Eosinophils: Offenders or General Bystanders in Allergic Airway Disease and Pulmonary Immunity?

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Key Words

Eosinophils • Asthma • Allergy • Pulmonary immunity

Abstract

Eosinophils have long been noted to be present in asthma and other forms of pulmonary inflammation, but whether they act as true offenders or merely as bystanders has been a point of uncertainty. However, in recent years, there has been increasing evidence suggesting that eosinophils are not passive cells in the respiratory system, acting only as markers of allergic inflammation. This review discusses key evidence from animal models and human clinical trials that support the importance of eosinophils as active and necessary, rather than passive and unnecessary, to the pathogenesis of allergic airway disease. Analyses that are supportive of important immunoregulatory roles of eosinophils in allergic pulmonary inflammation are also reviewed. Data indicating that eosinophils contribute to viral, bacterial, and mycobacterial defense and clearance are detailed. Continually increasing evidence has supported a new conception of eosinophils as being multifaceted immune cells with complex interactions with other immune cells and their local environment.

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Introduction

Despite decades of study since Ehrlich first identified eosinophils in the 1870s, the role of eosinophils in health and disease remains enigmatic [1]. Similarly, mechanisms of eosinophilic inflammation and the purpose of eosinophilic infiltration into tissues continue to be a relatively poorly understood aspect of immunology. The role of eosinophils in the respiratory system is emblematic of the controversies surrounding this cell. Eosinophils have long been noted to be present in asthma and other forms of pulmonary inflammation, but whether they act as true offenders or merely as bystanders has been a point of uncertainty. However, in recent years, there has been increasing evidence suggesting that eosinophils are not passive cells in the respiratory system, acting only as markers of allergic inflammation. In fact, regarding eosinophils as only end-stage effectors is insufficient as a model [2]. Studies of eosinophils in animal models of allergic airway inflammation as well as in clinical studies of human asthma have converged to the point where eosinophils must be considered to be active cytotoxic and immunomodulatory entities. This review will discuss key evidence from animal models and human clinical trials

that support the importance of eosinophils as active and necessary, rather than passive and unnecessary, to the pathogenesis of allergic airway disease. Analyses that are supportive of important immunoregulatory roles of eosinophils in allergic pulmonary inflammation will also be reviewed. Though eosinophils have not been traditionally thought to play a role in pulmonary immunity against respiratory pathogens, their status in pulmonary immunity is evolving to encompass a potentially new role as cells important to antimicrobial defense against a variety of pathogens. Data indicating that eosinophils contribute to viral, bacterial, and mycobacterial defense and clearance will be detailed.

Eosinophils in Allergic Airway Inflammation: Animal Models

While the true effector function of eosinophils in allergic airway inflammation has been an area of ongoing controversy, a series of murine studies has established that eosinophils are more than bystander cells in asthma models. Two reports appeared in 2004, describing the phenotypes of 2 different strains of eosinophil-deficient mice in ovalbumin challenge asthma models [3, 4]. Humbles et al. [3] reported that eosinophil-deficient Δ dbl GATA mice had decreased airway remodeling as assayed by collagen deposition compared to wild-type animals. These mice are selectively deficient in eosinophils due to the deletion of a high-affinity GATA binding site in the GATA-1 promoter. While mice that are completely deficient in GATA-1 demonstrate embryonic lethality with erythrocyte and megakaryocyte deficiencies, those with the deletion of the dbl GATA high-affinity site are viable mice with no hematopoietic deficiencies other than the loss of eosinophils [5, 6]. In the study of Δ dbl GATA mice by Humbles and colleagues [3], airway hyperresponsiveness and other measures of obstructive physiology were not significantly different between Δ dbl GATA mice and wild-type controls [3]. Lee et al. [4] described that a second murine strain lacking eosinophils known as *PHIL* mice also had attenuated responses to allergic airway challenge. *PHIL* mice are selectively deficient in eosinophils due to the expression of diphtheria toxin A in eosinophil lineage cells under the control of the promoter for eosinophil peroxidase. Unlike Δ dbl GATA mice, *PHIL* mice not only showed decreases in airway remodeling, but less mucous production and decreased airway hyperresponsiveness as well. Though the observations from Δ dbl GATA and *PHIL* mice were not identical, this pair

of studies powerfully suggested that eosinophils are indeed active offenders in animal models of allergic airway inflammation. Further studies have been performed in Δ dbl GATA mice suggesting that differences in the background strains of the experimental animals may be partially responsible for the differences in phenotype. Walsh and colleagues [7] studied the phenotype of Δ dbl GATA mice on a C57BL/6 background in an allergic airway disease model rather than the Δ dbl GATA BALB/c background reported in the earlier study. They found that Δ dbl GATA C57BL/6 mice showed both decreased airway hyperresponsiveness and decreased Th2 cytokine production in response to sensitization and challenge with ovalbumin. Significantly, adoptive transfer of eosinophils restored airway hyperresponsiveness and Th2 inflammation in these animals. Eotaxin-1 also appeared necessary to induce T-cell recruitment to the airways and airway hyperresponsiveness, as intranasal administration of eotaxin-1 restored these responses in Δ dbl GATA C57BL/6 mice independent of the presence of eosinophils. The modulated phenotype in asthma models of Δ dbl GATA C57BL/6 mice and *PHIL* mice is depicted in figure 1.

Several subsequent animal studies have supported the general conclusion that eosinophils are a necessary active component of allergic airway disease. Fulkerson and colleagues [8] examined the effect of blocking eosinophil recruitment to the lung using knockout mice deficient in the eotaxin receptor CCR3, as well as knockout mice deficient in both eotaxin-1 and eotaxin-2. They compared the response to repeated challenge with intranasal *Aspergillus fumigatus* extract to that obtained in Δ dbl GATA mice. Whether eosinophil recruitment was impaired by lack of eotaxin or by lack of eotaxin receptor, these mice had a reduction in mucous production. In addition, CCR3-deficient mice also had a decrease in the Th2 cytokines interleukin (IL)-4 and IL-13 present in homogenized lung specimens. Similarly, as with the ovalbumin challenge, Δ dbl GATA mice also showed decreased mucous as well as decreased IL-4 production. In another study using Δ dbl GATA mice that had inducible overexpression of IL-13, these investigators found that the leukocyte recruitment to the lung, the mucous cell metaplasia, and the airway remodeling attributable to IL-13 overexpression was partially dependent on eosinophils [9].

Jacobsen et al. [10] further characterized the responses of *PHIL* mice to ovalbumin sensitization and challenge in a series of experiments showing that eosinophils are not only effector cells but that they have an active immunoregulatory role in allergic airway inflammation. They

Fig. 1. Attenuated allergic inflammation in eosinophil-deficient mice. In the ovalbumin airway antigen challenge model, eosinophil-deficient Δ dbl GATA mice and *PHIL* mice experienced significant downregulation of airway hyperresponsiveness (AHR) and remodeling as compared to wild-type mice.

found that ovalbumin-primed and -challenged *PHIL* mice have decreased Th2 cytokine production in the lungs as well as decreased accumulation of lymphocytes to the airways, to the pulmonary parenchyma, and to draining lymph nodes. In a series of elegant adoptive transfer experiments using a combination of intravenous administration of OT-II T cells, which express T-cell receptor specific to ovalbumin, and intratracheal administration of eosinophils, they demonstrated that OT-II cells alone were insufficient to restore lymphocyte accumulation in the respiratory system of ovalbumin-challenged *PHIL* mice. Rather, the administration of intratracheal eosinophils was an absolute requirement to promote the accumulation of lymphocyte subsets and the production of the Th2 cytokines IL-5 and IL-13 in the lungs.

IL-33 has recently been identified as a potential key contributor to the multifaceted effects of eosinophils in allergic airway inflammation [11]. In mice deficient in a component of the IL-33 receptor, adoptive transfer of wild-type eosinophils to mice who were subsequently stimulated with IL-33 induced airway inflammation [11]. While the importance of IL-33 in allergic airway disease remains an ongoing field of investigation, this study provides additional key evidence showing the importance of eosinophils as an active inflammatory cell as opposed to a bystander cell.

Kanda and colleagues [12] reported that intratracheal adoptive transfer of activated eosinophils to wild-type recipient mice induced inflammatory cell accumulation to the airways and induced airway hyperresponsiveness.

When eosinophils were transferred to mice with severe combined immunodeficiency, inflammatory cell recruitment and airway hyperresponsiveness were still observed, indicating that eosinophils exerted an effect independent of lymphocytes. Surprisingly, they also found that the effect of eosinophils was dependent on interferon (IFN)- γ from the donor eosinophils, as eosinophils that were deficient in IFN- γ did not induce airway hyperresponsiveness.

Through the use of animal models deficient in eosinophils and by taking advantage of the adoptive transfer of eosinophils, evidence has now emerged that convincingly argues that eosinophils are a necessary component of allergic inflammation.

Eosinophils in Human Allergic Airway Disease

Eosinophil counts in the blood and in the lungs have long been correlated with disease severity in human asthma, but whether they are truly active in disease pathogenesis has been an ongoing point of controversy [13]. Cationic granule proteins released from eosinophils cause bronchoreactivity and airway remodeling in experimental models [14–16], and as emphasized throughout this review, a wealth of experimental evidence suggests that eosinophils have a complex immunoregulatory function in allergic disease. From a clinical perspective, a series of studies of eosinophil depletion in human subjects with monoclonal antibody against IL-5 has added depth to the

argument that eosinophils have an active, non-bystander role in pulmonary disease. IL-5 has a central role in eosinophil differentiation, bone marrow egress, and survival [17]. The first randomized, double-blinded, placebo-controlled study of eosinophil depletion with anti-IL-5 therapy in asthma actually supported the hypothesis that eosinophils act only as non-active markers of inflammation in asthma [18]. The patients studied in this trial had a clinical diagnosis of asthma with persistent symptoms despite the use of inhaled corticosteroids. While blood and sputum eosinophils were reduced after treatment with anti-IL-5 therapy, this study failed to find any statistically significant improvement in clinical outcomes with a course of therapy with a monoclonal antibody against IL-5. However, because human asthma is a heterogeneous disease that represents a final common phenotype to several pathways of airway inflammation, 2 subsequent studies sought to study the effect of eosinophil depletion with anti-IL-5 therapy in subjects with asthma characterized by eosinophilic inflammation [19, 20]. In both studies, subjects with refractory asthma were selected based on the finding of eosinophils in their sputum. Unlike the earlier trial in unselected patients, both of these trials did indeed show an improvement in clinical variables with treatment with anti-IL-5 monoclonal antibody. From a mechanistic perspective, these studies strongly suggest that, at least in selected forms of asthma, eosinophils have an active role in disease pathogenesis and not just a bystander role as previously suggested. Eosinophil depletion with anti-IL-5 therapy has also proven effective in clinical trials studying patients with Churg-Strauss syndrome and hypereosinophilic syndrome, both of which can feature prominent components of pulmonary eosinophilic infiltration [21, 22]. Because the target of anti-IL-5 therapy is specific to eosinophils, these clinical studies have provided important insight into the contribution of eosinophils to human disease and support the mechanistic hypothesis that eosinophils are true offenders in allergic airway disease.

Immunoregulation by Eosinophils in Allergic Airway Disease

As previously written, eosinophils have been increasingly recognized as having immunoregulatory function, with multiple lines of evidence from models of allergic airway inflammation [23]. Human eosinophils have been revealed as having preformed stores of multiple Th1 and Th2 cytokines that are available for rapid release [24]. The

presence of these pools of cytokines provides a potential mechanism to explain the multifaceted immunoregulation that eosinophils have demonstrated in a growing number of published studies. Of particular interest are novel findings that IL-4 in eosinophil granules is bound to IL-4 receptor α chain and that this complex is mobilized into secretory vesicles upon eotaxin stimulation of eosinophils [25]. Chen et al. [26] showed in an ovalbumin murine allergic inflammation model that eosinophils are the dominant source of IL-4, providing strong evidence for the immunoregulatory importance of eosinophils in Th2 allergic airway inflammation. Eosinophils have been demonstrated to produce IL-4 and IL-5 in a ragweed antigen challenge model as well, providing yet further support for eosinophils as orchestrators of Th2 polarization [27]. While the above data are of particular interest in addressing the issue of the active role of eosinophils specifically in pulmonary allergic inflammation, a large quantity of data has accumulated that demonstrate protean immunoregulation of Th2 immunity in general by eosinophils [28].

There is also increasing evidence that eosinophils have important roles as 'professional' major histocompatibility complex (MHC) class-II-restricted antigen-presenting cells (APCs) [23, 29]. Much of this evidence comes from the study of eosinophils in models of allergic pulmonary inflammation and from observations made in human disease. The increasing recognition of APC capability of eosinophils in pulmonary inflammation suggests that eosinophils are active immune cells in the respiratory system rather than bystanders. While unstimulated peripheral blood eosinophils do not express MHC class II, airway eosinophils from several pulmonary disease states have been noted to have the requisite MHC class II expression necessary for antigen presentation [30–32]. Sputum eosinophils in asthma and bronchoalveolar lavage eosinophils in chronic eosinophilic pneumonia have both been observed to have upregulation of MHC class II [30, 31]. In addition, eosinophils recovered from bronchoalveolar lavage from allergic subjects subjected to bronchoscopic segmental allergen challenge express increased MHC class II [32]. In murine models of allergic airway inflammation, airway eosinophils not only express increased MHC class II but also increased levels of the costimulatory proteins CD80 and CD86 [33–35]. Eosinophils have now been demonstrated in murine studies to functionally present antigen in models of allergic airway inflammation [34, 36]. Our laboratory has found that eosinophils can be considered 'professional' APCs, with the ability to stimulate naïve T cells [36]. In this set of ex-

periments, ovalbumin-loaded eosinophils intratracheally administered to naïve mice who received injections of ovalbumin-specific CD4⁺ T cells induced CD69 upregulation, proliferation, and IL-4 production in the antigen-specific T cells [36]. While the question of the relative importance of eosinophil APC function in human asthma remains open, it has now been unequivocally established in murine models and in vitro studies using human cells that eosinophils have the capability to perform APC functions [29].

Eosinophils in Pulmonary Immunity

Recent evidence indicates that eosinophils may have a protective role in the defense against certain respiratory infections rather than only a pathogenic role mediated by release of preformed granule contents. Human eosinophils express several Toll-like receptors (TLRs), an observation suggesting that eosinophils may have a more important role in the host defense against a variety of organisms than previously believed [37, 38]. Stimulation of eosinophils with TLR7 ligands causes them to assume an activated phenotype, with increased expression of cell surface adhesion molecules, prolonged survival, and increased production of superoxide [37]. Stimulation with ligands of TLR2 and TLR5 has also been observed to activate human eosinophils [38]. The array of receptors expressed by eosinophils for pathogen-associated molecular patterns, including the TLR family, has recently been reviewed elsewhere [39].

As noted in previous reviews [40, 41], though the influx of eosinophils seen in respiratory syncytial virus (RSV) infection has long been thought to be exclusively pathogenic, eosinophilia appears to have a potentially protective role in models of RSV infection. Human eosinophils have been shown to inhibit the growth of RSV in culture, with inhibition of eosinophil ribonuclease reversing the antiviral properties of eosinophils [42]. In addition, the same study demonstrated that recombinant human eosinophil-derived neurotoxin, the ribonuclease found in eosinophil granules, also inhibited viral replication [42]. Others have demonstrated that eosinophils can inhibit the spread of RSV to uninfected cells in cultures of epithelial cells [43]. Phipps et al. [44] observed that eosinophils promote clearance of RSV in murine infection through a TLR7-dependent mechanism. IL-5 transgenic mice, which have high numbers of circulating eosinophils, were found to have enhanced clearance of RSV. They observed that signaling occurred through MyD88,

as transfer of MyD88-deficient eosinophils to infected mice did not promote viral clearance, as opposed to transfer of wild-type eosinophils, which did promote viral clearance. Eosinophils have additionally been found to act as APCs of viral antigens to T cells [45]. Human eosinophils are able to stimulate proliferation and IFN- γ production of T cells specific to an RSV antigen [45].

While most of the evidence supporting a role for eosinophils in anti-viral immunity is from studies of RSV, additional evidence suggesting a role for eosinophils in viral immunity comes from a study by Adamko et al. [46] of guinea pigs infected with parainfluenza. They found that animals that were sensitized with ovalbumin prior to infection with parainfluenza had a reduction in viral content in the lungs that was reversed when eosinophils were depleted with anti-IL-5 antibody [46].

Eosinophils may have a role in pulmonary immunity against bacterial infections as well, given recent in vitro findings showing the ability of eosinophils to participate in bacterial killing. Eosinophils have been observed to secrete granule contents in response to in vitro exposure to several species of Gram-positive and Gram-negative bacteria and exhibit chemotaxis to several bacterial species as well [47]. One recent study found that eosinophils release mitochondrial DNA and granule content in a catapult-like fashion in response to stimulation with lipopolysaccharide [48]. Furthermore, in co-culture with *Escherichia coli*, bacterial killing occurred in a DNA-dependent manner. In another study, mouse eosinophils were found to kill *Pseudomonas aeruginosa* in vitro [49]. While both of these studies included in vivo experiments demonstrating bacterial clearance, neither used respiratory infection as an experimental model. However, one may speculate that eosinophils may play a role in clearance of certain respiratory infections, given that Gram-negative bacteria, though not the most common perpetrators, are implicated in pulmonary infectious processes.

Eosinophils have also been studied in vitro with respect to their ability to kill mycobacterial organisms, another type of bacteria with a propensity for pulmonary infection [50]. Driss et al. [50] reported that human eosinophils exhibit chemotaxis toward *Mycobacterium bovis* and have increased reactive oxygen species production, eosinophil cationic protein release, and tumor necrosis factor- α secretion when incubated with mycobacteria. Furthermore, they demonstrated that eosinophils respond to mycobacteria in a TLR2-dependent manner and kill mycobacteria through the action of α -defensins. Interestingly, a prior report describes that

TLR2-deficient mice have a reduction in the characteristic eosinophil accumulation seen in pleural space infection with *M. bovis* [51]. It has also been observed that human eosinophils upregulate expression of $\gamma\delta$ T-cell receptor when exposed to mycobacterial antigens and that they have $\gamma\delta$ T-cell receptor-dependent reactive oxygen species generation and release of granule contents and cytokines in response to mycobacteria [52].

Though the story remains incomplete, the above data provide a strong argument that eosinophils have important function in pulmonary immunity against specific viral and bacterial pathogens.

Conclusion

Continually increasing evidence has supported a new conception of eosinophils as multifaceted immune cells with complex interactions with other immune cells and their local environment. The role of eosinophils in allergic airway disease as being fundamentally active in nature has been brought into sharp relief by animal models

of genetic eosinophil depletion, human studies of eosinophil depletion with monoclonal antibody against IL-5, and a series of investigations that establish eosinophils as immunoregulatory cells with key roles in orchestrating Th2 airway inflammation and in functioning as APCs. In pulmonary immunity, several lines of evidence suggest that eosinophils have the ability to promote clearance of certain viral, bacterial, and mycobacterial organisms. In short, the question posed by the title of this review can now be definitively answered, albeit with an important modification. Eosinophils are neither offenders nor bystanders in allergic airway disease and pulmonary immunity. They are active, integral immune cells that have myriad effects on the respiratory system in allergic inflammation and infection.

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