Advances in mechanisms of allergy

Bruce S. Bochner, MD,[a] and William W. Busse, MD[b] *Baltimore, Md, and Madison, Wis*

This review summarizes selected Mechanisms of Allergy articles appearing between 2002 and 2003 in the *Journal of Allergy and Clinical Immunology*. Articles chosen include those dealing with human airways disease pathophysiology, pharmacology, cell biology, cell recruitment, and genetics, as well as information from allergen challenge models in both human and nonhuman systems. When appropriate, articles from other journals have been included to supplement the topics being presented. (J Allergy Clin Immunol 2004;113: 868-75.)

Key words: *Asthma, rhinitis, sinusitis, surrogate markers, eosinophils, basophils, mast cells, T cells, epithelial cells, chemokines, cytokines, prostaglandins, leukotrienes, animal models, smooth muscle, pathophysiology*

The use of animal models, human allergen challenge studies, and biopsy and lavage studies that generate human samples for ex vivo analysis continues to expand our understanding of the role of various mediators, inflammatory molecules, and cellular and physiologic aberrations associated with allergic disorders. Apropos to this, articles published in the Mechanisms of Allergy section of the *Journal of Allergy and Clinical Immunology* (JACI) from late 2002 through late 2003 shed light on many of these issues. This review summarizes selected articles that focus on the pathophysiology of allergic and eosinophilic diseases of the upper and lower airways (Table I). Articles include measurements of associated molecules and surrogate markers, using human samples to assess the development, diagnosis, disease severity, or response to therapy. Other studies using human cells relevant to allergic diseases or cell lines derived from human cells provide further mechanistic information on cellular responses. In addition, studies predominantly using human specimens that have identified a wide variety of genetic contributions to allergic disease responses are reviewed. When appropriate, articles from other journals have been included to supplement the topics being presented.

Abbreviations used
ADAM33: A disintegrin and metalloprotease 33
BAL: Bronchoalveolar lavage
CpG-ODN: CpG-oligodeoxynucleotide
CysLT1: Cysteinyl leukotriene receptor type 1
JACI: *Journal of Allergy and Clinical Immunology*
MBL: Mannose-binding lectin
MDC: Macrophage-derived chemokine (CCL22)
MMP-9: Matrix metalloprotease 9
RSV: Respiratory syncytial virus
SNP: Single nucleotide polymorphism
TARC: Thymus and activation−regulated chemokine (CCL17)
U-EPX: Urinary eosinophil protein X
VEGF: Vascular endothelial cell growth factor

PREDICTORS AND PREVENTION OF ALLERGIC DISEASE DEVELOPMENT

Three studies in the JACI focused predominantly on young children and infants, attempting to identify surrogate markers in this population that would be predictive of allergic disease development. Gore et al[1] showed that urinary eosinophil protein X (U-EPX) levels, measured prospectively from birth through the first 3 years of life, were slightly increased in nonatopic children with wheezing or eczema compared with those in nonatopic asymptomatic children. However, children who had allergic diseases early in life had significantly higher U-EPX levels at age 3 years. The highest U-EPX levels were found in atopic children with a history of wheezing and eczema, although levels did not correlate with lung function. In another study by Neaville et al,[2] PBMCs were studied from umbilical cord blood and again from peripheral blood 1 year later for mitogen-induced T_H1 and T_H2 cytokine production. Cytokine production was also compared with levels of eosinophils and specific IgE to selected inhalants and foods at birth and at 1 year. From birth to 1 year, IL-5 production in response to stimulation increased dramatically, whereas IL-13 and IFN-γ production decreased. Production of T_H2 cytokines was associated with blood eosinophilia and increased total IgE levels by age 1 year. Taken together, these studies point to patterns of immunologic responses that are established early in life. Such markers could be useful to monitor the success of future treatments designed to prevent allergic sensitization or at the very least to suggest when such interventions should be initiated.

Regarding disease prevention, a study by von Berg and the German Infant Nutrition and Intervention Study

From [a]the Department of Medicine, Division of Allergy and Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, and [b]the Department of Medicine, Division of Allergy and Immunology, University of Wisconsin Medical School, Madison.

Received for publication February 8, 2004; revised February 11, 2004; accepted for publication February 12, 2004.

Reprint requests: Bruce S. Bochner, MD, Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Room 2B.71, Baltimore, MD 21224-6801.

0091-6749/$30.00

doi:10.1016/j.jaci.2004.02.016

Group[3] examined whether different formulations of hydrolyzed cow's milk formula given to children at high risk for the development of atopic diseases could prevent its development. Interestingly, the incidence of allergic manifestations was significantly reduced (by approximately 50%) by using extensively hydrolyzed casein formula compared with cow's milk formula in these high-risk infants. The extensively hydrolyzed casein formula also reduced the incidence of atopic dermatitis at 1 year of age by about the same amount. This adds to the growing list of intriguing interventions focusing on allergy prevention.[4-6]

TABLE I. Advances in mechanisms of allergy

- Predictors and prevention of allergic disease development in infants and young children
  - U-EPX levels, cytokine production from PBMCs
  - Use of modified cow's milk formula
- Development of allergic sensitization and airway inflammation
  - Role of cytokines, chemokines, and endotoxin
  - Upper airway–lower airway connections and disease manifestation
- Genetics and asthma
  - Cigarette smoke exposure, *ADAM33* polymorphisms
- Pathophysiology of allergic diseases
  - Surrogate markers of disease severity and effects of therapeutic interventions
  - Chronic rhinosinusitis-nasal polyposis pathophysiology and comparison with allergic rhinitis and asthma
- Respiratory infections and their relationship to asthma
  - *Chlamydia pneumoniae*, RSV, rhinovirus
- Eosinophil, basophil, and mast cell biology
  - Activation, release of preformed and newly generated substances, apoptosis, adhesion and migration, signal transduction

DEVELOPMENT OF ALLERGIC SENSITIZATION AND AIRWAY INFLAMMATION

A first step in the development of allergic diseases and inflammation is allergen sensitization and the production of IgE. The factors regulating these processes provide insight into mechanisms of disease at many levels, including initiation, progression, and persistence. Furthermore, these processes become eventual therapeutic targets.

With the development and availability of mAbs against TNF-α, this cytokine has gained importance and interest. Iwasaki et al[7] evaluated the effect of TNF-α on allergic reactions in sensitized mice. TNF-α knockout mice could be sensitized to antigen (ovalbumin in this case). Once sensitized, mice were then nasally challenged, and the intensity of the allergic reaction (ie, sneezes and nasal rubs) was significantly reduced in the TNF-α knockout animals. There was also a reduction in mRNA for IL-4, IL-10, and eotaxin. The intensity of eosinophilia was also reduced. Thus TNF-α appears to be a pivotal mediator and major component of the allergic inflammatory response.

Endotoxin has multiple effects in allergic processes: protection against sensitization or as a cause of airway hyperreactivity. Gerhold et al[8] in Berlin used BALB/c mice to evaluate the effect of a continuous exposure to endotoxin (LPS), allergen, or both on the development of allergen-induced immune and inflammatory responses. Animals were either pre-exposed to LPS and then exposed to allergen or a combination of LPS and allergen was given to assess this interrelationship. Pre-exposure to LPS did not prevent sensitization to allergen in this model system. A continuous exposure to ovalbumin reduced sensitization (ie, tolerance). Finally, when both LPS and ovalbumin were given continuously, sensitization, inflammation, and airway hyperresponsiveness were inhibited. These studies point out how important the timing, sequence, order, and combination of airway-altering agents are on the eventual airway response to antigen.

A number of experimental models have been developed to identify the mechanisms by which airway inflammation develops after antigen challenge. Until recently, antigen provocation to study allergic inflammation has used a single challenge and the subsequent appearance of an early and late response. Liu et al[9] extended the application of antigen provocation responses to a more physiologic exposure with repetitive daily low-dose antigen challenges and compared these responses with those elicited by large single challenges. The objective of this study was to compare the effect of repetitive low doses, as might occur naturally, with the effects of single, large-dose antigen provocation. By administering small repetitive doses of antigen, the immediate decrease in lung function and generation of sputum eosinophilia required less cumulative antigen dose. These data suggest that there is a cumulative effect in giving antigen with loss in lung function and generation of airway inflammation gradually achieved with small and more continuous exposures. This gradual worsening in asthma symptoms seen with prolonged antigen might be more reflective of how inhaled antigen normally provokes the disease.

The connection between nasal allergic disease and provocation of asthma has been of considerable interest. To understand further how an upper airway allergic reaction provokes asthma, McCusker et al[10] evaluated the effect of a nasal allergen challenge on lower airway responses in a murine model. When lavage samples were collected from the lower airway 24 hours after antigen challenge of the nose, increases in IL-5 and granulocytic cells were found, despite an absence of allergen entering the lower airway. Furthermore, eosinophilia of both the upper and lower airway tissues was found. The development of lower airway inflammation to a nasal antigen challenge was similar whether sensitization was systemic or local to the nasal tissues. The mechanism of this linkage remains to be defined.

Erpenbeck et al[11] used bronchoscopy and segmental airway allergen challenges to determine the effect of an antigen challenge on IL-9 expression in asthma. IL-9 is a member of the T_H2 cytokine family, and the human gene has been mapped together with other T_H2 cytokines, such

as IL-4, IL-5, and IL-13. IL-9 has also been found in association with severe asthma and has been found to cause airway hyperresponsiveness. In mild asthma allergen challenge caused an eosinophilic inflammatory response. Twenty-four hours after antigen challenge, IL-9 protein levels increased, as did lymphocytes that were IL-9 positive. These data show striking correlations between the generation of IL-9 and the appearance of eosinophils in the airway lavage fluid, suggesting that IL-9 is specifically upregulated in the airway after allergen challenge. Recruited lymphocytes are likely the major source of this cytokine, and given the properties of IL-9, this cytokine is likely important to the development of inflammation in asthma.

As indicated by the preceding cited articles, the allergic airway response to antigen is complex and contributes to and is regulated by a myriad of proinflammatory mediators. To illustrate this expanding process, Bochner et al[12] used segmental allergen challenge to evaluate the role of chemokines in the recruitment of T_H2 lymphocytes to the airway. Chemokines acting through the receptors CCR3, CCR4, and CCR8 are predominantly associated with T_H2 lymphocytes, whereas chemokines interacting with CXCR3 are associated with T_H1 cells. The investigators hypothesized that T_H2 chemokines, mainly CCR4-active chemokines, such as macrophage-derived chemokine (MDC) and thymus and activation—regulated chemokine (TARC), as well as the CCR8-active chemokine I-309, would be generated in response to an allergen challenge. Lavage fluid obtained 24 hours after segmental allergen challenge contained significant increases in the CCR4 receptor chemokines MDC and TARC. The CXCR3 chemokine interferon-inducible protein 10 was also detected, but the CCR8-active chemokine I-309 was not. Thus the inflammatory response to allergen includes CCR4 and CXCR3, but not CCR8, chemokines. Because recruited lymphocytes belong predominantly to T_H2 types, it is likely that the CCR4 chemokines MDC and TARC contribute to the late inflammatory response to antigen.

Like the complexity associated with lymphocyte recruitment to sites of allergic inflammation, regulation of eosinophil migration to the airway is also influenced by many factors, including eotaxins and IL-5. Using wild-type, IL-5—transgenic, IL-13— and signal transducer and activator of transcription 6-deficient, and IL-4Rα—deficient mice, Yang et al[13] were able to begin to dissect apart these complexities and contrast the roles of eotaxin-2, IL-5, and IL-13 in the development of airway eosinophilia and hyperresponsiveness to allergen provocation. Using a mouse model, the investigators showed that eotaxin-2 and IL-5 act in a cooperative fashion to recruit eosinophils to the lung. Associated with the eotaxin-2 and IL-5 actions was the generation of IL-13 and increased airway reactivity. Second, eosinophils appear to be the source of IL-13 that then acts through the IL-4R α-chain and signal transducer and activator of transcription 6 to cause increases in airway responsiveness. Thus the process of allergic inflammation and changes in airway function include multiple cell recruitment, activation, generation of a putative mediator to alter airway physiology (ie, IL-13), and signaling through a distinct pathway, leading to the eventual increase in airway responsiveness.

To extend our insight into the contribution of lymphocyte subpopulations to the regulation of inflammation, Isogai et al[14] examined the hypothesis that $CD8^+$ subtypes of γδ T cells inhibit antigen-induced late-phase responses and airway eosinophilia. Using the Brown Norway rat, the investigators adoptively transferred $CD8^+$ γδ naive or sensitized T cells into the animals. The naive γδ cells from naive but nonsensitized rats suppressed the late-phase reaction to antigen (ovalbumin) in the sensitized recipient animals. Although the concentrations of IgE and the early phase response were unaffected (presumably because of mast cell activation), T-cell profiles showed a shift toward T_H1 status, which was probably the result of increased IFN-γ generation. In addition, macrophages had an increased number of phagocytosed eosinophils, indicating another mechanism to reduce the presence of eosinophils. The results of these studies also suggest that the suppression of eosinophils was IFN-γ dependent; in contrast, inhibition of the late-phase response was not IFN-γ dependent. A key finding of this study is the possibility of an independence of the late-phase reaction and eosinophilia.

A review article by Umetsu et al[15] on regulatory T cells and their role in the control of the development of allergic disease and asthma discusses the spectrum of lymphocyte populations and how these subpopulation cells might regulate both sensitization and the eventual inflammatory process.

GENETICS AND ASTHMA

Gene-by-environment interaction is an important component in the eventual expression of asthma. To illustrate, Colilla et al,[16] in the Collaborative Study for the Genetics of Asthma, examined the hypothesis that environmental tobacco smoke exposure might interact with susceptible genes to determine the expression of asthma. These areas were identified with nominal evidence for linkage and showed a significant increase from baseline lod score; 2 other areas not meeting nominal significance also showed a significant increase from baseline, 1q and 9q. These data are important because they show how environmental exposure can determine the genetic capacity to express asthma.

The association of a disintegrin and metalloprotease 33 *(ADAM33)* gene with asthma has been of considerable interest. Howard et al[17] extended this observation through the study of polymorphisms in *ADAM33* to asthma or closely related phenotypes in 4 distinct asthmatic populations: African American, US White, US Hispanic, and Dutch White. Significant associations were observed to at least one of the 8 single nucleotide polymorphisms tested with each population of asthmatic subjects. However, no single nucleotide polymorphism (SNP) was consistently associated with a specific asthma phenotype

in all groups. Their data support the previously reported associations for *ADAM33* but were unable to determine the primary SNP involved with this association.

PATHOPHYSIOLOGY OF ALLERGIC DISEASES

One of the problems faced by clinicians and scientists is the issue of disease heterogeneity. It would thus be useful, both for research and clinical purposes, to have other ways of classifying disease activity or severity in such a way as to predict pathophysiology and response to specific classes of medications. An example of one such study is the article by Prosperini et al,[18] which examined the effects of an inhaled steroid, budesonide, on induced sputum cytology and airway hyporesponsiveness, the latter being measured by means of both methacholine and adenosine-5′ monophosphate inhalation in a double-blind, placebo-controlled crossover study. As expected, inhaled steroids reduced the numbers of sputum eosinophils and epithelial cells. Perhaps more striking, however, was the observation that compared with other surrogate markers, improvements in PC_{20} values to inhaled adenosine-5′ monophosphate appeared to be the best marker of detecting the early inflammatory improvement seen during steroid treatment. The exact mechanism responsible for this effect was not determined.

Nakano et al[19] found that in patients with acute severe asthma, for whom peak flow rates were less than 50% of predicted value, plasma concentrations of the complement-derived protein C3a were on average about 50% higher in plasma from subjects admitted to the hospital than from those patients with stable, chronic asthma. They were also higher than those in asthmatic patients who were discharged from the emergency department rather than admitted. In contrast, in this same article there was no significant difference in serum eosinophilic cationic protein concentrations between any of these patient groups.

Kim et al[20] performed bronchoalveolar lavage (BAL) to analyze cytokine levels in BAL fluid obtained from children with acute asthma without respiratory syncytial virus (RSV) infection and those with acute bronchiolitis caused by RSV. They found increased levels of IL-5 by using ELISA in the asthmatic group without RSV infection, as well as in the subgroup of RSV-infected children who also had BAL eosinophilia. Furthermore, there was a correlation between the percentages of BAL eosinophils and the levels of IL-5 in the asthma and bronchiolitis groups.

Strunk et al,[21] reporting for the Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute, identified exhaled nitric oxide as a useful surrogate marker in children aged 6 to 17 years with mild-to-moderate persistent asthma only taking albuterol as needed. In this study exhaled nitric oxide correlated with peripheral blood eosinophilia, serum eosinophilic cationic protein, and PC_{20} to methacholine but not with urinary leukotrienes or peak flow measurements.

Taken together, these studies suggest that a number of noninvasive or minimally invasive surrogate markers could be used to monitor and categorize disease activity in asthmatic patients. Whether knowledge of such markers could be used longitudinally to better manage their disease, as was recently shown, for example, with sputum eosinophils versus standard asthma guidelines,[22] remains unknown.

Although only correlative, investigating various cellular or tissue markers can be useful in implicating those substances in disease pathophysiology. Together with pharmacologic intervention, these molecules can be directly implicated in a causative fashion. The latter often requires specific drugs that might or might not yet exist. Before such drugs can be generated and tested in allergic disease, it is important to know that there are aberrant responses or aberrant levels of a given molecule in the disease of interest. This was the focus of numerous articles in the JACI Mechanisms of Allergy section over the past year.

Rosenwasser et al[23] reported results of a multicenter, Phase I, single-dose clinical trial of an antibody to CD23. CD23 is an IL-4–inducible cell-surface protein that, in both its surface and soluble forms, has been implicated in a variety of biologic responses, including IgE production and T_H2 and dendritic cell function. The anti-CD23–primatized IgG1 antibody consists of cynomolgous macaque variable regions and human constant regions. In this dose-escalation study, single doses of the antibody were generally well tolerated. Most impressive were sustained, dose-dependent decreases in mean serum IgE concentrations by as much as 40% for at least 3 months, despite an antibody half-life of no more than 10 days. On the basis of data with omalizumab,[24] this is unlikely to represent a sufficient decrease in IgE levels to result in clinical efficacy. However, the sustained duration of the effect and the fact that only a single dose of antibody was administered suggest that more profound reductions of IgE levels might be achieved with a longer dosing duration. Also of note was an association of treatment with a slight reduction in circulating B-cell numbers, but its magnitude does not solely explain the reduction of IgE levels.

Ponikau et al[25] used histology and immunohistochemistry to show that eosinophilic inflammation and airway remodeling, already known to exist in asthma, are also present in chronic rhinosinusitis. This thickening of the lamina reticularis, with epithelial damage and shedding down to the basal epithelial layer, is not seen in patients with allergic rhinitis, suggesting that chronic rhinosinusitis pathophysiology might more closely resemble that of asthma than that of allergic rhinitis. Fritz et al[26] used gene chip arrays to examine 12,000 genes using mRNA generated from nasal mucosal biopsy specimens of subjects with allergic rhinitis with and without nasal polyps. They found that 34 genes, such as those involved in inflammation and cell growth, were

differentially expressed between the patient groups. One such growth-related gene overexpressed in polyp tissues was mammaglobin, and its overexpression was confirmed by using quantitative RT-PCR and immunohistochemistry. Approaches such as these might provide us with new, often unanticipated insights into disease pathophysiology. Steinke et al[27] documented increased cysteinyl leukotriene concentrations in tissues obtained from subjects with chronic hypoplastic sinusitis compared with healthy sinus tissue. This finding is in addition to a previous report of increased cysteinyl leukotriene type 1 receptor (CysLT1)−bearing cells in the nasal mucosa of patients with aspirin-sensitive rhinosinusitits.[28] Separately, Espinosa et al[29] found that when human bronchial smooth muscle cells were exposed in vitro to TGF-β, IL-13, or IFN-γ, expression of the CysLT1 receptor was significantly augmented, both at the protein and mRNA level. These cytokine-treated cells exhibited enhanced responsiveness to leukotriene D4, as well as augmented proliferation that was CysLT1 dependent.

Sandrini et al[30] compared pathophysiologic links between the upper and lower airways in subjects with allergic rhinitis. In this study intranasal triamcinolone acetonide was used in a double-blind parallel-group design to assess the effect of steroid treatment of the upper airway on markers of lower airway inflammation, namely exhaled nitric oxide and hydrogen peroxide, in exhaled breath condensates. In the subgroup of patients with allergic rhinitis who also had asthma, they reported that 4 weeks of treatment with triamcinolone resulted in a significant decrease in exhaled nitric oxide levels. In subjects with allergic rhinitis without asthma, exhaled breath condensate levels of hydrogen peroxide were higher and decreased with steroid treatment, regardless of whether they had asthma. However, no changes were seen in symptoms or methacholine reactivity. Nevertheless, this study suggests that treatment of the upper airway with a topical steroid can decrease markers of lower airway inflammation. Exactly how this occurs remains unclear.

Numerous studies identified additional markers of airway inflammation and other abnormalities in asthma. For example, Nakao et al[31] found that expression of SMAD-7, a cytoplasmic antagonist of TGF-β signaling, inversely correlated with basement membrane thickness and airway hyperresponsiveness in bronchial biopsy specimens obtained from subjects with asthma compared with those from control subjects.

Wenzel et al[32] found a higher proportion of patients with severe asthma had matrix metalloprotease 9 (MMP-9) staining in the subepithelial basement membrane zone than control subjects and also had higher levels of MMP-9 in BAL fluids. Interestingly, MMP-9 staining of the tissues correlated best with submucosal neutrophils, whereas the presence of subepithelial basement membrane MMP-9 staining was associated with higher levels of lavage and tissue eosinophils.

Asai et al[33] studied endostatin and vascular endothelial cell growth factor (VEGF), a cytokine known to induce endothelial proliferation, migration, and other functions. They determined that levels were higher in induced sputum from asthmatic subjects compared with those seen in control subjects. After 8 weeks of inhaled beclomethasone therapy, VEGF levels were reduced significantly, but endostatin levels were not.

In a related study using cultured human airway smooth muscle cells, Wen et al[34] showed that T_H2 cytokines and TGF-β enhanced production of VEGF. Both glucocorticoids and IFN-γ inhibited this effect.

In another steroid study, a variety of cytokines and tissue matrix proteins were studied by Chakir et al[35] before and after 2 weeks of oral corticosteroids. In biopsy specimens taken from these subjects, baseline expression of the profibrogenic cytokines IL-11 and IL-17 were increased, and oral steroids decreased these levels. Expression of both TGF-β and collagen was higher in all groups with asthma, but neither decreased with oral steroids.

All of these studies further implicate profibrogenic cytokines and pathways in the asthmatic airway. Whether efforts to target such pathways would reverse airways remodeling in established disease must await specific clinical trials.

Animal models have proved an avenue to explore new approaches to therapy and to define mechanistic effects of various interventions. For example, there has been considerable interest in the components of the allergic response to antigen that result in the development of eosinophilic inflammation and enhanced airway responsiveness. Eum et al[36] at the Meakins-Christie Laboratories in Montreal compared the effects of glucocorticosteroids (dexamethasone) versus antibodies to eotaxin and IL-5. Antibodies against eotaxin and IL-5 inhibited the development of antigen-provoked eosinophilia but had no effect on airway responsiveness. These data, at least in the rat, begin to dissect the regulation of allergic inflammation and suggest that effects of corticosteroids on airway responsiveness are not explained by their effects on eosinophils, eotaxin, IL-5, or IL-13 and have other effects that not only alter eosinophil recruitment but also modulate airway responsiveness.

Kumar et al[37] explored the possibility of extending the bronchodilator actions of atrial natriuretic peptide by administering it to mice through a plasmid. The treated mice had a significantly blunted increase in airway responsiveness to antigen challenge.

CpG-oligodeoxynucleotides (CpG-ODNs) have been effective in shifting the cytokine profile to T_H1 and thus preventing the development of eosinophilia and changes in lung function after antigen challenge. Jain et al[38] gave sensitized C57BL/6 mice repetitive antigen challenges for 6 weeks to cause changes in airway remodeling. CpG-ODNs inhibited eosinophilia, airway hyperresponsiveness, and changes of airway remodeling (ie, subepithelial collagen deposition and goblet cell hyperplasia). Associated with these suppressive actions was an increase in airway fluid TGF-β levels, suggesting the actions of CpG-ODNs might include effects on regulatory cells.

RESPIRATORY INFECTIONS AND THEIR RELATIONSHIP TO ASTHMA

Respiratory infections are a major cause of asthma exacerbations. Although these attacks are usually caused by respiratory viruses, *Chlamydia pneumoniae* has recently also been identified as a causative agent. Little is known, however, about host factors that lead to patient susceptibility to these consequences of respiratory infections. To address this void, Nagy et al[39] measured the immune response to *C pneumoniae* infection in 139 children with asthma compared with that in 174 healthy control subjects. In particular, they evaluated the contribution of mannose-binding lectin (MBL) in this process. MBL is a complement-activated innate immune defense serum protein that binds to mannose and acetylglucosamine sugar groups on different microorganisms. MBL inhibits infection of epithelial cells, suggesting that this molecule might protect against *C pneumoniae* infection. The study subjects were genotyped for 3 variants of MBL, and the presence of these genotypes was then compared with the IgA, IgG, and IgM antibody response to *C pneumoniae*. Children with variant MBL alleles were found to have a higher risk for asthma than children with the normal MBL genotype, and these variants were more pronounced in children with chronic or recurrent infection. These new findings raise the possibility that a patient's host defense mechanism to *C pneumoniae* contributes to the likelihood of asthma, and this might arise as the consequence of the infection because the bacteria is not normally cleared.

Zambrano et al,[40] at the University of Virginia, tested the hypothesis that airway inflammation at the time of a virus inoculation might be a risk factor for the severity of an asthma exacerbation with an infection. Before an experimental infection in the subjects, the presence of allergy was determined by means of skin tests and measurement of total serum IgE levels. After an experimental inoculation with rhinovirus, asthmatic patients had more pronounced upper and lower respiratory symptoms than healthy subjects. Furthermore, patients with high IgE levels had greater upper respiratory symptoms. The authors concluded that increased levels of IgE might be a risk factor in determining the severity of a respiratory infection. Both publications point out the importance of host risk factors in determining the particular response to a respiratory infection and the corollary that such patient features enhance the likelihood of an asthma exacerbation.

EOSINOPHIL, BASOPHIL, AND MAST CELL BIOLOGY

As recently reviewed in the JACI,[41,42] the role of the eosinophil in asthma pathophysiology remains controversial. The disappointing lack of therapeutic benefit from IL-5–directed therapies in asthma must be tempered with the understanding that this therapy failed to completely eliminate eosinophils from the airways. Indeed, very recent reports with IL-5 antibody show promise for treatment of other hypereosinophilic disorders.[43,44] Given the continued correlation of eosinophils and eosinophil-derived mediators in many studies of asthma and allergic upper airway disease, the eosinophil remains of interest as a cellular constituent of the chronic allergic inflammatory response. Not unexpectedly, a number of articles published over the last year focused on issues related to eosinophil activation, recruitment, and survival. Kessel et al[45] report that GM-CSF production by human eosinophils, a cytokine frequently implicated in autocrine survival of these cells, was inhibited by ligation of intercellular adhesion molecule 3 with specific antibodies. Another study that identified proapoptotic stimuli for eosinophils focused on nitric oxide, which reduced eosinophil survival through effects involving c-Jun N terminal kinase.[46]

Separate from studies on eosinophil apoptosis, Duffy et al[47] report inhibition of human mast cell proliferation and survival with tamoxifen. This study was performed because human lung mast cells and mast cell lines express a strongly outwardly rectifying chloride current, and tamoxifen is a chloride-channel blocker. Because tamoxifen inhibits mast cell proliferation in association with inhibition of the outward chloride current, such an agent might be useful in the treatment of mast cell–mediated hyperproliferation disorders, including mastocytosis.

Several articles examined novel aspects of eosinophil adhesion and migration responses. For example, myosin light chain kinase was implicated in eosinophil chemotactic responses.[48] Eotaxin induced phosphorylation of myosin light chain kinase, and this was inhibited by drugs that block extracellular signal-related kinase 1/2 and p38 mitogen-activated protein kinases. Studies of CCR3, the eotaxin family receptor, on eosinophils were performed by Zimmermann and Rothenberg,[49] who found that internalization of the receptor was necessary for some functional responses (eg, shape change and actin polymerization) but not all eotaxin effects (eg, desensitization and calcium mobilization). The focus on CCR3 and its ligands is consistent with an increasing literature implicating these molecules in eosinophilic inflammation of the gastrointestinal tract, airways, and other organs.[50] Furthermore, therapies targeting CCR3 or its ligands, such as eotaxin, are entering clinical development.

Several studies examined basophil and mast cell activation and their release of preformed and newly synthesized mediators. Mochizuki et al[51] studied release of basogranulin, a basophil-specific granule protein recognized by mAb BB1, which was previously used to identify basophils in tissues. These investigators showed that basogranulin is secreted along with histamine in response to IgE-dependent and IgE-independent activation, with a very high level of correlation seen between relative amounts of each mediator. Chen et al[52] showed that IFN-γ inhibits release of IL-13 from IL-3–primed basophils but not release of leukotriene C4 or histamine. Higa et al[53] showed that the flavonol fisetin inhibited IL-4

and IL-13 synthesis in human basophils induced by IgE-dependent pathways. Although less active, other more well-known flavonoids, such as quercetin, also showed inhibitory activity. Lorentz et al,[54] using mast cells isolated from intestinal tissues, showed that IgE-dependent generation of multiple cytokines, including TNF-α, IL-3, and IL-13, involved downstream signaling through extracellular signal–related kinase 1/2, protein kinase C, activation protein 1, NFAT, and nuclear factor κB. Perhaps information gained from these studies will be useful for developing a new generation of basophil and mast cell stabilizing drugs.

CONCLUSION

This review summarized selected articles appearing between 2002 and 2003 in the Mechanisms of Allergy section of the JACI (and a few other selected journals). Articles chosen include those dealing with predictors and prevention of allergic disease development, development of allergic sensitization and airway inflammation, genetics and asthma, pathophysiology of allergic diseases, respiratory infections and their relationship to asthma, and eosinophil, basophil, and mast cell biology (Table I).

REFERENCES

1. Gore C, Peterson CG, Kissen P, Simpson BM, Lowe LA, Woodcock A, et al. Urinary eosinophilic protein X, atopy, and symptoms suggestive of allergic disease at 3 years of age. J Allergy Clin Immunol 2003;112:702-8.
2. Neaville WA, Tisler C, Bhattacharya A, Anklam K, Gilbertson-White S, Hamilton R, et al. Developmental cytokine response profiles and the clinical and immunologic expression of atopy during the first year of life. J Allergy Clin Immunol 2003;112:740-6.
3. von Berg A, Koletzko S, Grubl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP, et al. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. J Allergy Clin Immunol 2003;111:533-40.
4. Warner JO, for the ETAC study group. A double-blinded, randomized, placebo-controlled trial of cetirizine in preventing the onset of asthma in children with atopic dermatitis: 18 months' treatment and 18 months' posttreatment follow-up. J Allergy Clin Immunol 2001;108:929-37.
5. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy 2001;31:1392-7.
6. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet 2003;361:1869-71.
7. Iwasaki M, Saito K, Takemura M, Sekikawa K, Fujii H, Yamada Y, et al. TNF-alpha contributes to the development of allergic rhinitis in mice. J Allergy Clin Immunol 2003;112:134-40.
8. Gerhold K, Bluemchen K, Franke A, Stock P, Hamelmann E. Exposure to endotoxin and allergen in early life and its effect on allergen sensitization in mice. J Allergy Clin Immunol 2003;112:389-96.
9. Liu LY, Swenson CA, Kelly EA, Kita H, Jarjour NN, Busse WW. Comparison of the effects of repetitive low-dose and single-dose antigen challenge on airway inflammation. J Allergy Clin Immunol 2003;111:818-25.
10. McCusker C, Chicoine M, Hamid Q, Mazer B. Site-specific sensitization in a murine model of allergic rhinitis: role of the upper airway in lower airways disease. J Allergy Clin Immunol 2002;110:891-8.
11. Erpenbeck VJ, Hohlfeld JM, Volkmann B, Hagenberg A, Geldmacher H, Braun A, et al. Segmental allergen challenge in patients with atopic asthma leads to increased IL-9 expression in bronchoalveolar lavage fluid lymphocytes. J Allergy Clin Immunol 2003;111:1319-27.
12. Bochner BS, Hudson SA, Xiao HQ, Liu MC. Release of both CCR4-active and CXCR3-active chemokines during human allergic pulmonary late-phase reactions. J Allergy Clin Immunol 2003;112:930-4.
13. Yang M, Hogan SP, Mahalingam S, Pope SM, Zimmermann N, Fulkerson P, et al. Eotaxin-2 and IL-5 cooperate in the lung to regulate IL-13 production and airway eosinophilia and hyperreactivity. J Allergy Clin Immunol 2003;112:935-43.
14. Isogai S, Rubin A, Maghni K, Ramos-Barbon D, Taha R, Yoshizawa Y, et al. The effects of CD8+gammadelta T cells on late allergic airway responses and airway inflammation in rats. J Allergy Clin Immunol 2003;112:547-55.
15. Umetsu DT, Akbari O, Dekruyff RH. Regulatory T cells control the development of allergic disease and asthma. J Allergy Clin Immunol 2003;112:480-8.
16. Colilla S, Nicolae D, Pluzhnikov A, Blumenthal MN, Beaty TH, Bleecker ER, et al. Evidence for gene-environment interactions in a linkage study of asthma and smoking exposure. J Allergy Clin Immunol 2003;111:840-6.
17. Howard TD, Postma DS, Jongepier H, Moore WC, Koppelman GH, Zheng SL, et al. Association of a disintegrin and metalloprotease 33 (ADAM33) gene with asthma in ethnically diverse populations. J Allergy Clin Immunol 2003;112:717-22.
18. Prosperini G, Rajakulasingam K, Cacciola RR, Spicuzza L, Rorke S, Holgate ST, et al. Changes in sputum counts and airway hyperresponsiveness after budesonide: monitoring anti-inflammatory response on the basis of surrogate markers of airway inflammation. J Allergy Clin Immunol 2002;110:855-61.
19. Nakano Y, Morita S, Kawamoto A, Suda T, Chida K, Nakamura H. Elevated complement C3a in plasma from patients with severe acute asthma. J Allergy Clin Immunol 2003;112:525-30.
20. Kim CK, Kim SW, Park CS, Kim BI, Kang H, Koh YY. Bronchoalveolar lavage cytokine profiles in acute asthma and acute bronchiolitis. J Allergy Clin Immunol 2003;112:64-71.
21. Strunk RC, Szefler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, et al. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. J Allergy Clin Immunol 2003;112:883-92.
22. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002;360:1715-21.
23. Rosenwasser LJ, Busse WW, Lizambri RG, Olejnik TA, Totoritis MC. Allergic asthma and an anti-CD23 mAb (IDEC-152): results of a phase I, single-dose, dose-escalating clinical trial. J Allergy Clin Immunol 2003;112:563-70.
24. Hochhaus G, Brookman L, Fox H, Johnson C, Matthews J, Ren S, et al. Pharmacodynamics of omalizumab: implications for optimised dosing strategies and clinical efficacy in the treatment of allergic asthma. Curr Med Res Opin 2003;19:491-8.
25. Ponikau JU, Sherris DA, Kephart GM, Kern EB, Gaffey TA, Tarara JE, et al. Features of airway remodeling and eosinophilic inflammation in chronic rhinosinusitis: is the histopathology similar to asthma? J Allergy Clin Immunol 2003;112:877-82.
26. Fritz SB, Terrell JE, Conner ER, Kukowska-Latallo JF, Baker JR. Nasal mucosal gene expression in patients with allergic rhinitis with and without nasal polyps. J Allergy Clin Immunol 2003;112:1057-63.
27. Steinke JW, Bradley D, Arango P, Crouse CD, Frierson H, Kountakis SE, et al. Cysteinyl leukotriene expression in chronic hyperplastic sinusitis-nasal polyposis: importance to eosinophilia and asthma. J Allergy Clin Immunol 2003;111:342-9.
28. Sousa AR, Parikh A, Scadding G, Corrigan CJ, Lee TH. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. N Engl J Med 2002;347:1493-9.
29. Espinosa K, Bosse Y, Stankova J, Rola-Pleszczynski M. CysLT1 receptor upregulation by TGF-beta and IL-13 is associated with bronchial smooth muscle cell proliferation in response to LTD4. J Allergy Clin Immunol 2003;111:1032-40.
30. Sandrini A, Ferreira IM, Jardim JR, Zamel N, Chapman KR. Effect of nasal triamcinolone acetonide on lower airway inflammatory markers in patients with allergic rhinitis. J Allergy Clin Immunol 2003;111:313-20.

31. Nakao A, Sagara H, Setoguchi Y, Okada T, Okumura K, Ogawa H, et al. Expression of Smad7 in bronchial epithelial cells is inversely correlated to basement membrane thickness and airway hyperresponsiveness in patients with asthma. J Allergy Clin Immunol 2002;110:873-8.
32. Wenzel SE, Balzar S, Cundall M, Chu HW. Subepithelial basement membrane immunoreactivity for matrix metalloproteinase 9: association with asthma severity, neutrophilic inflammation, and wound repair. J Allergy Clin Immunol 2003;111:1345-52.
33. Asai K, Kanazawa H, Otani K, Shiraishi S, Hirata K, Yoshikawa J. Imbalance between vascular endothelial growth factor and endostatin levels in induced sputum from asthmatic subjects. J Allergy Clin Immunol 2002;110:571-5.
34. Wen FQ, Liu X, Manda W, Terasaki Y, Kobayashi T, Abe S, et al. TH2 Cytokine-enhanced and TGF-beta-enhanced vascular endothelial growth factor production by cultured human airway smooth muscle cells is attenuated by IFN-gamma and corticosteroids. J Allergy Clin Immunol 2003;111:1307-18.
35. Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, et al. Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. J Allergy Clin Immunol 2003;111:1293-8.
36. Eum SY, Maghni K, Hamid Q, Eidelman DH, Campbell H, Isogai S, et al. Inhibition of allergic airways inflammation and airway hyperresponsiveness in mice by dexamethasone: role of eosinophils, IL-5, eotaxin, and IL-13. J Allergy Clin Immunol 2003;111:1049-61.
37. Kumar M, Behera AK, Lockey RF, Vesely DL, Mohapatra SS. Atrial natriuretic peptide gene transfer by means of intranasal administration attenuates airway reactivity in a mouse model of allergic sensitization. J Allergy Clin Immunol 2002;110:879-82.
38. Jain VV, Kitagaki K, Businga T, Hussain I, George C, O'Shaughnessy P, et al. CpG-oligodeoxynucleotides inhibit airway remodeling in a murine model of chronic asthma. J Allergy Clin Immunol 2002;110:867-72.
39. Nagy A, Kozma GT, Keszei M, Treszl A, Falus A, Szalai C. The development of asthma in children infected with Chlamydia pneumoniae is dependent on the modifying effect of mannose-binding lectin. J Allergy Clin Immunol 2003;112:729-34.
40. Zambrano JC, Carper HT, Rakes GP, Patrie J, Murphy DD, Platts-Mills TA, et al. Experimental rhinovirus challenges in adults with mild asthma: response to infection in relation to IgE. J Allergy Clin Immunol 2003; 111:1008-16.
41. Bochner BS. Verdict in the case of therapies versus eosinophils: the jury is still out. J Allergy Clin Immunol 2004;113:3-9.
42. Alam R, Busse WW. The eosinophil—quo vadis? J Allergy Clin Immunol 2004;113:38-42.
43. Koury MJ, Newman JH, Murray JJ. Reversal of hypereosinophilic syndrome and lymphomatoid papulosis with mepolizumab and imatinib. Am J Med 2003;115:587-9.
44. Garrett JK, Jameson SC, Thomson B, Collins MH, Wagoner LE, Freese DK, et al. Anti-interleukin-5 (mepolizumab) therapy for hypereosinophilic syndromes. J Allergy Clin Immunol 2004;113:115-9.
45. Kessel JM, Gern JE, Vrtis RF, Sedgwick JB, Busse WW. Ligation of intercellular adhesion molecule 3 inhibits GM-CSF production by human eosinophils. J Allergy Clin Immunol 2003;111:1024-31.
46. Zhang X, Moilanen E, Lahti A, Hamalainen M, Giembycz MA, et al. Regulation of eosinophil apoptosis by nitric oxide: Role of c-Jun-N-terminal kinase and signal transducer and activator of transcription 5. J Allergy Clin Immunol 2003;112:93-101.
47. Duffy SM, Lawley WJ, Kaur D, Yang W, Bradding P. Inhibition of human mast cell proliferation and survival by tamoxifen in association with ion channel modulation. J Allergy Clin Immunol 2003;112:965-72.
48. Adachi T, Stafford S, Kayaba H, Chihara J, Alam R. Myosin light chain kinase mediates eosinophil chemotaxis in a mitogen-activated protein kinase-dependent manner. J Allergy Clin Immunol 2003;111:113-6.
49. Zimmermann N, Rothenberg ME. Receptor internalization is required for eotaxin-induced responses in human eosinophils. J Allergy Clin Immunol 2003;111:97-105.
50. Hogan SP, Rothenberg ME, Forbes E, Smart VE, Matthaei KI, Foster PS. Chemokines in eosinophil-associated gastrointestinal disorders. Curr Allergy Asthma Rep 2004;4:74-82.
51. Mochizuki A, McEuen AR, Buckley MG, Walls AF. The release of basogranulin in response to IgE-dependent and IgE-independent stimuli: validity of basogranulin measurement as an indicator of basophil activation. J Allergy Clin Immunol 2003;112:102-8.
52. Chen YH, Bieneman AP, Creticos PS, Chichester KL, Schroeder JT. IFN-alpha inhibits IL-3 priming of human basophil cytokine secretion but not leukotriene C4 and histamine release. J Allergy Clin Immunol 2003;112:944-50.
53. Higa S, Hirano T, Kotani M, Matsumoto M, Fujita A, Suemura M, et al. Fisetin, a flavonol, inhibits TH2-type cytokine production by activated human basophils. J Allergy Clin Immunol 2003;111:1299-306.
54. Lorentz A, Klopp I, Gebhardt T, Manns MP, Bischoff SC. Role of activator protein 1, nuclear factor-kappaB, and nuclear factor of activated T cells in IgE receptor-mediated cytokine expression in mature human mast cells. J Allergy Clin Immunol 2003;111:1062-8.