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What targeting the eosinophil has taught us about their role in diseases

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Abstract

Eosinophil-associated disease is a term used to encompass a range of disorders from hypereosinophilic syndrome to asthma. Despite the longstanding belief that eosinophils can be primary contributors to disease pathophysiology, it is only in recent years that direct and selective reduction or elimination of eosinophils can be achieved in animals or in humans. These developments have been made possible in mice through clever targeting of eosinophil production. Use of antibodies and other agents that target soluble eosinophil-related molecules such as interleukin-5 (IL-5) or cell surface structures such as CCR3 has also proved useful in reducing blood and tissue eosinophils. In humans, the only eosinophil-selective agents tested in clinical trials so far are neutralizing antibodies to IL-5, with promising but mixed results. At the very least, such forms of pharmacologic hypothesis testing of the role of eosinophils in certain airway, gastrointestinal and hematologic diseases has finally provided us with new insights into disease pathogenesis. At its optimistic best, these and other targeted agents may someday become available for those afflicted with eosinophil-associated disorders. This review summarizes what has been learned in vivo in both preclinical and clinical studies of eosinophil-directed therapies, with an emphasis on recent advances.

Keywords

eosinophil; granules; asthma; hypereosinophilic syndrome; interleukin-5; chemokines; airways hyperactivity; Churg Strauss syndrome

Introduction

The role of the eosinophil in disease has long been a mystery. Paul Ehrlich, who discovered the eosinophil about 130 years ago and named it, believed that the prominent granules were storage sites, but why and for what purpose was unknown.¹ Nearly 100 years ago Schlecht and Schwenker observed that eosinophils infiltrated tissues of guinea pigs recovering from anaphylaxis, and they speculated that the eosinophil was recruited in response to damage.²

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Samter et al. in the 1950's transferred pieces of lung from guinea pigs having undergone severe anaphylaxis into the peritoneum of naïve guinea pigs. Such animals rapidly developed peritoneal eosinophilia, hinting at release from the transplanted lung of an eosinophil recruitment factor³ that about 40 years later was discovered to include eotaxin.⁴ The concept that the eosinophil functioned in tissue repair was supported by results showing that eosinophils and their products neutralized mast cell derived mediators of anaphylaxis.⁵ However, a direct test of this hypothesis failed to show any difference between the severity of hypersensitivity reactions in the presence and absence of the eosinophil.⁶ Analyses of eosinophil granule proteins revealed that they are cationic toxins able to mediate damage to tissues, and subsequent studies supported that view.^{7–11} Neutralization of MBP1 protected guinea pigs from development of airways hyperreactivity.¹² However, this and other such supportive evidence is circumstantial, and, in the eyes of many critics, unconvincing. In the case of human diseases, the problem was an inability to specifically ablate the eosinophil and thus to determine its role in disease. While glucocorticoids abolish eosinophils from blood and tissues, they possess so many pharmacological effects that one cannot ascribe any specific or unique anti-eosinophil role to them.¹³ The discovery of interleukin-5 (IL-5) as a specific eosinophil growth factor, the demonstration that the stimulatory effect of IL-5 on mouse B cells does not occur on human B cells^{14, 15} and the selective ability of eotaxins to promote eosinophil recruitment stimulated the development of humanized monoclonal anti-IL-5 drugs and antagonists of the eotaxin receptor CCR3.^{16, 17} While the latter molecules are still in development, initial clinical trials of anti-IL-5 in asthma failed to show beneficial effects^{18–22}, and reports on these results discouraged belief that the eosinophil played a significant role in this condition.²³ Nonetheless, additional studies in patients with marked eosinophilia revitalized the possible role of the eosinophil in disease, and within the past two years several seminal studies have shown that administration of anti-IL-5 to patients with the hypereosinophilic syndrome and asthma benefits them.^{24–26} Meanwhile, studies of murine eosinophils have progressed with production of transgenic animals overexpressing IL-5 and others lacking IL-5, CCR3, Siglec-F and other eosinophil-associated molecules.^{27–36} These mice have been used to explore the contribution of eosinophils and their mediators to a wide variety of immune and inflammatory responses. The purpose of this review is to discuss recent advances in animals and humans and thereby attempt to define the role of the eosinophil in various diseases as we know it at this time.

Molecules critical to eosinophil hematopoiesis, trafficking to and accumulation within tissues, and survival (see Table 1)

In the bone marrow, eosinophils differentiate from stem cells in response to a specific panel of cytokine growth factors. While the most specific is IL-5, it was initially discovered in the mouse as both an eosinophilopoietic cytokine and activator of B cell differentiation and proliferation. It is now known that IL-5 in humans does not act on B cells.³⁷ Other related cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3, share eosinophil growth factor activity but act more broadly. Indeed, a mouse strain deficient in the common CD131 beta chain shared by IL-3/IL-5/GM-CSF receptors was profoundly impaired in its lung eosinophilia in asthma models.³⁸ An advantage for those studying eosinophil biology is the ability to grow these cells *in vitro* from either human or mouse bone marrow using protocols in which IL-5 is the most critical cytokine for eosinophil maturation and terminal differentiation.^{39, 40} While these effects are mediated through the IL-5 heterodimeric receptor (CD125/CD131) expressed by eosinophils, basophils also express the IL-5 receptor⁴¹ and therefore IL-5 receptor-based therapies (e.g., MEDI-563, a humanized monoclonal antibody purportedly possessing antibody-dependent cellular cytotoxicity capabilities, see below), have the potential to affect basophil biology too.⁴² The critical role of IL-5 in eosinophil differentiation is underscored in the mouse in models involving its transgenic over-expression, where animals develop profound eosinophilia and splenomegaly.^{27, 28}

Similarly, mice genetically deficient in IL-5 develop little to no blood or tissue eosinophilia, yet they maintain low basal levels of eosinophils in the bone marrow.^{29, 30} In asthma or parasite infection models, these IL-5 deficient mice tend not to develop lung injury, remodeling or airways hyperreactivity, implicating IL-5 and eosinophils in these processes.^{29, 30}

Eosinophil differentiation occurs as a result of the collective effects of various transcription factors, such as GATA-1, FOG-1 (friend of GATA-1), C/EBP α (CCAAT enhancer-binding protein α) and the ets (E-twenty six) family transcription factor PU.1.⁴³ The role of GATA-1 is primarily in facilitating the differentiation of granulocyte-macrophage progenitors into eosinophils, whereas FOG-1 must be downregulated for eosinophil development to occur.^{40, 44} Indeed, GATA-1 deficient mice do not develop eosinophils and deletion of a specific GATA binding site of the mouse GATA-1 promoter (so-called Δ dblGATA mice) results in strains of mice in which terminal differentiation of eosinophils is prevented.^{45, 46} Similarly, mice deficient in C/EBP α are devoid of all granulocytes⁴⁷ and mice congenitally deficient in PU.1 are unable to generate terminally differentiated eosinophils.⁴³ Not surprisingly, many of these transcription factors are required for generation of eosinophil lineage-specific granule proteins, such as major basic protein (MBP1).⁴⁸ The highly eosinophil-specific expression of eosinophil peroxidase (EPO) has been exploited by developing a strain of eosinophil-deficient mice (so-called PHIL mice) in which expression of a toxin is molecularly linked to EPO expression, so as eosinophils undergo bone marrow differentiation, they die before ever leaving the bone marrow.⁴⁹ These eosinophil-less mice have subsequently been employed in various models of disease, including asthma, often with striking findings as discussed below.

Pathways regulating mature eosinophil departure from the bone marrow are not well understood, but it appears that certain surface markers associated with migration responses and terminal differentiation, such as CCR3, are involved.^{50–52} Exit from the bone marrow also appears to be influenced by IL-5.^{53, 54} While eosinophil-selective expression of Siglec-F plays an important role in their accumulation and survival, mice deficient in Siglec-F have normal levels of bone marrow and circulating eosinophils at baseline.³⁶ Exit from the circulation into tissue sites by eosinophils is mediated by the interaction of a variety of cell adhesion molecules expressed on the eosinophil and on endothelium and is further influenced by eosinophil-selective chemoattractants. Mouse studies indicate that among various adhesion molecules the following interactions are the most critical and selective: 1) between α 4 integrins (CD49d paired as a heterodimer with CD29 or β 7 integrin chains) with either VCAM-1 (CD106) or MAdCAM-1, 2) between LFA-1 (CD11a/CD18) and ICAM-1 (CD54), and 3) between P-selectin (CD62P) and P-selectin ligand (CD162). In comparison, interactions between E-selectin (CD62E) and its ligand, L-selectin (CD62L) and its ligand, or CD33 (platelet-endothelial cell adhesion molecule-1) contribute less if at all to processes of eosinophil recruitment.^{55–57} Among the chemoattractants most prominently involved in selective eosinophil recruitment, those active on eosinophil CCR3, such as eotaxin-1 (CCL11) eotaxin-2 (CCL24), eotaxin-3 ((CCL26) in humans but non-existent in mice), RANTES (CCL5) and MCP-4 (CCL13), are likely to be most important and selective.¹⁷ This is based on the fact that mice deficient in each of these molecules show impaired trafficking to the skin, airway and/or gut.⁵⁸ Separate studies showed that blockage of cytosolic phospholipase A2 effectively prevents eosinophil homing to the lungs⁵⁹ while another study implicated non-lymphoid myeloid cells in the lung that help to facilitate eosinophil recruitment via STAT6-inducible chemokines.⁶⁰ For intestinal homing of eosinophils, β 7 integrins are particularly important.⁶¹ A recent observation that mice deficient in the common γ chain that associates with receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 have a unique and selective alteration in gastrointestinal but not lung eosinophils suggests that cytokines active via this receptor pathway play a specific role in eosinophil homing and survival in the gastrointestinal tract.⁶² Other studies have revealed that CCR3 is essential for maintaining basal levels of eosinophils in the small intestine.^{63, 64} Once in tissues, eosinophil survival is primarily controlled by the presence or absence

of pro-survival factors generated locally, such as IL-5 and GM-CSF.⁴³ There may also be pathways regulating apoptosis as well. For example, mice deficient in Siglec-F show exaggerated blood, bone marrow and tissue eosinophilia following allergen sensitization and airways challenge, strongly suggesting the presence of a pro-apoptotic Siglec-F ligand in the lung.³⁶ Targeting Siglec-F with specific antibodies selectively induces eosinophil apoptosis and depletes eosinophils from the blood, gastrointestinal tract and lung and reduces fibrosis and inflammation in mouse models of asthma and food-associated eosinophilic gastroenteritis.^{65–67} So in summary, our knowledge of the factors that control the birth, travel, activation and lifespan of eosinophils has been greatly expanded, and this sets the stage for manipulating these molecules for therapeutic benefit.

Role of eosinophils in animal models of disease

A number of mouse models have been developed in which eosinophil-active cytokines or chemokines have been selectively over-expressed within a specific tissue, for example, in the airways⁶⁸ or the skin.⁶⁹ Over-expression of IL-13 at such sites results in profound airway remodeling associated with eosinophilia, although eosinophils in these models may not be necessary for airways remodeling. This conclusion differs from conclusions drawn from asthma models using IL-5-deficient mice, the eosinophil-deficient Δ dblGATA or PHIL mouse strains, or by targeting Siglec-F, where eosinophils are strongly associated with airways remodeling.^{30, 49, 67, 70–74} Furthermore, over-expression of IL-5 in the lung⁷⁵ and even more profoundly with co-expression of eotaxin⁷⁶, leads to physiologic and histologic changes resembling that of severe asthma. Indeed, the dual transgenic IL-5 and lung eotaxin animals develop pulmonary pathologies remarkably similar to severe asthma, including epithelial desquamation and mucus hypersecretion leading to airway obstruction, subepithelial fibrosis, airway smooth muscle hyperplasia, and striking methacholine-induced airway hyperresponsiveness, and these changes are accompanied by extensive eosinophil degranulation. Data from other mouse models suggest that eosinophils play a role in local antigen presentation and subepithelial fibrosis, and may even be required for T cell activation.^{77–84} Most of the animal models implicate the eosinophil as a source of profibrotic cytokines such as TGF β .⁷⁴ The conclusions regarding the contribution of eosinophils in tissue remodeling are not unlike the conclusions from human studies using anti-IL-5.^{21, 85}

Although eosinophils have long been associated with anti-helminth responses, experiments have not only implicated eosinophil granules in this response but have begun to suggest that these cells may play a much broader role in immune responses. Mice deficient in eosinophils or certain eosinophil granule proteins have impaired abilities to clear a variety of helminths.^{35, 86, 87} More recent data, however, suggests that eosinophils also play an antibacterial role based on the ability of eosinophils to release substances with potent antibacterial and antiviral activities.^{88–90}

Mouse models of hypereosinophilic syndrome and eosinophilic gastrointestinal disorders have been developed and have suggested a variety of pathobiologic mechanisms controlling these disorders. Models of hypereosinophilic syndrome include the IL-5 transgenic mouse, although in general this mouse is relatively healthy with little evidence of eosinophil degranulation, despite its profound eosinophilia.^{27, 28, 37} Also recently developed are mouse models of the FIPIL1/PDGFR α fusion gene driving hypereosinophilic syndrome, as this has been used to study chronic eosinophilic leukemia and its response to various therapies.^{65, 91} Regarding eosinophilic gastrointestinal disorders, eosinophil-derived EPO was implicated in a mouse model of ulcerative colitis using a strain deficient in EPO.⁹² Oral sensitization and repetitive challenge models have been developed that yield eosinophilic inflammation of the esophagus and small intestine. Such models have highlighted the roles of chemokines, IL-13, IL-5, and β 7 integrins in the development of eosinophilia, as well as a role for Siglec-F in its resolution.

61, 63–66, 93–95 Other molecules implicated in tissue eosinophilia, primarily through studies of deficient mice or with the use of antagonists, include prostaglandin D2 and its receptors^{96–98}, leukotrienes B4 and its receptors^{99–102}, the H4 histamine receptor^{103, 104} and thymic stromal lymphopoietin (TSLP)¹⁰⁵, although for some of these molecules, it is not clear whether their effects on eosinophils are direct or indirect. Unfortunately, while there is a long list of eosinophil-associated molecules to study, and while there are clever ways to utilize eosinophil-deficient or eosinophil-depleted mice in models of disease, none fully recapitulate the human disease and thus may not be predictive of the role played by the eosinophil in its human counterpart.

Studies in Human Disease

Asthma

Prior efforts to establish eosinophils as critical mediator cells in disease using anti-IL-5 failed, and most striking was the failure of mepolizumab, a humanized mouse IgG1 monoclonal anti-IL-5 antibody, to benefit patients with asthma.^{18, 19} Other studies conducted on patients with mild asthma also failed, although analyses of bronchial biopsies showed that mepolizumab only reduced the numbers of eosinophils by about 50% and did not appreciably reduce the degree of eosinophil granule protein deposition (as judged by localization of granule MBP).^{20, 21} These negative results cast a pall over the efforts of investigators concerned with eosinophil investigation, and for a time it appeared that the negative view of the eosinophil in disease, as expressed in the editorial accompanying the Leckie et al. article in the *Lancet*¹⁸, was likely correct. However, continuing studies of anti-IL-5 employing both mepolizumab and reslizumab (the latter differing from mepolizumab by being a humanized rat IgG4 anti-IL-5 monoclonal antibody) showed that reslizumab reduced eosinophils in the blood of patients with hypereosinophilic syndromes (HES)⁵³ and that mepolizumab was able to abolish eosinophils from tissues of patients with eosinophil-associated diseases, including HES and especially in the presence of cutaneous manifestations.^{106, 107} The latter paper demonstrated that mepolizumab strikingly reduced the severity of cutaneous disease in concert with reduction of blood eosinophils, eosinophils in skin biopsies and deposition of granule eosinophil cationic protein (ECP, RNase3). These findings encouraged belief that the negative results obtained with mepolizumab treatment of asthma might not hold for other eosinophil-associated diseases.

An important clinical experiment tested the hypothesis that measurement of sputum eosinophilia might be useful for asthma management.¹⁰⁸ This study compared the results of asthma management using either measurement of sputum eosinophils or standard asthma British Thoracic Society ((BTS) treatment guidelines. Seventy-four patients were recruited with moderate to severe asthma and randomly allocated to a sputum management group or the BTS management group, and the patients were treated for 12 months. If sputum eosinophilia was greater than 3%, treatment was increased (mainly utilization of inhaled or oral glucocorticoids); if less than 1%, treatments were decreased. The most interesting outcomes at the end of the 12 month period were a reduction in severe exacerbations in the sputum management group with 109 exacerbations in the BTS group and 35 exacerbations in the sputum management group ($p=0.01$) and fewer rescue courses of oral glucocorticoids with 73 courses in the BTS group and 24 in the sputum management groups ($0=0.008$). Further, change in methacholine PC₂₀ favored the sputum management group with an overall reduction in methacholine responsiveness at 12 months ($p=0.015$). In their discussion, the authors stressed the following key benefits of mepolizumab: 1) reducing severe exacerbations that require courses of oral glucocorticoids, 2) preventing asthma-related hospital admissions, morbidity and mortality, and 3) the value of using sputum eosinophils as a guide to treatment. Their results also supported a critical role for the eosinophil in the pathogenesis of asthma. In this regard it is interesting that they recruited all patients with asthma needing continued hospital follow-up and not a selected group with elevated sputum eosinophils.

The above results set the stage for two studies of asthma treatment with anti-IL-5.^{24, 25} While using different outcome measurements, both came to the same conclusion, namely that mepolizumab treatment benefits patients with sputum eosinophilia with reduction in prednisone doses or asthma exacerbations and improvement of asthma quality of life. Nonetheless, these studies stirred further controversy concerning the prevalence of patients with asthma and sputum eosinophilia.

The study by Nair et al. was based on the assumption that a rare subgroup of asthma patients demonstrates persistent sputum eosinophilia despite treatment with oral prednisone.²⁵ Twenty adults were recruited from a population of 800 patients with severe asthma, and all but two had more than 3% sputum eosinophilia in spite of daily treatment with prednisone at doses from 5 to 25 mg per day for four weeks (in addition to inhaled fluticasone at 600 to 2000 µg per day). The study period lasted up to 26 weeks, and patients were treated with 750 mg mepolizumab or saline intravenous infusions at weeks 2, 6, 10, 14 and 18 in a randomized double-blind fashion. Oral prednisone was reduced using a pre-established protocol, and exacerbations defined as increased use of albuterol, nocturnal awakenings, a decrease of 15% in FEV₁, or worsening of cough. The most striking outcome was a reduction in exacerbations with 12 in the placebo group and two in the mepolizumab group ($p=0.008$). FEV₁ increased significantly, and both cough and Juniper asthma control questionnaire scores improved in the mepolizumab group but not in the placebo group. Prednisone was reduced by $83.8\pm33.4\%$ in the mepolizumab group and by $47.7\pm40.5\%$ in the placebo group ($p=0.04$). As expected, eosinophils were significantly reduced in both blood and sputum in the mepolizumab treated group, and eosinophil levels remained within normal limits after prednisone reductions. In contrast, reductions in prednisone in the placebo group were accompanied by significant increases in the numbers of eosinophils in sputum and blood. Adverse events in the groups appeared comparable. One limitation of this study was a higher sputum eosinophil count at baseline in the mepolizumab group, raising the possibility that patients in this group who responded were those with the greatest contribution of eosinophils to asthma. Another limitation was a failure to show a significant difference in final prednisone dosage between the groups. A final debatable shortcoming was the apparent conclusion that the favorable outcome achieved in the mepolizumab group only pertained to a rare subset of patients with asthma. Nonetheless, the authors interpreted their study as highlighting the importance of selecting patients with airway eosinophilia.

The study by Haldar et al. focused on asthma exacerbations²⁴ and is an extension of the earlier work by the same group on the use of sputum eosinophils for asthma management (summarized above).¹⁰⁸ Patients studied had sputum eosinophil counts greater than 3% on at least one occasion in the prior two years despite high-dose glucocorticoid treatment and at least two exacerbations in the prior 12 months. All patients were treated with oral prednisolone at 0.5 mg per kilogram (maximum dose 40 mg per day) at the beginning and end of the study. Patients received 12 monthly 750 mg mepolizumab or saline infusions. At baseline patients were well matched, and there were no significant differences between the groups, including eosinophil counts in sputum (in contrast to the Nair et al. report). Over the treatment period (348 days for the mepolizumab group and 340 days for the placebo group) 57 severe exacerbations occurred in the mepolizumab group for a mean of 2.0 per patient and 109 in the placebo group for a mean number of 3.4 ($p=0.02$). Patients in the mepolizumab group had three hospital admissions and placebo patients had 11 ($p=0.07$). The total number of days in the hospital was significantly less in the mepolizumab patients than those receiving placebo (12 days vs. 48 days, $p<0.001$). Sputum eosinophils were significantly reduced in the mepolizumab patients, even during an exacerbation ($p=0.005$), although sputum eosinophils still rose in 36% of mepolizumab treated patients during exacerbations. The mean asthma quality of life questionnaire scores favored the mepolizumab treated patients ($p=0.02$), but FEV₁ and methacholine sensitivity did not change significantly between the groups. Interestingly, computerized tomographic analyses

obtained before and after treatment showed a significant difference in the airway wall area changes ($p=0.02$). Concerning safety, the numbers of adverse events were 29 in the mepolizumab patients and 32 in the placebo patients; one patient was withdrawn from the study because of a transient rash 24 hours after the first mepolizumab infusion. The authors suggest that there is dissociation between the mechanisms of exacerbations and those responsible for asthma symptoms and lung function. However, the rises in sputum eosinophils during exacerbations in the mepolizumab treated patients suggest that eosinophil-mediated pathogenetic stimuli were not completely suppressed.

Editorial comment on the studies by Nair et al. and Haldar et al. emphasized the rarity of eosinophil associated asthma, its occurrence in patients with adult-onset asthma, the relatively minimal benefit of mepolizumab therapy, even in these selected patients, and the possibility that alternative cells, presumably mast cells and neutrophils and their mediators, might play critical presently underappreciated roles in asthma.¹⁰⁹ Overall, the editorial assumed that mepolizumab therapy sufficiently suppressed eosinophil-mediated inflammation so that it represented an adequate test of the role of the eosinophil in asthma. However, an alternative view is that mepolizumab only partially suppresses eosinophil mediated inflammation and therefore the effects of mepolizumab only partially show what might be expected if eosinophilic inflammation were totally abolished.¹¹⁰ It is the firm opinion of the authors of this present review article that the clear benefit of an eosinophil-specific therapy in reducing exacerbations in selected patients with asthma, especially in a population where about one-third also had nasal polyposis, is so striking and exciting that one cannot escape the conclusion that eosinophils are critical to the pathogenesis of disease in this cohort.

The Hypereosinophilic Syndrome

Several relatively small, uncontrolled trials with anti-IL-5 antibodies in eosinophilic disorders involving the skin, esophagus and other organs have been reported.^{106, 107, 111–114} The reader is referred to the accompanying article in this issue of the *Journal* by Simon et al. for additional details on these topics.¹¹⁵ Instead, this section will focus on the one controlled trial published to date.

The effect of mepolizumab on HES was tested in an international, randomized, multi-center, placebo-controlled, double-blind study.²⁶ Patients entering the study required at least 20 mg of prednisone to control HES manifestations and to satisfy the Chusid criteria for HES diagnosis, including blood eosinophilia equal to or greater than 1.5×10^9 , duration of disease greater than six months and absence of other eosinophil-associated diseases such as allergic or parasitic causes. A total of 85 patients were enrolled in the study and were divided into placebo and mepolizumab treatment groups. During a run-in period of up to six weeks, prednisone (or equivalent glucocorticoid) was tapered to the lowest level able to reduce blood eosinophils to $<1 \times 10^9/L$, and patients requiring 20 mg per day or more were randomized. Prednisone reduction to 10 mg per day or lower was the primary endpoint, and the dose was reduced using a pre-established algorithm. Patients in whom blood eosinophilia could not be controlled were rolled over into an open mepolizumab trial. The results of the study showed that mepolizumab was an effective steroid sparing drug and that it reduced both blood eosinophilia and the level of eosinophil participation by also reducing serum levels of the eosinophil-derived neurotoxin (EDN, RNase2). Furthermore, mepolizumab was very well tolerated, and the spectrum of adverse events in the treated patients did not differ significantly from the placebo group. Another study involving several types of patients with eosinophilic disorders (e.g., HES, eosinophilic gastrointestinal disorders) treated with mepolizumab found a consistent and prolonged suppressive effect on circulating eosinophil numbers and markers of eosinophil activity.¹¹⁶ Finally, in a retrospective study, Ogbogu et al. reported on a variety of treatments used for the treatment of HES, including anti-IL-5 antibodies mepolizumab and reslizumab.

¹¹⁷ The majority of those treated with anti-IL-5 showed favorable responses at one month, and discontinuation due to intolerance was rare. Overall these results supported the beneficial effects of anti-IL-5 in disease and a role for the eosinophil in tissue dysfunction. Regrettably, applications for registration of mepolizumab for the treatment of HES have foundered on concerns by regulators that the HES trial was not adequately blinded (the investigators should not have been aware of the eosinophil levels) and that steroid reduction was not a proper endpoint.¹¹⁸ Presently, in spite of its demonstrated usefulness and safety, it is unlikely that mepolizumab will be approved for the treatment of HES. The status of reslizumab approval at the present time is also uncertain.¹¹⁹

Churg Strauss Syndrome (CSS)

Scant information on the role of eosinophils in CSS exists in spite of their strong association with CSS and their presence as a criterion for the diagnosis of CSS. Two studies have probed the effect of mepolizumab in CSS. One is a case report of a 28 year old female with marked peripheral blood eosinophilia, eosinophilic pneumonia, myocarditis, peripheral neuropathy who was treated with glucocorticoids, methotrexate, interferon- α , cyclophosphamide, intravenous immunoglobulins, azathioprine and etoposide.¹²⁰ In spite of these treatments disease activity was not controlled, and a trial of mepolizumab begun. Improvement was noted within a month and by six months pulmonary infiltrates had cleared. By 15 months all evidence of disease activity disappeared. However, reduction of mepolizumab resulted in a recurrence of disease, suggesting that the therapeutic benefit was not due to disease remission. The second study administered mepolizumab to seven CSS patients for four months to assess its safety and to determine whether systemic glucocorticoids could be reduced.¹²¹ The results showed that mepolizumab reduced eosinophil blood counts, was well tolerated and permitted reduction of glucocorticoids in all patients. After cessation of mepolizumab treatment, CSS manifestation recurred, necessitating increased glucocorticoid treatment. These studies encourage belief that anti-IL-5 may be a beneficial treatment of CSS. Clearly, additional studies in larger numbers of patients are needed to determine the place of anti-IL-5 in CSS treatment.

Implications and future directions regarding IL-5-directed therapies

The beneficial effects of mepolizumab on asthma can be regarded as a partial test of the eosinophil's role in disease. For example, sputum eosinophils still rose during exacerbations²⁴, and mepolizumab did not reduce deposition of eosinophil granule MBP1 in patients with mild asthma.²⁰ Therefore, the Nair et al. and Haldar et al. studies may have shown the effects of partial reduction of eosinophil participation and efficacy. One presumption is that more robust eosinophil suppression would show a greater degree of therapeutic benefit. This was achieved in the double transgenic murine asthma model when these asthmatic mice were mated to eosinophil deficient animals, and the resultant triple transgenic animals became normal.⁷⁶ Fortunately, a potential medication that might more strikingly reduce eosinophils is on the horizon. MEDI-563 is a humanized anti-IL-5 receptor alpha (IL-5R α) monoclonal antibody that is presently in clinical trials.⁴² This antibody binds with high affinity $K_D=46nM$ and mediates the lysis of IL-5R α positive cells, including eosinophils and basophils. A single intravenous dose of MEDI-563 was well tolerated by patients with mild asthma and decreased circulating eosinophils below detection limits within 24–48 hours for 8–12 weeks. Because this antibody mediates the lysis of eosinophils, it may be the best reagent to test the role of eosinophils in disease.

Conclusions

Despite the recent advancements in our understanding of the contribution of eosinophils to disease as summarized above, it remains unclear as to whether we could live without these

cells. Teleologically, something has allowed this cell type to persist. Nearly all in the eosinophil field would likely agree that the primary role of these cells is in helminth infestation, yet we still do not know if they are essential to protecting the host from such organisms. It now appears that eosinophils can be selectively targeted with therapeutic agents, and this seems to provide clinical benefit in a subgroup of asthmatics with persistent airways eosinophilia. Most subjects with hypereosinophilic syndrome given anti-IL-5 reduced their daily steroid requirements, but we do not know if this provides safe and effective control of their disease long-term. A variety of other disorders associated with tissue eosinophilia have yet to be treated with selective eosinophil-lowering agents, so we can only continue to speculate on the role this cell plays in each of these conditions. Nevertheless, if such agents ever garner FDA approval, we will be one step closer to understanding the role of this beautiful, yet enigmatic, cell in human health and disease.

What do we know?

- Eosinophils remain an enigmatic cell.
- We have learned a great deal about how to make an eosinophil from early precursor cells.
- Eosinophils have a unique pattern of cell surface receptors, including adhesion molecules and chemokine receptors, which impart it with distinct homing capabilities.
- Survival and death signals, especially those involving cytokines and inhibitory receptors, appear to regulate the persistence of eosinophils at inflammatory sites.
- Mouse strains have been developed that result in mice that are truly and specifically deficient in the eosinophil lineage, and these are yielding valuable insights into the role of the eosinophil in various mouse models of disease.
- Only since the recent development of IL-5-directed therapies have we now been able to dissect the unique, specific role of the eosinophil in asthma, hypereosinophilic syndrome, and related disorders.
- Studies with anti-IL-5 antibody therapies show consistent, profound and prolonged suppression of eosinophil hematopoiesis and blood eosinophilia and these treatments seem well tolerated.
- Studies with anti-IL-5 antibody therapies show improved asthma control in a subset of difficult-to-control asthmatics with persistent airways eosinophilia.
- Studies with anti-IL-5 antibody therapies show steroid-sparing activity in patients with hypereosinophilic syndrome.

What is still not known?

- Are there diseases that are purely eosinophil-mediated?
- Is suppression of eosinophils safe long-term and would it provide sustained improvement in asthma and other eosinophilic diseases beyond our current treatment approaches?
- Does chronic reduction in eosinophils result in any permanent improvements in asthma parameters such as lung function or airway remodeling?
- Will anti-IL-5 directed therapies get FDA approval? If so, for what indication?
- Would it be better, from an efficacy standpoint, to develop eosinophil-directed therapies that also target other cells?

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Abbreviations used

BTS	British Thoracic Society
C/EBP α	CCAAT enhancer-binding protein α
CSS	Churg Strauss syndrome
Δ dblGATA mice	mice deficient in eosinophils due to a deletion of a specific GATA binding site of the mouse GATA-1 promoter
ECP	eosinophil cationic protein, or RNase3
EDN	eosinophil-derived neurotoxin, or RNase2
EPO	eosinophil peroxidase
FOG-1	friend of GATA-1
GM-CSF	granulocyte-macrophage colony-stimulating factor
HES	hypereosinophilic syndromes
IL	interleukin
IL-5R	interleukin-5 receptor
MBP1	major basic protein-1
RNase	ribonuclease
PHIL mice	mice deficient in eosinophils due to expression of a toxin linked to EPO expression

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Table 1

The contribution of various molecules to eosinophil biological responses. Organ-specific conclusions are based primarily on data from animal models

	Bone marrow maturation and/or egress	Skin homing	Lung homing	Gut homing
Intracellular factors				
<i>GATA-1</i>	+	?	?	?
<i>C/EBPα</i>	+	?	?	?
PU.1	+	?	?	?
FOG-1	–	?	?	?
<i>FIP1L1/PDGFR</i>	+	?	?	?
Soluble mediators				
<i>IL-5</i>	+	+	+	+
IL-13	0	+	+	+
TSLP	0	+	+	+
<i>Eotaxins</i>	+	+	+	+
Prostaglandin D2	+	+	+	?
Leukotriene B4	0	?	+	?
Surface molecules				
<i>IL-5 receptor</i>	+	+	+	+
Common γ c chain	0	?	0	+
α 4 integrins/ <i>VCAM-1</i>	+	+	+	+
β 7 integrin/ <i>MAdCAM-1</i>	0	0	0	+
β 2 integrins/ <i>ICAM-1</i>	0	+	+	+
P-selectin	0	+	+	+
L-selectin	0	0	0	0
E-selectin	0	+ (weak)	0	0
CCR3	+	+	+	+
CD33	0	?	0	?
CD44	0	?	+	?
Siglec-F	0	?	+	+
Histamine H4 receptor	0	+	+	?

+, directly involved in a positive way; –, directly involved in a negative way, 0, no impact, ?, not known or not applicable

Those molecules clearly proven to be critical for the biology of human eosinophils, either based on in vitro data or in vivo pharmacology, are shown in *italics*.