Published in final edited form as:

Curr Mol Med. 2008 September ; 8(6): 585–590.

Targeting eosinophils in asthma

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Abstract

Recruitment of eosinophils has long been recognized as a hallmark of the inflammatory response in asthma. However, the functions of this population of cells in host defense remain poorly understood. Eosinophils play an important part in the inflammatory response and have key regulatory roles in the afferent arm of the immune response. More recently, eosinophils have been demonstrated to participate in host defense against respiratory viruses. The specific contributions of eosinophils to the pathophysiology of asthma remains controversial. However, the balance of evidence indicates that they have a significant role in the disease, suggesting that they may be appropriate targets for therapy. Towards this end, a novel intervention of considerable potential interest is the use of an antibody directed against the β -common chain of the receptor for interleukin-3, interleukin-5 and granulocyte-macrophage colony-stimulating factor. However, eliminating eosinophils may not be a risk-free therapeutic strategy, as there is potentially an increased likelihood of respiratory viral infections. This may predispose to the development of acute exacerbations of asthma, an outcome that would have significant clinical implications.

Keywords

asthma; eosinophils; antiviral defense; interleukin-5 receptor; beta-common subunit

Recruitment of eosinophils (Figure 1) has long been recognized as a hallmark of the inflammatory response in asthma [1]. However, to borrow from Winston Churchill, the eosinophil remains “a riddle, wrapped in a mystery, inside an enigma” [radio broadcast, 1939]. The overall role of this population of cells in host defense is still poorly understood. The conventional view, that eosinophils are primarily effector cells which are recruited in response to parasitic infection and that Th2-biased allergic inflammation such as in asthma represents this mechanism of host defense gone awry, is clearly an oversimplification. While in vivo studies have demonstrated that eosinophils can play a protective role in immunity against certain specific helminth infections e.g. *Strongyloides* spp., the mechanisms underlying these species-specific responses remain unclear [2]. Curiously, induction of a

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strong Th2-biased immune response to helminth proteins may actually serve to suppress allergic inflammation [3].

The eosinophil in host defense

Eosinophils contribute to the non-specific acute inflammatory response. Of the cationic granule proteins, which are uniquely secreted by eosinophils, major basic protein (MBP) and eosinophil cationic protein (ECP) are direct-acting, potent inducers of increased microvascular permeability; eosinophil peroxidase (EPO) increases permeability primarily via its enzymatic activity; while eosinophil-derived neurotoxin (EDN) is much less effective [4]. The first three proteins are also able to amplify inflammatory responses indirectly via interaction with mast cells [5]. Furthermore, eosinophils synthesize inflammatory mediators such as leukotrienes, which are important in asthma (reviewed in [6]).

There is now increasing evidence for a crucial role for eosinophils in the afferent arm of the immune/allergic response and these cells clearly have important regulatory functions (reviewed in [6, 7]). Recent investigations have emphasized that eosinophils are part of the innate response to potential allergens [8] and that they may drive allergic inflammation via secretion of pro-inflammatory cytokines such as IL-18 [9] or the Th2-enhancing cytokine IL-25 [10]. Eosinophils also express co-stimulatory molecules that regulate or amplify the effector Th2 response, notably class II major histocompatibility complex proteins, CD80/CD86 and various molecules of the leukocyte immunoglobulin-like receptor family [6, 7]. In addition, these cells secrete a variety of cytokines that could influence the pattern of immune response that is induced, notably including IL-4 and IL-13, as well as IL-10, IL-12 and transforming growth factor- β 1 (TGF- β 1) [7]. Eosinophils may therefore contribute to the cross-talk between cellular networks of host innate defense and the antigen-specific immune response, which has recently acquired the label “contiguous immunity” [11]. Such interactions are suggested to be particularly relevant to complex allergic disorders such as asthma.

More recently, the ribonuclease activity of ECP and EDN has received attention in the context of host defense against viral infections. A protective role for eosinophils in infection by respiratory syncytial virus (RSV) has been demonstrated in a mouse model [12]. The significance of this finding, which provides new evidence that these cells play an important part in the innate response to microbial infection, is discussed later in this review.

Pathogenetic role of eosinophils in asthma

The contribution of eosinophils to the pathogenesis of asthma remains controversial. Experimental studies have attempted to attribute specific independent roles to the cationic granule proteins. Initial reports emphasized the presence of MBP and other granule proteins in airway biopsies from asthmatics (reviewed in [13, 14]) and evidence of toxicity of individual granule proteins for respiratory epithelial cells in vitro (reviewed in [15]). More recently, the notion that epithelial injury by these proteins might be responsible for changes such as airway hyperresponsiveness (AHR) has been questioned, because targeted deletion of the MBP or EPO genes had little impact in an acute model of ovalbumin sensitization and challenge [16, 17]. Whilst species differences and the short-term nature of the experimental model employed may in part account for the lack of effect, the contribution of these mediators to the pathophysiology of asthma is likely to be more subtle than was first thought.

Data from human studies have not helped to resolve the controversy. Clinical trials of short-term therapy using monoclonal antibodies against interleukin-5, the primary eosinophil differentiation and survival factor [18], successfully decreased numbers of eosinophils in

blood and sputum, but failed to reverse allergen-induced airflow obstruction or to suppress AHR to histamine [19–21]. The findings were interpreted as indicating that eosinophils did not have an important role in the development of clinical manifestations of the disease, but this conclusion may have been inappropriate [22] because anti-IL-5 treatment does not adequately deplete tissue eosinophils [23]. Moreover, anti-IL-5 does have beneficial effects on airway remodeling [24] although it is unclear whether this relates directly to the secretory activities of eosinophils or to an indirect effect via other cell populations e.g. epithelial cells or myofibroblasts.

A recent Cochrane review confirms that tailoring asthma interventions to evidence of eosinophilic inflammation (as assessed by sputum analysis) is beneficial in reducing exacerbations [25]. However, the picture has been complicated by the demonstration that in controlled asthmatics in whom the dose of inhaled corticosteroids is progressively reduced, clinical deterioration precedes any evidence of eosinophilic airway inflammation (assessed in sputum, bronchoalveolar lavage or biopsies) [26]. Furthermore, a prominent role for neutrophilic inflammation is now recognized both in exacerbations of asthma and, potentially, in resistance to therapy with inhaled corticosteroids [27–29].

The role of eosinophils in asthma has therefore been assessed in animal models using eosinophil-ablated mice. These have included both short- and long-term challenge models of allergic bronchopulmonary inflammation. In one such study, mice lacking eosinophils were created on a BALB/c background by targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter region. These Δ dblGATA mice were protected against changes of airway remodeling, including peribronchiolar collagen deposition and increased airway smooth muscle, in a chronic challenge model of murine allergic airways disease [30], but there was no apparent impact of eosinophil ablation on AHR or mucus secretion. However, depletion of eosinophils in mice on a C57BL/6 background, by transgenic expression of diphtheria toxin A chain under control of the EPO promoter, yielded contrasting results, as these PHIL mice did not develop AHR in response to pulmonary allergen challenge [31]. The disparate findings have variously been attributed to the strain differences, a potential residual pool of eosinophils, or unexplored effects induced by the Δ dblGATA mutation or the PHIL transgene [6, 32–34].

To investigate these issues further, we employed our long-term allergen challenge model, which reproduces many of the features of mild chronic human asthma, including chronic inflammation and remodeling of the airways as well as airway-specific AHR [35, 36]. We demonstrated that in Δ dblGATA mice, there was significant diminution of the subepithelial fibrosis and epithelial hypertrophy that developed in allergen-challenged wild-type mice (Yang M, Herbert C, Foster PS, Kumar RK; unpublished results). Furthermore, there was a modest reduction in AHR to methacholine, as assessed by forced oscillation methodology. Interestingly, although eosinophil-derived TGF- β 1 has been proposed to be important in the pathogenesis of airway wall remodeling [37, 38], we found that eosinophils were not a significant source of TGF- β 1 in our chronic challenge model. Instead, this mediator appeared to be primarily a product of airway epithelial cells [39]. However, it is worth emphasizing that eosinophils are able to stimulate epithelial cells to produce a variety of factors relevant to remodeling [40].

Adding to the complexity of this picture, we obtained differing results in a related model of an allergen-induced acute exacerbation of chronic asthma [41]. In this model, in which a single-moderate level challenge with inhaled antigen is used to induce an exacerbation of inflammation on a background of changes of chronic asthma, AHR is assessed by transpulmonary resistance and dynamic compliance. Remarkably, in Δ dblGATA mice, development of AHR was independent of eosinophil recruitment. However, AHR in this

model, as in short-term challenge models, may be primarily related to early airway closure, resulting from airway wall thickening by inflammatory edema or changes in surfactant function [42, 43], rather than a specific abnormality in the active response of the airways [44]. This offers an alternative explanation for the discrepancies between the findings with respect to AHR in the different strains of eosinophil-deficient mice using different challenge protocols, but further emphasizes the risks of extrapolating data from assessment of AHR in animal models to human asthma.

Thus although a definitive answer is not available at this point, the balance of evidence suggests that eosinophils have a significant role in the pathophysiology of asthma. In our opinion, therefore, the question is not whether these cells should be targeted, but rather how best to do so.

Novel molecular approaches to targeting eosinophils

Given the redundancy of cytokine signaling in asthma, targeting single mediators or signaling molecules is unlikely to be successful in management [45, 46]. The focus therefore needs to be on approaches that act on multiple relevant cell types or pathways. Here, we will review only those approaches that are directly targeting the eosinophil leukocyte, emphasizing novel experimental strategies not yet in clinical use. There are several recent more general reviews of new anti-asthma therapeutic agents, currently at various stages of clinical development, to which the reader is referred [47–49].

A substantial amount of effort went into the development of humanized monoclonal antibodies directed against IL-5 [22, 50, 51]. In a randomized, double-blind study of mepolizumab, the anti-IL-5 monoclonal antibody from GlaxoSmithKline (UK), peripheral blood eosinophils were dramatically decreased in patients with asthma but clinical manifestations were largely unaffected. Remarkably, despite repeated administration of anti-IL-5 therapy, eosinophils persisted both in the lung tissue and the airways. An independent trial performed with the anti-IL-5 monoclonal antibody SCH55700 (Schering-Plough, USA) likewise resulted in a depletion of peripheral blood eosinophils without improvement in clinical features.

Among various alternative strategies under consideration, several groups have focused on the chemokine eotaxin (CCL11), an important contributor to eosinophil recruitment to the lungs following allergen provocation. Eotaxin is an eosinophil-specific chemokine which interacts with the CC-chemokine receptor-3 (CCR3). Strategies targeting CCR3 have resulted in successful attenuation of eosinophil recruitment to the lung in experimental models of allergic airways disease. Nevertheless, this approach may have some of the same limitations as the aforementioned anti-IL-5 strategy, given that key features of asthma, such as production of allergen-specific IgE and functional activity of Th2 cells, still persist [52–54].

Other therapeutic approaches under consideration involve strategies that result in eosinophil ablation, rather than approaches that focus on inhibiting their recruitment to sites of allergic inflammation. The only recognized mediators capable of inducing eosinophil differentiation from bone marrow progenitors are IL-3, IL-5 and GM-CSF, which regulate eosinophil activation, migration and survival [55–57]. The actions of these cytokines are mediated by receptor heterodimers comprising an unique α receptor subunit (IL-3R α , IL-5R α and GM-CSFR α) and a common β receptor subunit (β c) [58, 59]. One approach that is currently under investigation centers on the α -chain of the IL-5 receptor (IL-5R α , CD125). Blockade of expression of IL-5R α , by intravenous administration of specific antisense oligonucleotides or short hairpin RNA molecules, prevents IL-5-induced eosinophilopoiesis in vitro and suppresses numbers of eosinophils in bone marrow, peripheral blood and tissues

in response to allergic stimuli [60–62]. In a chronic challenge model, IL-5R α null mice exhibit diminished eosinophil recruitment and reduced airway fibrosis [63]. However, the impact of IL-5R α suppression on the development of the pathogenetic features of allergic airways disease has not yet been reported. It has been proposed that clinical administration of specific antibodies targeting IL-5R α may have the benefit of both inhibiting functional interaction of IL-5 with its receptor and actively depleting IL-5R α -expressing cells (eosinophils, possibly mast cells and basophils) through augmented antibody-dependent cellular cytotoxicity (Kozhich A, Reed JL, Erjefalt JS et al.; unpublished results). However, the cellular distribution and pattern of expression of IL-5R α on eosinophils isolated from asthmatics is still the subject of investigation [64, 65].

Several groups have explored strategies that might promote apoptosis of airway eosinophils, as a method for reducing inflammation and perhaps also the clinical manifestations of asthma [66, 67]. Although issues relating to eosinophil apoptosis in vivo remain controversial [68], at least one study has shown a negative correlation between the severity of asthma symptoms and the degree of eosinophil apoptosis found in the airways [69]. Traditional glucocorticoid therapy is believed to exert anti-asthmatic effects in part by inducing eosinophil apoptosis in blood and in tissue [70]. Interestingly, treatment of asthmatics with the anti-IgE monoclonal antibody, omalizumab, resulted in increased eosinophil apoptosis in the peripheral blood, in association with a reduction in the number of IL-13-producing lymphocytes [71]. Similarly, administration of the cysteinyl-leukotriene receptor inhibitor, montelukast, resulted in augmented apoptosis of eosinophils in sputum [72]. Finally, an evaluation of dextrosimendans, an agent used to treat heart failure, revealed augmented eosinophil apoptosis in the airways of ovalbumin-sensitized and challenged mice, which persisted even in the presence of elevated concentrations of IL-5 [73].

An intriguing possibility for future consideration is that of eosinophil-suppressive therapies based on specific microRNA (miRNA) expression signatures. This is an approach which has already shown potential for inhibition of macrophage function [74, 75]. Its application to eosinophils may lead to the development of a novel class of therapeutic agents that will be effective for the full spectrum of eosinophil-associated inflammatory diseases.

Targeting the β c subunit

Although the shared IL-3/IL-5/GM-CSF β c subunit is clearly functionally important, because its deletion leads to impaired eosinophilopoiesis and impaired recruitment of these cells in an inflammatory response [76–78], it has received little attention as a potential target for asthma therapy. We have recently demonstrated, for the first time, that the β c molecule has a pivotal role in regulating all phases of allergic airways disease [79]. Unlike in human cells, an additional β receptor chain specific for IL-3 (β_{IL-3}) is present in mice, which provides a pathway for IL-3 signaling independently of the β c subunit [80]. We therefore employed mice deficient in both β c and β_{IL-3} (designated as β c $^{-/-}$) to demonstrate that allergen-induced expansion of the eosinophil pool and the subsequent accumulation of eosinophils in the lung was abolished when β c signaling was eliminated. Moreover, β c ablation resulted in inhibition of other hallmark features of asthma, including AHR and mucus hypersecretion. Unexpectedly, we also identified a critical role for this receptor in regulating type 2 immunity, manifested as impaired ability of Th2 cells in the lungs of allergen-challenged β c $^{-/-}$ mice to proliferate, to secrete cytokines or to migrate to effector sites [79].

Our findings provide strong support for the concept of developing therapies directed against the β c subunit, which has the benefit of acting at multiple levels for the control of allergic inflammation. Firstly, targeting this common receptor subunit rather than individual ligands

addresses potential signaling redundancy, by blocking all three eosinophilopoietic cytokines. Secondly, eliminating eosinophil differentiation from CD34⁺ IL-5Rα⁺ progenitors as well as targeting the function of the mature cell is likely to be a highly effective strategy for the attenuation of allergic disease, particularly as immature progenitors are now known to traffic to inflamed tissue where they subsequently differentiate into mature effector cells [81, 82]. Thirdly, the pleiotropic role of cytokines signaling via the βc subunit, which drive mast cell, basophil and granulocyte responses, suggests that anti-βc therapies may have an impact on both early and late phases of the allergic response. Consistent with this, we have demonstrated suppression of antigen-specific IgE production in βc^{-/-} mice, which may be explained in terms of attenuation of early IL-4 production by cells of the innate host response, the function of which is regulated by cytokines signaling through βc. Further studies employing mast-cell dependent models of asthma (e.g. [83]) may help to delineate the specific contribution of the βc subunit to the early phase of the allergic response. Finally, the demonstration that eosinophils contribute to the development of lesions of remodeling associated with chronic asthma, and that lesions are diminished by treatment with anti-IL-5 [24, 84], raises the possibility that blocking the βc subunit may have a favourable impact on the development of chronic allergic airway disease. This issue is the subject of ongoing studies in our laboratories.

Eosinophils and host defense: the negative impact of eosinophil ablation?

Eliminating eosinophils may not be a risk-free therapeutic approach. The potential problems associated with targeting inflammatory cells in asthma are rarely if ever considered in the literature, because the focus is usually on their contribution to pathogenesis. Recent evidence suggests that eosinophils interact with respiratory viruses and may be an important component of innate antiviral defense. Indeed recruitment, activation and degranulation of eosinophils is observed in response to respiratory viruses [85, 86] and such infections have long been recognized as playing a major role in exacerbations of asthma [87, 88]. In studies performed in vitro, isolated human eosinophils as well as the granule proteins EPO and EDN reduced the infectivity of viral pathogens for target respiratory epithelial cells (reviewed in [89]). Furthermore, two studies have addressed the question of eosinophil-mediated antiviral activity in vivo. In the first of these, Adamko and colleagues [90] infected ovalbumin-sensitized guinea pigs with Sendai virus and found that the titer of respiratory virus in infected lung tissue correlated inversely with the presence of eosinophils. More recently, using several strains of genetically modified mice, Phipps and colleagues [12] demonstrated that clearance of respiratory syncytial virus in response to primary challenge correlated directly with the presence of pulmonary eosinophils, and that the eosinophil-mediated antiviral response depended in large part on an intact TLR7-MyD88 signaling pathway.

These findings will require additional exploration, as well as confirmation that they can be extrapolated to the human situation. However, they raise the disturbing possibility that targeting eosinophils may in fact predispose to the development of acute exacerbations of asthma. These might then be neutrophil-dominated exacerbations, which would therefore be more likely to be steroid resistant [29]. An intriguing question is whether this situation already exists in patients on long-term high-dose therapy with inhaled steroids, who subsequently develop exacerbations. The clinical implications of this issue indicate that it is clearly worthy of further investigation.

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Figure 1.

(A) Human eosinophils: note the large, intensely stained refractile cytoplasmic granules, which contain cationic secretory mediators (photographed with $\times 100$ objective). (B) Fatal asthma: note the eosinophils beneath the zone of subepithelial collagenization at left, the epithelial detachment, and the numerous eosinophils in the intraluminal mucus plug at right (photographed with $\times 60$ objective). (Image for panel A provided by Dr. Jennifer Moser and Dr. Kimberly Dyer, Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA).