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Biomedicine & Diseases: Review Eosinophils in the pathogenesis of allergic airways disease

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Abstract

Eosinophils are traditionally thought to form part of the innate immune response against parasitic helminths acting through the release of cytotoxic granule proteins. However, they are also a central feature in asthma. From their development in the bone marrow to their recruitment to the lung via chemokines and cytokines, they form an important component of the inflammatory milieu observed in the asthmatic lung following allergen challenge. A wealth of studies has been performed in both patients with asthma and in mouse models of allergic pulmonary inflammation to delineate the role of eosinophils in the allergic response. Although the long-standing association between eosinophils and the induction of airway hyper-responsiveness remains controversial, recent studies have shown that eosinophils may also promote airway remodelling. In addition, emerging evidence suggests that the eosinophil may also serve to modulate the immune response. Here we review the highly co-ordinated nature of eosinophil development and trafficking and the evolution of the eosinophil as a multi-factoral leukocyte with diverse functions in asthma.

Keywords

Eosinophil; asthma; airway hyper-responsiveness; remodelling

Introduction – eosinophils and asthma

Eosinophils were first described by Paul Ehrlich in 1879. These bilobed granulocytes possess secondary granules containing four primary cationic proteins namely, major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPO) all of which can be toxic. Eosinophils are thought to have evolved as part of the innate immune response against parasitic helminths. These multi-cellular parasites display distinct developmental phases with changing antigenicity during infection and invasion of host tissue. Classically, helminth infection can induce an IgE-mediated response leading to eosinophilia together with a Th2 cytokine response. While eosinophils have been shown to effectively kill parasites *in vitro* [1], they have been associated with both protective immunity [2] and pathology [3] in response to helminth infection *in vivo*. The complexity of the immune response against helminth infection is demonstrated by the fact that infection can be associated with long periods of latency, due to the ability of helminths to antagonise and regulate various cells of the immune system. The intricate nature of the immune response against helminth infection is reviewed in detail elsewhere [4, 5].

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The pathology of asthma is thought to represent an aberration in the defence response to helminth infection. The prevalence of asthma is increasing in the developed world and current anti-inflammatory treatment cannot completely control symptoms [6]. Asthma is a chronic disease affecting the airways whereby progressive inflammation together with structural changes known as airway remodelling lead to reversible airway hyper-responsiveness (AHR) and obstruction. Although the aetiology of asthma remains unclear, both environmental and genetic factors are thought to contribute. Genes that regulate both airway remodelling and inflammation have been associated with susceptibility to asthma [7]. The coordinated action of numerous leukocytes and their mediators then manifest the diverse pathology seen in the lung.

Commonly, susceptible individuals mount an immune response to otherwise innocuous agents. This begins with the IgE-mediated activation of mast cells, basophils and macrophages, which leads to the rapid release of pro-inflammatory mediators including histamine, eicosanoids and reactive oxygen species. These induce microvascular leakage, resulting in oedema within the airway wall, which contributes to the narrowing of the airway lumen. Such plasma exudation may also collect in the airway lumen, damaging the epithelium and preventing mucus clearance. In addition, mucus secretion and smooth muscle cell contraction are also induced by these early phase pro-inflammatory mediators. The production of various chemoattractants then leads to the recruitment of numerous leukocytes, including CD4 T cells and eosinophils. The persistent activation of these cells and their production of pro-inflammatory mediators, particularly Th2 like cytokines, plays an integral role in the pathogenesis of asthma [8].

In addition to this chronic inflammatory state within the airway, the aberrant regeneration and repair processes following tissue damage are thought to result in airway remodelling. This is characterised by goblet cell hyperplasia, subepithelial fibrosis and smooth muscle hypertrophy. The precise mechanisms leading to airway remodelling are still unknown. While it was believed that airway remodelling occurred as a result of ongoing inflammatory processes, much evidence suggests that inflammation and remodelling may indeed occur as parallel and independent events [9]. As such alternative ideas regarding the evolution and treatment of asthma unfold, the central role of the eosinophil in the pathogenesis of asthma is of particular interest. The diversity of eosinophil secretory products (Table 1), together with the range of receptors expressed on eosinophils (Table 2) is reflective of their broad range of functions beyond the role of a basic granulocyte. Eosinophils are able to modulate immune mechanisms that are important in asthma and contribute to inflammation, AHR and the remodelling processes. This review focuses on the changing views of the eosinophil in asthma. The role of eosinophils in homeostasis and mucosal immunity as well as asthma has also been recently reviewed elsewhere [10].

Eosinophil development

Eosinophils predominately reside within the gastrointestinal tract, and represent only 1–3% of circulating leukocytes. They are derived from $CD34^+$ haematopoietic progenitor cells within the bone marrow. Their differentiation is induced by the co-ordinated actions of the transcription factors GATA-1, PU.1 and C/EBP members (CCAAT/enhancer-binding protein family). The contribution of other transcription factors and subtypes may also be important but is less well established [11]. Although GATA-1, PU.1 and C/EBP are commonly expressed by a variety of haematopoietic lineages, their unique co-ordination together with specific cytokine stimulation results in the selective development of eosinophils.

Varied levels of expression of PU.1 ensure the specific differentiation of lymphocytic or myelocytic cells [12]. Furthermore, while the actions of PU.1 and GATA-1 have been antagonistic with regard to other cell types, these transcription factors act in synergy for eosinophil differentiation. *In vitro* studies show that the transfection of PU.1 amplified GATA-1 mediated gene transcription in the eosinophil lineage. This synergism was dependent on the level of PU.1 expression relative to that of GATA-1 [13].

Various types of C/EBP have differing roles in eosinophil development. C/EBPα knockout (KO) mice lack both neutrophils and eosinophils, suggesting that C/EBPα is required for the early induction of myeloid lineage-specific genes [14]. Both C/EBPα and C/EBPβ physically interact with GATA-1 and enhance transactivation of the eosinophil-specific MBP promoter, while two isoforms of human C/EBPε act to limit myeloid gene expression by antagonising GATA-1/PU.1 synergy and the functions of C/EBPα and C/EBPβ [13]. This further demonstrates the highly regulated process of eosinophil development.

While C/EBP is crucial for the development of both eosinophils and neutrophils [14], GATA-1 selectively promotes eosinophil development. The enforced expression of GATA-1 in human primary myeloid progenitor cells leads to eosinophil lineage commitment, via a mechanism dependent upon the COOH-terminal zinc finger of GATA-1 [15]. Furthermore, foetal liver cells from GATA-1-deficient mice did not support the development of eosinophils [15]. In eosinophils, GATA-1 uniquely acts via a high-affinity palindromic (or double) GATA site, which is present in the downstream GATA-1 promoter and also in regions of eosinophil-specific genes such as CCR3, MBP and IL-5 receptor alpha (IL-5Rα) [16]. Moreover, ΔdblGATA mice, which harbour a deletion of this palindromic GATA site, are devoid of eosinophils, while other haematopoietic cells are unaffected [16, 17].

In addition to highly regulated transcriptional control, eosinophil development is also governed by the actions of the cytokines IL-3, IL-5 and GM-CSF [18]. In line with the common use of transcription factors, all three cytokines are associated with the development of various cells of the myeloid lineage. IL-3, IL-5 and GM-CSF function by signalling through receptors that share a common beta chain but have cytokine-specific alpha chains [18]. Therefore, all three cytokines contribute to eosinophil development, endothelial adhesion, activation and survival; they act on progenitor cells within the bone marrow as well as mature cells at distal sites.

IL-5 is the most selective for differentiation, proliferation and maturation of eosinophils within the bone marrow as IL-5 expression is restricted to eosinophils and basophils, while various cell types express receptors for IL-3 and GM-CSF. The administration of IL-3 and GM-CSF in animal models and in clinical trials has shown that their ability to stimulate eosinophil production is relatively low compared to their ability to enhance the production of neutrophils and macrophages [18]. Furthermore, the expression of IL-5R on eosinophils is closely regulated depending upon the activation state and anatomical location of the cell. For instance stimulation of mature human eosinophils with IL-3, IL-5 and GM-CSF down-regulates their expression of IL-5Rα and reduces their responsiveness to IL-5 [19]. Interestingly, stimulation of human cord blood-derived $CD34^+$ cells with IL-3, IL-5 and GM-CSF led to the exclusive up-regulation of the IL-5Rα; an important early step in eosinophil lineage commitment [20]. Eosinophil development resulting from the culture of these cells with IL-3 and GM-CSF was associated with increases in IL-5 mRNA, and blocking this endogenously produced IL-5 prevented eosinophil development [20]. These data demonstrate the essential role for IL-5 in eosinophil development within the bone marrow. However, the observation that mice that are unresponsive to IL-3, GM-CSF and

IL-5 are able to produce eosinophils albeit in low numbers indicates the presence of unidentified factors that may play an important role in eosinophil development [21].

Eosinophil production within the bone marrow is an important feature in asthma. The numbers of $CD34^+CD45^+$IL-5Rα eosinophil progenitors are increased in the peripheral blood and bone marrow of atopic asthmatics [22] and upon allergen challenge in asthmatics [23] and in murine models of airway inflammation [24]. Therefore, upon allergen challenge, signals from the bronchial mucosa are relayed to the bone marrow to increase the pool of mature eosinophils available for rapid mobilisation. Further evidence suggests that progenitor cells are also able to migrate from the bone marrow to the site of allergic inflammation and then differentiate *in situ*. Increased numbers of $CD34^+$/IL-5Rα $mRNA^+$ cells were found in the bronchial mucosa of atopic asthmatic individuals [25]. Recently, $CD34^+CD45^+$IL-$5Rα^+$ eosinophil progenitors were found to be elevated in murine lung tissue 6 hours after allergen challenge preceding an increase in total progenitor cells and bronchoalveolar lavage (BAL) eosinophils [26]. This study identifies a possible contribution of resident progenitor cells that are able to differentiate *in situ*. The trafficking of both mature eosinophils and progenitor cells are thought to effectively provide a continual source of inflammatory cells.

Eosinophil trafficking

The trafficking of eosinophils from the bone marrow to the inflammatory site is dependent on the co-ordinated actions of numerous cytokines, chemokines and adhesion molecules. However, only IL-5 and the eotaxin family of chemokines selectively regulate eosinophil trafficking.

Eosinophilic chemoattractants – Eotaxin

Eotaxin was the first eosinophil-specific chemokine to be discovered after being identified as the major chemoattractant found in the BAL fluid taken from guinea pigs that had been sensitised and challenged with ovalbumin (OVA) [27, 28]. Eotaxin was then identified in mouse [29, 30], rat [31], and human tissue [32]. Subsequently, two more CC chemokines with functional similarities to eotaxin were cloned and named eotaxin-2 and -3 [33, 34]. Eotaxin was then referred to as eotaxin-1. A functional murine homologue of human eotaxin-3 has not yet been characterised. Studies using mouse models of airway inflammation have determined a functional role for the eotaxin family of chemokines in mediating the pulmonary recruitment of eosinophils [35, 36].

The eotaxins are up-regulated upon allergen challenge in mouse models [37] and in asthmatics [38]. They can be produced by mast cells, alveolar macrophages, eosinophils, airway smooth muscle and vascular endothelial cells but are mainly produced by epithelial cells [39]. Recently, it has been shown that monocyte/macrophage-derived eotaxin-2 predominates over eotaxin-1 in the BAL fluid of allergic mice [40]. However, allergen-induced pulmonary eosinophilia was shown to be significantly reduced in eotaxin-1 and -2 double-KO mice when compared to eotaxin-1 or eotaxin-2 single-KO mice, demonstrating the importance of the synergism between these eotaxins in the induction of eosinophilia [40]. Indeed, human studies reveal that eotaxin-1 maybe important immediately after allergen challenge, whereas eotaxin-2 and possibly eotaxin-3 may account for the persistence of eosinophilia [41].

Unlike most chemokines that bind to numerous receptors, the eotaxins signal exclusively through the CCR3 receptor [42, 43]. CCR3 is primarily expressed on basophils [44], mast cells [45] and Th2 cells [46] as well as both mature eosinophils [43] and $CD34^+$ progenitor cells [47]. Although CCR3 is constitutively expressed on eosinophils, its expression is

further increased in response to inflammatory stimuli [47]. Typical of chemokine receptors, CCR3 is a seven transmembrane spanning, G protein coupled receptor. Ligation of eosinophil CCR3 by eotaxin stimulates calcium mobilisation, actin polymerisation, shape change and chemotaxis, and also induces inhibitory events such as desensitisation of the receptor [48]. CCR3 undergoes prolonged ligand-induced receptor internalisation [49]. Certain functional responses, such as actin polymerisation and shape change, are dependent on such receptor internalisation, whereas signals that regulate chemotaxis are largely dissociated from those that regulate receptor internalisation [48, 49]. Understanding the biology of the CCR3 receptor may identify new approaches to target-specific eosinophil function. Although CCR3 also recognises RANTES, MIP-1α and MCP-2, -3, and -4, these chemokines act via a variety of receptors and are not as selective as eotaxin for the recruitment of eosinophils. Therefore, the eotaxin/CCR3 axis remains central in the pathology of asthma. The expression of CCR3 on some Th2 cells and the unique synergism between T cell-derived IL-5 and eotaxin in regulating various stages of eosinophil trafficking further highlight the importance of the CCR3 receptor.

Eosinophil mobilisation

Mobilisation of eosinophils from the bone marrow represents the first stage of eosinophil trafficking and IL-5 plays a critical role in this. The importance of IL-5 in regulating eosinophil maturation and release is demonstrated by the fact that IL-5 transgenic mice exhibit eosinophilia [50], while IL-5 KO mice show a reduction in eosinophils in blood and lungs after challenge [51]. The systemic administration of IL-5 to guinea pigs reduced bone marrow eosinophil numbers while increasing circulating eosinophils, indicative of a role in inducing mobilisation [52]. Using an *in situ* perfusion system of the guinea pig hind limb, infusion of IL-5 was shown to dose dependently induce the selective release of eosinophils from the bone marrow [53]. This release was dependent on the actions of β2-integrin and regulated by the antagonistic actions of α4-integrin [53]. IL-5 also synergises with eotaxin to enhance mobilisation of eosinophils.

Although eotaxin is primarily produced by epithelial cells and therefore largely associated with the recruitment of eosinophils into lung tissue, it also has a role in the release of eosinophils from the bone marrow. Allergic airway inflammation in guinea pigs showed that levels of eotaxin in the lung peak as rapidly as 6 hours post challenge, and correlated with blood and lung eosinophilia together with a reduction in bone marrow eosinophil numbers [36]. The inhibition of IL-5 abolished these effects without altering the production of eotaxin in the lung [36], thereby demonstrating the importance of the co-ordinated actions of eotaxin and IL-5 in the development of eosinophilia. Furthermore, neutralisation of eotaxin during the development of murine airway inflammation prevented the production of myeloid progenitor cells within the bone marrow, while administration of eotaxin enhanced myelopoiesis [54]. In contrast, systemic administration of eotaxin in unsensitised mice induced blood eosinophilia without any changes in bone marrow eosinophil numbers [55]; however, the administration of eotaxin was sufficient to mobilise eosinophils in unsensitised guinea pigs [56]. Moreover, the infusion of eotaxin into the arterial supply of guinea pig femoral bone marrow resulted in the selective release of eosinophils and eosinophil progenitor cells, while the infusion of IL-5 followed by eotaxin significantly enhanced the number of mature eosinophils being released from the bone marrow [56].

Eosinophil priming and trafficking through the vasculature

Following the release of eosinophils from the bone marrow their recruitment into the lung is also governed by the synergism between IL-5 and eotaxin. Systemic administration of eotaxin dose dependently induced blood eosinophilia but had no effect on increasing the chemotactic response to eotaxin given subcutaneously; however, systemic IL-5 significantly

increased tissue eosinophilia and this effect was further amplified when eotaxin and IL-5 were co-administered intravenously [55]. The essential and synergistic role of IL-5 seen here could in part be attributed to its ability to prime circulating eosinophils, which increases their capacity of migration, adhesion and degranulation.

Amongst asthmatics, levels of IL-3, IL-5 and GM-CSF are raised within the blood [57]; these together with mediators such as platelet-activating factor (PAF) and complement factor 5a (C5a) may act to prime circulating eosinophils [58, 59]. *In vitro* studies using human eosinophils showed that PAF may act on eosinophils in an autocrine manner. Furthermore, stimulation of these eosinophils with IL-5 or IgG resulted in the production of PAF, and the specific antagonism of PAF inhibited both superoxide production and eosinophil degranulation [60]. Further support for the circulation of primed eosinophils comes from studies that show that peripheral blood eosinophils obtained from asthmatics display differing patterns of adhesion and chemotaxis compared to eosinophils from normal subjects [58, 59]. In addition, eosinophils obtained from BAL fluid of allergen-challenged asthmatics were primed to a greater extent than those in the peripheral blood, showing that eosinophil priming begins in the circulation but peaks within the lung [61].

The migration of these partially primed eosinophils from the vasculature into tissue depends on the binding of several adhesion receptors including eosinophil P-selectin glycoprotein ligand-1, $\alpha_4\beta_1$, and $\alpha_A\beta_2$ to endothelial P-selectin, VCAM-1 and ICAM-1, respectively [62]. Eosinophilia was significantly reduced following peritoneal challenge with ragweed in both P-selectin- and ICAM-deficient mice when compared to wild-type (WT) mice [63]. Specifically intra-vital microscopy revealed that eosinophil rolling and firm adhesion was impaired in P-selectin-KO mice, while only firm adhesion of leukocytes in general was investigated and found to be inhibited in ICAM-KO mice [63]. In contrast to neutrophil recruitment, eosinophil recruitment was not completely abolished in P-selectin/ICAM-1 double-KO mice, but pre-treatment of these mice with an antibody against VCAM-1 resulted in an almost complete inhibition of eosinophil recruitment [63]. In support of these studies, Gonzalo et al. [35] showed the requirement of P-selectin in the early stages of eosinophil recruitment to the lung following allergen challenge. In addition, mice that were genetically deficient for VCAM-1 and ICAM-1 did not develop pulmonary eosinophilia following allergen challenge. Given that pulmonary eosinophilia is a T cell-dependent process [35] and lymphocyte accumulation in the lung was also reduced in VCAM-1- and ICAM-1-KO mice, VCAM-1 and ICAM may regulate tissue eosinophilia by directly regulating endothelial-eosinophil interactions and also by enhancing T cell responses [35]. Evidence from CCR3-KO mice indicates that the binding of eotaxin with eosinophil CCR3 is mainly responsible for the migratory process beyond the sub-endothelial space [64].

Eosinophil recruitment into the lung

Mediators such as PAF, LTB4 and C5a were amongst the first eosinophil chemoattractants to be identified [65]; however, these were found to be non-selective, having potent actions on neutrophils [66]. Interestingly, incubation of human eosinophils with IL-5 selectively enhanced their migratory response to PAF, LTB4 and FMLP, while having no such effect on neutrophils [58]. Therefore, the increase in these mediators within the asthmatic lung may contribute to eosinophil recruitment in the presence of IL-5. Unlike chemokines, which induce direct chemotaxis along a gradient, these mediators have stimulatory and chemoattractant properties. Cytokines such as IL-4 and IL-13 produced by Th2 cells and epithelial cells [67] and also the recently described acidic mammalian chitinases [68] are increased upon allergen challenge and may contribute to eosinophil recruitment by enhancing the production of eotaxin.

Within the lung, the combined actions of eotaxin and IL-5 are crucial in the specific recruitment of eosinophils. IL-5 has been shown to display chemokinetic properties [56]. In addition, subcutaneous administration of IL-5 induced concentration-dependent eosinophilia in mice [55], suggesting that it may act as a chemoattractant. Therefore, IL-5 may act to enhance the local chemotactic response to eotaxin. Interestingly, tissue eosinophilia using eotaxin could not be induced in IL-5-KO mice, and restoration of eosinophil homing ability in these mice could not be restored by the intravenous transfer of eosinophils. Tissue eosinophilia could only be induced in these IL-5 KO mice by transplanting a device that continually delivered IL-5 for 72 hours [55]. This demonstrates an essential role of IL-5 in tissue homing of eosinophils. In addition to the direct action of eotaxin on eosinophil mobilisation and recruitment into tissue, its actions on Th2 cells further enhances eosinophil recruitment to the lung. Eosinophilic inflammation seen in asthma is dependent on the antigen-specific activation of Th2 cells. T cell-deficient mice are protected from lung eosinophilia and AHR [35, 69] and Th2 cells are increased in the BAL fluid of asthmatic patients [70]. In a murine model of allergen-induced airway inflammation, eotaxin was responsible for the early recruitment of Th2 cells, a response that was later maintained by the actions of monocyte-derived chemokine [71]. Cytokines produced by Th2 cells, including IL-4 and IL-13, stimulate and enhance the production of eotaxin, which then synergises with Th2 cell-derived IL-5 in the selective recruitment of eosinophils [39, 72]. A summary of eosinophil trafficking from the bone marrow to the lung is shown in Figure 1.

Blockade of eotaxin using neutralising antibodies [37] and single eotaxin-KO animals [73] have shown significant yet incomplete reduction in eosinophilic inflammation, suggesting that other factors may contribute. The combined actions of the CC chemokines RANTES, MCP-1 and MCP-5 together with eotaxin led to the development of OVA-induced lung eosinophilia [37]. While these chemokines do not act specifically on eosinophils, it is noteworthy that the neutralisation of RANTES completely abolished OVA-induced lung eosinophilia [37]. The CCL1/CCR8 axis has also been shown to preferentially induce the recruitment of eosinophils to the lung following allergen challenge [74]. The complexity of chemokine function *in vivo* is discussed in detail elsewhere [75], but collectively these studies indicate that chemokines other than eotaxin may also have an important role in eosinophil recruitment.

Furthermore, eosinophils also express the chemokine receptor CCR1, which binds MIP-1α as well as RANTES and MCP. Asthmatic patients exhibit increased mRNA expression of MCP-1, RANTES, and MIP-1α [76]; moreover, these chemokines are increased at sites of endobronchial allergen challenge [77]. In addition, blockade of MIP-1α *in vivo* reduced lung eosinophils by 20% [37], and MIP-1α also induced a potent chemotactic response in eosinophils from a subgroup of asthmatic patients [78]. Therefore, CCR1 may also be an important target in blocking eosinophil responses. Human eosinophils also express the IL-8 receptors CXCR1 and CXCR2 [79]. Given the potency of IL-8 for neutrophils its significance in eosinophil trafficking is less clear. Generally, while numerous other chemokine networks may act in concert to regulate eosinophil trafficking, the importance of eotaxin acting via CCR3 was demonstrated by the substantial reduction in eosinophilia seen in both CCR3 and eotaxin double KO mice [40].

Inflammation in the lung

Eosinophils have a central role in influencing the inflammatory networks that are established within the asthmatic lung. Here, primed eosinophils are further activated by numerous stimuli including IL-5, GM-CSF, IL-3 and secretory IgA. Activation is dependent on β2-integrins and results in degranulation [80].

Eosinophils are able to selectively release stored mediators via three highly regulated mechanisms (classical exocytosis, compound exocytosis or piecemeal degranulation) as well as cytolysis where the entire contents of the cell are released following rupture [81]. Classical exocytosis involves the extrusion of single secretory granules but this has not yet been demonstrated in airway tissue *in vivo* [82]. Compound exocytosis entails the fusion of multiple intra-cellular granules followed by focused secretion onto the target cell at the site of adhesion [83]. Piecemeal degranulation allows the partial and selective release of granule contents by transferring them into smaller vesicles, which are subsequently released by exocytosis. This is demonstrated by the selective release of RANTES from IFN-γ-stimulated human eosinophils independently of both MBP and EPO release [84]. The release of mediators by exocytosis is regulated by the formation of a docking complex composed of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), which are located on both the vesicle and the target cell [85, 86]. Piecemeal degranulation appears to be the predominant mechanism of granule release in allergic inflammation, since examination of nasal biopsies following allergen challenge revealed 67% of eosinophils undergoing piecemeal degranulation with the remainder undergoing cytolysis [87]. Furthermore, mucosal tissue obtained from patients suffering from various allergic conditions including asthma, rhinitis and colitis contained similar numbers of tissue eosinophils but these displayed differing levels of piecemeal degranulation [88].

Although mechanisms of exocytosis act to limit tissue damage, eosinophil granule proteins are cytotoxic and damage the epithelium [89]. In addition, they are able to increase vascular permeability *in vivo* at concentrations that are observed in pathological conditions [90]. Importantly, they also activate mast cell release of pro-inflammatory mediators including histamine, eicosanoids and cytokines [91]. In addition, eosinophils produce stem cell factor and nerve growth factor, which further supports the growth and survival of mast cells [91]. Eosinophils can also produce pro-inflammatory cytokines such as IL-4 [92] and IL-13 [93], which potently stimulate the release of eotaxin and also increase production of RANTES and MCP-1 [67], thereby further enhancing eosinophil recruitment. Eotaxin can induce the respiratory burst and actin polymerisation in eosinophils and therefore directly contribute to tissue damage as well as orchestrate the continual recruitment of both eosinophils and T cells [94]. Altogether, eosinophils have an essential role in the perpetuation of the inflammatory response.

Following allergen challenge, eosinophils are recruited to the lung as part of a protective inflammatory response after which cells are cleared from the tissue. This process of inflammatory resolution is a highly co-ordinated and active event and mechanisms exist to limit eosinophilic inflammation such as the actions of the regulatory cytokine IL-10 [95]. Defects in these resolution pathways may cause inflammation to persist. The down-regulation of IL-3 may be a potentially important mechanism in limiting airway inflammation. The cytokines IL-3, IL-5, and GM-CSF promote eosinophil activation and survival in all compartments. However, in contrast to their effects within the bone marrow, the stimulation of mature human eosinophils with IL-3, IL-5, and GM-CSF down-regulates the IL-5Rα and up-regulates the IL-3Rα [19]. In a murine model of acute transient AHR, IL-3 was elevated in the lung prior to the resolution of inflammation, which was associated with reduced levels of IL-3 together with increased leukocyte apoptosis in the peri-bronchiolar regions. In addition, inhibition of IL-3 in a model of sustained AHR resulted in a reduction in eosinophilia and AHR together with an increase in the number of apoptotic cells within the lung [96]. Therefore, IL-3 may act to promote inflammation by preventing apoptosis but also pro-resolving signals may exist to down-regulate IL-3 and promote cell clearance by apoptosis. It has also been proposed that, in addition to apoptosis, eosinophils could be more effectively removed by promoting egress into the airway lumen. The

resolution of murine allergic airway inflammation following steroid treatment was associated with the transepithelial egression of eosinophils into the lumen [97].

Eosinophils and AHR

AHR refers to the increased ability of the airways to narrow after exposure to non-specific stimuli. It is a classical feature of asthma and its severity correlates with the severity of disease [98]. Similarly, eosinophilia is also a characteristic feature of asthma, and eosinophil number and activation state broadly correlate with disease severity [8]. Eosinophils are recruited and activated during allergen challenge in both human subjects [23] and animal models of disease [24]. Activated eosinophils are thought to contribute to airway inflammation and AHR by the direct release of basic granules, leukotrienes and other mediators and also indirectly by their interactions with numerous cells types.

Although eosinophils release an array of mediators, some of the basic granule proteins are specific to eosinophils. These are highly toxic and present within the lung of asthmatic patients at concentrations that are able to damage the airway epithelium and increase smooth muscle cell reactivity [99]. Smooth muscle cell contraction is controlled via M3 muscarinic receptor stimulation with acetylcholine released from parasympathetic nerves [100]. The release of acetylcholine and, therefore, smooth muscle cell contraction are limited by the M2 receptor [101]. Evidence suggests that eosinophils may be responsible for the loss of M2 receptor function. Eosinophils can be seen clustered around the vagal nerve ganglia in lung sections taken from asthmatic patients [102]. Furthermore, MBP is an allosteric antagonist for the M2 receptor and has been shown to induce bronchoconstriction by dysregulating vagal muscarinic M2 and M3 receptor function [103]. In guinea pigs sensitised and challenged with OVA, eosinophil depletion prevents the loss of M2 receptor function [104].

Eosinophils are also a rich source of cysteinyl leukotrienes, which directly contribute to bronchoconstriction but also increase vascular permeability, and contribute to inflammatory cell recruitment. Furthermore, clinical trials of two leukotriene receptor antagonists have shown that they are able to control the symptoms of asthma [105]. In addition, eosinophils indirectly contribute to the development of AHR by the induction of mast cell and basophil degranulation, leading to the production of prostaglandins, leukotrienes and histamine, all of which can induce AHR [65].

Many studies support the long-standing view of the eosinophil as a central effector cell in airway disease. The thorough depletion of lung eosinophils in C57BL/6J mice using an antibody against CCR3 abolished allergen-induced AHR while having no direct effect on other cell types [106]. Other studies highlight the importance of eosinophil interactions with T cells. The transfer of eosinophils to OVA-sensitised IL-5 KO mice resulted in the development of eosinophilia, Th2 cytokine production and the development of AHR in a similar magnitude to that in WT mice; however, treatment of KO mice with anti-CD4 antibody diminished the effect of adoptive transfer of eosinophils on AHR [107]. While IL-13 alone has been shown to induce AHR independently of eosinophilia [108], the ablation of eosinophils using IL-5- and eotaxin-double KO mice abolished AHR by reducing the ability of T cells to produce IL-13 [109]. This study also showed that the transfer of IL-13-producing T cells could induce AHR in naïve eosinophil-deficient mice, suggesting a more indirect role for the eosinophil in the induction of AHR [109]. While these studies indicate an important role of the eosinophil in the development of AHR, they also suggest the significant and independent contributions of other cells and mediators.

Despite the fact that eosinophilia is a common feature of asthma, its precise role in the pathology of the disease remains unclear. The overlapping actions of various cells that infiltrate the asthmatic lung make it difficult to assess the contribution made by eosinophils

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alone. The current literature shows that there is much controversy surrounding the role of the eosinophil in the induction of AHR. As with human disease, animal models have also shown the association of eosinophilic inflammation with AHR [110]. Numerous *in vivo* studies use eosinophilia as an indicator of pathology, but human studies show that the correlation between eosinophilia and the severity of asthma as measured by AHR remains weak, and amongst non-atopic asthmatics some studies show no correlation at all [111]. Observations that eosinophilic bronchitis presents with a similar distribution of tissue eosinophils to that seen in asthma but shows no signs of AHR further suggests that mast cells may be more important than eosinophils in manifesting AHR [112]. Furthermore, while eosinophil-derived MBP is thought to directly contribute to AHR, MBP-1-KO mice were not protected from airway inflammation or AHR [113]. Other studies also indicate that eosinophils may not be necessary for the induction of AHR. In a model of OVA-induced airway inflammation the tracheal administration of recombinant CCL1 led to the recruitment of eosinophils in the absence of T cells or AHR. Additionally, neutralisation of CCL1 specifically blocked eosinophil recruitment but had no effect on AHR [74].

The specific blockade of eosinophils mainly by targeting CCR3 and IL-5 in animal models have played a crucial role in trying to define the importance of the eosinophil in the induction of AHR. Although CCR3 is expressed on numerous cell types including Th2 cells [46], basophils [44] and mast cells [45], CCR3 is the principle chemokine receptor involved in the attraction of eosinophils into inflamed tissue [43]. Allergen-challenged CCR3-KO mice showed that eosinophils could migrate through the endothelium but could not pass any further through the blood vessel, indicating that the eotaxin/CCR3 axis is essential for the final step of migration into inflamed tissue [64]. However, CCR3 also has a role in mast cell homing, with CCR3-KO mice displaying increased numbers of tracheal mast cells, possibly accounting for the increase in AHR seen in allergic mice [64]. This, together with the fact that differing methods of sensitising CCR3-KO mice give opposing effects on AHR [64, 114], indicates a complex role for the eotaxin/CCR3 axis in airway inflammation. Therefore, the role of eosinophils in the induction of AHR may be difficult to interpret from studies based on the blockade of CCR3, particularly due to the fact that CCR3 is not exclusively expressed on eosinophils. Recently, treatment with a low molecular weight CCR3 antagonist resulted in a 50–60% reduction of tissue eosinophilia and led to the reduction of AHR and prevention of airway remodelling [115]. While infiltration of lymphocytes and macrophages and the local production of IL-5 and IL-13 remained unchanged, this reduction in AHR could be accounted for by CCR3 antagonism on mast cells. Furthermore, the partial blockade of eosinophils observed after antagonism of CCR3 [115] and IL-5 [116] suggest that interplay and possibly compensation by other factors may be important.

Despite this, IL-5 is undoubtedly a major contributing factor in the induction of eosinophilia due to its central role in eosinophil trafficking. However, the relationship between IL-5 blockade and AHR remains controversial. AHR was observed in transgenic mice that overexpress IL-5 [117], and reduced in IL-5-KO mice [51], but this reduction was strain specific. The reduction in AHR was less pronounced or non-significant in IL-5-KO mice on a BALB/c background depending upon the mode of sensitisation used [118]. In addition, the inhibition of mast cell responses by blocking IL-4 *in vivo* could not control AHR in BALB/c mice [118]. These data suggested that multiple T cell-mediated mechanisms may exist to regulate AHR in BALB/c mice. BALB/c mice have been shown to have greater airway responses than C57BL/6 mice [119]. Furthermore, using similar protocols of i.p sensitisation with OVA followed by challenge, the number of BAL eosinophils was increased by tenfold in BALB/c mice [118] when compared to C57BL/6 mice [51]. Therefore, the direct or indirect impact of residual eosinophils may also account for the strain-specific differences in AHR seen in IL-5-KO mice. Similarly, amongst asthmatic patients anti-IL-5 therapy has also produced some conflicting data as discussed later.

Further to the difficulties in interpreting data obtained following the use of both CCR3- and IL-5-KO animals and blocking antibodies, eosinophil-deficient mice were generated and used to better assess the role of eosinophils in the induction of AHR. Eosinophils were genetically ablated in mice by the deletion of a high-affinity GATA-binding site in the GATA-1 promoter [16]. Following the induction of airway inflammation, these ΔdblGATA mice showed no significant differences in AHR when compared to WT mice [17]. In contrast, mice in which eosinophils were depleted by the transgenic expression of the diphtheria-toxin A chain under the control of the eosinophil peroxidase promoter (PHIL), were protected against AHR [120]. More recently, a study using chronic allergen challenge to model the acute exacerbation of allergic asthma also used these ΔdblGATA mice and also found that the recruitment of eosinophils was not obligatory for the development of AHR [121].

The differences between these studies could be due to the fact that the ΔdblGATA mice were on a BALB/c background, whereas the PHIL mice were on a C57BL/6 background. Furthermore, the diphtheria toxin-mediated deletion of maturing eosinophils by inducing apoptosis resulted in a significant increase in the number of peripheral blood leukocytes in these mice [120], which could alter their immune response. In addition, increased levels of apoptosis seen in the PHIL mice may cause a generalised immunosuppressive effect [122], which may also contribute to the suppression in AHR seen in these mice. Three independent studies have failed to detect any residual eosinophils in these ΔdblGATA mice, which may account for the insignificant differences in AHR observed between WT and ΔdblGATA mice [17, 121, 123]. In addition, ΔdblGATA mice do not develop eosinophilia, even when backcrossed to the IL-5 transgenic mice [16]. Recently, a minor population of eosinophil-like cells was reported in BAL fluid of ΔdblGATA mice following chronic intra-nasal challenge with *Aspergillus fumigatus* allergic extract [124]. However, lung tissue from these mice was essentially devoid of eosinophils and BAL eosinophil numbers were significantly reduced in these ΔdblGATA mice to the same degree as that observed in both CCR3-KO and eotaxin-double KO mice [124]. It may therefore be unlikely that such a population could account for the induction of AHR seen by Humbles et al. [17]. Although much controversy surrounds the role of eosinophils in the induction of AHR, emerging evidence suggests that eosinophils may have an important role in airway remodelling that is less recognised.

Eosinophils and remodelling

Airway remodelling refers to structural changes in the asthmatic airway thought to occur as a result of dysfunctional regeneration and repair processes within the lung. It is characterised by the increased deposition of extracellular matrix (ECM) proteins such as collagen I and tenascin within the reticular basement membrane and bronchial mucosa, increases in airway smooth muscle mass, and goblet cell hyperplasia [125]. Airway remodelling may contribute to AHR and fixed airway flow obstruction and also contribute to the loss of lung function over time [9, 126]. Patients with chronic asthma have shown a decline in lung function despite aggressive anti-inflammatory therapy [9, 126]. Furthermore, airway remodelling has been seen in children prior to the symptoms of asthma [127]. Therefore, targeting airway remodelling represents an important strategy in controlling asthma.

Although eosinophils can secrete a range of mediators that could contribute to airway remodelling, an association between eosinophils and airway remodelling has only recently been described. The essential role for eosinophils in airway remodelling was shown by a study in which eosinophils were genetically ablated in mice by the deletion in the high-affinity GATA-binding site in the GATA-1 promoter. Interestingly, after a period of prolonged allergen challenge using a well-established model [110], WT mice exhibited prominent features of airway remodelling, namely increased subepithelial deposition of

collagen together with airway smooth muscle cell hyperplasia and proliferation, both of which were significantly reduced in Δdbl-GATA mice [17]. Importantly, the observations seen using the Δdbl-GATA mice [17] compliment those seen in human studies where anti-IL-5 treatment protects against remodelling [116]. This signifies the importance of targeting the eosinophil in asthma regardless of its debated role in the induction of AHR.

Eosinophils may contribute to the induction of airway remodelling by synthesising a variety of profibrotic mediators (Fig. 2). Eosinophils are thought to be an important source of the potent pro-fibrotic cytokine TGF-β [128, 129], although numerous other cells types including platelets, fibroblasts, smooth muscle and epithelial cells can also produce TGF-β [130]. TGF-β is able to induce ECM protein production [131, 132], and also contributes to the accumulation of fibroblasts below the reticular basement membrane by stimulating fibroblast proliferation [133]. It further contributes to airway remodelling by promoting the differentiation of myofibroblasts from resident fibroblasts [134] and also from circulating precursor cells known as fibrocytes [135]. The differentiation of myofibroblasts into smooth muscle cells [136] and their proliferation [137] may also be governed by TGF-β.

Many studies implicate eosinophil-derived TGF-β in promoting mechanisms of remodelling. IL-5-KO mice expressing significantly reduced BAL eosinophils in a model of chronic allergen challenge were protected from airway remodelling [129, 138]. Both studies point to a role for eosinophil-derived TGF-β in the propagation of airway remodelling. Furthermore, administration of an anti-TGF-β antibody in mice that underwent sensitisation followed by prolonged allergen challenge prevented the progression of airway remodelling without altering inflammation [137]. These animal experiments are supported by the findings of Flood-Page et al. [116], who showed that treatment of asthmatic patients with an anti-IL-5 antibody reduced the deposition of ECM proteins within the lung in association with a reduction in BAL TGF-β. However, the precise role of eosinophil-derived TGF-β in airway remodelling is complicated by the fact that TGF-β may induce the expression of other fibrotic factors such as plasminogen activator inhibitor [139] while being able to act both synergistically and antagonistically with other factors such as epidermal growth factor [130]. In addition, TGF-β itself is produced by numerous cell types. Eosinophils could be the primary source of TGF-β production at the early stages of disease, while other sources such as myofibroblasts may become more important at later stages [138]. In a chronic model of OVA-induced airway remodelling, monocytes/macrophages were found to be the primary source of lung TGF-β [110], and using the same model Δdbl-GATA mice were protected against airway remodelling via a TGF-β-independent pathway [17].

Numerous other eosinophil-derived fibrogenic factors may play an important role in airway remodelling as summarised in Table 1; however, many of these are not specifically produced by eosinophils [140]. Recently, the incubation of normal human bronchial epithelial cells with subcytotoxic concentrations of the eosinophil granule proteins MBP or EPO increased the mRNA expression of endothelin-1 and platelet-derived growth factor (PDGF), as well as the matrix metalloproteinases MMP1 and MMP9, all of which are known to contribute to airway remodelling [141]. MMP-9 also has a critical role in eosinophil recruitment [142] and can therefore amplify both inflammation and remodelling. In addition, eosinophils are a rich source of the cysteinyl leukotriene LTC_4 [143], and mice that were unable to produce cysteinyl leukotrienes due to the targeted disruption of the LTC_4 synthase gene were protected against alveolar septal thickening and collagen deposition [144]. A novel study using microarray analysis to compare lung tissue from allergen-challenged WT, CCR3-KO and Δdbl-GATA mice revealed that the induction of genes for the adenosine A3 receptor and the coagulation-associated genes PAI-2, factor 5 and factor 10 was significantly reduced in eosinophil-deficient and CCR3-KO mice when compared to WT mice [124]. These genes have a role in regulating mucus production and fibrin

deposition. Furthermore, this study also described eosinophil-dependant genes that have an important role in modulating the immune response [124].

Immunomodulation

Eosinophils are a classical feature of the inflammatory infiltrate subsequent to allergen challenge and also contribute to airway remodelling. Being granulocytes, their function has been largely attributed to the release of granule proteins. However, the effectiveness of granule release as a solely important mechanism in host defence has been questioned by the observations that granule release may be responsible for eliminating a finite number of pathogens and may also destroy the cell itself [145]. From an evolutionary perspective, while it is now thought that eosinophils may have evolved to combat parasitic infection, eosinophils existed prior to the emergence of helminths that had adopted a parasitic lifestyle [145]. Eosinophils also existed prior to the evolution of acquired immune responses, such as the Th2 response that they are now associated with for the clearance of parasites. Furthermore, the functions of granule proteins are not conserved and eosinophils show species-specific differences with regard to granulation [145]. Such observations led these authors to suggest that perhaps the role of eosinophil degranulation may stem from the requirement of large multi-cellular organisms to amplify the signals generated during cellular stress and damage, thereby acting to regulate host defence and facilitating tissue remodelling and the clearance of cellular debris to maintain homeostasis. Indeed, the role for eosinophils in immunomodulation is evident from the fact that eosinophils are able to synthesise, store, and secrete a variety of cytokines. These include not only the Th2 cytokines IL-4 [92] and IL-13 [93] that have been shown to have a central role in the propagation of asthma, but also Th1 cytokines IL-12 [146] and IFN-γ [147] and regulatory cytokines such as IL-10 [147, 148].

Eosinophils express MHC class II and co-stimulatory molecules for lymphocytes. Human eosinophils are able to function as antigen-presenting cells after cytokine stimulation *in vitro* [149]. Furthermore, *in vivo* studies show that eosinophils are also able to traffic to T cell areas of the paratracheal lymph nodes and stimulate lymphocyte proliferation [150]. Antigen-loaded eosinophils were also able to prime for Th2-driven allergic disease of the lung when transferred to naïve animals [151]. They may therefore influence T cell proliferation, differentiation and apoptosis *in vivo*. Eosinophils may also effect T cell polarisation by their constitutive production of indolamine 2,3-dioxygenase (IDO), a substance that converts tryptophan into kynurenines; these induce the apoptosis of Th1 cells [152]. Additionally, gene transcripts of the leukotriene B_4 receptor, IL-4, Egr-2 and the IL-1 receptor-related protein ST2, all of which are important in regulating T cell activation and recruitment, were significantly reduced in eosinophil-deficient and CCR3-KO mice [124]. Such varied functions of eosinophils suggest that their role in asthma may be more diverse than initially anticipated.

Targeting eosinophils for therapy

Given the potentially important role of eosinophils in both airway hyper-responsiveness and remodelling, eosinophils have been targeted as a novel therapeutic strategy for asthma, particularly via IL-5. However, the use of anti-IL-5 antibody therapy in animal models has produced some conflicting data. While some studies show that treatment with anti-IL-5 antibodies abolished eosinophilia and AHR in both BALB/c and C57BL/6 mice [153], anti-IL-5 antibodies abolished eosinophilia but had no effect on AHR when treating established airway disease [154].

Subsequently, two monoclonal antibodies against IL-5, SCH55700 (Schering-Plough Research Institute) and Mebolizumab/SB-240563 (Glaxo SmithKline) were used in clinical

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trials. Treatment with SB240563 prevented the maturation of eosinophils in the bone marrow and significantly reduced the number of eosinophil progenitors in the bronchial mucosa [155]. In a double-blind, randomised, placebo-controlled trial, allergen-challenged atopic asthmatics received a single infusion of SB240563 and showed a significant reduction in both blood and sputum eosinophils up to 30 days post treatment. Despite this, no improvement was seen in the late asthmatic reaction or AHR after inhaled allergen [156]. This led these authors to conclude that eosinophils may not be required for the induction of AHR. In support of this study, the administration of another anti-IL-5 antibody, SCH55700, to patients with severe persistent asthma showed a long-lasting reduction in blood eosinophils without any significant improvement in lung function [157].

The interpretations from these studies remain inconclusive as the SCH55700 was tested in a small Phase I study and the study using SB240563 has also been criticised for lack of statistical power as well as for improper baseline measurements [158]. In addition, a later study found that even after multiple infusions of SB240563 eosinophil numbers in the bronchial mucosa were only reduced by 55% [116]; however, this was sufficient to significantly reduce parameters of airway remodelling including the deposition of tenascin, lumican and procollagen III, and the percentage of tissue eosinophils expressing mRNA for TGF-β [116]. Therapies based on targeting the CCR3/eotaxin axis may be more beneficial in the treatment of asthma as these will not only prevent eotaxin-mediated eosinophil recruitment but also target numerous other cell types, such as Th2 cells and mast cells that play an important role in the pathogenesis of asthma. Recently the administration of a low molecular weight CCR-3 antagonist after the establishment of disease in a murine model of allergic airway inflammation resulted in a reduction of AHR and prevention of airway remodelling [115]. A number of CCR3 antagonists are being developed by pharmaceutical companies, but at present the most advanced data come from compound DPC168, which has completed Phase I clinical trials for asthma and allergic rhinitis [159]. Antibody therapy has also been developed to target this chemokine/receptor axis. A neutralising monoclonal antibody directed to CCR3 blocks chemotaxis and calcium flux induced by all CCR3 ligands in human eosinophils *in vitro* [160]. *In vivo*, anti-CCR3 antibodies led to an improvement in allergic pathology in a mouse model [103]. The most advanced anti-chemokine antibody for treatment of allergic diseases in patients is Bertilimumab (CAT213), a human IgG4 monoclonal antibody specific for CCL11 (eotaxin-1), which is under development by Cambridge Antibody Technology. Phase I/IIa clinical trials have determined that Bertilimumab is well tolerated with no adverse events reported, and the compound is currently in Phase II trials for allergic rhinitis and in preclinical investigation for asthma [161].

The delay in development of specific eosinophil therapy for asthma may, in part, stem from nervousness in the industry over the failure of therapies such as anti-IL-5 antibodies to abolish eosinophil recruitment after allergen challenge [151] as well as an indication that eosinophils may not control AHR [16]. However, further trials are needed as well as the development of combination therapies designed to antagonise both CCR3 and IL-5 before the eosinophil can confidently be deprioritised as a candidate target for asthma therapy. This point is of particular importance when one considers the potential role of the eosinophil in the long-term aspects of the asthmatic reaction – airway remodelling.

Concluding remarks

Emerging evidence suggests that eosinophils have a broad range of functions beyond that of a basic granulocyte. They remain an important therapeutic target in the management of asthma due to their role in the perpetuation of airway inflammation, their potential in the induction of AHR and their recently described role in airway remodelling. Evidence also

suggests that eosinophils have important immunomodulatory functions. Further *in vivo* studies examining the mechanisms of eosinophil-induced airway remodelling and also examining the importance of the potential of eosinophils to modulate immune function will improve our understanding of the extended role of the eosinophil in the pathogenesis of asthma and thereby identify the best methods of targeting this cell.

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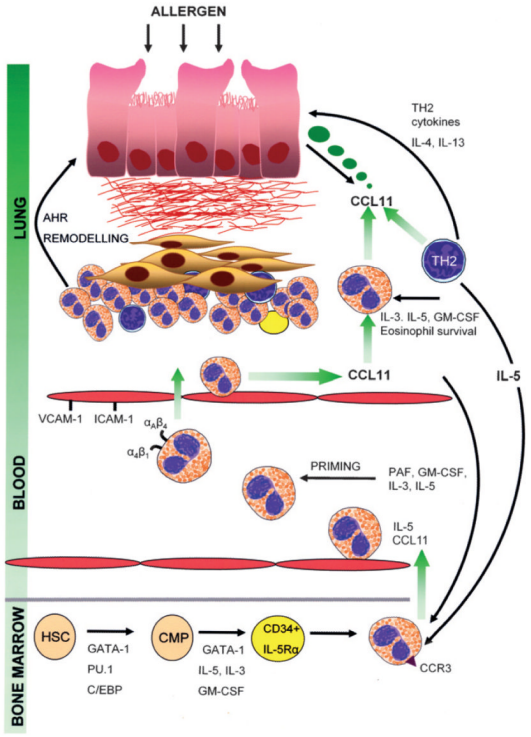

Figure 1.
Eosinophil development and trafficking. The differentiation of hematopoietic stem cells (HSC) and then common myeloid progenitor cells (CMP) into eosinophil progenitor cells ($CD34^+/IL\text{-}5R\alpha^+$) is governed by the transcription factors PU.1, C/EBP and GATA under the influence of IL-3, IL-5 and GM-CSF. IL-5 and eotaxin (CCL11) generated following allergen-induced inflammation within the lung signal the increase in eosinophil production within the bone marrow. Mature eosinophils are specifically mobilised from the bone marrow by the combined actions of IL-5 and eotaxin. Eosinophils are primed in the circulation, increasing their migratory capacity and adhesiveness. This facilitates their specific integrin-mediated trafficking across the vasculature, after which their recruitment into the lung is largely dependent on eotaxin. Eosinophil accumulation within the lung contributes to both AHR and airway remodelling. This is perpetuated by the actions of Th2 cells that can accumulate partly due to the actions of eotaxin; they then act to further increase the production of both eotaxin and IL-5 and thereby enhance eosinophil recruitment.

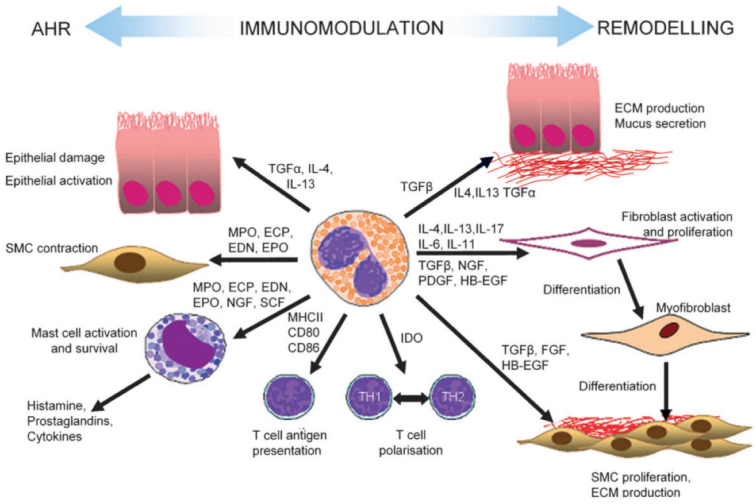

Figure 2.

Mechanisms by which eosinophils may contribute to airway hyper-responsiveness, airway remodelling and modulation of the immune response. ECM, extracellular matrix; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; FGF, fibroblast growth factor; HB-EGF, heparin-binding epidermal growth factor; IDO, Indoleamine 2,3-dioxygenase; MBP, major basic protein; NGF, nerve growth factor; SCF, stem cell factor; TGF-β, TGF-α, transforming growth factor; VEGF, vascular endothelial growth factor.

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Table 1

Eosinophil-derived secretory products.

	Mediator	General function	References
Basic granule proteins.	Major basic protein (MBP)	Cell and parasite toxicity.	[99]
		Stimulation of mast cells, basophils and neutrophils.	[91, 162]
		Binding to M2 inhibitory muscarinic receptor leading to bronchoconstriction	[163] [103]
	Eosinophil peroxidase. (EPO)	Cell and parasite toxicity. Generation of oxygen radicals as part of respiratory burst	[164, 165]
	Eosinophil-derived Neurotoxin (EDN)	Ability to cause neuronal damage. Anti-viral activity as it is a ribonuclease	[166] [167]
	Eosinophil cationic protein (ECP)	Cell and parasite toxicity. Anti-viral activity as it is a ribonuclease . Suppression of lymphocyte responses. Stimulation of mast cells and fibroblasts	[168] [167] [169] [170, 171]
Lipids	Cysteinyl leukotrienes, . PAF, thromboxane, PGD_2, PGE_2,	Activation of eosinophils, mast cells, basophils, Smooth muscle cell contraction	[60, 143, 172]
Chemokines	CCL11, CCL5, . CCL2, IL-8, CCL3	Migration of eosinophils, T cells, monocytes, macrophages	[173–177]
Cytokines	IL-3, IL-5, GM-CSF	Eosinophil development, activation and survival	[18]
	IFNγ, TNFα, IL-2, . IL-4, IL-6, IL-13, . IL-16, Il-17	Pro-inflammatory	[92, 93, 147, 177–181]
	IL-2, IL-8.	Enhances migration of primed eosinophils	[176, 182, 183]
Regulatory . mediators	IL-10, IL-12, . indoleamine 2,3-dioxygenase (IDO)	Anti-inflammatory, immunomodulatory and T cell polarising effects	[146, 148, 152]
Fibrogenic factors	IL-1β, IL-4, IL-11,. IL-13, Il-17	Activation of fibroblasts	[92, 93, 181, 184–187]
	TGF-β	ECM production, myofibroblast development	[188, 189]
	TGF-α	Epithelial cell proliferation	[190, 191]
	NGF	Fibroblast migration and differentiation	[192]
	NGF, SCF	Mast cell survival	[91]
	PDGF	Myofibroblast development, smooth muscle cell proliferation	[193–195]
	FGF	Fibroblast proliferation, angiogenesis, smooth muscle cell proliferation	[140, 195]

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	Mediator	General function	References
	VEGF	Induction of vascular permeability and angiogenesis	[140]
	HB-EGF	Fibroblast and smooth muscle cell proliferation	[196, 197]
	MMP9, MMP17	Degradation of matrix proteins, Cell migration through tissue, remodelling	[198–200]
	TIMP-1,-2	Inhibitors of MMPs	[198]

CCL2,monocyte chemotactic peptide; CCL3,macrophage inflammatory protein; CCL5, regulated on activation, normal T cell expressed and secreted; CCL11, eotaxin; ECM, extracellular matrix; FGF, fibroblast growth factor; HB-EGF, heparin-binding epidermal growth factor; IFN, interferon; MMP, matrix metalloproteinase; NGF, nerve growth factor; PAF, platelet-activating factor; PGD_2/PGE_2, prostaglandin D_2/E_2; TGF-β, TGF-α, transforming growth factor; TIMP, tissue inhibitor of metalloproteinase; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

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Table 2

Receptors expressed by eosinophils and their ligands..

	Receptor	Ligand
Chemokine	CCR1	CCL3, CCL5
	CCR2	CCL2
	CCR3	CCL2, -5,- 11, -24, -26
	CCR6	CCL20
	CRTH2	PGD_2
	CXCR1 and CXCR2 (h)	IL-8,
Activation factors and chemotactic agonists	C3aR, C3bR, C5aR	Complement factor C3 and C5
	PAF receptor	Platelet-activating factor
	LTB4R	Leukotriene B4
	CysLT1R, CysLT2R	Cysteinyl Leukotrienes
	fmLPR	Bacterial *N*-formyl peptide FMLP
	Histamine 4R	Histamine
	CD156 (ADAM8)	CD23 and various other ligands [201], [202]
Cytokine	CD25, CD116, CD119,	IL-2, GM-CSF, IFNγ, TNFα
	CD120,.	IL-3, IL-4, IL-5, IL-13
	CD123, CD124, CD125,	IL-9, TGFβ
	CD213,.	
	IL-9R, TGFβR	
Adhesion molecules	CD11a/CD18 ($\alpha_L\beta_2$)	ICAM-1,-2,-3
	CD11b/CD18 ($\alpha_M\beta_2$)	ICAM-1, fibrinogen, C3bi
	CD11c/CD18 ($\alpha_X\beta_2$)	Fibrinogen, C3bi
	CD49d/ CD29 (VLA-4/β_1)	VCAM-1, fibronectin
	CD49f / CD29 (VLA-6 β_1)	Laminin
	CD62L (L-selectin)	MAdCAM-1, CD34
	CD162 (P-selectin glycoprotein-1)	P-selectin
	$\alpha_d\beta_2$	VCAM
	$\alpha_4\beta_7$	MAdCAM-1, VCAM, fibronectin

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	Receptor	Ligand
	Siglec F (m) or Siglec 8 (h)	Sialic acid-containing glycans 6′-sulfo-sialyl Lewis X [203]
Signalling molecules	Toll like receptors-7, -8	Oligonucleotide-based pattern associated membrane proteins (PAMPs)
	MHCII	T Cell receptor
	CD80, CD86	CD28
	Leukocyte Ig-like receptor (LIR) . LIR -1,-2,-3, -7	MHC I and HLA-G molecules [204]
	CD244 (2B4) – (h)	CD48 [205]

(m) and (h) indicate exclusive expression on mouse or human eosinophils, respectively.

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