

Advances in mechanisms of allergy

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This review summarizes selected "mechanisms of allergy" articles appearing between 2001 and 2002 in the *Journal of Allergy and Clinical Immunology* (JACI). Papers chosen include those dealing with human eosinophil and basophil biology from life to death, as well as studies with animal models of allergic disease, including knock-out mice, Brown Norway rats, and ovalbumin (OVA)-sensitized and challenged BALB/c mice, that further our understanding of mechanisms of allergic diseases. When appropriate, articles from other journals have been included to supplement the topics being presented. (*J Allergy Clin Immunol* 2003;111:S819-23.)

Key words: Eosinophils, basophils, mast cells, T cells, epithelial cells, chemokines, cytokines, mouse models, airway hyperreactivity, anaphylaxis, CpGS

In asthma and other chronic allergic diseases, eosinophils, basophils, and Th₂ cells through release of preformed and newly generated mediators, granule proteins, and cytokines, are believed to be key effector cells. These cell types all originate within the bone marrow and eventually travel through the circulation to destinations in tissues. This review summarizes articles appearing between 2001 and 2002 in the *Journal of Allergy and Clinical Immunology* (JACI) dealing with human eosinophils and basophils, as well as studies with animal models of allergic disease that further our understanding of mechanisms of allergic diseases (Table I). When appropriate, articles from other journals have been included to supplement the topics being presented.

EOSINOPHIL HEMATOPOIESIS

Treatment of patients with cysLT1 receptor antagonists results in an almost 20% reduction in circulating eosinophil counts.¹ The mechanism by which this occurs is not clear. Braccioni et al² suggest that one effect may be at the level of the bone marrow. They showed that eosinophil-basophil colony forming units, generated in the presence of GM-CSF and IL-5, were increased by the addition of LTD₄ to the cultures. Further evidence for a functional role of cysLT1 receptors on eosinophils was provided by Nagata et al,³ who demonstrated that eosinophil adhesion molecule expression and function in vitro are enhanced by LTD₄. Bandeira-Melo et al⁴

Abbreviations used

AHR: Airways hyperreactivity
BAL: Bronchoalveolar lavage
JACI: *Journal of Allergy and Clinical Immunology*
IHES: Idiopathic hypereosinophilic syndromes
Ova: Ovalbumin
SHIP: Src-homology 2 domain containing inositol 5' phosphatase
STAT: Signal transducer and activator of transcription

demonstrated that eosinophils respond to cysteinyl leukotrienes by producing IL-4. In each case, cysLT1 antagonists blocked the response. These papers suggest an important biological role of cysteinyl leukotrienes, acting through cysLT1 receptors, on human eosinophil hematopoiesis, recruitment, and activation, and may help to explain the eosinophil-reducing effects seen in vivo when patients are treated with cysLT1 antagonists.

Eosinophils originate in the bone marrow from CD34⁺ progenitors under the influence of cytokines such as IL-5. In an early phase II clinical trial, Leckie et al⁵ showed that a single dose of IL-5 antibody had a profound inhibitory effect on the number of circulating eosinophils and allergen-induced sputum eosinophils, but no effect on allergen-induced late phase physiology or airways hyperreactivity (AHR). This cast doubt on the role of the eosinophil in asthma. However, a follow-up study comparing levels of eosinophils in lung parenchyma and bone marrow with those in blood and bronchoalveolar lavage (BAL) has made a startling observation. Eosinophil numbers in blood and BAL were reduced 80% or more after 3 months of anti-IL-5 therapy, but levels of eosinophils in bone marrow and bronchial biopsies were only reduced 50% to 60%.⁶ These data suggest that IL-5 antibody therapy effectively reduces circulating and airway luminal eosinophils, but is less effective at reducing bone marrow and lung parenchymal eosinophils. This may explain its lack of clinical efficacy to date. Whether this form of therapy will be effective for other hypereosinophilic disorders is currently being evaluated. Other papers report promising preliminary data treating patients suffering from idiopathic hypereosinophilic syndromes (IHES) with relatively low doses of imatinib mesylate (Gleevec).^{7,8} This new drug is used for the treatment of chronic myeloid leukemia and other malignancies because it inhibits several tyrosine kinases, including the BCR-ABL oncogene involved in cell proliferation.⁹ This drug was tried in IHES patients because treatments used for chronic myeloid leukemia (eg, hydroxyurea, IFN- α) can be effective. Few patients with

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TABLE I. Advances in mechanisms of allergy

Human systems
CysLT1 and its role in eosinophil hematopoiesis and activation
Antieosinophil therapies targeting IL-5 or tyrosine kinase-dependent growth factor receptors
Alteration of eosinophil or basophil phenotype (FcγR, chemokine and PGD2 receptors, activation markers)
Mechanisms of eosinophil apoptosis and survival
Murine systems
Role of Th2 cytokines and STAT6 in AHR
Mechanisms of anaphylaxis
Use of CpG oligodeoxynucleotides and H1 antihistamines to downregulate Th2 responses

IHES have been treated so far, but these reports suggest that imatinib mesylate may be a useful treatment for some IHES patients.

Finally, given the fact that IL-5-based strategies do not completely eliminate eosinophils in mice or humans, attempts have been made to identify other pathways for their elimination. This was successfully achieved by Yu et al¹⁰ based on studies suggesting that GATA-1, a transcription factor, is essential for myeloid cell differentiation into erythroid, megakaryocyte, and eosinophil lineages. By targeting a high affinity GATA binding site in the GATA-1 promoter, these investigators generated a new strain of mice with a selective and complete loss of the eosinophil lineage, yet they appear otherwise healthy. These mice should be useful in defining the role of eosinophils in mouse models.

CIRCULATING EOSINOPHILS AND BASOPHILS AND THEIR ACTIVATION

Immunomagnetic techniques are commonly used for purification of leukocyte subtypes. One such application involves the depletion of neutrophils from granulocyte fractions to generate pure eosinophils. In this method, antibodies are employed to CD16 (FcγRIII), expressed on neutrophils but not eosinophils. Activated eosinophils can express CD16,^{11,12} but Davoine et al¹³ now demonstrate constitutive expression of CD16 on circulating eosinophils of allergic and asthmatic subjects. The median differences were relatively small (7% to 8% positive in allergic or asthmatic subjects compared with 3% in nonallergic controls); however, levels of CD16 varied widely and reached as much as 48% positive in one allergic donor. Therefore, investigators using CD16 antibody-based methods to purify eosinophils may unknowingly be removing a subpopulation of eosinophils during the procedure.

Recruitment of eosinophils and basophils into tissues is felt to be mediated through a complex interplay of adhesion molecules, chemokines, and other chemoattractants.^{14,15} Several papers in the JACI focused on potential differences and functions of chemoattractant receptors on circulating eosinophils, as well as activation markers for these cells. For example, among chemokine receptors relevant to allergic inflammation, CCR3 (a ligand for chemokines such as eotaxins and MCP-4) has

received much attention because of its relatively selective expression on eosinophils and basophils. Some¹⁶ but not all studies¹⁷ using CCR3 knock-out mice provide support for its role in allergic airways inflammation. Nagase et al¹⁸ showed that in addition to CCR3, human eosinophils express CCR1, CXCR1, CXCR2, and CXCR4 but do not express CCR2, CCR4, or CCR5. They demonstrated that surface levels of CCR3 were decreased and CXCR4 (a ligand for the chemokine SDF-1α) were increased on eosinophils in the lung, whereas other receptors were unchanged, implicating CCR3 and CXCR4 in the process of eosinophil recruitment. These data are somewhat in conflict with work by Dunzendorfer et al,¹⁹ because they were able to detect CCR2 mRNA, surface expression, and functional responses using MCP-1, a ligand for CCR2. Resolution of these contradictions will require further studies.

Another recently identified 7-transmembrane, G protein-coupled receptor is CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells). This receptor was originally believed to be specific for Th2 cells. Subsequent studies determined that CRTH2 is also expressed on human eosinophils and basophils, and one of its major ligands is the mast cell product PGD₂.²⁰ Gervais et al²¹ have confirmed that eosinophils express CRTH2 as well as another PGD₂ receptor called DP. They showed that PGD₂ has a number of rapid effects on eosinophils, including induction of shape change, chemokinesis, and degranulation. Furthermore, PGD₂-like agonists delayed the onset of apoptosis in cultured eosinophils. These data suggest that CRTH2 and perhaps DP may be good targets for development of novel therapies for asthma, although studies in mice targeting PGD₂ were only modestly impressive.²² Nevertheless, these data provide a possible link between mast cell production of PGD₂ and eosinophil recruitment, activation, and survival.

Several studies focused on novel aspects of circulating basophil biology. Foster et al²³ demonstrated that human basophils express tryptase, challenging the long-held notion of mast cell specificity for this enzyme. The amount of tryptase per basophil varied over a 2-log range among donors, although some basophils contained levels approaching those found in mast cells. Besides realizing that not all serum tryptase is coming from mast cells, another implication of this work is that antitryptase antibodies used in flow cytometry or immunohistochemistry may not distinguish mast cells from basophils.

Previous studies have identified so-called activation markers on eosinophils and basophils.²⁴ For example, structures such as CD44 and CD69 are considered activation markers for eosinophils.²⁵ In a paper by Yoshimura et al,²⁶ CD69, along with CD63, were rapidly induced after activation of human basophils *in vitro* and *in vivo*. Hauswirth et al²⁷ reported that CD203c, an ectonucleotide pyrophosphatase involved in the clearance of extracellular nucleotides, is also rapidly increased on basophils after cross-linking of FcεRI by allergens. Further studies are needed to understand the biological consequences of these findings.

Two additional papers explored novel aspects of Fc ϵ RI signaling in human basophils. Vonakis et al²⁸ suggest that the enhanced histamine release responses seen in histamine-releasing factor responder basophils may be the result of unusually low levels of Src-homology 2 domain containing inositol 5'phosphatase (SHIP) in these cells, and implicate SHIP as an additional regulator of secretion in human basophils. Besides histamine, basophils produce IL-4, and on a per-cell basis release more than any other leukocyte. Because the NFAT (nuclear factor of activated T-cells) family of transcription factors are critical for induction of IL-4 gene expression in T-cells, Schroeder et al²⁹ examined the role of NFAT proteins in Fc ϵ RI-mediated IL-4 production in basophils. They discovered that human basophils constitutively express one particular family member, NFAT2, and presented data suggesting that it may play a role in signal transduction events leading to basophil production of IL-4.

LIFE AND DEATH OF EOSINOPHILS IN TISSUES

Several papers in the JACI focused on eosinophil survival and apoptosis pathways. Sexton et al³⁰ showed that human alveolar epithelial cells can engulf apoptotic eosinophils by using structures previously implicated in engulfment of apoptotic cells by macrophages, such as α v integrins and the phosphatidylserine receptor.

Bureau et al³¹ explored mechanisms by which engagement of CD40 enhances eosinophil survival. CD40 is a member of the TNF receptor superfamily. Its binding partner is CD40 ligand (CD154) and is primarily found on T-cells. CD40 is not expressed on normal eosinophils but appears on eosinophils in tissue sites or after culture. These authors report that CD40 ligation delays eosinophil apoptosis and that this is likely because of one member of the inhibitor of apoptosis protein (IAP) family, namely c-IAP2, inside the cell. These data suggest a possible role for c-IAP2 in prolonging eosinophil survival *in vivo*.

Wedi et al³² describe the ability of staphylococcal exotoxins to activate eosinophils and inhibit their apoptosis. One potential relevance of this finding is that staphylococci often colonize the skin in atopic dermatitis. The authors demonstrated that culture of eosinophils with staphylococcal exotoxins led to phenotypic and functional changes consistent with eosinophil activation and prolonged survival. Finally, Temple et al³³ used microarray "gene chip" technology to compare mRNA isolated from fresh eosinophils with mRNA obtained from IL-5 activated eosinophils to look for induction of survival-related genes. Of 73 genes found to be upregulated at least 2-fold, 2 in particular, Pim-1 and SLP-76, were found to be relatively restricted to eosinophils. Pim-1 is known to act as a survival factor in myeloid cells by inhibiting apoptosis, whereas SLP-76 is an adapter protein that functions during IL-5 receptor-mediated signal transduction. Targeting of Pim-1 and SLP-76 may, therefore, represent useful strategies for selectively inducing eosinophil apoptosis.

ANIMAL MODELS AND MECHANISMS OF ALLERGY

Pope et al³⁴ used eotaxin/IL-5 double-deficient mice to demonstrate that IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. This model is particularly useful for studying the interactions of more than one mediator in inflammation. This group has also shown that mucous production is independent of eosinophil recruitment.

Several groups have used Brown Norway rats over the years to study the role of CD4⁺ T cells. They have now been used to dissect the role of CD8⁺ T cells in asthma. Suzuki et al³⁵ demonstrated that CD8⁺ T cells inhibit allergen-induced airway eosinophilia but not late AHR. In addition, they have shown that inhibition of eosinophilia is dependent on secretion of IFN- γ by CD8⁺ T cells. Interestingly, this study found no correlation between eosinophils and AHR.

Most of the publications using animals that have appeared in the JACI over the last year have primarily concentrated on the use of OVA-sensitized and challenged BALB/c mice to answer many questions, particularly the relation of inflammation to lung physiology. Tomkinson et al³⁶ demonstrated the importance of signal transducer and activator of transcription 6 (STAT6) in allergen-induced AHR by using STAT6 deficient mice. They showed that transfer of STAT6 +/+ T cells from OVA-sensitized mice to naïve STAT6 -/- mice transferred AHR when the naïve mice were later challenged with OVA. Furthermore, the development of AHR was not associated with significant BAL fluid or tissue eosinophilia. Previously, these investigators showed that sensitization of STAT6 +/+ mice induced a Th2-type immune response in mice, whereas the STAT6 -/- mice developed a predominantly Th1 type-immune response.³⁷ These latest results are important because they demonstrate that priming of T cells by sensitization in STAT6 -/- and STAT6 +/+ donor mice might result in a different skewing of the T-cell populations toward either Th1- or Th2-type responses.

In line with allergen-induced responses in BALB/c mice, pathways of anaphylaxis have been further investigated in the mouse. Classically, anaphylaxis is believed to involve IgE, Fc ϵ RI, mast cells, and histamine. Prior studies in mice, however, suggest that IgE is not always essential³⁸ and IgG can substitute for IgE,³⁹ although the latter still appears to require Fc ϵ RI.⁴⁰ Strait et al⁴¹ used a mouse model characterized by mastocytosis and a high level of antigen-specific IgE and showed that IgG, Fc γ RIII, macrophages, and the mediator platelet-activating factor contribute to anaphylaxis in mice. The importance of IgG-mediated anaphylaxis in humans remains unknown.

ANIMAL MODELS AS TOOLS TO INVESTIGATE NEW THERAPIES

Several studies in JACI explored potential new therapies for the treatment of asthma. Consistent with the hygiene hypothesis, Gerhold et al⁴² showed that adminis-

tration of endotoxin prevented most manifestations of allergic asthma (production of IgE, Th2 cell cytokines, and airway eosinophilia), but not all of them (AHR). Another study looked at the effect of *Amb a 1*-linked CpG oligodeoxynucleotides in an animal model of allergen-induced inflammation. Previously, it was shown that immunostimulatory DNA sequences containing CpG motifs are effective in preventing the development of allergic inflammation in mice, preferentially inducing a Th1 response and downregulating the Th2 response to *Amb a 1* antigen.⁴³ In vitro studies have also demonstrated that *Amb a 1*-linked CpG oligodeoxynucleotides can promote a Th2 to Th1 switch in peripheral blood mononuclear cells from ragweed-allergic patients.⁴⁴ Furthermore, animal experiments showed these agents to preferentially induce a Th1 response and downregulate the Th2 response to *Amb a 1* antigen.⁴³ The study by Santeliz et al⁴⁵ provides additional evidence to suggest that *Amb a 1*-linked CpG oligodeoxynucleotides are effective in reversing allergen-induced AHR and eosinophilia in sensitized BALB/c mice. They also showed that this inhibitory effect was associated with a significant increase in IFN- γ , Th1-directed IgG2a responses, and inhibition in Th2-mediated IgG1 responses. These data support the view that delivery of immunostimulatory DNA sequences linked to common aeroallergens may provide a safe and effective therapy for asthma.

Luccioli et al⁴⁶ published an important contribution characterizing the early events after allergen challenge in mice. They showed that IL-4 mRNA is detectable as early as 100 minutes after allergen challenge in sensitized animals. Cellular analysis revealed the presence of early IL-4 protein coming exclusively from myeloid cells with characteristics of basophils. Inhibition of this early production of IL-4 attenuated the recruitment of eosinophils to the lungs, suggesting that it may be necessary to target basophils in order to treat early allergic lung inflammation.

Finally, the role of antihistamines in asthma has been examined for more than 5 decades. Returning to the usefulness of histamine (H₁)-receptor antagonists in treatment of asthma, Gelfand et al⁴⁷ found that the third-generation antihistamine fexofenadine was effective in downregulating AHR, BAL and tissue eosinophilia, lymphocyte numbers, and Th2 cytokine production in sensitized and OVA-challenged mice. This suggests that antihistamines can have anti-inflammatory effects, although there is limited evidence for this in humans.

CONCLUSION

This review highlighted recent publications in the Mechanisms of Allergy section of the JACI (and other selected journals) that focus on human eosinophil and basophil immunobiology. Information from studies using mouse and rat models to explore asthma and allergic disease pathogenesis and treatment have also been discussed. A wide range of topics were covered (Table I). Ultimately, it is the successful translation of these find-

ings from the laboratory to the clinic that may result in new treatments for asthma and allergic diseases.

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