The LTRAs may interfere with the capacity of T_H2 cells to release critical cytokines (eg, IL-3, IL-5, GM-CSF) as well as chemokines (eg, RANTES) required for bone marrow stimulation of eosinopoiesis. Additionally, LTRAs may downregulate receptors or critical elements for these cytokines. More likely, LTRAs may exert their effect on the growth, differentiation, and efflux of bone marrow eosinophil progenitors (Fig 4).¹³¹ A currently ongoing study is aimed at examining the LTRAs' mode of action on sequential growth and differentiation steps from CD34⁺ progenitor to the fully differentiated and activated cell. In addition, the effects of these agents on bone marrow levels of eosinophil-sensitive chemokines (eg, RANTES and eotaxin) are to be ascertained. The results of this study will determine whether there is a potential blocking action of LTRAs on the proximal arm of cell accumulation in tissue and related events associated with the egress of eosinophils from hematopoietic sites.

Montelukast inhibits eosinophil transmigration across human umbilical vein endothelial cells.¹¹⁵ In addition, the demonstration that human eosinophils express mRNA for the CysLT₁ receptor strongly supports the hypothesis that these inflammatory cells may accumulate and traffic to sites of allergic inflammation as a result of chemotactic activity exerted by CysLTs. Thus, LTRAs may interfere with eosinophil recruitment by way of effects on adhesion molecules that facilitate rolling, tethering, flattening, and transmigration by diapedesis (Fig 4).

Previous studies have concluded that LTC₄, LTD₄, and LTE₄ have little if any chemotactic activity for human eosinophils^{132,133} and no upregulatory effects on eosinophil effector function (including cytotoxicity)¹³⁴ in comparison with leukotriene B₄. In light of the fact that these results achieved more concrete evidence for a chemotactic function of CysLTs, it is crucial that these data be revisited to appreciate the magnitude and mechanisms regulating and reducing eosinophils in the sputum of LTRA-treated asthmatic subjects. In addition, the effect of LTRAs may be the outcome of an indirect effect on bystander immune, inflammatory, and structural cells in the bronchial mucosa. For instance, the lower numbers of eosinophils in sputum may occur as a result of puta-

tive CysLT receptor-mediated effects on epithelial cells, which in turn may influence the synthesis, storage, and release of eosinophilotactic chemokines, including RANTES and eotaxin.^{71,135} These proteins have been shown to be present in the bronchial tissue in asthma.^{136,137} In addition, epithelial cell-induced eosinophil chemotaxis also may be influenced by cytokines such as IL-16, a potent T-cell and eosinophilotactic cytokine and a major product of bronchial epithelial cells. IL-16 has been shown to use CD4 receptors expressed on eosinophils to exert its chemotactic activity,^{138,139} and IL-16 expression has been shown to be a pathologic feature of human bronchial asthma.¹⁴⁰

Little is known about the influence of LTRA treatment on IL-5 bioactivity both locally and systemically, but inhalation of LTD₄ causes an increase of IL-5.³⁰ This cytokine is a critical factor in eosinophil terminal differentiation and, together with IL-3 and GM-CSF, prolongs eosinophil survival in the tissue. Production of IL-5 by mononuclear cells under stimulation with mite antigen was markedly suppressed when they were exposed to the LTRA pranlukast. The data may provide clues to the mechanism by which LTRAs, including zafirlukast and montelukast, can reduce airway, sputum, and blood eosinophil counts in clinical asthma.¹⁴¹ Such data could provide further support for the possibility that LTRAs may exert their influence on eosinophilic responses by interfering with IL-5 protein synthesis and turnover in asthmatic airways.

A well-recognized function of chemotactic factors relates to their ability to upregulate inflammatory cell function by increasing the expression of various receptors as well as inducing and enhancing their capacity to synthesize and release their de novo synthesized and preformed, stored mediators. Eosinophils are major secretory cells in airway inflammation, and a better understanding of the effects of LTRAs on eosinophil degranulation and exocytosis and on eosinophil mediator secretion is needed (Fig 4).

Among the inflammatory cells, eosinophils may be targets for various pharmacologic activities of LTRAs through the ability of these agents to downregulate a number of extracellular and intracellular events that may be key to the effector function of these cells. Much remains to be studied in the pursuit of a clearer understanding of the range of activities of these anti-inflammatory agents. The spectrum of their anti-inflammatory effects may range from dampening chemotactic activities that are key to cell trafficking to and accumulation in relevant tissues to the interruption of intracellular events regulating granule and secretory vesicle exocytosis and mediator release.

CONCLUSIONS

The sensitized reaction to an allergen includes responses from T cells, mast cells, basophils, macrophages, and eosinophils. In the case of asthma and allergic rhinitis, the mediators released from these cells perpetuate the asthmatic inflammatory response, and the CysLTs have emerged as one of the important mediators.

In human beings, the 5-LO pathway is expressed only in myeloid cells, including mast cells, basophils, neutrophils, eosinophils, and alveolar macrophages. Eosinophils from asthmatic subjects produce more LTC₄ than do those from healthy control donors, and there is an overexpression of LTC₄ synthase in bronchial biopsy samples obtained from asthmatic patients. Mast cells and basophils are primary cells that bear a high affinity IgE receptor and, during their key involvement in the early and late phases, CysLTs are released. Basophils produce as much LTC₄ as do mast cells and much more than do eosinophils.

The CysLTs have the potential to cause the cardinal symptoms of asthma: mucus hypersecretion, increased microvascular permeability, and edema, as well as impaired ciliary activity, inflammatory cell recruitment, and neuronal dysfunction. They are able to induce smooth muscle hypertrophy and hyperplasia, and their increased production can be measured in BAL and sputum. With their selective eosinophilotactic activity, the CysLTs can also promote transmigration of eosinophils through endothelial barriers.

Clinical trials with LTRAs have shown them to exert significant control over asthma symptoms and to modulate cytokine function. Consequently, these agents have been implicated in the pathophysiology of asthma, acting through multiple mechanisms. Basic and clinical studies will continue to deepen our understanding of the complex inflammatory processes in asthma and related conditions. The results of such studies hold important therapeutic implications.

QUESTIONS AND DISCUSSION

Marc Peters-Golden: Qutayba, is the epithelium capable of differentiating into mesenchymal-like cells, as they appear to do in the kidney? Could the smooth muscle cells that appear to be pushing up into the epithelial layer actually be the basal epithelial cells that are differentiating into smooth muscle cells instead?

Qutayba Hamid: We did not see any evidence that epithelial cells go to something that is not a cytocuritin-positive cell or what would probably look like a smooth muscle. Epithelial cells from asthmatic patients certainly behave differently from normal epithelial cells in the way that they are differentiated. I have not seen any evidence that they differentiate into mesenchymal cells.

Stephen Holgate: There is a most remarkable organization underneath the epithelium. People talk about leukocytes coming up through the epithelium into the lumen as if the leukocytes are eating their way through. As you strip off the epithelium and look down at it, you see that it is full of channels. From electron micrographs, we can see leukocytes and dendritic cells traffic through these channels. These channels are highly organized, and nothing is eating its way through. What we have shown recently is that the attenuated fibroblasts, or whatever we want to call them, actually extend tendrils through so that they are in contact with the basement membrane underneath the epithelium itself. These "flat cells" extend their

feelers up through to the epithelium and form an integrated unit, just as is seen in the developing fetal lung.

Qutayba Hamid: Not to be forgotten are the neuroendocrine cells in the lung epithelium, which can secrete a number of growth factors. They appear to change with age and to regulate the repair process.

Stephen Holgate: In a children's biopsy study we did collaboratively with colleagues in northern Siberia, there were cells that stained like these neuroendocrine cells, and children with asthma had many more of these. They may be relevant to the differentiation process that you described.

Redwan Moqbel: How does the presence or absence of brush border in airway epithelial cells compared with gut epithelium impinge on their functions?

Stephen Holgate: The gut is designed to shed its epithelium and the whole machinery of the crypt, and the way the epithelium turns over is to get rid of it all the time. It has got a fantastic turnover rate. The airway epithelium is not designed for that. There is a fundamental difference in the turnover rate of epithelial cells. I think that it would be quite dangerous to extrapolate too much from the gut, even though the lung is an outgrowth of the gut.

Qutayba Hamid: When you take stem cells from subjects with severe asthma and grow them, they differentiate into epithelial cells that do not have cilia. If you take them from healthy individuals, the cells have a lot of cilia. The cells from the patients with the most severe asthma have changed their phenotype so that they cannot produce any more cilia. It is difficult to say whether there are any structural changes in the cell, but if there are any changes, they are phenotypic rather than structural.

Stephen Holgate: Christopher Britling in Leicester recently had quite a nice study showing that patients who have asthma with variable air flow obstruction and bronchial hyperresponsiveness had the same number of eosinophils and mast cells in the submucosa and epithelium, but it was the smooth muscle that showed a marked increase in the mast cell population. Is studying BAL and mucosal specimens actually looking at the right compartment if we are interested in smooth muscle responses? Mast cells or other inflammatory cells that are in the smooth muscle may be more important, and the mast cells that are present in the smooth muscle are ones that contain kinase rather than tryptase and seem to be resistant to steroids. What do you think?

Mark Liu: Cells that are on the airway surface or within the epithelium initially can respond and change the surface permeability so that the antigen can get to the cells that are deeper in the tissue. Whether the mechanism has to do with directly activating those deeper mast cells that are next to smooth muscle or whether neural phenomena or other factors are involved in the bronchoconstriction is not clear to me. We are clearly limited in terms of what biopsy specimens tell us. In the Kepley study of fatal asthma,⁶⁴ many sections were available. There were basophils all through the lung, in alveoli or alveolar structures and around smooth muscle, not just lined up along the surface of the airway.

Redwan Moqbel: Mark, how does the fact that the IgE receptors are expressed beyond basophils and mast cells affect your conclusions about how these cells function?

Mark Liu: Whether IgE receptors are functionally significant or not is open to discussion. There is no question that mast cells and basophils are the most responsive cells to antigen and to IgE-mediated stimuli. I am not even sure what the physiologic stimulus is for the eosinophil, for example, or for the macrophage for that matter. Even though the receptors are there in other cells, I just do not know what to make of them in terms of mediator release.

Qutayba Hamid: Why does the release of mediators from mast cells seem to be resistant to steroids?

Mark Liu: The mast cell is not responsive to steroids directly, but products from the mast cell and mechanisms that the mast cell may initiate would be responsive to steroids. If you look only at the mast cell and its mediator release, cytokine generation, and those sorts of effects, they are not affected by treatment of steroids. But many of the cascades are initiated by the mast cell, such as cell recruitment, the consequences of TNF- α action on the endothelium, histamine release, and permeability changes. All these would be steroid responsive, because the steroids would affect the downstream events initiated by the mast cell.

Anthony Sampson: Redwan, do you think that the leukotrienes released from the eosinophils act on the CysLT₁ receptors of eosinophils to control their maturation, proliferation, and migration?

Redwan Moqbel: I do not know. Eosinophils contain lipid bodies that are a major source of many of the cysteinyl phospholipids, and eosinophils can generate their own chemotactic factors. Whether these function in an autocrine manner through the CysLT₁ or CysLT₂ receptors is important to know but has not yet been studied.

Qutayba Hamid: Are the cells obtained in sputum fully activated cells? Do they reflect cells that have gone all the way through the activation processes and are capable of pouring out all the mediators?

Redwan Moqbel: Yes. Most, but not all, of the cells are measurable, because as we section the sputum plugs, a high percentage of the cells show activation.

Emilio Pizzichini: Can we be sure that the major source of the degranulation products is eosinophils? For example, we have observed neutrophilic exacerbations in which the size of inflammation is 50 million cells/mL, whereas the stable state is 4 million cells/mL. Could the neutrophils be secreting the MBP and eosinophil cationic protein that is damaging the epithelium?

Redwan Moqbel: We have made observations similar to what you have described. The MBP is stored primarily in eosinophils, and to a lesser extent basophils, whereas neutrophils have been shown to express eosinophil cationic protein and eosinophil-derived neurotoxin. The only other MBP-like source is found in trophoblasts during pregnancy. The MBP from eosinophils acts on neutrophils, and there is always the possibility that once eosinophils undergo cytolysis or necrosis, the MBP may

become sequestered in neutrophils. We use MAb BMK-13 to detect the human MBP.

Qutayba Hamid: What are the half-lives of these eosinophil products in the tissue?

Redwan Moqbel: These products have long-term effects, and because of their cationic nature they bind strongly to the target cells, which makes it difficult to get rid of them. These basic proteins are extremely "sticky" and rich in arginine. Peroxidase and MBP are the two that are most cytotoxic. Eosinophil cationic protein and eosinophil-derived neurotoxin have potent ribonuclease activity and lesser cytotoxic capacity. I am not aware of any studies on the half-lives of these proteins in the tissue, but I assume that they may be long.

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