# Eosinophils in asthma and other allergic diseases

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A hallmark of allergic disease is infiltration of the tissues with increased numbers of eosinophils. This is the result of the co-ordinated action of cytokines, particularly IL-5, CCR3 binding chemokines and the adhesion molecules P-selectin and VCAM-1, acting in concert to cause selective trafficking of eosinophils into allergic tissue. This process is orchestrated by the Th-2 allergen specific lymphocyte. While there is little data to support the view that eosinophils ameliorate the allergic process, although they could have an important role in the disordered repair that leads to permanently impaired function in some allergic diseases, the evidence that they cause many of the pathophysiological features of allergic disease, while strong, remains circumstantial. Much of the data could be interpreted just as easily to suggest that eosinophils are bystander cells; markers of a certain type of pathological process, but not impinging upon it. The most direct evidence for a pathological role rests on the toxicity of the eosinophil granule proteins for bronchial epithelium and the bronchoconstrictor actions of the sulphidopeptide leukotrienes. The actions of LT antagonists in asthma which are certainly beneficial, but in most cases are not as effective as glucocorticoids, could be interpreted both for and against the eosinophil. In this paper we have focused on the studies that ask most directly the question of whether eosinophils are important effector cells in the pathogenesis of allergic disease. We conclude with a qualified affirmative. Even if they are only bystander cells they remain clinically important as diagnostic markers and a guide to the management of allergic disease.

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Eosinophils are a characteristic feature of the pathology of asthma and related diseases such as atopic dermatitis and rhinitis. Ideas about their role in these diseases have fluctuated with a view in the 1970s that they were an ameliorating influence, followed by a consensus since the 1980s that they are important pro-inflammatory cells. This view evolved from evidence that eosinophils were potentially cytotoxic. In the 1970s it was shown that eosinophils could effectively kill the larval stage of helminthic parasites[1]. This was followed by evidence demonstrating a toxic effect on mammalian cells, particularly bronchial epithelium, mediated by the eosinophil specific basic proteins[2]. Eosinophils were

shown to produce large amounts of the sulphidopeptide leukotrienes (LTC4/D4 and E4) and platelet activating factor (PAF) which were thought to be involved in causing bronchospasm in asthma. Furthermore, it had long been known that one of the most noticeable effects of glucocorticoids in asthma was their ability to reduce the blood and airway eosinophilia. Further impetus for the hypothesis that eosinophils were inextricably involved in the asthma process (and by extension other allergic diseases) was provided by the development of the hypothesis in the 1990s that allergic disease is due to inappropriate activation of allergen specific Th-2 lymphocytes[3]. Th-2 cells through elaboration of IL-4 and IL-5 are inextricably linked with the development of blood and tissue eosinophilia with IL-5 being the major eosinophil specific growth factor[4] and IL-4 causing selective up-regulation of eosinophil specific endothelial adhesion and chemo-attractant pathways[5]. The view of asthma as a disease caused by an airway eosinophilia leading to bronchial hyper-responsiveness (BHR) has become firmly embedded in the literature to the extent that the widely used mouse model of asthma has been defined in terms of generation of an airway eosinophilia and BHR after antigen challenge.

The relevance of eosinophils to allergic disease has generated considerable interest in their biology which has been regularly reviewed in recent years[6–10]. Here we will give only a brief overview. Similarly the literature on eosinophils and allergic disease is extensive with over 3500 papers on *Medline* for asthma and eosinophils alone, most of which have been published in the last decade. It is not possible to do justice to all these papers, many of which deal with animal models of allergic disease. This review focuses on the key issue as to whether eosinophils really can be regarded as key effector cells in allergic disease. Attention is concentrated on studies of clinical disease rather than animal models which, while useful in dissecting out the possible role of individual molecules, particularly in trafficking, are of limited relevance to the disease process itself. Similarly the allergen challenge models in humans are of questionable value in understanding the pathogenesis of chronic allergic disease and these will be mentioned only in passing.

## The biology of eosinophils

Eosinophils are end stage cells derived from the bone marrow under the influence of GM-CSF, IL-3 and the late differentiation factor IL-5, which in humans is only active on eosinophils and basophils[11]. In terms of their ontogeny, they are more closely related to the basophil than the neutrophil or monocyte. After leaving the bone marrow, eosinophils circulate briefly in tissue before migrating to the gastrointestinal mucosa under

the direction of the eosinophil specific chemokine eotaxin and the gut homing adhesion receptor α4β7 which binds to the gut expressed MAdCAM-1[12,13]. Once in the tissues, eosinophils survive for up to 2 weeks through autocrine production of GM-CSF as a result of α4 mediated interactions with fibronectin[14]. Teleologically, it has been presumed that this is a surveillance mechanism related to the gut orientated life cycle of helminthic parasites, which are thought to be the major host defence target of eosinophils. In allergic disease, eosinophils migrate to extra-intestinal sites in a relatively selective fashion, with up to 100-fold enrichment of eosinophils over neutrophils in tissue. This is mediated by a multi-step process directed by Th-2 cytokine producing T-cells[5]. The first step is increased production and release of eosinophils from the bone marrow under the influence of the IL-5 and specific chemo-attractants such as eotaxin. Secondly, the target organ vasculature has increased adhesiveness for eosinophils through the specific effects of locally generated IL-4 and IL-13. These cytokines induce expression of VCAM-1 which binds eosinophils through VLA-4, a receptor not expressed by neutrophils, and P-selectin to which eosinophils bind with greater avidity than neutrophils[15–17]. CC chemokines such as eotaxin which bind CCR3, a receptor almost exclusively expressed by eosinophils and basophils, attract eosinophils into tissue where they survive for prolonged periods as a result of locally generated IL-5. The mechanisms by which IL-5 cause prolonged eosinophil survival (or delayed eosinophil apoptosis), are still imperfectly understood. IL-5 survival associated signalling is dependent on phosphorylation of Lyn and Jak2 kinases which bind to the common β chain of the IL-5 receptor, as well as Raf-1[18]. However survival is not dependent on activation on P-13 kinase, a pathway that mediates many IL-5 (and other agonist) mediated effects on eosinophils including increased adhesiveness and superoxide generation (personal observation). IL-5 prevents translocation of the pro-apoptotic Bcl-2 family member BAX, which is well expressed by eosinophils to the mitochondrial outer membrane. BAX translocation results in increased mitochondrial permeability, cytochrome c release and subsequent activation of downstream caspases, particularly caspase 3[19].

The relatively eosinophil specific basic proteins which are stored in the distinctive secondary granules are major basic protein (MBP) eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil derived neurotoxin (EDN). All of these proteins are toxic to the larval stages of many helminthic parasites as well as bronchial epithelial cells. Inhaled MBP can also induce BHR in primates[20], and inhibit the actions of down-regulatory M2 muscarinic receptors[21]. Eosinophils, with mast cells and basophils, are the most prominent source of sulphidopeptide leukotrienes produced mainly by enzymes present in lipid bodies[22]. They also produce significant amounts of PAF although this is not specific to

the eosinophil. Many of the diverse range of cytokines produced by eosinophils are released only in small amounts which may, nonetheless, have important autocrine effects[23]. For example, the small amounts of GM-CSF produced by the eosinophil is sufficient to prolong its own survival. The production of cytokines such as TGF-α and TGF-β by eosinophils has broadened the range of eosinophil functions emphasising their potential importance in, for example, wound healing.

The physiological triggers which lead to eosinophil mediator release in allergic disease remain uncertain. Engagement of Fcγ and Fcα receptors are the most reliable physiological triggers for degranulation of eosinophil basic proteins, especially when presented on the surface of a large particle, or in the context of co-engagement of the adhesion receptor Mac-1[24]. However, there is little evidence that immunoglobulin-mediated release is involved in allergic disease. Priming with eosinophil active growth factors such as IL-5 greatly enhances degranulation via Fc receptors and also causes degranulation directly[25]. PMA is an effective, albeit non-physiological, stimulus for superoxide production but not for degranulation or cytokine release. Calcium ionophore is a good stimulus for lipid mediator production. Chemo-attractants binding via G protein linked serpentine receptors stimulate superoxide production, but are not a good stimulus for other mediators. Cytochalasin B which inhibits cytoskeletal assembly has been used in many studies to enhance release and the relevance of this to a physiological setting is unclear. Eosinophils undergo piecemeal degranulation in most *in vivo* settings[26]. This involves the eosinophil releasing its granule products through pores in the plasma membrane without leading to cell death. With more vigorous stimuli, for example after allergen challenge, cytolysis is often prominent[27].

## The immunopathology of asthma and other allergic diseases

Although there is a significant body of work detailing the presence of eosinophils in the tissue in all allergic diseases, most work has been undertaken in the context of asthma, particularly those studies relating disease severity to the degree of tissue eosinophilia. For this reason, most of the papers reviewed below relate to asthma. The pathology of asthma comprises infiltration of the bronchial submucosa and epithelium with eosinophils, monocytes and lymphocytes although the latter two cell types are relatively abundant in the normal airway. Epithelial desquamation, or at least fragility, has been considered a hallmark of the disease, although detailed studies of the pathology of asthma deaths has called this dogma into question[28], and it is striking that induced sputum from asthmatics does not contain an increased number of bronchial epithelial cells. Thickening of the basement membrane beneath the

epithelium is a constant feature as is increased smooth muscle hypertrophy leading to thickening of the bronchial wall, as well as increased vascularity. Mucus hypertrophy and mast cell degranulation have also been reported. In this review, attention will focus on the eosinophil, although it is important to note that this cell type is just one part of a distinctive mucosal inflammatory response which is the underlying cause of asthma and rhinoconjunctivitis.

## *Bronchoscopy studies*

The observation that increased numbers of eosinophils were a feature of asthma has been known for many decades. Eosinophils and their granule products, including Charcot Leyden crystals were a hallmark of spontaneously induced sputum and were plentiful in the airways in *post-mortem* specimens[29]. Subsequent studies have confirmed this association[30], at the same time pointing out that a small proportion of patients dying of asthma, particularly those with sudden death, do not have an airway eosinophilia[31]. The inflammatory response in asthma deaths has been noted to effect both the large and small airways[32]. *Post-mortem* studies inevitably suffer from small numbers, lack of appropriate controls, a paucity of clinical details and the difficulty in controlling for the effects of treatment. It also represents an extreme end of the asthma severity spectrum. In the early 1980s, it was appreciated that bronchoscopy in asthma could be carried out safely as long as appropriate precautions were taken[33]. It then became apparent that even in very mild asthma there was evidence of airway inflammation and that the most obvious feature of the inflammatory response was the increased number of eosinophils in BAL fluid without an increase in neutrophils[34], as reviewed by Djukanovic and colleagues[35]. These studies demonstrated a considerable variability in the degree of BAL eosinophilia ranging from 1% to up to 30% (the normal being less than 1%). There was evidence that the eosinophils were actively secreting mediators with increased amounts of the eosinophil granule proteins and leukotrienes in BAL fluid[36,37] (and subsequently sputum[38]), although data regarding mediators in BAL fluid should always be treated with caution because of the lack of a good denominator to control for recovery of airway lining fluid. BAL eosinophils express the putative activation receptor CD69, have reduced expression of L-selectin, and increased expression of Mac-1 and ICAM-1 when compared to peripheral blood eosinophils. These changes in phenotype can be mimicked *in vitro* by treatment with IL-5 (for 24 h)[39], and suggest an activated phenotype.

Studies of BAL were followed by biopsy studies which allowed a more detailed immunopathological analysis of the inflammatory response in

the bronchial tree[40–42]. One advantage of biopsy studies is that they allow accurate quantification of bronchial lymphocytes and mast cells which are anchored within the epithelium and do not migrate into the lumen so do not appear in BAL fluid (or sputum) in representative numbers. These studies confirmed the consistent increase in the number of eosinophils in the airway submucosa without an increase, in most cases, in neutrophils. Eosinophils which are very infrequent in the normal airway, are enriched by up to 100-fold in the airways of asthmatic subjects compared to neutrophils. All these studies involve a cross-sectional analysis and say little to nothing about the kinetics of migration of these cells into the airways. Endobronchial biopsy studies also demonstrated increased numbers of CD4 T-cells in asthmatic airways. Although most bronchoscopy studies have been undertaken in young adults with mild atopic asthma, increased numbers of eosinophils have also been reported in intrinsic asthma[43], and occupational asthma due to Western Red Cedar and toluene di-isocyanate[44,45], as well as aspirin sensitive asthma where more eosinophils were seen than in non-aspirin sensitive asthmatics[46]. In severe corticosteroid dependent asthma two patterns were observed. Out of 34 severe oral glucocorticoid dependent asthmatics, in 14 patients eosinophils were absent in endobronchial biopsies whereas in 20 subjects eosinophils were increased. Neutrophils were increased in both groups. The eosinophilic group had been intubated more often[47]. Few bronchoscopy studies have been undertaken in children with asthma, although increases in both eosinophils and neutrophils have been reported[48,49]. As well as asthma, eosinophils are also a characteristic feature of seasonal and perennial rhinitis[50]. Nasal polyposis is usually associated with an intense eosinophilia[51] as is non-atopic rhinitis with eosinophils (NARES). Atopic dermatitis is characterised by increased numbers of eosinophils and deposition of eosinophil basic proteins in the affected skin[52,53]. Eosinophils in bronchial biopsies often have a partially degranulated appearance[54] and after nasal allergen challenge were shown to have undergone degranulation through a combination of cytolysis and piecemeal degranulation[27]. Tissue eosinophils express a range of cytokines as shown by both *in situ* hybridization and immunohistochemistry[55]. The anti-ECP antibody EG2 has been widely used as a marker of eosinophil activation, although the validity of this has recently been called into question[56]. An airway eosinophilia is a consistent feature of the late response to allergen challenge in the bronchial and nasal mucosa as well as the skin[57–59]. This is often more marked than the often modest number seen in chronic disease. However, the extent to which allergen challenge is a model for clinical disease remains controversial.

### *Induced sputum*

Bronchoscopy is invasive, potentially hazardous and expensive. Small numbers of individuals with generally mild disease are usually studied and repeat measurements are difficult. Measurement of the eosinophil count in induced sputum has overcome some of these problems. Although analysis of sputum in asthma has a long pedigree, it was hampered as an investigative tool by lack of standardization and the ability to obtain a sample in only a minority of cases. This problem was overcome by inducing sputum using hypertonic saline, a technique which is safe and reproducible. The sputum plugs are generally selected to reduce contamination with upper respiratory tract material (although not all groups do this), and the cells dissociated from mucus using a reducing agent such as dithiothreitol (DTT). Good quality cytospins can be routinely obtained and a reliable cell differential generated[60–62]. Eosinophil counts in sputum correlate reasonably well with bronchial biopsies, washings and BAL with the eosinophilia being often more marked in sputum[63,64]. The normal value for non-smokers has been reported as 0.4% with a 90th percentile of up to 1.1% with atopics higher than non-atopics[65]. We have found similar values. The range of eosinophil counts in asthma is wide from 1–50% or more. We have found that most, though not all, asthmatics have a raised eosinophil count (Fig. 1). Taking a cut off of 1% as indicating a raised sputum eosinophil count when compared with normal airways, a sputum eosinophilia as a test for asthma (defined by a Pc20 of < 8.0 mg/ml or a significant improvement in FEV1 after β2 agonists) gives a sensitivity of over 80% and a specificity of 95%. These values fall to 70% and 80%, respectively, when compared with subjects with respiratory symptoms which have led to a diagnosis of asthma but who subsequently turn out to have other conditions. In both cases this is considerably better than peak flow variability and much more sensitive than improvements in FEV1. Interestingly, in our experience, while the majority of patients with acute severe asthma have a very high eosinophil count, a small proportion had no eosinophils. Raised numbers of neutrophils in acute severe asthma have been reported by others[66,67], although in the Fahy study about a third had been on oral glucocorticoids which may have influenced the findings. Up to 64% of neutrophils are found in the normal airway which makes interpretation of a raised neutrophil count in asthma more difficult. A sputum eosinophilia is a feature of occupational asthma and may be useful in diagnosis[68]. As discussed below, the use of induced sputum has allowed a much more detailed assessment of the relationship between asthma and airway inflammation in terms of specificity, severity, asthma phenotype, bronchial hyperresponsiveness and response to treatment. For example, bronchoscopy

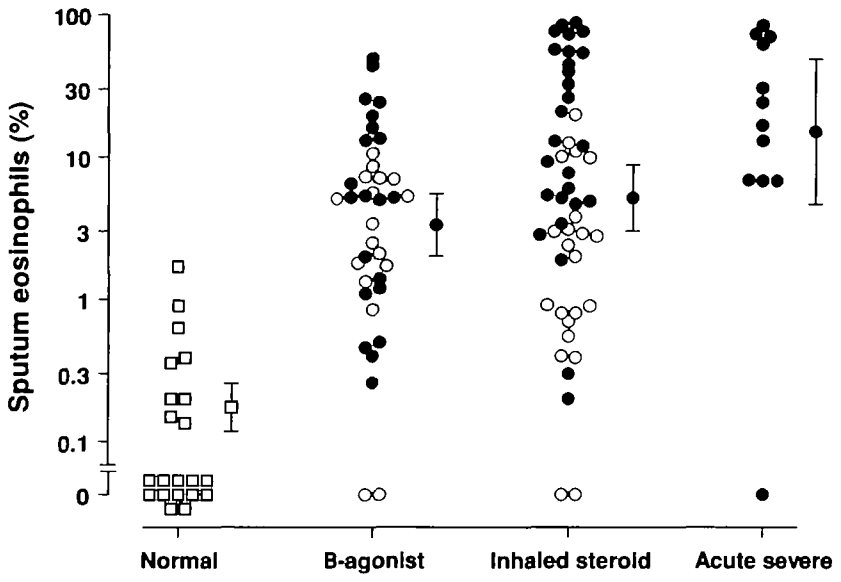


**Fig. 1** Percentage of eosinophils in induced sputum from patients with asthma attending an out-patient clinic or admitted to hospital with acute severe asthma compared with normal non-smoking controls. The normal sputum eosinophil count was less than 1%. Closed symbols represent patients requiring a step-up in treatment. Most asthmatics had increased numbers of sputum eosinophils with numbers being greatest in the patients with acute severe asthma.

studies have suggested that, whereas asthma is characterised by an airway eosinophilia, COPD is associated with a neutrophilia[69], although eosinophils were noted to be present during exacerbations[70]. However, it is clear from sputum studies in which larger numbers of more severe patients can be studied that up to a third of patients with stable irreversible smoking associated COPD have a significant airway eosinophilia[71,72]. In addition, an airway eosinophilia is commonly associated with chronic cough (a condition labelled eosinophilic bronchitis) which occurs in the absence of variable airflow obstruction and BHR[73,74]. Increased numbers of eosinophils compared to controls are seen in the airways of atopics without asthma although the increase is less marked than in asthmatics[54,65,75].

## Eosinophils and relationship to disease severity

### *Symptoms and lung function*

If eosinophils were important in causing the pathophysiology of asthma and related diseases, then a correlation between the degree of tissue

eosinophilia and disease severity might be expected. Early studies of peripheral blood eosinophil counts had suggested a correlation with lung function both in clinical disease[76] and after allergen challenge[77]. However, blood eosinophils are an insensitive and imprecise marker of tissue inflammatory responses[78]. In asthma deaths, the eosinophilic inflammation, particularly in the proximal bronchial tree, is more intense than in mild-to-moderate asthma[79]. In the early bronchoscopy studies, the presence of eosinophils in BAL fluid was associated with symptomatic asthma and was not seen in asthmatics in remission[34]. There was also a broad correlation between clinical severity and degree of airway eosinophilia[80]. In 17 patients with mild asthma, Walker *et al.* reported a correlation between the degree of BAL eosinophilia, lung function and BHR. This study also found a correlation between the numbers of eosinophils and activated T cells as did a bronchial biopsy study by Bradley *et al.* emphasising the relationship between T-cells and eosinophils in allergic disease[81]. Lim *et al.* found that there was a weak correlation between mucosal eosinophils and lung function in 16 asthmatics half of whom were taking inhaled glucocorticoids[82]. Most bronchoscopy studies have investigated mild asthma so it is difficult to get a spectrum of asthma severity in terms of symptoms and lung function. Induced sputum can be obtained safely even in moderately severe asthmatics and has allowed a more detailed analysis of the relationship between eosinophilic airways inflammation and asthma[83].

In a relatively early sputum study, Pin *et al.* found an inverse correlation between FEV1 and sputum eosinophil counts[84]. In contrast, another study of 20 asthmatics after an 8 week course of high dose inhaled steroids, no correlation between sputum eosinophils and clinical markers of severity was observed although there was a weak correlation with BHR[85]. In 20 atopic asthmatic children on inhaled steroids with moderate to severe chronic asthma, a weak correlation was observed between sputum eosinophil numbers and asthma severity in terms of lung function and an asthma severity index[86]. In a study which compared the degree of airways inflammation in mild and moderate asthma, the number of eosinophils, as well we other markers of inflammation, were more marked in the more severe disease group[87]. In a study of induced sputum in 74 asthmatics ranging from mild to severe persistent disease, asthma severity as assessed by lung function, symptoms scores and BHR, correlated with the degree of airway eosinophilia. A weak correlation was also seen between sputum neutrophilia and symptom scores[88]. In a study of 43 mild-to-severe asthmatics, the sputum eosinophil count was greater in the severe compared to the mild and moderate asthmatics as defined by the clinical Aas score, but only weak correlations with FEV1 and BHR were observed[89]. In an interesting study by Jatakanon *et al.*, asthma

exacerbations were induced by withdrawing inhaled steroids. Baseline sputum eosinophilia was a predictor of subsequent exacerbations and the degree of sputum eosinophilia correlated with falls in PEF and FEV1[90]. Probably more important than numbers of eosinophils is the amount of mediators they are generating. In a study of 36 patients with asthma ranging in severity from mild to severe there was a higher concentration of ECP in the severe asthmatics compared to the mild-to-moderate patients. Taking all the subjects ECP levels correlated with symptom scores and inversely with PEF[91]). Similarly Virchow *et al.* found that, in 14 patients with asthma not taking corticosteroids, sputum ECP was a better marker of severity in terms of lung function than sputum eosinophil counts[92].

Studying the relationship between various markers of asthma severity and airway inflammation is difficult. Symptoms are not objective and peak flow measurements are unreliable. Cross-sectional studies involving a single measurement of FEV1 and eosinophil count (the usual study design) in a disease like asthma which is defined in terms of its variable severity are crude, particularly when the sampling errors involved in measuring the degree of airway eosinophilia are taken into account. There is a paucity of longitudinal studies correlating asthma severity with airway inflammation on an individual basis. Most of the reported studies are relatively under-powered and treatment with anti-inflammatory drugs adds another important variable.

### *Eosinophils and bronchial hyper-responsiveness (BHR)*

BHR refers to the increased sensitivity of asthmatics to irritant inhaled stimuli. This is non-specific in the sense that it not antigen dependent and can be caused by a number of diverse agents ranging from smoke and dust through to cold air, exercise and perfumes. It can, therefore, be caused by agents such as methacholine and histamine that act directly on smooth muscle to cause bronchoconstriction or agents such as cold air that are thought to act indirectly possibly through a neural reflex. BHR is a hallmark of asthma and measurement of BHR, for example by constructing a histamine or methacholine dose response curve, is the most sensitive and specific test for asthma. BHR is regarded as a necessary ingredient for the integrity of asthma models either in humans or animals. However, the extent to which the increased bronchial reactivity seen after allergen challenge and in animal models is related to the BHR seen in clinical disease is debatable. Despite intensive research over the last 20 years the cause of BHR is still unknown. In particular, the extent to which BHR is caused by, or interacts with, airway inflammation and especially eosinophilic inflammation remains

contentious. BHR and eosinophilic inflammation generally occur together. In the early bronchoscopy studies, although there was a clear association between BHR and an airway eosinophilia, there was little evidence of a correlation between the severity of the BHR and the number of BAL eosinophils[34]. Similarly, in the study by Foresi *et al.* quoted above of 15 asthmatics and 30 patients with seasonal allergic rhinitis, a good correlation ($P<0.005$) was seen between sputum eosinophils and BHR, but this was skewed by the inclusion of patients with seasonal rhinitis without asthma[75]). In a much larger study of 71 asthmatics, no relationship was seen between sputum eosinophilia and BHR although the eosinophil count did inversely correlate with lung function[93]. Some studies have seen a correlation. For example, Jatakanon *et al.* found a weak negative correlation (r –0.4) between the sputum eosinophil count and Pc20 in 35 stable asthmatics taking only β2 agonists. A stronger inverse correlation was seen with NO concentrations in exhaled air[94]. In our own experience of over 200 stable asthmatics attending our routine out-patient clinics (46% atopic and 44% taking inhaled steroids), there was no relationship between log sputum eosinophil count and log Pc20 in the non-atopic group but there was a significant inverse correlation in the atopic group ($P < 0.002$) in both patients taking inhaled steroids and those on β2 agonists alone, although the correlation was weak (r –0.365). A dissociation between BHR and airway eosinophilia has been observed in animal studies. For example, Henderson *et al.* found that an antibody against the α4 integrin when given intraperitoneally was able to inhibit eosinophil migration into the airways after ovalbumin challenge, but had no effect on BHR, whereas both BHR and the airway eosinophilia were inhibited by nasal delivery of the antibody[95]. One issue is that the Pc20 threshold may not be the best marker of BHR to use. Moller *et al.* found a good correlation between the plateau of the methacholine dose response curve (which in the majority of patients had to be modelled from a calculated sigmoid curve) and the airway eosinophil count in 20 asthmatics, but no correlation with Pc20[96].

Current evidence supports the idea that BHR and eosinophilic airway inflammation are independently regulated but closely interrelated, a view supported by a factor analysis undertaken by Rosi *et al.* in 99 mild asthmatics[97]. This would predict that in a cross-section of patients, for a given degree of inflammation, marked differences in BHR can result. This is consistent with the observation that eosinophilic inflammation can occur without BHR as in eosinophilic bronchitis and marked BHR can occur in the context of minimal airway eosinophilia. It would also suggest, however, that within an individual, changes in BHR may mirror changes in eosinophilic airway inflammation to the extent that airway inflammation could be used longitudinally to guide asthma management.

## *Eosinophils and anti-inflammatory drugs in asthma*

If eosinophils are to be plausibly implicated in causing allergic disease, drugs which are effective in their treatment should also reduce eosinophil numbers and mediator release. With the exception of β2 agonists and anti-cholinergic bronchodilators, this appears to be the case as is most clearly seen with glucocorticoids (GCs), the most effective anti-inflammatory treatment for asthma. GCs cause a marked eosinopenia when given orally and both oral and topical GCs reduce the tissue eosinophilia in a wide range of eosinophilic conditions in a dose-dependent manner. In contrast, they have little effect on neutrophils, actually increasing the peripheral blood neutrophil count, and are ineffective in most diseases characterised by neutrophilic inflammation. GCs increase the rate of eosinophil apoptosis, although this effect is modest in degree and only seen at relatively high concentrations of steroids with an $IC_{50}$ of about $10^{-6}$ M whereas they prolong neutrophil life-span[98]. The molecular basis for this difference is unknown. It is likely that the principle action of steroids in asthma is to inhibit cytokine and chemokine production by both leukocytes and resident airway cells, such as epithelial cells, fibroblasts and bronchial smooth muscle[99]. The evidence that GCs reduce eosinophil counts in asthma is consistent. For example, 6 days of treatment with oral glucocorticoids reduced the sputum eosinophil count (and ECP level) in 24 asthmatics from 14% to 1%, whereas no change was observed in the placebo group. Moreover, the increase in peak flow associated with steroid treatment correlated with the fall in the eosinophil count[100]. Oral prednisolone caused a fall in the sputum eosinophil count and ECP level in patients with severe exacerbations of their disease. The improvement in sputum eosinophils and ECP levels correlated with improvement in lung function[101]. In a bronchial biopsy study of 10 asthmatics, treatment with 2 mg of beclomethasone diproprionate for 6 weeks resulted in an improvement in lung function, symptoms, Pc20 and a reduction in markers of inflammation including the eosinophil count[102]. Inhaled budesonide resulted in a fall in eosinophil counts which correlated with the improvement in Pc20 in 14 asthmatics[103]. Oral prednisolone given for 2 weeks caused a significant fall in eosinophil counts which was not seen in the placebo limb of the study[99]. In all these studies, comparable effects were also seen on mast cell and T-cell counts emphasising the broad spectrum of anti-inflammatory actions of GCs, although neutrophil numbers were unaffected. However, the eosinophil count does appear to be a useful marker of steroid responsiveness in both asthma, COPD and rhinitis[71,104–108]. In all these studies, the airway eosinophilia was not abolished and in some patients only a modest fall was seen. It was striking that in our clinic population many patients still had a significant

sputum eosinophilia despite being on inhaled GCs, although whether this was due to poor compliance, insufficient dose or GC resistance is not clear. As well as GCs, leukotriene antagonists modestly reduce the eosinophil count in asthma. Interestingly, they also promote eosinophil apoptosis[109,110]. Cyclosporin and a thomboxane $A_2$ antagonist have been shown to reduce eosinophil counts in asthmatic airways although whether this is related to their benefit in asthma is not clear as they have a wide range of other actions[111,112].

## Novel anti-eosinophil therapies

The evidence supporting an important role for the eosinophil in causing allergic disease has led to a number of pharmaceutical companies developing specific anti-eosinophil therapies. The most advanced of these, and the only one where clinical studies have been reported, involved the use of two different anti-IL-5 antibodies. One was an allergen challenge study in mild asthma[113] and one was a study in severe asthma (not yet published). Both were designed primarily as dosing and safety studies and were not really powered for their clinical effects. Nonetheless, some interesting data have emerged. In both cases, the antibodies were well tolerated and effective in reducing the peripheral blood and, to a lesser extent, sputum eosinophilia. In the allergen challenge study, no affect was seen on either the early or late response or on the severity of BHR in these very mild subjects. Caveats to this study are the extent to which allergen challenge is a good model for asthma and the reliance on sputum so that we can not be sure that the eosinophil count in the bronchial submucosa was also inhibited. In the severe asthma study, there was a trend towards an improvement in the active group but this was not clear cut and the numbers of patients were too small to draw any firm conclusions.

## Conclusions

The hypothesis that eosinophils are important effector cells in asthma and allergic disease rests on three main pillars of evidence. Firstly, that eosinophils are found in asthmatic airways (and relevant tissues in other allergic diseases); secondly, that their mediators are relevant to the disease process; and thirdly, that removal of eosinophils is associated with an improvement in the disease. Examining each of these in turn, there is a considerable body of literature documenting in detail the close relationship between the presence of eosinophils in the airway submucosa and asthma to the extent that it is a useful diagnostic marker.

Broadly speaking, the degree of eosinophilia correlates with disease severity, and in atopics at least BHR, although the correlation with BHR is weak suggesting an indirect relationship. Eosinophil specific mediators certainly appear relevant to asthma and to a lesser extent other allergic diseases, but their importance has been challenged by recent studies showing lack of evidence for epithelial damage in asthma and by the ineffectiveness of PAF antagonists and the modest benefits of LT antagonists. GCs undoubtedly have a profound effect on eosinophils and their beneficial effects in allergic disease appear to go hand-in-hand with their inhibition of tissue eosinophilia, but they are of course broad spectrum anti-inflammatory drugs.

Taking the evidence together, there is no doubt that eosinophils are intimately associated with asthma and the other atopic diseases. As there is virtually no evidence that eosinophils can ameliorate disease, we presume they must either be bystander cells or actively involved in pathogenesis. The current evidence is consistent with a role for eosinophils simply as markers of the inflammatory process, but not impinging upon it. Even if they are just markers, eosinophils are so closely related to at least asthma that they remain extremely useful as indicators of diagnosis and response to treatment in allergic diseases. The evidence would also be entirely consistent with eosinophils being important effector cells. Perhaps the most likely answer is that eosinophils are part of a complex inflammatory process in which they favour one aspect of the pathophysiology, cough for example or airway wall remodelling. If this is the case, using anti-eosinophilic drugs to assess their role may be more difficult than simply measuring FEV1 or BHR in short-term studies.

There is clearly a great deal more work that can be done to determine the extent to which the airway eosinophilia might guide management. In particular, in diagnosis and in longitudinal studies of individual patients to assess the value of the sputum eosinophil in guiding treatment with GCs. In contrast, getting closer to the question as to whether eosinophils cause asthma requires detailed studies with specific anti-eosinophilic therapies. We await these with interest.

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