

Role of Eosinophil Granulocytes in Allergic Airway Inflammation Endotypes

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Abstract

Eosinophil granulocytes are intriguing members of the innate immunity system that have been considered important defenders during parasitic diseases as well as culprits during allergy-associated inflammatory diseases. Novel studies have, however, found new homeostasis-maintaining roles for the cell. Recent clinical trials blocking different Th2 cytokines have uncovered that asthma is heterogeneous entity and forms different characteristic endotypes. Although eosinophils are present in allergic asthma with early onset, the cells may not be essential for the pathology. The cells are, however, likely disease causing in asthma with a late onset, which is often associated with chronic rhinosinusitis. Assessment of eosinophilia, fraction exhaled nitric oxide (FeNO) and periostin are markers that have emerged useful in assessing and monitoring asthma severity and endotype. Current scientific knowledge suggests that eosinophils are recruited by the inflammatory environment, activated by the innate interleukin (IL)-33 and prevented from apoptosis by both lymphocytes and innate immune cells such as type two innate immune cells. Eosinophils contain four specific granule proteins that exhibit an array of toxic and immune-modulatory activities. The granule proteins can be released by different mechanisms. Additionally, eosinophils contain a number of inflammatory cytokines and lipid mediators as well as radical oxygen species that might contribute to the disease both by the recruitment of other cells and the direct damage to supporting cells, leading to exacerbations and tissue fibrosis. This review aimed to outline current knowledge how eosinophils are recruited, activated and mediate damage to tissues and therapies used to control the cells.

Introduction

Eosinophils, cells with homeostasis-maintaining functions

Eosinophil granulocytes have since long been associated with T helper type 2 (Th2) responses such as parasitic and helminthic diseases, and pathology in allergic asthma and rhinitis. Although ever-increasing knowledge is gained of what is the function of the cells, it is still not known exactly how and when they confer such pathology. This review aims to detail the recent insight that has been gained of the eosinophil granulocytes and how such aspects fit with the increasingly heterogeneous aetiology of allergic disease of the respiratory tract.

Over a hundred years ago, the first report of the presence of eosinophils in nasal polyps and in asthmatic sputum was published. When the German physician Paul Ehrlich 1879 stained human blood smears, he discovered cells that avidly bound acidic aniline dyes [1]. Studies have subsequently

been performed to characterize the cells, and increasingly during the recent years to identify novel functions for eosinophils at different anatomical locations apart from the ones associated with the Th2 response. Eosinophils are formed in the bone marrow where they constitute a small but stable subpopulation. This population consists of both mature eosinophil cells and eosinophil lineage precursors at various stages of development. The cells have a role in the bone marrow where they augment plasma cells survival [2]. Three cytokines have growth factor activities for eosinophils: granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3 and IL-5. IL-5 is the most important cytokine in mediating eosinophil proliferation and activation [3–5]. Production of the cytokine is induced during Th2 response leading to increased production and release of the cells from the bone marrow. Mice deficient in the *IL5* gene cannot expand their eosinophil population in response to inflammation [6]. IL-5, IL-3 and GM-CSF all also recruit eosinophils from the blood and enhance

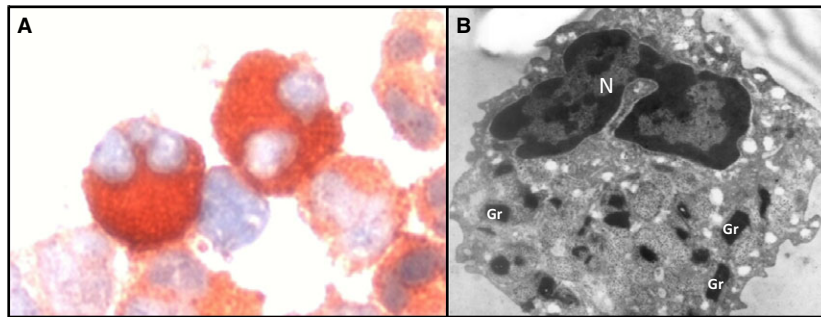


Figure 1 (A) Presence of major basic protein (MBP) in peripheral blood leucocytes. MBP is localized to specific granules and cytoplasmic vesicles in human eotaxin-stimulated eosinophils. Of the peripheral blood cells, only eosinophils are stained by the antibody. The leukocyte population was fixed and permeabilized with permeafix prior to incubation with primary and secondary antibodies. Antibody staining was visualized using phosphatase anti-alkaline phosphatase (APAAP) with fast red substrate, and the slides were counterstained with Mayer's haematoxylin. Original magnification ($\times 800$). (B) Presence of MBP in the core of eosinophil specific granules. An eosinophil from an allergic subject during pollen season was immunolabelled for MBP by monoclonal antibodies conjugated with 15-nm gold particles. MBP was found present in the crystalloid core of the specific granules. Many specific granules show alterations of ultrastructure ranging from loss of matrix and core to complete lucency of matrix. MBP is observed as well in the matrix of unaltered specific granules and in the cytoplasm both in the vicinity of granules and in the granule-free area. The cells were fixed in paraformaldehyde and embedded in Lowicryl KM4 prior to addition of the antibody. Original bar is 480 nm. N, nucleus; Gr, specific granules.

eosinophil survival in the periphery. At homeostasis, IL-5 is produced by tissue-resident innate lymphoid cells in the gastrointestinal tract and adipose tissue, maintaining homeostatic levels of the eosinophils at those locations [7–9]. The cells promote development of and conversion of white to brown fat in the adipose tissue [10, 11] and support IgA production and thereby bacterial homeostasis in the intestine [12]. Small number of eosinophils are found extravascularly also in the spleen, lymph nodes, thymus, mammary glands and uterus [13].

One identifying feature for eosinophils is their bilobed nuclei (Fig. 1A). Further, the eosinophils contain a number of potent mediators that can be released upon stimulation of the cells. The crystalloid granules present in the cells cytoplasm can be visualized by the dye eosin, by immunohistochemistry (Fig. 1A) or by electron microscopy (Fig. 1B) using antibodies detecting specific proteins. The granules contain substances that are toxic to many organisms and probably also to the patient's own tissues [14, 15]. Four distinct cationic proteins are present in the granules that exert a range of biological effects on host cells and microbial targets (Table 1). The proteins are the two cationic proteins eosinophil cationic protein (ECP) [16, 17] and eosinophil protein X (EPX) [18], the latter also called eosinophil-derived neurotoxin (EDN). EPX/EDN is structurally related to ECP and both are arginine-rich, but EPX/EDN shares few of ECPs cytotoxic properties, except the non-physiologic neurotoxicity for which it was named. Both proteins are also ribonucleases with less such activity than the related RNase1 [19]. The other two granule proteins are the eosinophil peroxidase (EPO) [20] and the major basic protein (MBP). EPO catalyses the reaction of hydrogen peroxide with halogens to hypohalides and has a spectrum of *in vitro* cytotoxicity that is very similar to that of MBP [21]. Figure 1B shows an eosinophil stained with

Table 1 The major proteins of human eosinophil granules.

Protein	Molecular weight (kDa)	Localization
Eosinophil cationic protein (ECP)	18–21	Matrix of eosinophil granules
Eosinophil peroxidase (EPO)	67	Matrix of eosinophil granules
Eosinophil protein X/eosinophil-derived neurotoxin (EPX/EDN)	18	Matrix of eosinophil granules, neutrophils
Major basic protein (MBP)	14	Core of eosinophil granules, neutrophils

The granule proteins present in the eosinophil granulocyte secondary granules. Presented is the proteins, its molecular size and localization within the granule (either outer matrix or core).

an anti-MBP antibody. The proteins show a range of properties and are implicated in tissue remodelling and asthma exacerbations. Apart from granule proteins, the eosinophils release cytokines, lipid mediators and oxygen radicals in response to stimulation. The cells produce the cytokines IL-1 β , IL-6, IL-8 and IL-4 and lipid bodies within the cells contain the 15-lipoxygenase products 15-HETE and the cystinyl leukotriene C₄ (CTL₄) [22, 23]. By utility of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, the cells can produce more radical oxygen species (ROS) per cell basis than what neutrophils can do [24]. Clearly, the cells are equipped for miscellaneous insults but if not controlled can cause damage to the self. The cells do, however, have ability to regenerate damage tissue, which has been shown during regeneration of the liver. By virtue of their ability to produce IL-4, the rapidly influxing eosinophils stimulated

hepatocytes to proliferate for this aim. This process has features similar to parasitic infection [25, 26]. Eosinophils have also been found to infiltrate tumours. In some malignancies, tumour-homing eosinophils secreted CCL5, CXCL9 and CXCL10, which guided T cells into the tumour, resulting in tumour eradication and survival [27, 28]. In other studies, however, eosinophils were found to have malignancy-promoting properties [29].

Although it has been known for a long time that eosinophils are expanded in response to Th2 driven parasitic and helminthic diseases, it is still unclear how the cells contribute to such pathology. A pathognomonic function has been established in the hypereosinophilic syndrome (HES). This disorder is characterized by more than 1.5×10^6 eosinophils/ml blood during a time period of at least 6 months for unknown reason. Symptoms include atopic dermatitis, endomyocardial fibrosis and lung fibrosis [30]. Anti-IL-5 antibodies (mepolizumab and reslizumab) were used to remove the cells from these individuals reducing symptoms such as dermatitis and myocardial fibrosis. Neither these individuals, devoid of eosinophils in response to treatment nor individuals with naturally low levels or absence of the cells, or mice genetically engineered to lack eosinophils, showed an altered phenotype. This suggests that the cells contribution at homeostasis is limited to, at least in mice, adipose tissues and control of certain pathogens in the gut. The fact that a few eosinophil-deficient individuals developed thymoma or autoimmune diseases suggest that, whatever additional contribution of the cells is to the organism, it might be of importance for maintenance of its homeostasis [31]. Below, eosinophils role in asthma will be described.

Several endotypes of asthma have similar symptoms

Asthma is an inflammatory disease of the respiratory tract. If the disease is left untreated, inflammatory and structural cells will release pro-inflammatory mediators and thus induce an acute inflammatory response that includes thickening of the bronchial mucosa with signs of epithelial injury, subepithelial basement thickening, smooth muscle hypertrophy and increased vascularization [32, 33]. Asthma was previously thought to be either an allergic or a non-allergic disease entity. It has, however, emerged differentiating into several subgroups with similar aetiology, but involving different cell types and with varying responsiveness to treatment [34–37]. Inflammatory cells such as eosinophils, mast cells, T lymphocytes and innate lymphoid cells are present in the inflammatory mucosa in various proportions. Inflammation in the airway guided by environmental factors and genetic predisposition results in interactions between these cells, epithelial and other stromal cells and the characteristic pathophysiology and symptoms of asthma. The heterogeneity of the disease was

established through transcriptomic profiling of cell in the lungs and through blockage of different cytokines unravelling different disease endotypes [38–42]. Although such endotypes are now established, a significant overlap exists.

Eosinophils contribute in varying degree to pathology of these different disease endotypes. It has been established that one asthma endotype is driven by Th2 cytokines and can have an onset early in life. It is driven by Th2 cytokines IL-4 and IL-13, inducing IgE antibodies to various allergens, which activate mast cells, degranulation and recruitment of other immune cells [41, 43]. Eosinophils are present, and their contribution can be varying dependent of the severity of the disease. This corticosteroid responsive asthma phenotype with subepithelial fibrosis often develops from or is comorbid to allergic rhinitis and can be associated with obesity [44, 45]. Adult-onset asthma endotype on the other hand is often more severe, eosinophil dominated and can be associated with rhinosinusitis [41, 46]. Although also Th2 cytokines associate to some degree, this asthma phenotype is less responsive to corticosteroid treatment and evidence indicates that severe forms might be driven by IL-33-producing epithelial cells sometimes in combination with interleukin (IL)-13- and IL-5-producing innate lymphoid cells [47, 48]. In about 50% of asthmatic patients (5% of children), the asthma disease is allergen independent [38, 42]. These patients' disease is relatively steroid non-responsive and also heterogeneous. The disease might be driven by neutrophilic inflammation IL-17, IL-1 β , intrinsic defects in barrier function or by chronic subclinical infection by viruses or atypical intracellular bacteria [38, 42]. Although this type of asthma rarely involves eosinophils, it has many features similar to eosinophil-rich asthma.

The aim of this review was to discuss the contribution of the eosinophils to asthma endotypes. We will review our previous studies assessing the eosinophil granulocyte in allergic diseases of the respiratory tract in the context of the novel knowledge of the diseases. In these disorders, eosinophil activation, degranulation and granule protein deposition have been implicated causing tissue damage [3, 40].

Eosinophils in different asthma endotypes

The heterogeneity of asthma was established when transcriptional profiling of epithelial cells from patients and correlation with fraction exhaled nitric oxide FeNO identified several different subgroups [49]. Asthma can present during childhood triggered by allergen exposure [50, 51]. Patients with early-onset asthma have more skin prick test-positive reactions and poorer lung function [52]. This endotype is often associated with rhinitis [36]. Th2 lymphocytes are central to the pathogenesis, producing IL-4, IL-5 and IL-13. IL-4 promotes B cells antibody class switching to the IgE subtype, and the cytokine activates

T cells. IL-13's activity overlap with IL-4's, as the cytokine has a shared receptor subunit but IL-13 do not activate B cells. Both cytokines also induce goblet cell hyperplasia and airway hyper-reactivity [53, 54]. Genomewide association studies (GWAS) indicate that also an innate immune response is important for the development of asthma. The genes for the alarmin IL-33 and its receptors, *IL33* and *IL1RL1*, and polymorphisms in those genes were linked to the disease [55–57]. Furthermore, the IL-33 receptor ST2L has been detected in the plasma of asthmatics associated with blood eosinophilia and IL-33 [58, 59]. IL-33 originates from damaged epithelial cells, fibroblast and other cells. Animal studies have shown that stimulated epithelial stem cell progenitors can acquire altered epigenetic state and could thereby by the production of IL-33 drive a chronic airway disease independent of the immune system [60]. Group 2 innate lymphoid cells (ILC2) are present in the respiratory tract responding to IL-33 [61, 62]. ILC2 constitute only a small portion of the innate cells in lung, but they are centrally located along the airways [63]. Eosinophils entering from the blood are also responding to IL-33 and are a constituent of the early-onset Th2 asthma endotype. Also haematopoietic stem cell progenitors have been identified in the lung that can differentiate to eosinophils [64]. Progenitors proliferate in response to IL-5, and mature eosinophils maintain survival by cytokine. IL-5 is secreted by Th2 lymphocytes, mast cells and ILC2 [7]. ILC2 produce IL-13 as well and can therefore promote both eosinophilia and goblet cell hyperplasia [65].

Use of Th2 targeting biologics questioned the role of eosinophils in asthma

One of the most effective treatments of allergic asthma today is locally administered corticosteroids. As this treatment has shown to reduce eosinophil numbers in mucosa it was thought that eosinophils were the target for the treatment [66–68]. However, several clinical trials targeting Th2 effector molecules have come to question this assumption. One study using mepolizumab resulted in reduced peripheral eosinophil numbers, but the treatment showed no anti-asthmatic efficacy (Table 2) [69]. Another study that enrolled and treated asthmatic patients with elevated eosinophils using an anti-IL-4 receptor alpha antibody (dupilumab) showed improved lung function in response to allergen inhalation but no change in eosinophil numbers [70] (Table 2). Corren *et al.* [39] employed the anti-IL13 antibody lebrikizumab, and blood eosinophil numbers increased in response to treatment, which was explained by induced inhibition of migration of eosinophils to the lungs. Lebrikizumab showed modest but significant improvement in forced expiratory volume in one-second (FEV₁) and favourable changes in FeNO in a subgroup of patients with high periostin. The response did,

however, not correlate with IgE or atopy [39] (Table 2). In a second study with two Lebrikizumab trials pooled, a non-significant improvement in FEV₁ and exacerbations could be found again in a subgroup of patients with high periostin levels [71]. Periostin influence many aspects of allergic inflammation, such as eosinophil recruitment, airway remodelling, development of a Th2 phenotype and increased expression of inflammatory mediators [72]. Periostin levels in serum correlate well with number of eosinophils present in bronchi [73].

Taken together, these studies suggest that the contribution of eosinophils, although correlating with the level of periostin and FeNO in some but not all studies [49, 71, 74], may therefore be limited or at least varying for this early-onset asthma endotype. This conclusion is also based on the fact that mepolizumab not was efficacious for patients with mild asthma (Table 2) [69]. Studies indicate that the disease is driven by interplay between Th2 lymphocyte cytokines, epithelial cells and innate cells such as ILC2, perpetuating the inflammation in the asthmatic lung [75]. However, eosinophils may be used as marker of disease severity and response to treatment [76]. Findings from these studies highlight the difficulties sometimes encountered to discriminate the different endotypes.

Targeting IL-5 identified eosinophil-dependent asthma endotype

Use of biologic therapy targeting IL-5 has discriminated an endotype of asthma different from early-onset allergic asthma, which might constitute of up to 50% of cases and is less dependent of Th2 response [42]. This endotype has an average onset later in life than Th2 type asthma, and is more severe and often corticosteroid resistant. Although IgE antibodies might be present, the allergic reaction which is taking place is different from that in the early-onset asthma. High number of the eosinophils in lungs and sputum has been shown to be a key indicator for this type of asthma [77–80]. The bone marrow-derived eosinophils migrate from the blood to the respiratory tract in response to chemotactic signals or are differentiating from haematopoietic progenitors *in situ* [64]. The cells remain there prevented from apoptosis by IL-5 and other factors [3, 6]. Treatment of patient cohorts with severe asthma (unlike patients with mild asthma) using mepolizumab or the anti-IL5RA ab benralizumab reduced their exacerbations, suggesting an important pathological role of the eosinophils in this disease endotype [40, 69, 80] (Table 2). The level of eosinophils was found to be a good marker for responsiveness being reduced during course of the treatment. The treatment with mepolizumab for 1 year also resulted in reduced airway wall thickness as determined by CT scan [40]. Re-analysis of our old electron microscopy results indicates that eosinophils from patients with eosinophil-rich asthma are activated with morphological

Table 2 Key studies assessing biologics therapy for asthma- and eosinophil-associated responses. Modified from Murkherjee *et al.* [124].

Biologic	Dosage	Inclusion criteria, eosinophils	Eosinophil changes	Outcome	Reference
Mepolizumab	10 mg/kg once	Mild atopic asthma, blood eos $>0.2 \times 10^9/l$	Blood eos at day 29, $0.04 \times 10^9/l$, placebo $0.29 \times 10^9/l$	No effect	[69]
	750 mg, iv/month, 20 weeks	Severe persistent asthma with eosinophilia, sputum eos $>3\%$, (blood eos $0.35\text{--}0.66 \times 10^9/l$, ***)	Blood eos reduced to $0.05\text{--}0.08 \times 10^9/l$, sputum eos reduced to $0\text{--}1.3\%$, $P < 0.05$	Reduced exacerbations $P < 0.01$, FEV ₁ and ACQ-5 improved, $P = 0.01$	[77]
	750 mg, 12 iv/month, 50 weeks	Refractory eosinophilic severe asthma, sputum eos 3% (blood eos $0.34 \times 10^9/l$ ***)	Blood eos reduced 6.6-fold sputum eos reduced 7.1-fold, $P < 0.0002$	Reduced exacerbations, $P = 0.02$, AQLQ improved, $P = 0.02$	[40]
	75–750 mg, sc/month, 52 weeks	Severe refractory asthma with >2 exacerbations/year, blood eos $0.2 \times 10^9/l$ or sputum eos $>3\%*$	Blood eos reduced to $0.22\text{--}0.12 \times 10^9/l$ $P < 0.0001$, sputum eos ratio $0.68\text{--}0.12**$, $P = 0.0082$	Reduced exacerbations, $P = 0.0005$ or less	[78]
	75 mg iv or 100 mg sc/month, 32 weeks	Severe asthma with ≥ 2 exacerbations, blood eos $0.15 (0.3)^a \times 10^9/l$	iv: 83% reduction, sc: 86% reduction, $P < 0.001$	Reduced exacerbations $P < 0.001$, FEV ₁ and asthma scores improved, $P < 0.05$	[125]
	100 mg, sc/month, 20 weeks	Severe eosinophilic asthma eos $0.15 (0.3)^a \times 10^9/l$	Blood eos reduced, $P < 0.001$	50% reduced use of OCS, $P < 0.007$, exacerbations reduced by 32%, $P = 0.04$, improved ACQ-5 score, $P = 0.004$	[126]
Reslizumab	30 mg, sc/month, 12 weeks	Poorly controlled asthma high dose of ICS, sputum eos 3% (blood eos $0.5 \times 10^9/l$ ***)	Blood and sputum eos, reduced, $P < 0.0001$ and $P = 0.0068$ (38.7%), respectively	Trend towards reduced exacerbations, $P = 0.083$, reduced FEV ₁ , $P = 0.002$, improved ACQ-5 in patients with polyps, $P = 0.01$	[91]
Benralizumab	2–100 mg, sc/month, 28 weeks	Uncontrolled asthma ≥ 2 exacerbations/year, FeNO >50 ppm	Blood eos reduced to $0.05 \times 10^9/l$ for all doses	Subgroup of patients with blood eos $>0.3 \times 10^9/l$ had reduced exacerbations in response to 20 and 100 mg dose, $P = 0.049$	[80]
	Single 0.3–1 mg/kg iv infusion	Subjects attending ED with asthma exacerbation and partial response to treatment ≥ 1 exacerbations/year	Reduced blood eos, Serum ECP decline from 26.1 to 8.6 $\mu g/l$	Reduced exacerbation rate, $P = 0.02$, visits to ED reduced, $P = 0.02$	[81]
Lebrikizumab	250 mg, sc/month, 24 weeks	Uncontrolled asthma ACQ5 1.5 or higher Th2 hi: more than $0.14 \times 10^9/l$ eos or IgE 100 IU/ml	Blood eos increased, $P = 0.01$	Periostin-positive patients had increased FEV ₁ , $P = 0.03$ Th2 hi subgroup had reduced exacerbations, $P = 0.03$	[39]
	37.5–250 mg, sc/month, 24 weeks	Uncontrolled asthma ACQ-5 ≥ 1.5 and FEV ₁ 40–80% of predicted	Blood eos increased to $0.24 \times 10^9/l$	In periostin hi group 60% reduced exacerbations and 9.1% improved FEV ₁	[71]
Dupilumab	300 mg, sc/month, 12 weeks	Persistent moderate-to-severe asthma, blood eos $0.3 \times 10^9/l$ or sputum eos $3\%*$	No change	87% reduction in exacerbations, $P = 0.001$, FEV ₁ improved, $P < 0.0001$, ACQ5 improvement, $P = 0.001$	[70]

sc, subcutaneous; iv, intravenous; ICS, inhaled corticosteroids; OCS, oral corticosteroids; FEV₁, forced expiratory volume in 1 s; AQLQ, Asthma Quality of Life Questionnaire; ACQ-5, Asthma Control Questionnaire-5; ED, emergency department.

^aOr 0.3×10^9 eos at least once during the previous year. *** Not inclusion criteria

changes. Figure 1B shows an activated eosinophil from an allergic patient whose granules show signs of disintegration. Granule proteins released from the activated eosinophils can be measured in the bronchoalveolar lavage

fluid (BALF) [34]. The released granule proteins might contribute to the symptoms seen in this endotype (see the next section). As benralizumab target IL-5RA, it might facilitate cell killing by antibody-dependent cell-mediated

cytotoxicity or act on basophils that also have the IL-5RA [80]. Further evidence that eosinophils are responsible for asthmatics exacerbations was gained in a study where patients with asthma and severe exacerbations encountering an emergency department improved in response to benralizumab [81]. Although the eosinophil-rich asthma phenotype has been found to be associated with FeNO [49], when mepolizumab was used to improve disease, no reduction in FeNO was achieved [40]. Anti-inflammatory therapy guided by FeNO was also shown to reduce only moderate but not severe exacerbations [82]. Furthermore, we have shown that that high level of blood eosinophils better discriminated patients with visits to the emergency department than what FeNO can do, suggesting that the specific severe character of eosinophil-dominated asthma is not directly related to FeNO level (Fig. 3) [74].

Eosinophil-dependent asthma endotype might be driven by abhorrent response to pathogens

As is the case with early-onset asthma, the epithelium probably plays an important role and released IL-33 might activate eosinophils, ILC2 and other innate immune cells during severe asthma. A GWAS correlating SNPs with level of IL-5 in the blood identified an association with the IL-33 receptor gene, highlighting the important linkage between eosinophils innate response and ILC2s [83]. Furthermore, IL-33 stimulated ILC2 from peripheral blood from asthmatic patients where more active than ILC2 from rhinitis patients and healthy controls, responding with production of IL-5 and IL-13 [48]. The lack of response to corticoid treatment in eosinophil-dominated late-onset asthma might be explained by the inflammation driven by IL-33 as this cytokine is produced by epithelial cells and smooth muscle cells that do not respond to such treatment [60, 84].

It is still not unclear how the eosinophil-dominated asthma endotype develops. It might be sequelae of an abhorrent response to an infection. The presence of rhinovirus was for example correlated with increased levels of peripheral blood eosinophils as well as eosinophilic infiltration of the mucosal tissue of asthmatics. It was suggested rhinovirus infection under certain conditions can stimulate local cytokine production of eotaxin, eotaxin-2, RANTES (regulated upon activation, normal T cell expressed and secreted), and GM-CSF to attract and stimulate eosinophils [85]. ILC2 is involved in viral pathogen fighting [86]. It has been proposed that a dysregulated immune response to the viral infection can lead to asthma development [63].

Severe eosinophil-dominated asthma is also associated with chronic rhinosinusitis (CRS) and aspirin sensitivity [41]. One study found that ILC2 frequencies were associated with the presence of nasal polyps and tissue eosinophilia. In the CRS with nasal polyps population,

ILC2s were increased in patients with coexisting asthma [87, 88]. There were no significant associations found between ILC2s and serum total IgE and allergic disease. The correlation between CRS and asthma is, however, not universally agreed upon [89]. Mepolizumab showed possibly efficacy in reducing polyp size of CRS patients [90]. Reslizumab was also most effective in patients with polyps (Table 2) [91].

Evidence therefore supports that eosinophil-rich asthma can be induced by abhorrent responses to pathogens such as respiratory viruses and develop subsequent to occurrence of polyps. The aetiology associated with this endotype is surprisingly similar to non-allergic asthma driven by neutrophils [38, 42].

Pathologic features of eosinophils, accumulation and granule release

The clinical studies have shown that eosinophils in eosinophil-rich asthma are responsible for exacerbations, probably fibrosis and in certain cases reduced FEV₁ [40, 80]. It not known what specific signals that are attracting eosinophils in the eosinophil-rich endotype. The mechanism by which the cells confer pathology is also largely unknown. The accumulation of eosinophils is a consequence of the release of pro-inflammatory and chemotactic mediators, such as prostaglandin D₂, leukotrienes and eotaxin during the acute phase (Fig. 2) [13, 92–94]. Interaction between very late activation antigen (VLA)-4 on eosinophils and its ligand, vascular cellular adhesion molecule (VCAM)-1, on endothelial cells induces the selective recruitment of the cells into the airways [95–98]. Immunohistochemical studies have shown increased expression of VCAM-1 in asthmatic airways [96, 98]. Also basophils have the IL-5 receptor and might be influenced by the cytokine. A recent study has shown that basophils upregulate VCAM-1 expression in the endothelial cells, which thereby facilitate eosinophil transmigration. When endothelium-transmigrating basophils bound IgE, the cells slowed down and released IL-4. This induced VCAM-1 expression on endothelial cells and facilitated eosinophil influx [99]. Hence, basophils might exacerbate to tissue eosinophilia.

Although eosinophils can release acute phase reactants such as CTL4 they are foremost considered part of the late-phase response to allergic stimulation. Patients with this asthma endotype benefited from treatment with mepolizumab, reslizumab or benralizumab with reduced exacerbations [39, 40, 78, 91]. Eosinophil-dependent exacerbations can result from localized damage to the respiratory epithelium and IL-33 release, which is typically seen in biopsies from lungs of asthmatic patients (Fig 2) [100, 101]. Released granule proteins have been proposed to cause such damage to cells of the airways. The granule proteins can be liberated from their granules both during

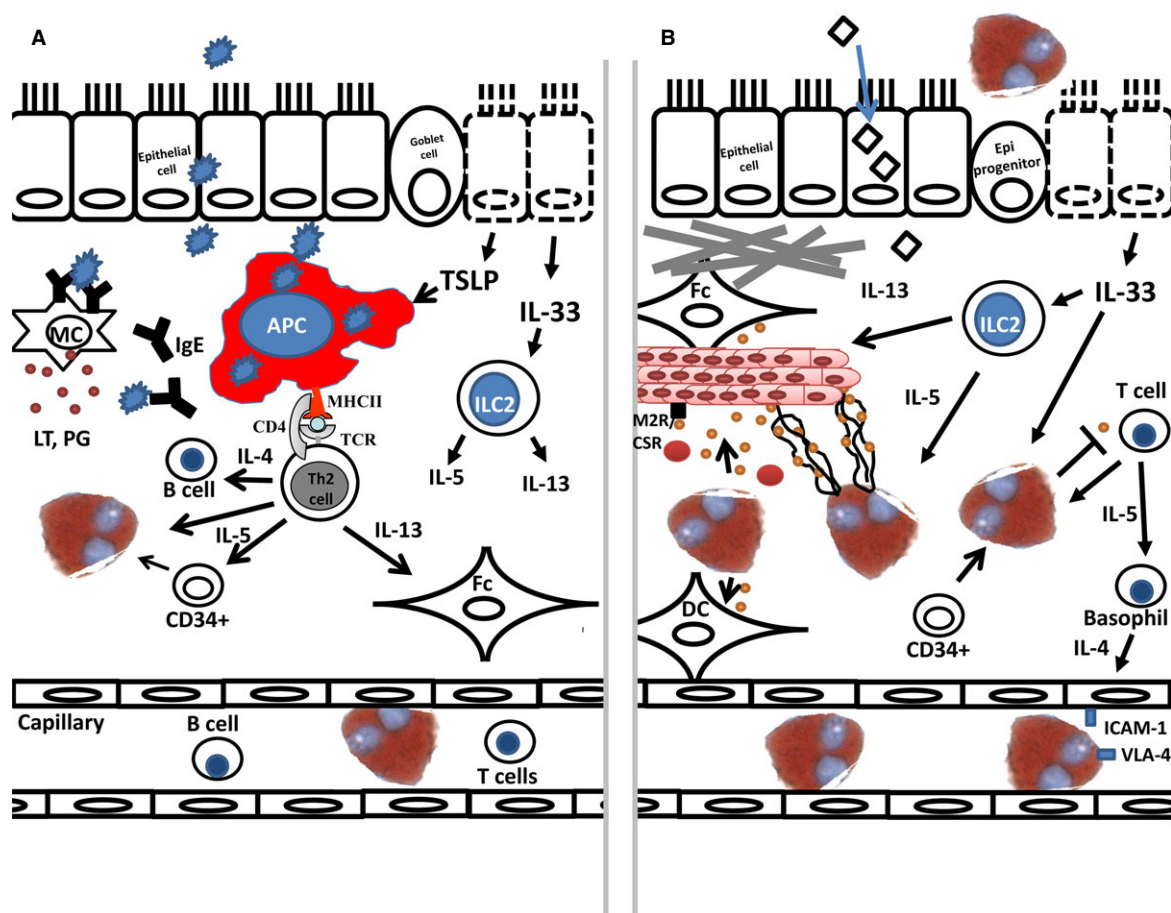


Figure 2 Schematic description of basic cellular interactions during inflammation in the lung mucosa in two asthma endotypes. (A) The early-onset asthma endotype. Asthma is induced by allergens presented by antigen-presenting cells to recognizing Th2 cells. These cells release IL-4, IL-13 and IL-5. IL-4 stimulate B cell class switch to allergen-specific IgE production. IgE binds Fc receptors on mast cells and when cross-linked by allergens induce release of cytokines and lipid mediators. IL-5 stimulates eosinophil survival and maturation from CD34+ progenitors. IL-13 stimulates fibrosis and mucus production. Also ILC2 releases IL-5 and IL-15. Damaged epithelial cells release IL-33 and TSLP that stimulate innate immune cells perpetuating disease development. (B) The eosinophil-rich asthma endotype. It is hypothesized that the disease develops as a consequence of an abhorrent response to a viral infection or subsequent to CRS with polyps. Due to epigenetic modifications, epithelial cell progenitors can expand and drive disease independent of allergen stimulation. IL-33 released from such cells and damaged epithelial cells stimulate ILC2 and eosinophils. The eosinophils are activated (by interactions with cytokines, lipid mediators, immunoglobulins, and complement fragments) and release DNA and granule content. Degranulation can take place by piecemeal degranulation, release of whole granules or by cytolysis of the cells. Degranulation products (e.g. granule proteins, lipid mediators, proteases and cytokines) are augmenting and disrupting cells in the bronchial epithelium. MBP binds muscarinic M2 receptor while ECP binds calcium-sensing receptor (CSR) on smooth muscle cells. High level of ECP influences fibroblasts inducing fibrosis while low level inhibit T lymphocytes. EPX/EDN has shown to influence dendritic cell migration. Eosinophils are maturing in the bone marrow or differentiating from CD34+ progenitors *in situ*. The cells undergo transmigration from the endothelial cell layer into the connective tissues. Basophils can facilitate eosinophil migration by inducing specific adhesion molecules on endothelial cells.

the process of piecemeal degranulation, or by rupture of cells, cytolysis [32, 102, 103]. Whole granules have been found released from eosinophils in allergic airway disease correlating with severity of disease [104]. Another type of release described for eosinophils is the expulsion of mitochondrial DNA. The DNA which is coated with granule proteins is thought to be an antimicrobial defence mechanism trapping the pathogens but has been reported as well in allergic skin and airway diseases [105, 106]. The released DNA protein complexes might cause damage to epithelial cells in the airways. Recently, also the

simultaneous release of eosinophil chromosomal DNA and granules was reported. This process was found to be NADPH dependent and was termed eosinophil extracellular DNA trap cell death [107]. Once released, a vast array of effects by the granule proteins on the different cells in the respiratory mucosa have been described that could all contribute to asthmatic exacerbations, remodelling and hyper-reactivity.

MBP has been reported localized to damaged sites of bronchial epithelium in patients of asthma, with its concentration correlated with the severity of bronchial

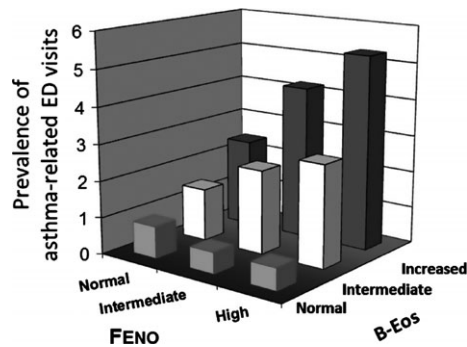


Figure 3 Prevalence of ED visits according to normal, intermediate, or high FeNO and blood eosinophil values. Permission to reprint this figure was given by Elsevier Limited.

hyper-reactivity [108]. Epithelial damage, as well as mucus production and increased vascular permeability, has been reported [109–112] as well as modulation of the production of mediators from epithelial cells (Fig. 3). Loss of epithelial integrity due to eosinophilic mediators could lead to exposure of sensory nerves [112, 113]. MBP has been suggested to possess pro-angiogenic activity and directly altering the airway smooth muscle contraction response by binding to and antagonizing response of vagal muscarinic M2 receptor [108, 114, 115]. Furthermore, instillation of human MBP and EPO provokes bronchoconstriction, and MBP increases airway responsiveness to inhaled methacholine [116]. EPX/EDN on the other hand has been suggested to attract dendritic cells to the airways [117]. Similarly, ECP has been shown to influence a number of cell types present in the respiratory tract. Low level of the protein was shown to be immunomodulatory while high level was found to damage the epithelium [16]. ECP activate a calcium-sensing receptor on airway smooth muscle cells. Asthmatic patients and allergen-sensitized mice expressed high level of this receptor [118]. ECP was also shown to induce fibrosis by acting on fibroblasts, which might relate to reduced air way wall thickness reported in response to mepolizumab [40, 119, 120]. The cytokines and lipid mediators produced by the eosinophils can contribute to the asthma and rhinosinusitis disease pathology and have been suggested to act in autocrine fashion [121, 122]. There is therefore an abundance of evidence, indicating that the presence of eosinophils can cause pathology leading to exacerbations in eosinophil-dominated airway disease.

Conclusions

In this review, the focus has been the contribution of eosinophils to the airway disease. While eosinophilic inflammation is one of the features of the Th2 inflammation which is best associated with symptoms and treatment response in early-onset allergic asthma paradoxically, the eosinophil seems to be of limited importance for the

pathology of this disease endotype (Fig. 2A). In eosinophil-dominated late-onset asthma endotype on the other hand, the pathology is probably to a large extent driven by eosinophils. After recruitment, the cells likely induce damage to the airway mucosa and the associated nerves by the release of cytotoxic granule proteins and lipid mediators (Fig. 2B). Eosinophils contain several basic and cytotoxic granule proteins that are released upon activation. A number of effects related to these granule proteins, including damaging endothelial cells, attraction of dendritic cells, inhibition of a muscarinic receptors and induction of fibrosis, have been described. That this may lead to hyperactivity, exacerbations and airway wall thickness is supported by the results of several clinical studies using biologic therapies targeting IL-5. New therapeutic approaches for the treatment of allergic diseases, such as asthma and rhinitis, could be aided by the development of agents that target the eosinophils and their ability to release their toxic contents.

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Conflict of interest

The authors declare no conflict of interests.

Author contributions

Kawa Amin, Christer Janson and Jonas Bystrom contributed equally with ideas and writing for the completion of this manuscript.

References

- 1 Erlich P. Ueber die spezifischen Granulationen des Blutes. *Arch Anat Physiol* 1879;3:571–9.
- 2 Chu VT, Fröhlich A, Steinhäuser G *et al.* Eosinophils are required for the maintenance of plasma cells in the bone marrow. *Nat Immunol* 2011;12:151–9.
- 3 Carlson M, Peterson C, Venge P. The influence of IL-3, IL-5, and GM-CSF on normal human eosinophil and neutrophil C3b-induced degranulation. *Allergy* 1993;48:437–42.

- 4 Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. *Blood* 1989;73:1504–12.
- 5 Clutterbuck EJ, Sanderson CJ. Regulation of human eosinophil precursor production by cytokines: a comparison of recombinant human interleukin-1 (rhIL-1), rhIL-3, rhIL-5, rhIL-6, and rh granulocyte-macrophage colony-stimulating factor. *Blood* 1990;75:1774–9.
- 6 Foster PS, Hogan SP, Ramsay AJ *et al*. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195–201.
- 7 Nussbaum JC, Van Dyken SJ, von Moltke J *et al*. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* 2013;502:245–8.
- 8 Wu D, Molofsky AB, Liang HE *et al*. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* 2011;332:243–7.
- 9 Molofsky AB, Nussbaum JC, Liang HE *et al*. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med* 2013;210:535–49.
- 10 Lee MW, Odegaard JI, Mukundan L *et al*. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell* 2015;160:74–87.
- 11 Qiu Y, Nguyen KD, Odegaard JI *et al*. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell* 2014;157:1292–308.
- 12 Chu VT, Beller A, Rausch S *et al*. Eosinophils promote generation and maintenance of immunoglobulin-A-expressing plasma cells and contribute to gut immune homeostasis. *Immunity* 2014;40:582–93.
- 13 Hogan SP, Rosenberg HF, Moqbel R *et al*. Eosinophils: biological properties and role in health and disease. *Clin Exp Allergy* 2008;38:709–50.
- 14 Tai PC, Spry CJ, Peterson C *et al*. Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. *Nature* 1984;309:182–4.
- 15 Wagner JM, Franco M, Kephart GM *et al*. Localization of eosinophil granule major basic protein in paracoccidioidomycosis lesions. *Am J Trop Med Hyg* 1998;59:66–72.
- 16 Bystrom J, Amin K, Bishop-Bailey D. Analysing the eosinophil cationic protein – a clue to the function of the eosinophil granulocyte. *Respir Res* 2011;12:10.
- 17 Olsson I, Venge P. Cationic proteins of human granulocytes. II. Separation of the cationic proteins of the granules of leukemic myeloid cells. *Blood* 1974;44:235–46.
- 18 Peterson CG, Venge P. Purification and characterization of a new cationic protein–eosinophil protein-X (EPX) – from granules of human eosinophils. *Immunology* 1983;50:19–26.
- 19 Gleich GJ, Loegering DA, Bell MP *et al*. Biochemical and functional similarities between human eosinophil-derived neurotoxin and eosinophil cationic protein: homology with ribonuclease. *Proc Natl Acad Sci USA* 1986;83:3146–50.
- 20 Carlson MG, Peterson CG, Venge P. Human eosinophil peroxidase: purification and characterization. *J Immunol* 1985;134:1875–9.
- 21 Gleich GJ, Loegering DA, Mann KG *et al*. Comparative properties of the Charcot-Leyden crystal protein and the major basic protein from human eosinophils. *J Clin Invest* 1976;57:633–40.
- 22 Turk J, Maas RL, Brash AR *et al*. Arachidonic acid 15-lipoxygenase products from human eosinophils. *J Biol Chem* 1982;257:7068–76.
- 23 Cromwell O, Moqbel R, Fitzharris P *et al*. Leukotriene C4 generation from human eosinophils stimulated with IgG-*Aspergillus fumigatus* antigen immune complexes. *J Allergy Clin Immunol* 1988;82:535–43.
- 24 El-Benna J, Dang PM, Gougerot-Pocidalo MA. Priming of the neutrophil NADPH oxidase activation: role of p47phox phosphorylation and NOX₂ mobilization to the plasma membrane. *Semin Immunopathol* 2008;30:279–89.
- 25 Goh YP, Henderson NC, Heredia JE *et al*. Eosinophils secrete IL-4 to facilitate liver regeneration. *Proc Natl Acad Sci USA* 2013;110:9914–9.
- 26 Todd R, Donoff BR, Chiang T *et al*. The eosinophil as a cellular source of transforming growth factor alpha in healing cutaneous wounds. *Am J Pathol* 1991;138:1307–13.
- 27 Rivoltini L, Viggiano V, Spinazze S *et al*. In vitro anti-tumor activity of eosinophils from cancer patients treated with subcutaneous administration of interleukin 2. Role of interleukin 5. *Int J Cancer* 1993;54:8–15.
- 28 Carretero R, Sektioglu IM, Garbi N *et al*. Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. *Nat Immunol* 2015;16:609–17.
- 29 von Wasielewski R, Seth S, Franklin J *et al*. Tissue eosinophilia correlates strongly with poor prognosis in nodular sclerosing Hodgkin's disease, allowing for known prognostic factors. *Blood* 2000;95:1207–13.
- 30 Ogbogu PU, Bochner BS, Butterfield JH *et al*. Hypereosinophilic syndrome: a multicenter, retrospective analysis of clinical characteristics and response to therapy. *J Allergy Clin Immunol* 2009;124:1319–25.
- 31 Gleich GJ, Klion AD, Lee JJ *et al*. The consequences of not having eosinophils. *Allergy* 2013;68:829–35.
- 32 Amin K, Ludviksdottir D, Janson C *et al*. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. *Am J Respir Crit Care Med* 2000;162:2295–301.
- 33 Amin K, Rinne J, Haahtela T *et al*. Inflammatory cell and epithelial characteristics of perennial allergic and nonallergic rhinitis with a symptom history of 1 to 3 years' duration. *J Allergy Clin Immunol* 2001;107:249–57.
- 34 Nair P. What is an "eosinophilic phenotype" of asthma? *J Allergy Clin Immunol* 2013;132:81–3.
- 35 Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* 2008;372:1107–19.
- 36 Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;18:716–25.
- 37 Rondon C, Campo P, Togias A *et al*. Local allergic rhinitis: concept, pathophysiology, and management. *J Allergy Clin Immunol* 2012;129:1460–7.
- 38 Raedler D, Ballenberger N, Klucker E *et al*. Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. *J Allergy Clin Immunol* 2015;135:81–91.
- 39 Corren J, Lemanske RF, Hanania NA *et al*. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088–98.
- 40 Haldar P, Brightling CE, Hargadon B *et al*. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;360:973–84.
- 41 Haldar P, Pavord ID, Shaw DE *et al*. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008;178:218–24.
- 42 Woodruff PG, Modrek B, Choy DF *et al*. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;180:388–95.
- 43 Amin K. The role of mast cells in allergic inflammation. *Respir Med* 2012;106:9–14.
- 44 Desai D, Newby C, Symon FA *et al*. Elevated sputum interleukin-5 and submucosal eosinophilia in obese individuals with severe asthma. *Am J Respir Crit Care Med* 2013;188:657–63.
- 45 Spector SL. Overview of comorbid associations of allergic rhinitis. *J Allergy Clin Immunol* 1997;99:S73–80.
- 46 Jarvis D, Newson R, Lotvall J *et al*. Asthma in adults and its association with chronic rhinosinusitis: the GA2LEN survey in Europe. *Allergy* 2012;67:91–8.

- 47 Mjosberg JM, Trifari S, Crellin NK *et al.* Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR2 and CD161. *Nat Immunol* 2011;12:1055–62.
- 48 Bartemes KR, Kephart GM, Fox SJ *et al.* Enhanced innate type 2 immune response in peripheral blood from patients with asthma. *J Allergy Clin Immunol* 2014;134:671–8.
- 49 Modena BD, Tedrow JR, Milosevic J *et al.* Gene expression in relation to exhaled nitric oxide identifies novel asthma phenotypes with unique biomolecular pathways. *Am J Respir Crit Care Med* 2014;190:1363–72.
- 50 Ernst P, Ghezzi H, Becklake MR. Risk factors for bronchial hyperresponsiveness in late childhood and early adolescence. *Eur Respir J* 2002;20:635–9.
- 51 Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. *Birth Defects Res B Dev Reprod Toxicol* 2008;83:547–60.
- 52 Moore WC, Meyers DA, Wenzel SE *et al.* Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;181:315–23.
- 53 Solomon A, Puxeddu I, Levi-Schaffer F. Fibrosis in ocular allergic inflammation: recent concepts in the pathogenesis of ocular allergy. *Curr Opin Allergy Clin Immunol* 2003;3:389–93.
- 54 Perkins C, Wills-Karp M, Finkelman FD. IL-4 induces IL-13-independent allergic airway inflammation. *J Allergy Clin Immunol* 2006;118:410–9.
- 55 Moffatt MF, Gut IG, Demenais F *et al.* A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;363:1211–21.
- 56 Traister RS, Uvalle CE, Hawkins GA, Meyers DA, Bleecker ER, Wenzel SE. Phenotypic and genotypic association of epithelial IL1RL1 to human T2-like asthma. *J Allergy Clin Immunol* 2015;135:92–9.
- 57 Ramasamy A, Kuokkanen M, Vedantam S *et al.* Genome-wide association studies of asthma in population-based cohorts confirm known and suggested loci and identify an additional association near HLA. *PLoS One* 2012;7:e44008.
- 58 Kurowska-Stolarska M, Kewin P, Murphy G *et al.* IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol* 2008;181:4780–90.
- 59 Rogala B, Gluck J. The role of interleukin-33 in rhinitis. *Curr Allergy Asthma Rep* 2013;13:196–202.
- 60 Byers DE, Alexander-Brett J, Patel AC *et al.* Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. *J Clin Invest* 2013;123:3967–82.
- 61 Kim BS, Siracusa MC, Saenz SA *et al.* TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med* 2013;5:170ra16.
- 62 Kim BS, Wojno ED, Artis D. Innate lymphoid cells and allergic inflammation. *Curr Opin Immunol* 2013;25:738–44.
- 63 Barnig C, Cernadas M, Dutile S *et al.* Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci Transl Med* 2013;5:174ra26.
- 64 Radinger M, Bossios A, Sjostrand M *et al.* Local proliferation and mobilization of CCR3(+) CD34(+) eosinophil-lineage-committed cells in the lung. *Immunology* 2011;132:144–54.
- 65 Barlow JL, Peel S, Fox J *et al.* IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol* 2013;132:933–41.
- 66 Wardlaw AJ. Eosinophils in the 1990s: new perspectives on their role in health and disease. *Postgrad Med J* 1994;70:536–52.
- 67 Walters EH, Gardiner PV. Bronchoalveolar lavage findings and bronchial hyperreactivity. *Respiration* 1992;59:6–8.
- 68 Djukanovic R, Wilson JW, Britten KM *et al.* Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. *Am Rev Respir Dis* 1992;145:669–74.
- 69 Leckie MJ, ten Brinke A, Khan J *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144–8.
- 70 Wenzel S, Ford L, Pearlman D *et al.* Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med* 2013;368:2455–66.
- 71 Hanania NA, Noonan M, Corren J *et al.* Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies. *Thorax* 2015;70:748–56.
- 72 Li W, Gao P, Zhi Y *et al.* Periostin: its role in asthma and its potential as a diagnostic or therapeutic target. *Respir Res* 2015;16:57.
- 73 Jia G, Erickson RW, Choy DF *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012;130:647–54.
- 74 Malinovski A, Fonseca JA, Jacinto T *et al.* Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subjects. *J Allergy Clin Immunol* 2013;132:821–7.
- 75 Klein Wolterink RG, Hendriks RW. Type 2 innate lymphocytes in allergic airway inflammation. *Curr Allergy Asthma Rep* 2013;13:271–80.
- 76 Lacoste JY, Bousquet J, Chanez P *et al.* Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis, and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 1993;92:537–48.
- 77 Nair P, Pizzichini MM, Kjarsgaard M *et al.* Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985–93.
- 78 Pavord ID, Korn S, Howarth P *et al.* Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;380:651–9.
- 79 Chung KF. New treatments for severe treatment-resistant asthma: targeting the right patient. *Lancet Respir Med* 2013;1:639–52.
- 80 Castro M, Wenzel SE, Bleecker ER *et al.* Benralizumab, an anti-interleukin 5 receptor alpha monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. *Lancet Respir Med* 2014;2:879–90.
- 81 Nowak RM, Parker JM, Silverman RA *et al.* A randomized trial of benralizumab, an anti-interleukin 5 receptor alpha monoclonal antibody, after acute asthma. *Am J Emerg Med* 2015;33:14–20.
- 82 Syk J, Malinovski A, Johansson G *et al.* Anti-inflammatory treatment of atopic asthma guided by exhaled nitric oxide: a randomized, controlled trial. *J Allergy Clin Immunol Pract* 2013;1:639–48.
- 83 Gudbjartsson DF, Bjornsdottir US, Halapi E *et al.* Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009;41:342–7.
- 84 Lambrecht BN, Hammad H. Asthma: the importance of dysregulated barrier immunity. *Eur J Immunol* 2013;43:3125–37.
- 85 Wos M, Sanak M, Soja J *et al.* The presence of rhinovirus in lower airways of patients with bronchial asthma. *Am J Respir Crit Care Med* 2008;177:1082–9.
- 86 Kumar RK, Foster PS, Rosenberg HF. Respiratory viral infection, epithelial cytokines, and innate lymphoid cells in asthma exacerbations. *J Leukoc Biol* 2014;96:391–6.
- 87 Ho J, Bailey M, Zaunders J *et al.* Group 2 innate lymphoid cells (ILC2s) are increased in chronic rhinosinusitis with nasal polyps or eosinophilia. *Clin Exp Allergy* 2015;45:394–403.
- 88 Miljkovic D, Bassiouni A, Cooksley C *et al.* Association between group 2 innate lymphoid cells enrichment, nasal polyps and allergy in chronic rhinosinusitis. *Allergy* 2014;69:1154–61.
- 89 Uhlarova B, Kopincova J, Kolomaznik M *et al.* Comorbidity has no impact on eosinophil inflammation in the upper airways or on

- severity of the sinonasal disease in patients with nasal polyps. *Clin Otolaryngol* 2015;40:429–36.
- 90 Gevaert P, Van Bruene N, Cattaert T *et al.* Mepolizumab, a humanized anti-IL-5 mAb, as a treatment option for severe nasal polyposis. *J Allergy Clin Immunol* 2011;128:989–95.
 - 91 Castro M, Mathur S, Hargreave F *et al.* Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am J Respir Crit Care Med* 2011;184:1125–32.
 - 92 Nagata M, Saito K. The roles of cysteinyl leukotrienes in eosinophilic inflammation of asthmatic airways. *Int Arch Allergy Immunol* 2003;131 (Suppl. 1):7–10.
 - 93 Tager AM, Dufour JH, Goodarzi K *et al.* BLTR mediates leukotriene B(4)-induced chemotaxis and adhesion and plays a dominant role in eosinophil accumulation in a murine model of peritonitis. *J Exp Med* 2000;192:439–46.
 - 94 Hirai H, Tanaka K, Yoshie O *et al.* Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 2001;193:255–61.
 - 95 Schleimer RP, Sterbinsky SA, Kaiser J *et al.* IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. *J Immunol* 1992;148:1086–92.
 - 96 Bentley AM, Durham SR, Robinson DS *et al.* Expression of endothelial and leukocyte adhesion molecules intercellular adhesion molecule-1, E-selectin, and vascular cell adhesion molecule-1 in the bronchial mucosa in steady-state and allergen-induced asthma. *J Allergy Clin Immunol* 1993;92:857–68.
 - 97 Zangrilli JG, Shaver JR, Cirelli RA *et al.* sVCAM-1 levels after segmental antigen challenge correlate with eosinophil influx, IL-4 and IL-5 production, and the late phase response. *Am J Respir Crit Care Med* 1995;151:1346–53.
 - 98 Fukuda T, Fukushima Y, Numao T *et al.* Role of interleukin-4 and vascular cell adhesion molecule-1 in selective eosinophil migration into the airways in allergic asthma. *Am J Respir Cell Mol Biol* 1996;14:84–94.
 - 99 Cheng LE, Sullivan BM, Retana LE *et al.* IgE-activated basophils regulate eosinophil tissue entry by modulating endothelial function. *J Exp Med* 2015;212:513–24.
 - 100 Young JD, Peterson CG, Venge P *et al.* Mechanism of membrane damage mediated by human eosinophil cationic protein. *Nature* 1986;321:613–6.
 - 101 Trautmann A, Schmid-Grendelmeier P, Kruger K *et al.* T cells and eosinophils cooperate in the induction of bronchial epithelial cell apoptosis in asthma. *J Allergy Clin Immunol* 2002;109:329–37.
 - 102 Erjefalt JS, Greiff L, Andersson M *et al.* Allergen-induced eosinophil cytotoxicity is a primary mechanism for granule protein release in human upper airways. *Am J Respir Crit Care Med* 1999;160:304–12.
 - 103 Persson C. Lysis of primed eosinophils in severe asthma. *J Allergy Clin Immunol* 2013;132:1459–60.
 - 104 Neves JS, Weller PF. Functional extracellular eosinophil granules: novel implications in eosinophil immunobiology. *Curr Opin Immunol* 2009;21:694–9.
 - 105 Simon D, Hoesli S, Roth N, Staedler S *et al.* Eosinophil extracellular DNA traps in skin diseases. *J Allergy Clin Immunol* 2011;127:194–9.
 - 106 Dworski R, Simon HU, Hoskins A *et al.* Eosinophil and neutrophil extracellular DNA traps in human allergic asthmatic airways. *J Allergy Clin Immunol* 2011;127:1260–6.
 - 107 Ueki S, Melo RC, Ghiran I, Spencer LA, Dvorak AM, Weller PF. Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion-competent eosinophil granules in humans. *Blood* 2013;121:2074–83.
 - 108 White SR, Ohno S, Munoz NM *et al.* Epithelium-dependent contraction of airway smooth muscle caused by eosinophil MBP. *Am J Physiol* 1990;259:L294–303.
 - 109 Jeffery PK, Wardlaw AJ, Nelson FC *et al.* Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;140:1745–53.
 - 110 Allegra L, Fabbri LM, Picotti G *et al.* Bronchial epithelium and asthma. *Eur Respir J Suppl* 1989;6:460–8.
 - 111 Naylor B. The shedding of the mucosa of the bronchial tree in asthma. *Thorax* 1962;17:69–72.
 - 112 Gleich GJ, Flavahan NA, Fujisawa T *et al.* The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. *J Allergy Clin Immunol* 1988;81:776–81.
 - 113 Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989;83:1013–26.
 - 114 Jacoby DB, Gleich GJ, Fryer AD. Human eosinophil major basic protein is an endogenous allosteric antagonist at the inhibitory muscarinic M2 receptor. *J Clin Invest* 1993;91:1314–8.
 - 115 Verbout NG, Lorton JK, Jacoby DB *et al.* Atropine pretreatment enhances airway hyperreactivity in antigen-challenged guinea pigs through an eosinophil-dependent mechanism. *Am J Physiol Lung Cell Mol Physiol* 2007;292:L1126–35.
 - 116 Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000;105:651–63.
 - 117 Yang D, Chen Q, Su SB *et al.* Eosinophil-derived neurotoxin acts as an alarmin to activate the TLR2-MyD88 signal pathway in dendritic cells and enhances Th2 immune responses. *J Exp Med* 2008;205:79–90.
 - 118 Yarova PL, Stewart AL, Sathish V *et al.* Calcium-sensing receptor antagonists abrogate airway hyperresponsiveness and inflammation in allergic asthma. *Sci Transl Med* 2015;7:284ra60.
 - 119 Hernnas J, Sarnstrand B, Lindroth P *et al.* Eosinophil cationic protein alters proteoglycan metabolism in human lung fibroblast cultures. *Eur J Cell Biol* 1992;59:352–63.
 - 120 Zagai U, Dadfar E, Lundahl J *et al.* Eosinophil cationic protein stimulates TGF-beta1 release by human lung fibroblasts in vitro. *Inflammation* 2007;30:153–60.
 - 121 Bloemen K, Verstraelen S, Van Den Heuvel R *et al.* The allergic cascade: review of the most important molecules in the asthmatic lung. *Immunol Lett* 2007;113:6–18.
 - 122 Fuentes-Beltran A, Montes-Vizuet R, Valencia-Maqueda E *et al.* Chemokine CC-ligand 5 production and eosinophil activation into the upper airways of aspirin-sensitive patients. *Clin Exp Allergy* 2009;39:491–9.
 - 123 Venge P, Peterson CGB. Eosinophil biochemistry and killing mechanisms. In: Morley J, Colditz JI, eds. *Eosinophil in Asthma*. New York: Academic Press, 1989: 163–77.
 - 124 Mukherjee M, Sehmi R, Nair P. Anti-IL5 therapy for asthma and beyond. *World Allergy Organ J* 2014;7:32.
 - 125 Ortega HG, Liu MC, Pavord ID *et al.* Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014;371:1198–207.
 - 126 Bel EH, Wenzel SE, Thompson PJ *et al.* Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014;371:1189–97.