

Asthma 2005-2006: Bench to bedside

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Asthma is a prevalent and complex syndrome with several phenotypic variants. The central features are bronchial inflammation and airway hyperresponsiveness. Many aspects of asthma, such as control of airway hyperresponsiveness, causative factors, and variable responses to treatment, remain poorly understood. This review highlights some of the latest insights into the pathogenesis of asthma that might ultimately bear on the development or choice of treatment modalities. (J Allergy Clin Immunol 2006;118:582-6.)

Key words: Asthma, airway hyperresponsiveness, inflammation

The concept that asthma is associated with (and in part caused by) persistent inflammation of the bronchial wall is broadly accepted. Increasingly sophisticated animal models have helped to dissect molecular and cellular pathways leading to the cardinal features of asthma. At the same time, clinical, translational, and genetic approaches to studies of asthma in human subjects continue to uncover novel (and sometimes surprising) insights into pathogenesis. Some, but not all, of these insights recapitulate or validate findings made in animals. Many have the potential to challenge existing paradigms and change therapeutic approaches. This review will highlight recent advances in asthma in the past year (Table I). Additionally, some select animal studies with potential for rapid translation into human subjects are discussed.

EFFECTOR LYMPHOCYTES: A CHANGING PARADIGM

Since the recognition over a decade ago that bronchoalveolar lavage fluid samples from patients with

Abbreviations used

AA:	Arachidonic acid
AHR:	Airway hyperresponsiveness
BLT ₁ :	Type 1 receptor for leukotriene B ₄
cys-LT:	Cysteinyl leukotriene
CysLT ₁ :	Type 1 receptor for cysteinyl leukotrienes
CysLT ₂ :	Type 2 receptor for cysteinyl leukotrienes
eNO:	Exhaled nitric oxide
iNKT:	Invariant natural killer T
5-LO:	5-Lipoxygenase
LT:	Leukotriene
LXA ₄ :	Lipoxin A ₄
MC:	Mast cell
NK:	Natural killer
OVA:	Ovalbumin
VLA-4:	Very late antigen 4

asthma contained CD4-bearing T lymphocytes with a T_H2-like profile of cytokines,¹ the concept that asthma involves (and perhaps is caused by) helper T cells and their cytokines has become broadly accepted. Most animal models of allergen-induced airway disease support this dogma because the development of the histologic and physiologic signatures of “asthma” in these experiments require “T_H2” CD4 lymphocytes and their associated cytokines (IL-4, IL-5, and IL-13).² However, it is increasingly apparent that lymphocyte ontogeny and heterogeneity, as they relate to the pathophysiology of asthma in human subjects, are tremendously complex. Natural killer (NK) cells, inducers of antiviral immunity, can segregate into T_H1- and T_H2-like subsets based on their profiles of cytokines. Wei et al³ reported that peripheral blood from 8 asthmatic subjects contained substantially higher numbers of NK cells (as defined by expression of CD16 and CD56) that expressed a T_H2-like profile of cytokines than did peripheral blood from healthy control subjects. When cloned by limiting dilution cultures, these cells *ex vivo* showed constitutive activation of signal transducer and activator of transcription 6, the transcription factor essential for the transduction of gene expression through IL-4 and IL-13 receptors. Moreover, treatment of these human subjects for 10 weeks with oral erythromycin induced a shift toward IFN- γ generation by their NK cells. These observations suggest a potential link between antiviral immune responses and the generation of effector cytokines linked to airway dysfunction in

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asthma, although further study will be necessary to verify that such cells can be identified in the airway tissue.

Invariant NK T (iNKT) cells are CD4⁺ lymphocytes that differ from conventional class II-restricted helper T cells in their expression of an invariant rearrangement of the T-cell receptor (V α 14-J α 18 in mice and V α 24-J α 18 in human subjects). In many instances iNKT cells are specialized to recognize glycolipid antigens presented in the context of CD1d, rather than the peptide antigens presented by HLA class II that are recognized by CD4⁺ T helper cells. iNKT cells can generate both T_H1- and T_H2-type cytokines and are implicated in models of autoimmune disease. Some previous studies of iNKT-deficient mice had suggested that these cells were important sources of IL-4 and IL-13 in the induction of allergen-mediated airway disease.⁴ Two separate studies within the past year suggested that these specialized T cells might be involved in human asthma. One study reported that the peripheral blood from patients with asthma contained as many as 10-fold more V α 24⁺ iNKT cells than did blood from healthy control subjects. Moreover, in contrast to the iNKT cells from control subjects, the cells in the asthmatic subjects expressed high levels of CCR9 and responded chemotactically to CCL25, the ligand for CCR9.⁵ CCL25 mRNA was highly expressed in bronchial biopsy specimens from patients with asthma compared with that seen in control specimens. Although asthmatic iNKT cells in peripheral blood expressed higher levels of mRNA encoding IFN- γ , IL-2, IL-10, and TGF- β than cells from healthy control subjects, they were able to potently induce T_H2 cytokine generation by syngeneic CD3⁺ cells in a cell contact-specific manner involving cross-talk between CCR9 and CD226. Another study reported that the majority of CD4⁺ lymphocytes recovered from the bronchoalveolar lavage fluids of asthmatic subjects were iNKT cells rather than class II-restricted T helper cells. In this study the iNKT cells expressed intracellular IL-4 and IL-13 but not IFN- γ .⁶ Although these studies support disparate potential mechanisms for the functional role of iNKT cells in asthma, they collectively introduce the concept that a population of cells that recognize innate or exogenous glycolipid antigens might induce effector responses to airway hyperresponsiveness (AHR) and other features of asthma. These studies should prompt research to understand the roles of glycolipid antigens and cognate iNKT cells as inducers, amplifiers, and perpetuators of airway inflammation and AHR in asthma and might help to shed light on the nature of the imperfect correlation between asthma and classical atopy. Moreover, the finding that this unique T-cell subset selectively expresses CCR9 provides a potential opportunity for a relatively selective development of therapy.

5-LIPOXYGENASE PATHWAY PRODUCTS: OLD MEDIATORS, NEW FUNCTIONS

The involvement in asthma of mediators derived from arachidonic acid (AA) metabolism by 5-lipoxygenase

TABLE I. Advances in asthma: 2005-2006

- Demonstration of iNKT cells in human asthma
- Demonstration of T_H2-like NK cells in human asthma
- Role of eNO as a predictive monitoring tool for steroid responsiveness
- Role of sputum eosinophilia as a predictor of failure in steroid weaning
- Prognostic significance of rhinovirus-induced wheeze
- Efficacy of anti-TNF- α in severe asthma
- Lipoxin deficiency as a marker of severe asthma

(5-LO) has been recognized for decades. AA liberated from membrane phospholipids by cytosolic phospholipase A₂ is the substrate for conversion by 5-LO to 5-hydroperoxyeicosatetraenoic acid and subsequently to leukotriene (LT) A₄. LTA₄ either is hydrolyzed by cytosolic LTA₄ hydrolase to form LTB₄ or is conjugated to reduced glutathione to form LTC₄, the parent compound of cysteinyl leukotrienes (cys-LTs) LTD₄ and LTE₄. Macrophages and mast cells (MCs) generate both LTB₄ and LTC₄ when activated, whereas neutrophils and eosinophils are restricted to LTB₄ and LTC₄ generation, respectively. Although the role of cys-LTs as bronchoconstrictors in asthma is well known and broadly accepted, the development of mice bearing targeted disruptions of synthetic enzymes and receptors has uncovered unexpected potential disease-related functions for both classes of LTs. Mice lacking LTC₄ and therefore selectively unable to generate cys-LTs showed strikingly lower levels of AHR, goblet cell metaplasia, eosinophilia, and T_H2 cytokine production than wild-type BALB/c control mice when subjected to a protocol involving immunization with ovalbumin (OVA) without adjuvant, followed by repetitive intranasal OVA.⁷ Surprisingly, these mice also showed a profound defect in their ability to synthesize IgE, both at baseline and especially in response to OVA, and showed a marked deficiency in the numbers of MCs in the tracheobronchial mucosa. Thus cys-LTs, independently of their established effects on airway and vascular smooth muscle, might play an important role in the inductive events that control subsequent mucosal T_H2 responses. This finding raises the possibility that modulation of cys-LT generation might have therapeutic utility in preventing the development of sensitization to allergens. The cellular targets and receptors responsible for these effects remain to be defined.

Two cloned receptors, the type 1 (CysLT₁) and type 2 (CysLT₂) receptors for cysteinyl leukotrienes, respectively, mediated the biologic functions of the cys-LTs. Each of the orally active receptor antagonists (montelukast, zafirlukast, and pranlukast) is selective for the CysLT₁ receptor. Although animal studies suggest a distinct and critical role for CysLT₂ receptors in mediating experimental pulmonary fibrosis,⁸ the lack of specific antagonists has precluded a systematic analysis of this receptor's function in human subjects. A study by Corrigan et al⁹ used specific antibodies to compare the

distribution of CysLT₁ and CysLT₂ in nasal biopsy specimens from 21 subjects with nasal polyposis and aspirin-exacerbated respiratory disease, 11 subjects with nasal polyposis who were aspirin tolerant, and 9 healthy control subjects. Compared with the other groups, the biopsy specimens from subjects with aspirin-exacerbated respiratory disease showed increased percentages of CD45⁺ leukocytes expressing CysLT₁. Although the percentage of CysLT₂-expressing cells did not differ among the 3 groups, CysLT₂ dominated over CysLT₁ on epithelial cells and mucous glands. These findings suggest that CysLT₂ mediates responses of structural cells of the respiratory mucosa to cys-LTs. Further studies will be needed to define the distribution of CysLT₂ in the lower airway. Although selective antagonists of CysLT₂ are not yet available, it is tempting to speculate that this receptor might play a role in structural remodeling, an established signature of cys-LT functions.

In contrast to cys-LTs, LTB₄ is not a direct bronchoconstrictor but is a classical chemoattractant for certain leukocytes, vascular smooth muscle cells,¹⁰ and MC progenitors.¹¹ These chemotactic effects are mediated largely by the type 1 receptor for LTB₄ (BLT₁). Two groups reported that BLT₁-deficient mice showed deficient induction of AHR in response to sensitization and challenge with OVA.^{12,13} In both studies BLT₁-deficient mice showed decrements in IL-13 production in the lung. This outcome partly reflects an especially important role of LTB₄ and BLT₁ as a chemotactic axis for CD8⁺ effector/memory T cells,¹³⁻¹⁵ which, at least in mice, are potent producers of IL-13. Recently, the numbers of CD8⁺ cells in bronchial biopsy specimens was shown to correlate with a rapid decrease in lung function.¹⁶ Collectively, these observations support a role for LTB₄ in the generation and maintenance of AHR. It will be essential to validate whether CD8⁺ cells recruited during antiviral responses are an important causative link to asthma in human subjects. The development of BLT₁-selective antagonists for the treatment of arthritis and other inflammatory diseases in human subjects might permit proof-of-principle studies in asthma.

Although LTs induce and amplify inflammation, lipoxin A₄ (LXA₄) is a product of 5-LO-induced AA metabolism that dampens inflammatory responses through a specific receptor. Two groups independently reported that severe asthma is associated with reduced LXA₄ generation. Levy et al¹⁷ reported that whole peripheral blood from patients with severe asthma contained approximately 5-fold less LXA₄ than blood from patients with moderate asthma. In contrast, the ratios of cys-LTs were the inverse. Vachier et al¹⁸ reported substantially lower levels of LXA₄ in induced sputum from patients with severe asthma than in those with mild disease, whereas LTB₄ levels were higher in patients with severe disease. Thus LXA₄ production is impaired in patients with severe asthma and is inversely correlated with LT generation. Whether the deficiency in LXA₄ is causative or whether it is an association based on a relative increase in terminal LT-generating enzymes remains to

be seen. The reintroduction of zileuton, an inhibitor of 5-LO, for the treatment of asthma should permit identification of functions for each of these mediators in human subjects and validation of findings made in mice.

TARGETED TREATMENTS

The relative success of anti-IgE as a treatment for asthma¹⁹ and the finding that MC accumulation in airway smooth muscle is specific for asthma²⁰ have rekindled interest in the disease-associated pathways that are in part under the control of MCs. Among these pathways, MCs are a source of preformed TNF- α , a master regulator of inflammation and a potent inducer of gene expression in resident tissue cells and leukocytes. In mice TNF- α induces goblet cell metaplasia²¹ and is important for the allergen-induced late phase in a mouse model of asthma through a mechanism involving cytosolic phospholipase A₂ and AA products.²² A recent double-blind, crossover, placebo-controlled trial of etanercept, a TNF- α antagonist, strongly supports a role for TNF- α in the pathophysiology of asthma.²³ Ten patients with severe asthma, 7 of whom required daily oral corticosteroid therapy, participated. Treatment with etanercept for 10 weeks significantly increased FEV₁ compared with that seen with placebo and reduced AHR to methacholine by 3.5 doubling doses. These changes were associated with significant improvements in symptomatic control and quality of life. Although sputum cell counts, eosinophil counts, and neutrophil counts were not altered during etanercept treatment, sputum levels of histamine decreased by approximately 60%. The latter finding supports a role for TNF- α as an autocrine mediator of MC activation, as proposed previously based on *in vitro* studies.²⁴ Identification of the actual mechanism or mechanisms by which TNF- α antagonism induces its bronchoprotective effects might be useful for identifying additional targets and pathways for targeted therapy, especially in severe asthma.

All leukocytes adhere to vascular endothelial cells to migrate to target tissues. The α 4 β 1 integrin heterodimer (also known as very late antigen 4 [VLA-4]) is strongly expressed by lymphocytes, eosinophils, and basophils.²⁵ Interactions between VLA-4 and its endothelial counterligand, vascular cell adhesion molecule 1, are essential to the adhesion and migration of lymphocytes and eosinophils to the lung in animal models of allergen-driven pulmonary inflammation.²⁶ The potential therapeutic utility of an orally active VLA-4 antagonist, IVL745, was explored in a placebo-controlled, double-blind, 2-way crossover study.²⁷ Sixteen subjects with mild-to-moderate allergic asthma received IVL745 (administered twice daily for a week) or placebo, followed by allergen inhalation challenge. IVL745 was well tolerated and decreased the percentages of eosinophils recovered from induced sputum obtained 2 days after the allergen challenge by approximately 50%. IVL745 had no effect on either baseline lung function or the magnitude of decrease in airflow

during the early- and late-phase responses to the challenge and did not alter symptoms, β_2 -agonist use, AHR, or concentrations of exhaled nitric oxide (eNO). Although this preliminary study validates a role for VLA-4 in eosinophil recruitment in human subjects, larger and more highly powered studies are needed to determine whether it can be harnessed as a treatment for asthma.

BIOMARKERS AND ASTHMA TREATMENTS

The growing number of available asthma treatments increases the potential complexity in choosing appropriate therapy. Measurements of airflow do not correlate uniformly with other indices of drug efficacy or with measures of airway inflammation. A multicenter, 16-week crossover study compared intraindividual and interindividual responses of asthmatic children between the ages of 6 and 17 years to fluticasone (100 μ g twice daily) and montelukast (5 or 10 mg daily) as part of the Childhood Asthma Research and Education network.²⁸ Both treatments improved most clinical parameters, although fluticasone was superior to montelukast for inducing changes in asthma control days, airflow, and eNO. Levels of eNO at baseline predicted responses to fluticasone. Another crossover study by the same investigators sought to determine whether responses of baseline FEV₁ to fluticasone or montelukast were concordant or discordant among individuals.²⁹ Interestingly, 55% of the 126 individuals in this study failed to show improvement in FEV₁ with either drug (perhaps because the average lung function in this age group was close to normal). Responders to fluticasone alone, montelukast alone, or both drugs comprised 23%, 5%, and 17% of the study participants, respectively. Those with higher eNO values, lower baseline lung function, higher bronchodilator response, greater daily use of β_2 -agonists, higher serum levels of eosinophil cationic protein, and greater AHR had greater responses to fluticasone over montelukast. Favorable response to montelukast alone was predicted by younger age and shorter disease duration. These studies begin to provide a framework for the integration of eNO, along with more traditional parameters, as a guide to therapeutic choices.

Although current guidelines advocate the use of inhaled corticosteroids as primary treatment for persistent asthma, daily therapy might not be necessary in all patients with mild disease.³⁰ Investigators of the Asthma Clinical Research Network conducted a study in which a group of subjects with mild-to-moderate asthma, maintained on inhaled steroids, were transitioned to placebo or salmeterol alone or continued on inhaled steroids.³¹ Rates of deterioration in asthma control over a 16-week period were 9.3%, 24.1%, and 37.5% in the groups continued on steroids, transitioned to salmeterol, or switched to placebo, respectively. Importantly, neither eNO nor AHR predicted deterioration with transition to placebo when these parameters were measured at 2 weeks after steroid discontinuation. However, sputum eosinophil counts at 2 weeks after

cessation and changes in sputum eosinophil counts from baseline on steroid treatment both strongly predicted deterioration in control. Measurement of sputum eosinophils might be a useful and noninvasive measure of airway inflammation that could provide a monitoring tool to predict which patients might safely be weaned from corticosteroids.

CHILDHOOD WHEEZING AND RELATIONSHIP TO ASTHMA

Viral respiratory tract infections frequently cause wheeze in infants and young children, but the relationship of these episodes to asthma is incompletely understood. A prospective study analyzed the relationship of viral infections in infancy to the development of asthma in a cohort of 285 children at genetic risk.³² Serial nasal lavage was used to survey this population for infection with respiratory viruses correlated with the timing of clinical illnesses. Wheezing with viral infection in infancy was a strong predictor of wheezing in the third year of life. Of the viral pathogens, wheezing illnesses associated with rhinovirus infection in infancy was the strongest predictor of subsequent wheeze because 63% of the infants who wheezed during the rhinovirus season continued to wheeze at age 3 years compared with only 20% for the remaining infants. Thus although respiratory syncytial viruses are the most common cause of clinical bronchiolitis, wheezing with rhinoviruses carries greater prognostic significance for the development of asthma. These studies should promote further research into the nature of virus-host interactions that drive airway dysfunction.

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