

Editorial

The eosinophil—Quo vadis?

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Whither goest thou, dear eosinophil? Clues to mechanisms of disease and a cell's involvement in pathophysiology can be defined in a number of ways. In the movie *Casablanca*, for example, the police's attempt to solve a killing began with a "rounding up of the usual suspects." This approach usually works well also in the study of disease mechanisms in which highly suspicious cells and mediators are identified and then "interrogated" as to their involvement in the resulting pathophysiology in an attempt to "catch the killer red-handed." In asthma, parasitic diseases, and hypereosinophilic syndromes (HES), the eosinophil has always emerged as the "prime suspect" to explain altered airway function, parasite host defense mechanisms, and endomyocardial disease, for example. Despite these "guilt-by-association" situations, the role of eosinophils in the pathogenesis and pathophysiology of disease states has undergone a "quo vadis" experience in response to complexities and perplexities that have arisen, and because recent evidence suggests that the eosinophil is "not guilty" or might not be involved in various disease states. This conclusion is likely to be premature and overly reactive when current evidence is viewed without in-depth knowledge.

As a consequence, the ultimate role, participation, and contribution of eosinophils to asthma, parasitic infestations, and HES are being reappraised as a more comprehensive understanding of the pathophysiology of these conditions emerges. Right now there is considerable confusion as to whether the eosinophil is important to allergic diseases. If so, under what conditions? Because the eosinophil is so closely associated with allergic and immunologic processes and is a potential therapeutic target in these situations as well, it is important to have the most recent and critical information available to best judge the "guilt, innocence, or noninvolvement" of eosinophils in asthma, parasitic diseases, and HES. To accomplish this goal and to identify the right suspect in these situations, the Journal asked 4 expert groups to review the current status of the eosinophil in complex

Abbreviation used

HES: Hypereosinophilic syndromes

conditions and to bring to its readers a timely and critical reappraisal of this cell's role in disease.

Over the years we have learned much about the eosinophil's role in parasitic diseases and have extended this information to other conditions. The eosinophil is found around tissue-invasive parasites, and its granule proteins are toxic to these organisms, just as it is hypothesized that the same enzymes are toxic to airway tissues in asthma. As discussed by Klion and Nutman,¹ the eosinophil is important in host defense mechanisms against parasites, but possibly not indispensable. Their discussion sets the stage for a critical reappraisal of eosinophil biology in a complex and redundant host defense response in parasitic infestations, as well as other eosinophil-associated processes.

As reviewed by Bochner,² the eosinophil's biology is well-suited to cause airway inflammation and dysfunction in asthma, and, until recently, the evidence for a role of eosinophils in human asthma seemed convincing, albeit circumstantial. Similarly, strong but still circumstantial evidence implicates a role for eosinophils in eosinophilic esophagitis and gastroenteritis as reviewed earlier by Rothenberg.³ The importance of the eosinophil to gastrointestinal disease is now further substantiated by Garrett et al⁴ in this issue, in which the University of Cincinnati group presents preliminary evidence that treatment with anti-IL-5 might be effective in these hypereosinophilic conditions. In HES, Leiferman and Gleich⁵ describe and discuss the pleiotropic features of this syndrome and how the eosinophil appears inextricably linked to the clinical manifestations and responses to therapy of these diseases. The 4 reviews on eosinophils in this issue of the Journal are timely and informative and should provide direction on the pathway of this cell in pathobiology of disease. It is our hope that these 4 contributions can be a "quo vadis" (whither goest thou?) as we begin to emerge from the current confusion and methodically determine the biologic path of the eosinophil in disease.

HOW DID THE EOSINOPHIL'S IMPORTANCE IN PATHOBIOLOGY CHANGE WITH THE ANTI-IL-5 EXPERIENCE?

Animal studies have shown that anti-IL-5 antibodies profoundly reduced airway eosinophilia and airway dys-

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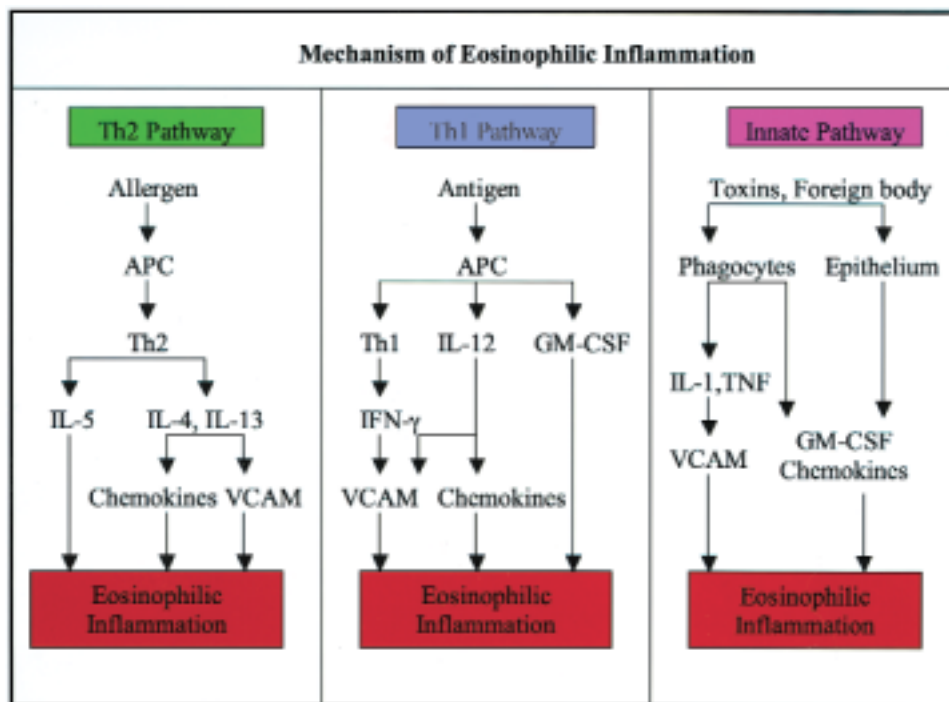


FIG 1. Mechanism of induction of eosinophilia by various types of the immune response. The T_H2 -type immune response is the dominant and most effective mechanism of eosinophilia. However, both T_H1 and innate mechanism are capable of inducing eosinophilia in the absence of a concomitant T_H2 response.

function in models for asthma. These observations heightened expectations that similar events would occur in asthma in humans, and anti-IL-5 would eradicate allergic inflammation in asthma. Anti-IL-5 antibody treatment did completely abrogate peripheral blood eosinophilia but had no effect on airway dysfunction. There are many possible explanations for this outcome. First, airway eosinophilia was reduced by only 55% by anti-IL-5.⁶ Second, there are also multiple mechanisms for the induction of eosinophilia. Although T_H2 -type immune responses (IL-5 is the major mediator) are the dominant mechanism for eosinophilia, both innate immunity⁷ and T_H1 immune responses⁸ are also capable of inducing tissue eosinophilia (Fig 1) in the absence of a T_H2 response. Chemokines play an important role in the induction of eosinophilia by all forms of the immune response. Indeed, maximal depletion of airway eosinophils has been reported to occur only in IL-5 and eotaxin double knockout mice in an allergic immunization model.⁹ The importance of IL-5 to asthma is further complicated by the fact that airway eosinophils lose IL-5R α and become more dependent on GM-CSF for survival and function.¹⁰ It is, therefore, quite possible that a significant number of airway eosinophils are not affected by the anti-IL-5 antibody. Consequently, therapeutic strategies directed at eliminating airway eosinophilia must target more than 1 regulator of eosinophils. As discussed by Bochner,² one such strategy might include a combination of the anti-IL-5 antibody and an inhibitor of the CC chemokine receptor-3 or blocking adhesion molecules.

The advantage of the foregoing strategy is that it is directed at 2 different phases of airway eosinophilia, eosinophil differentiation and eosinophil recruitment into the airways.

IF ASTHMA CAN EXIST WITH FEW EOSINOPHILS IN THE BLOOD AND AIRWAY LUMEN, WHAT ROLE DOES THIS CELL HAVE IN ASTHMA?

Asthma is a complex disease process with many phenotypes, which likely reflect variations on dominant cellular and molecular mechanisms involved in the clinical manifestations and therapeutic responses of this disease. In addition, there exist many features of asthma, including airflow obstruction, bronchial hyperresponsiveness, and airway remodeling, each likely to be regulated by distinct processes. Moreover, there are variable severities of asthma and different levels of disease—intermittent, chronic persistent, or undergoing an exacerbation. It is likely that the cellular and molecular mechanisms in each of these situations are distinct and variable. Therefore, to understand the eosinophil's involvement and participation, it is necessary to consider these multiple features and variabilities of asthma.

Airway eosinophilia has been found to correlate with asthma severity and airway hyperresponsiveness.^{11,12} This holds true not only for allergic asthma but also for nonallergic asthma, which is typically associated with

higher numbers of tissue eosinophils.¹³ When Leckie et al¹⁴ found that anti-IL-5 antibody did not protect against allergen-induced increases in bronchial hyperreactivity, the role of eosinophils in asthma was seriously questioned as noted earlier. However, the small sample size of this study did not allow any definitive conclusions to be drawn.¹⁵ There is, however, circumstantial evidence to link eosinophils with asthma exacerbations. Patients who had reduced eosinophil counts in the sputum achieved through rigorous adjustment of inhaled corticosteroids had fewer asthma exacerbations and hospitalizations.¹⁶ Because corticosteroids target not only eosinophils, a causal relationship with asthma exacerbations must await studies with more comprehensive anti-eosinophil treatment protocols.

Critical studies with anti-IL-5 have not been done to determine whether a highly selective reduction in eosinophils will reduce asthma exacerbations, as occurs with inhaled corticosteroids. Or is it possible that the eosinophils in exacerbations are merely a marker for other cell involvement such as the lymphocyte?

Chronic asthma is associated with airway remodeling and a loss of lung function in a subset of patients. Many prototypic fibrotic illnesses are associated with mild to moderate tissue eosinophilia.^{17,18} Fibrosis is facilitated by myofibroblast differentiation and subsequent production of essential tissue remodeling factors by this cell type. In vitro studies suggest that eosinophils are capable of inducing myofibroblast differentiation.¹⁹ In support of this hypothesis, a recent study with mepolizumab (anti-IL-5) showed reduced amounts of the tissue matrix proteins lumican, tenascin and procollagen III in the airways of the treated subjects,²⁰ suggesting a causal relationship between eosinophils and airway remodeling in asthma. Perhaps it is in airway remodeling that eosinophils exert their primary activity.

ARE THE ROLE AND CONTRIBUTION OF EOSINOPHILS IN DIFFERENT DISEASES DISTINCT?

As detailed by Leiferman and Gleich,⁵ the clinical features of HES can be explained, in large part, by the biology of the infiltrating eosinophils. However, like asthma, there are distinct syndromes within the HES class of diseases, and treatment effectiveness appears dependent on the genotype variant of HES.²¹ This possibility is perhaps best illustrated by the recent recognition of a novel kinase referred to as FIP1L1-PDGFR α ²¹ and the response to imatinib in some patients with HES. Furthermore, Garrett et al⁴ present very preliminary data in this issue that anti-IL-5 might be effective in some patients with HES. Increased numbers of tissue eosinophils have frequently been observed in various gastrointestinal diseases as detailed by Rothenberg³ in an in-depth review of eosinophil involvement in gastrointestinal diseases. The most intriguing observation in this new paradigm is an emerging link between respiratory allergen sensitization and eosinophilic esophagitis,⁴

at least in the animal model. The report by Garrett et al⁴ indicating that some HES patients respond to anti-IL-5 raises the possibility that different organs, ie, esophagus versus airways, might be affected by eosinophils and regulated by IL-5 in a distinct fashion.

IN THIS EMERGING PARADIGM, HOW DO EOSINOPHILS PARTICIPATE IN THE PATHOBIOLOGY OF DISEASE?

Eosinophils possess a myriad of mediators to induce airway injury, tissue remodeling, and immunoregulation.¹¹ The toxic and injurious effects of major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin on airway epithelial cells have been extensively described. There is a good correlation between the deposition of eosinophil-derived mediators in the airways and epithelial damage in asthmatic patients.²² Major basic protein is known to antagonize M2 muscarinic receptors in the airways and, thereby, could contribute to the neural mechanism of airway hyperreactivity.²³ Moreover, eosinophils are a major source of tissue remodeling factors such as TGF- β , TGF- α , heparin-binding-epidermal growth factor, platelet-derived growth factor- β , vascular endothelial growth factor, and IL-4.^{24,25} Indeed, eosinophil-induced myofibroblast differentiation is blocked by an antibody against TGF- β ,¹⁷ indicating a crucial role of this growth factor in airway remodeling.

Eosinophils also interact with lymphocytes. Although eosinophils are typically viewed as effector cells of the efferent limb of an immune response, emerging information suggests that eosinophils can also function in the afferent limb of the immune response and influence the function of lymphocytes. Eosinophils have been shown to be involved in antigen presentation, immunoregulation, and maintenance of a T_H2 response. For example, eosinophils appear, at least in animal models, to be capable of antigen internalization and presentation under some experimental conditions^{26,27} but not others.²⁸ Intratracheally administered eosinophils migrate into the paracortical area of the regional lymph nodes,²⁶ which is reminiscent of dendritic cells. Whether eosinophil-mediated antigen presentation is important in allergic diseases is unclear right now. The issue that needs to be clarified is what unique antigen-presenting function(s) do eosinophils have that cannot be performed by professional antigen-presenting cells of the airways such as dendritic cells? One possibility is that allergens and parasitic antigens are inherently different from typical antigens, and that eosinophils are specialized to present these antigens better. Another possibility is that dendritic cells are the initial presenter of allergens and parasitic antigens, but as stimulation of the immune system with these antigens continues in a repeated manner and for a sustained period of time, the role of antigen presentation is switched from dendritic cells to eosinophils. These possibilities need to be tested in future experiments. Interestingly, eosinophil-derived neurotoxin has been recently shown to be chemotactic for dendritic cells.²⁹ This effect

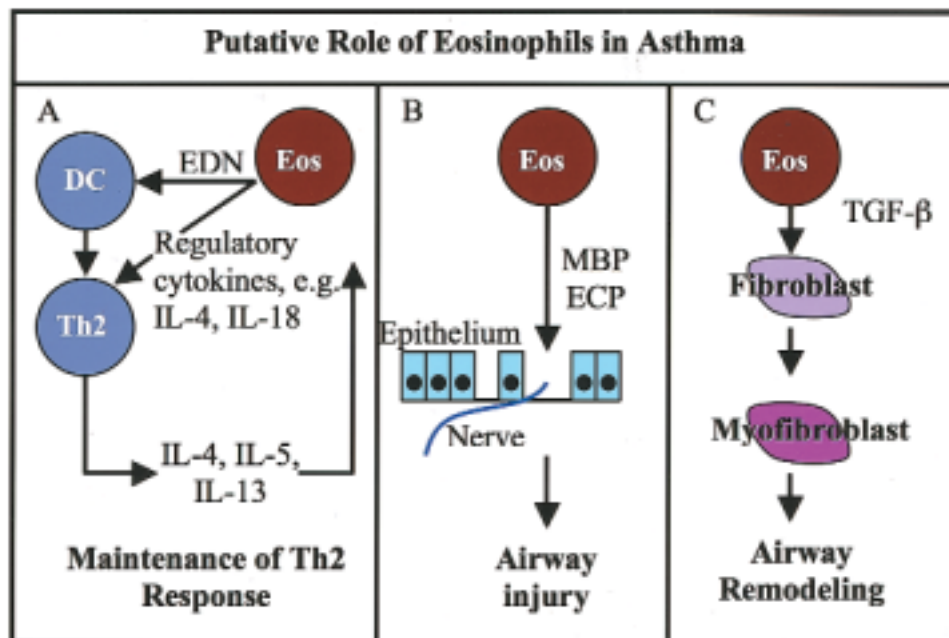


FIG 2. Putative role of eosinophils in asthma. **A**, Maintenance of a T_H2 response by eosinophils. Eosinophils help recruit dendritic cells through eosinophil-derived neurotoxin. T_H2 cells produce regulatory cytokines such as IL-5 and IL-13, which induce eosinophil differentiation and activation. Eosinophils, in return, produce IL-18, which is important for continued IL-13 production by T_H2 cells. **B**, Eosinophil-derived mediators are toxic to epithelial cells and antagonize M2 muscarinic receptors in the airways. **C**, Eosinophils induce myofibroblast differentiation through the production of TGF- β and contribute to airway remodeling.

appears to be specific for RNases of the eosinophil-derived neurotoxin family. Thus, eosinophils could contribute to the persistence of allergic inflammation by continued recruitment of dendritic cells into the airways.

There is also a growing body of evidence to suggest that eosinophils have modulatory effects on an ongoing immune response. Eosinophils produce a wide variety of cytokines,^{24,25} including T_H2 -type cytokines (IL-4, IL-5, IL-6, IL-13), T_H1 -type cytokines (IFN- γ , IL-12), nonpolarizing cytokines (IL-1, IL-2, IL-3, IL-16, TNF- α , GM-CSF), immunoregulatory cytokines (IL-10, IL-18), and chemokines (IL-8, RANTES, eotaxin, MIP-1 α). However, because most cells produce cytokines, this is not a unique function. Whether eosinophil-derived cytokines are important for specific pathophysiologic conditions needs further investigation.

There might also be some unique circumstances in which eosinophil-derived cytokines would be essential for sustaining a T_H2 -type response. Indeed, it has been shown that IL-13 production, which is needed for sustained allergic inflammation, requires the presence of eosinophils in the milieu.³⁰ T_H2 cells from mice deficient in IL-5 and eotaxin are unable to produce IL-13. Eosinophils are not the source of IL-13, but they can produce IL-18, which is needed for persistent production of IL-13 by T_H2 cells. This exciting observation needs to be corroborated in human diseases to understand its relevance fully.

In summary, new developments in eosinophil research point to an expanded and expanding role of this cell type in asthma and likely other diseases (Fig 2). The anti-IL-

5 trials suggest that the eosinophil biology is far more complex than that driven by IL-5 alone and has given us a tool to further determine the pathobiology of this cell in humans. To assess the role of eosinophils in asthma fully, we will need a comprehensive anti-eosinophil strategy targeting not only eosinophil differentiation but also eosinophil recruitment to the airways. It is also important to consider the possibility that in different sites (airway vs circulation) and organs (lung vs esophagus vs heart) the eosinophil functions are regulated in distinct ways. Therefore, although an "eosinophil" might always be an "eosinophil," its functions, interactions with target tissues, and, most importantly, its response to treatment will be influenced or determined by its genotype and environment. Although evidence has suggested a lessened role for eosinophils in parasitic and allergic diseases, its pathobiology and overwhelming presence in the above-discussed diseases strongly suggest that it is more than just a "usual suspect." Given new observations and understanding, it is now necessary to gather more evidence to establish the eosinophil's apparent guilt and hence involvement and contribution to the "crimes" found in its wake. The information in this issue is a first step in completing this detective work.

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