



Eosinophils in the pathogenesis of paediatric severe asthma

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Purpose of review

The importance and functional contribution of eosinophils to the pathogenesis of severe asthma was questioned when adult studies reported neutrophilic inflammation and Th17 cells were central to disease.

Recent findings

Eosinophilic inflammation, atopic sensitization and airway remodelling are the hallmark features of severe asthma in children. Contrary to adult reports, neutrophils, Th17 and Th2 cytokines are rarely detected in children with severe disease who are prescribed high-dose steroids. Type 2 innate lymphoid cells (ILCs), which are induced by the epithelial cytokine interleukin (IL)-33, and also release the Th2 cytokines IL-5 and IL-13, have been shown to be important in mediating asthma, and specifically, ILCs have a role in mediating eosinophil homeostasis. A direct link between IL-33, airway remodelling and steroid resistance in paediatric severe asthma suggests the steroid-resistant eosinophilia may be mediated by ILCs rather than classical Th2 lymphocytes.

Summary

Data from children with severe asthma have shown that findings from adults cannot be translated to children, and that steroid-resistant eosinophilic inflammation appears central to the pathogenesis of paediatric disease. Age-appropriate experimental models and use of airway samples from children are critical to understanding the underlying mechanisms and identifying novel therapeutic targets.

Keywords

eosinophils, innate lymphoid cells, interleukin-5, remodelling, severe asthma

INTRODUCTION

The importance and contribution of eosinophils to the pathogenesis of asthma have recently been debated. Eosinophils were once thought critical to the initiation of disease, and thus an important therapeutic target, but subsequently, their role was deemed less important than that of T lymphocytes which became the focus of research. Although data from murine models have shown generation of allergic airways disease in eosinophil-deficient comparable to that in wild-type mice, it remains certain that eosinophils are the predominant inflammatory cells present in asthmatic airways.

PAEDIATRIC SEVERE ASTHMA IS EOSINOPHILIC

In the context of severe asthma, it is apparent that eosinophils are the prominent inflammatory cell type. In a cohort of children in whom underlying modifiable factors for poor disease control, such as poor adherence and persistent allergen exposure,

had been excluded, the dominant inflammatory phenotype was eosinophilic [1^{••}]. This was true for both airway luminal samples [induced sputum and broncho-alveolar lavage (BAL)] as well as within the airway wall (endobronchial biopsy).

An important consideration when determining inflammatory phenotype in the context of severe disease in patients that are on high dose inhaled and/or oral steroid therapy is the relationship between pulmonary eosinophilia and peripheral blood eosinophils. Samples that reflect pulmonary inflammation either require invasive techniques

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Curr Opin Allergy Clin Immunol 2014, 14:143–148

DOI:10.1097/ACI.0000000000000045

KEY POINTS

- Eosinophils are the predominant inflammatory cell types present in the airway lumen and airway wall in children with severe asthma.
- Children with severe asthma have a steroid-resistant pulmonary eosinophilia.
- The tissue-specific location of eosinophils, for example pulmonary (airway lumen or wall) or within adipose tissue, is likely important in determining their effector function.
- Peripheral blood eosinophilia does not reflect pulmonary (airway or mucosal) eosinophilia in children with severe asthma.
- Recent findings demonstrating a relationship between type 2 ILCs and eosinophil homeostasis suggest further investigation of interactions between these two cell types in the lungs of asthmatic patients may shed light on eosinophil effector functions in severe disease.

(bronchoscopy) and/or a significant amount of resource utilization in processing (induced sputum). Thus their clinical utility needs significant justification, especially in children. However, when high-dose inhaled steroids are prescribed, and efforts have been made to ensure they are being taken, it is apparent that an absence of peripheral blood eosinophilia does not equate to the same either in the airways or tissue [2[¶]]. Similarly, an attempt to determine whether relatively less invasive markers of inflammation than induced sputum, such as exhaled nitric oxide, serum IgE and peripheral eosinophils, could be used as a surrogate for sputum eosinophil percentages in adults within the severe asthma research program showed none of the non-invasive markers individually, or in combination, could be used reliably to represent sputum eosinophils [3[¶]]. These observations in severe asthma have very important clinical implications. Although elevated blood eosinophils may reflect airway eosinophilia, normal blood counts do not reliably exclude airway eosinophilia. Thus it is clear that peripheral counts should not be used to guide therapy or determine phenotypes in children with severe disease. Moreover, in children with severe asthma, the use of sputum eosinophils to guide alterations in inhaled corticosteroid therapy was not beneficial [4[¶]]. In fact, in children with severe asthma, sputum inflammatory phenotype does not remain stable over time [5]. Therefore, improvement of control in patients after therapies targeting eosinophils have not been uniform and have been met with limited success, suggesting that a much more

complex relationship between eosinophils and induced lung pathology exists.

There are emerging data to suggest that markers of eosinophil activity, rather than numbers *per se*, may better dictate disease status [6,7]. The propensity of blood eosinophils to lyse and release free extracellular granules is increased with increasing asthma severity in children [8]. Blood eosinophil morphology was used as a marker of activated eosinophils which were higher in asymptomatic asthmatic patients compared with controls, and numbers were further increased during an exacerbation. In an adult study [9], patients with fewer granules in tissue eosinophils had better asthma control. It is possible that assessment of markers of eosinophil activity in both pulmonary tissue and airway lumen may better reflect disease control.

A limitation of induced sputum samples obtained from children is that they are not always of adequate quality for a reliable leukocyte count. A potential test that may address both the issue of measuring eosinophil activity and difficulty in obtaining an adequate cell count from sputum samples is an eosinophil peroxidase ELISA-based assay for sputum supernatants. This provided a reliable and eosinophil-specific surrogate for sputum eosinophil numbers and activity [10[¶]].

STERIOD-RESISTANT EOSINOPHILIA IN THE PATHOGENESIS OF SEVERE ASTHMA

Eosinophils have been viewed as omnipresent inflammatory cells whose role in asthma, although still not clearly defined, has been characterized as destructive. As a result, their presence in airways is suggested to increase and/or cause the symptoms occurring in asthma. Medicines that target eosinophils, such as steroids, are therefore the mainstay of therapy. However, paediatric severe asthma has an eosinophilic phenotype and is associated with significant airway remodelling [1^{¶¶}] despite high-dose steroid therapy. Thus, suggesting steroid-resistant eosinophilia may be central to the pathogenesis (Fig. 1). In the presence of relatively steroid-resistant eosinophilic inflammation, an alternative therapeutic approach may be to target the cytokine interleukin (IL)-5 which mediates the development, survival and recruitment of eosinophils to tissues. Although this approach has not yet been tested in clinical trials in children, its potential success is debatable, since IL-5 was rarely detected in either the airway mucosa or lumen of children with severe asthma [1^{¶¶}], highlighting potential pitfalls of direct translation of findings from adult studies to children. However, several adult studies have reported the efficacy of anti-IL-5 monoclonal antibody therapy

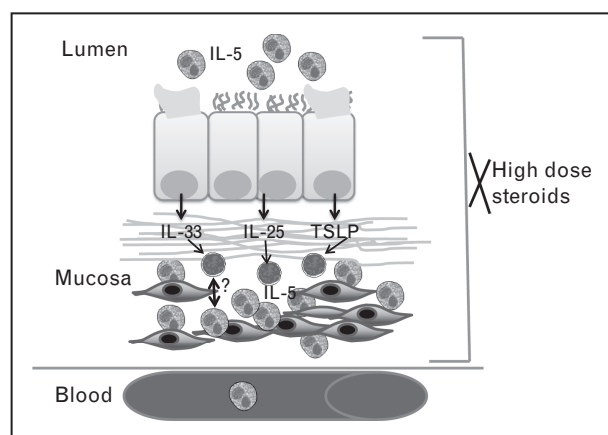


FIGURE 1. The role of eosinophils in paediatric severe asthma: paediatric severe asthma is characterized by eosinophilia in the airway lumen and bronchial submucosa, and airway remodelling with increased reticular basement membrane thickness and airway smooth muscle mass. Treatment with high-dose steroids does not impact this tissue-specific eosinophilic inflammation, although peripheral blood eosinophils are reduced. Innate cytokines released from the epithelium may directly induce the recruitment of innate lymphoid cells which secrete cytokines such as IL-5, a chemoattractant for tissue eosinophils, and this innate pathway may drive the steroid-resistant eosinophilia and remodelling. However, little IL-5 is detectable in the submucosa and lumen of children with severe asthma, suggesting alternative, and as yet unknown mediators released by innate lymphoid cells may contribute to the persistent inflammation.

in severe asthma. The refractory eosinophilic asthma phenotype is also recognized in adults, and these patients were eligible for inclusion in the Dose Ranging Efficacy And safety with Mepolizumab in severe asthma trial [11²²]. Interestingly, although evidence of eosinophilic inflammation was an inclusion criterion, tissue-specific inflammation was not necessary. Criteria for eosinophilic inflammation included sputum eosinophilia or an elevated exhaled nitric oxide or a blood eosinophilia. This is intriguing since it is now apparent that relationships between each of these compartments are unreliable in both paediatric [2²,4²] and adult severe asthma [3²]. There was a significant reduction in exacerbations in patients that received the anti-IL-5 antibody mepolizumab. Although there was no impact on daily symptoms or lung function [12], which has raised the possibility of mechanisms other than those driven by IL-5 resulting in persistent eosinophilia and persistent symptoms.

More recently, a two-cohort, randomized, double-blind, placebo-controlled trial of benralizumab, which is a monoclonal antibody directed

against IL-5 receptor α , has assessed effects on both airway (sputum) and tissue (biopsy) eosinophils [13²]. Since it was a phase I trial, numbers were small. There was a reduction in tissue and sputum eosinophils following the antibody treatment [13²]; however, this was only significant in a post-hoc analysis when biopsies from patients administered the antibody using two different routes were combined [14]. Thus, suggesting the reduction in tissue-specific pulmonary eosinophils was not as large as expected. Although disappointing, this may be a reflection of small patient numbers, or may be explained by the relative absence of detectable IL-5 levels in sputum, BAL and tissue previously reported in children with severe asthma [1²²]. Either way, the question of mechanisms or mediators other than IL-5 driving the eosinophilic inflammation in severe asthma is raised.

INNATE LYMPHOID CELLS AS PRESERVERS OF EOSINOPHIL HOMEOSTASIS AND MEDIATORS OF EOSINOPHILIA IN SEVERE ASTHMA

The epithelial cell-derived cytokines IL-33, IL-25 and thymic stromal lymphopoietin (TSLP) promote eosinophilia by inducing IL-5 production from the relatively recently described innate lymphoid cells (ILCs). All three innate epithelial cytokines directly induce the production of type 2 ILCs (ILC2), which release the Th2 cytokines IL-5 and IL-13 without the development of an adaptive immune response [15²], and are sufficient to initiate allergic airways disease in murine models [16,17]. Although Th2 cytokine expression was not increased in endobronchial biopsies from children with severe asthma, IL-33 expression was elevated, and was associated with increased airway remodelling and steroid resistance [18²²]. These data suggest that IL-33 may contribute to the induction of ILC2 cells in severe asthma, which may be predominantly responsible for disease pathogenesis (Fig. 1). However, to date, the role of ILC2 cells in allergic airways disease has only been shown in experimental models [16]. Although the phenotypic characteristics of human ILCs have been established [19–21], they have not yet been identified in the airway lumen of patients with asthma, but curiously, they have been found in lung tissue from five adult asthma patients [22²]. A very interesting association between ILC2 cells and eosinophil homeostasis has also been described [23²] which emphasizes the importance and function of tissue-specific eosinophils and circulating IL-5 levels. Serum IL-5 levels are maintained by long-lived ILC2s resident in peripheral tissues. ILC2 cells secrete IL-5 constitutively and are induced to co-express IL-13

during type 2 inflammation, resulting in localized eotaxin production and eosinophil accumulation.

TISSUE-SPECIFIC EOSINOPHILS, OBESITY AND THE METABOLIC SYNDROME

The lung is a site where eosinophils are rare in health, but accumulation is a characteristic of allergic disease. The importance of eosinophil location and the specific tissue that they are within in determining their effector function has been highlighted by data linking eosinophils within adipose tissue with glucose homeostasis and the metabolic syndrome [24]. Eosinophils present within healthy adipose tissue helped maintain metabolic homeostasis and glucose tolerance through the interaction with and regulation of the activity of alternatively activated macrophages in the fat, which was supported by ILC2s [25]. This suggests that the location of eosinophils within different tissues might influence their function and may shed light on their pathological function in asthma, particularly in patients who are overweight. In relation to this, an oral hypoglycaemic agent, metformin, which reverses obesity-associated insulin resistance, has been shown to reduce allergen-induced eosinophilic inflammation in obese mice [26[□]]. Treatment with metformin only reduced eosinophil accumulation within lung tissue, suggesting metformin may facilitate cell migration into the airway lumen, thus accelerating clearance of tissue eosinophilia in obese mice. In a similar manner, only submucosal tissue eosinophils (within the airway wall) have a relationship with BMI in adults with severe asthma, whereas sputum (representing airway lumen) or peripheral blood eosinophil counts had no relationship with BMI [27^{□□}]. Thus, suggesting lung tissue eosinophils may be functionally more important than those released into the airway lumen. Interestingly, a similar finding has been reported in children whereby obesity was not associated with either sputum or peripheral blood eosinophilia [28]. Although this was interpreted as a lack of association between asthma, obesity and inflammation by the authors, it may simply be that an association between mucosal eosinophils and obesity was missed since the patients did not undergo bronchial biopsy. Another study [29[□]] in children has shown similar blood levels of eosinophils in asthmatic obese and non-obese patients, but increased eosinophil activity, demonstrated by spontaneous chemotaxis, in the asthmatic obese patients compared with asthmatic non-obese patients.

In summary, these data demonstrating a role for eosinophils within adipose tissue in glucose and metabolic homeostasis, and a pathological role for

eosinophils when in specific tissue sites (such as the lung), have started to shed some light on their role in health, but also in driving disease in allergic asthma. It is becoming increasingly apparent that therapies that target tissue-specific eosinophils may be more effective than those that only reduce peripheral or airway luminal eosinophilia, and this seems especially important in the context of severe disease and obesity-associated disease. It may be that the steroid-resistant eosinophilia that is in paediatric severe asthma is mediated predominantly by eosinophils within the lung tissue, and mechanisms that aim to investigate the interactions between tissue eosinophils and lung structural cells, such as the bronchial epithelium or airway smooth muscle, are an important avenue to pursue.

EOSINOPHILS IN THE PATHOGENESIS OF AIRWAY REMODELLING

The two pathological characteristics of severe asthma in children are eosinophilic inflammation and structural changes in the airway wall (airway remodelling). Although both are present, mechanisms that directly show causation between the two are not well described. Previously, eosinophil-deficient mice had been shown to be protected from the development of ovalbumin-induced airway remodelling, but more recently, this was shown not to be the case using house dust mite to induce disease [30]. A mechanism linking eosinophilic inflammation and remodelling via epithelial to mesenchymal transition has been published using in-vitro and in-vivo techniques. Co-cultures of human peripheral blood eosinophils and bronchial epithelial cells were associated with increased expression of transforming growth factor beta 1 and SMAD3 phosphorylation in the bronchial epithelial cells [31]. However, both the epithelial cells and eosinophils were obtained from healthy donors, and it is difficult to know whether asthmatic cells would have similar functional characteristics. Fibroblasts cultured from bronchial biopsies from children with severe asthma release significantly more collagen when stimulated with rIL-33 compared with those from healthy controls [18^{□□}]. In addition to a pro-fibrotic role, a reduced resolution capability of eosinophils from asthmatic patients has been shown. Protectin D1 was identified as one of the main anti-inflammatory and pro-resolving lipid mediators synthesized by human eosinophils, and suppressed chemotaxis induced by CCL11 (eotaxin-1) [32[□]]. However, compared with eosinophils from healthy individuals, there was decreased biosynthesis of protectin D1 by

eosinophils from patients with severe asthma. Thus, eosinophils from asthmatic patients may have a reduced capacity to induce resolution of inflammation, and not just have direct pro-inflammatory effects in asthma. Not only do functional differences between eosinophils from healthy and asthmatic patients need to be considered, but equally, disparities in eosinophil function from patients of different ages may also be important. The findings from genome wide association studies that highlight very specific genes for childhood asthma serve to illustrate this. Orosomucoid like 3 (ORMDL3) has been identified as a candidate gene for susceptibility to childhood asthma, but to date, the functional mechanisms by which it may contribute to pathogenesis are unclear. An interesting link between ORMDL3 and eosinophils has recently been described, whereby eosinophils that were recruited to the airways of mice challenged with the fungal allergen *Alternaria alternata* expressed ORMDL3. Moreover, knockdown of ORMDL3 significantly inhibited cell shape changes, adhesion and recruitment to sites of inflammation *in vivo* [33]. ORMDL3 regulated eosinophil trafficking, recruitment and de-granulation, thus providing a potential mechanism of its effects in allergic disease.

CONCLUSION

Increasingly, the data suggest that just as severe asthma is phenotypically heterogeneous, we need to acknowledge that the pathogenesis in children is different to that in adults. Although a childhood-onset phenotype is recognized in adults, the disease in children is not necessarily the same. Mechanisms mediating severe asthma in children need to be investigated in age-appropriate experimental models, confirmed in airway luminal and tissue samples obtained from children and finally, therapeutic targets that arise from paediatric mechanistic studies need to be trialled directly in children. It is no longer acceptable to either directly translate findings from adult studies to children, or to test the targets that seem to work in adult patients in children, with the assumption that factors involved in pathogenesis in adult and paediatric disease are the same. A potential imminent pitfall is the automatic trialling of new monoclonal antibody therapies targeting IL-5 in children, having seen some efficacy in adults. It is a danger to assume that these will be effective in combating the steroid-resistant eosinophilia present in children. A big limitation at present is the lack of interest shown by the pharmaceutical industry in applying their drug development expertise to paediatric severe asthma research. This is despite the fact that asthma

is predominantly a disease of childhood, and is the most common chronic respiratory condition to affect children worldwide.

Acknowledgements

None.

Conflicts of interest

C.M.L. is a Wellcome Senior Research Fellow in Basic Biomedical Science.

The authors have no conflicts of interest to declare in relation to this review.

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- of special interest
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