



The Biology of Eosinophils and Their Role in Asthma

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This review will describe the structure and function of the eosinophil. The roles of several relevant cell surface molecules and receptors will be discussed. We will also explore the systemic and local processes triggering eosinophil differentiation, maturation, and migration to the lungs in asthma, as well as the cytokine-mediated pathways that result in eosinophil activation and degranulation, i.e., the release of multiple pro-inflammatory substances from eosinophil-specific granules, including cationic proteins, cytokines, chemokines growth factors, and enzymes. We will discuss the current understanding of the roles that eosinophils play in key asthma processes such as airway hyperresponsiveness, mucus hypersecretion, and airway remodeling, in addition to the evidence relating to eosinophil–pathogen interactions within the lungs.

Keywords: eosinophils, asthma, IL-5, eosinophil receptors, respiratory tract infections, asthma exacerbation

INTRODUCTION

The three main processes responsible for the clinical features of asthma are well recognized: bronchoconstriction, mucus hypersecretion, and airway inflammation. However, the underlying pathophysiology responsible for these processes is complex and nuanced, involving multiple cell types and cytokines (1). Furthermore, the activity and clinical impact of each cellular and subcellular component varies considerably between individuals and can change over time, as well as in response to drug therapy and environmental/lifestyle influences.

Among these myriad cellular interactions and this extremely heterogeneous patient group, it is possible to identify certain key cells that are commonly involved—of which, arguably, the eosinophil is the most important.

Eosinophil precursors originate in the bone marrow and following differentiation traffic to the lungs (among other sites) *via* the bloodstream (2). While high concentrations of circulating eosinophils are often measured in asthmatic patients, of more clinical relevance is the lung tissue eosinophilia that is also frequently present.

The phenotype of “severe eosinophilic asthma” refers to a subgroup of asthmatic patients with evidence of eosinophilia that often require high maintenance doses of oral corticosteroids to maintain reasonable disease control. The notoriously non-specific mechanisms of action of corticosteroid therapy give rise to numerous well-documented adverse effects (3), which have driven decades of research focused on the development of targeted anti-eosinophil drug therapies. In order to understand how to better assist this group of patients, who currently have an unmet clinical need, it is helpful to understand the eosinophil itself, and the role that it plays in asthma. Targeted anti-eosinophil therapies will be touched upon but will be covered in greater detail by other reviews in this Research Topic.

EOSINOPHIL CELL STRUCTURE

Eosinophils are granulocytes, typically measuring 10–16 μm in diameter. They possess segmented (usually bi-lobed) nuclei and their nucleus: cytoplasm ratio is approximately 30%. Eosinophils stain with acidophilic dyes—a feature noted in 1879 by Paul Ehrlich, who first described eosinophils and appreciated their increased presence in patients with asthma and helminth infections, among other conditions (4). See **Figure 1** for an overview of the eosinophil ultrastructure.

The presence of large specific granules, also known as secondary granules, is a characteristic feature that distinguishes eosinophils from other granulocytes (neutrophils and basophils). Specific granules consist of a dense crystalline core and a matrix, surrounded by a membrane (5). They contain a large number of mediators capable of inducing inflammation and/or tissue damage, including basic proteins, cytokines, chemokines, growth factors, and enzymes. The predominant substances are the proteins: major basic protein (MBP) is located in the core, while the matrix contains eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN) (6).

Primary granules tend to be smaller than specific granules. They are the principal location of Charcot–Leyden crystal protein (galactin-10): hexagonal bipyramidal crystals, which exhibit

lysophospholipase activity and have been identified in tissues subject to eosinophilic inflammation (7).

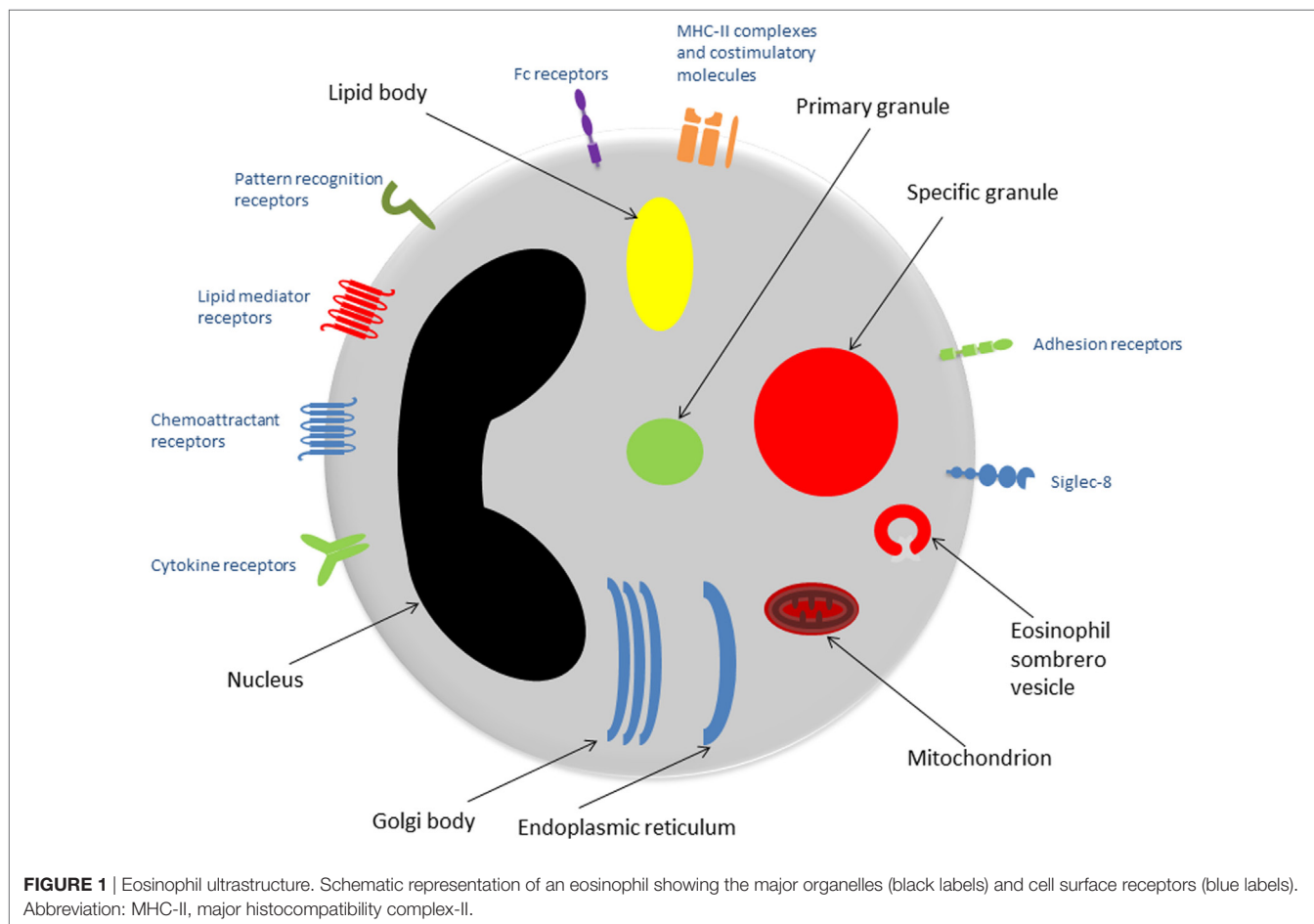
Lipid bodies are particularly important when considering the role of eosinophils in asthma, due to their involvement in the production of eicosanoids, including cysteinyl leukotrienes, prostaglandins, and thromboxane (2). Lipid bodies are a key site of arachidonic acid esterification and eicosanoid production due to their high concentrations of relevant enzymes such as cyclooxygenases, 5-lipoxygenase, and leukotriene C4-synthase (5).

Golgi bodies, endoplasmic reticulum, and mitochondria are also present and fulfill the fundamental duties of protein and adenosine triphosphate production within the eosinophil.

The histological appearance of eosinophils varies depending on the level of activation. For example, higher numbers of vesicles such as eosinophil sombrero vesicles may be seen when the cell is undergoing piecemeal degranulation (PMD), a process described in detail further on.

EOSINOPHIL SURFACE MOLECULES AND RECEPTORS

The varied roles of the eosinophil are reflected in its wide repertoire of surface molecules and receptors, which integrate eosinophils with both the innate and adaptive immune systems.



Cytokine and Growth Factor Receptors

The heterodimeric receptor for IL-5 is thought to be the most important cytokine receptor expressed by eosinophils. The beta-subunit is identical to the beta-subunit of the receptors for granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-3 (both of which are also present on eosinophil cell membranes). The alpha-subunit, IL-5R α , is specific to IL-5 and has been identified as a therapeutic target for severe eosinophilic asthma and other eosinophil-mediated conditions. The IL-5 receptor is also expressed by basophils.

Eosinophils also express receptors for multiple other cytokines and growth factors, including for IL-4, IL-13, IL-33, thymic stromal lymphopoietin, and transforming growth factor- β (TGF- β).

Chemoattractant Receptors

Chemokines are small cytokines, which stimulate the migration of specific subsets of leukocytes. Chemokines are divided into four groups, depending on the presence or absence of one or more interposing amino acid(s) between two cysteine residues (known as CXC-, CX3C-, and CC-chemokines), or the presence of only one cysteine residue (XC-chemokines) (8).

CC-chemokine receptor-3 (CCR3) is an important G protein-coupled receptor expressed on eosinophil cell membranes. CCR3 binds to all three subtypes of eotaxin (a variety of CC-chemokine that functions as a selective eosinophil chemoattractant). CCR3 also binds to several other chemokines including monocyte chemoattractant protein-3 (MCP-3) and MCP-4. The airways of patients with asthma have been shown to contain higher numbers of cells expressing mRNA for CCR3 and its ligands, compared to non-asthmatic control subjects (9). In mouse models of allergic airway inflammation, antigen-induced airway eosinophilia may be inhibited by the administration of either a monoclonal antibody against CCR3 (10) or a low molecular weight CCR3-antagonist (11).

CCR1 is another key chemokine receptor on eosinophils, which is activated by chemoattractant cytokine ligand-3 (CCL-3) and CCL-5 (also known as RANTES—regulated on activation, normal T cell expressed and secreted).

Lipid Mediator Receptors

Eosinophils possess cell surface receptors for lipid mediators such as leukotrienes, prostaglandins, and platelet-activating factor, all of which have been shown to have a role in asthma pathophysiology (12–14).

Pattern Recognition Receptors (PRRs)

Pattern recognition receptors react to microbial pathogen-associated molecular patterns (PAMPs) or host-derived damage-associated molecular patterns (DAMPs) and regulate the immune response to these indicators of infection and/or tissue damage (15).

Toll-like receptors (TLRs) are a family of PRRs, which are expressed by eosinophils, as well as multiple other cell lines. In humans there are 10 types of TLRs. TLRs are transmembrane glycoproteins, some of which are located at the cell surface and some in endosomes. The cytoplasmic domain resembles that of the IL-1 receptor, and the intracellular signals generated are therefore similar (16).

Eosinophils also express several other families of PRR, including retinoic acid-inducible gene-I-like receptors, nucleotide-binding oligomerisation domain-like (NOD-like) receptors, and the receptor for advanced glycation endproducts (RAGE) (15).

Fc Receptors

Fc receptors to IgA, IgD, IgE, IgG, and IgM are expressed on the surface of eosinophils, facilitating interaction with the adaptive immune system.

The high-affinity Fc ϵ R1 receptor binds IgE and signals *via* intracellular tyrosine kinases. On mast cells and basophils, where Fc ϵ R1 is expressed as a tetramer ($\alpha\beta\gamma_2$), stimulation by IgE results in degranulation. However, on eosinophils, Fc ϵ R1 is usually expressed in very small quantities as a trimer (without a β chain) and has no role in eosinophil activation (17). In contrast, cross-linking of Fc α RI and Fc γ RII, with IgA and IgG, respectively, has been shown to trigger eosinophil activation (18).

Major Histocompatibility Complex-II (MHC-II)

Eosinophils have an additional role as antigen-presenting cells, facilitated by the presence of MHC-II molecules and co-stimulatory molecules such as CD80 and CD86. In allergic patients, evaluated after segmental antigen challenge, expression of HLA-DR (a subtype of MHC-II molecule) was found to be approximately four times greater in lung eosinophils compared to blood eosinophils (19).

Adhesion Receptors

Adhesion receptors, as their name suggests, allow cells such as the eosinophil to adhere to the extracellular matrix (ECM) and to other cells. They also allow the eosinophil to sense its surroundings and respond accordingly. Adhesion receptors are divided into four main groups: integrins, cadherins, selectins, and immunoglobulin-like cell adhesion molecules (Ig-CAM). Integrins and selectins are the main forms of adhesion receptors expressed on eosinophil cell membranes.

Eosinophils express seven types of integrins, which are transmembrane glycoproteins, consisting of an α and a β chain (20). Examples include very late antigen-4 (VLA-4, CD49d/CD29) and the complement receptor CR3 (CD11b/CD18), which is otherwise known as macrophage-1 antigen (Mac-1).

Selectins are single-chain transmembrane glycoproteins with multiple domains. There are three families: E-, L-, and P-selectin; the latter two are expressed by human eosinophils, whereas E-selectin is present on activated endothelium (21).

Siglec-8

Siglec-8 is a sialic acid immunoglobulin-like lectin (a carbohydrate-binding protein) expressed by eosinophils, mast cells, and basophils. Its physiological role has not yet been identified, although it is thought to represent a potential therapeutic target for eosinophil-mediated disease, due to the observation that administration of an antibody targeted against Siglec-8 can induce selective eosinophil apoptosis and inhibit mast cell degranulation (22).

EOSINOPHIL DIFFERENTIATION, MATURATION, MIGRATION, ACTIVATION, AND DEGRANULATION

Eosinophils develop from pluripotent CD34+ granulocyte progenitor cells.

Differentiation and maturation occurs as follows:

Myeloblast → Promyelocyte → Eosinophil myelocyte
→ Eosinophil metamyelocyte → Eosinophil.

Allergen challenge of mild asthmatics results in increased expression of IL-5R α on CD34+ cells in the bone marrow, associated with blood and sputum eosinophilia (23). Eosinophil differentiation usually occurs in the bone marrow. However, eosinophil precursors have been isolated from the peripheral blood of atopic subjects at significantly higher concentrations compared to non-atopic controls (24). Increased numbers of CD34+/IL-5R α + eosinophil precursors have also been identified in bronchial biopsies of atopic asthmatics, compared to non-asthmatic control subjects (both atopic and non-atopic) (25). Eosinophil-lineage committed cells have also been identified in lung tissue in a mouse model of allergic airway inflammation (26). More recently, eosinophil progenitors isolated from the blood of patients with severe eosinophilic asthma have been shown to have an exaggerated clonogenic response to IL-5 *in vitro*, compared to eosinophil precursors from mild asthmatics, suggesting that *in situ* eosinophilopoiesis may have a clinically relevant role in severe eosinophilic asthma (27).

The differentiation of eosinophils is regulated by the transcription factors GATA-binding protein 1 (GATA-1), PU.1, and the CCAAT-enhancing binding protein (c/EBP) family. GATA-1 and PU.1 synergistically promote transcription of MBP (28). GATA-1 is thought to have the most important role, as disruption of the GATA-1 gene in mice results in a strain completely devoid of eosinophils (29).

The cytokines IL-3, IL-5, and GM-CSF also synergistically contribute to the development of mature eosinophils (30). IL-5 is the most eosinophil-specific and also promotes the release of eosinophils from the bone marrow to the bloodstream, acting synergistically with eotaxin (31, 32). Eosinophils are present in relatively low numbers in peripheral blood, usually accounting for no more than 5% of the total white blood cell count (33). They have a relatively short blood half-life of approximately 18 h (34). Migration to specific body sites, including the lungs and intestines, is mediated by eosinophil chemoattractants such as eotaxins. Eotaxins are a variety of CC-chemokines. There are three known subtypes: eotaxin-1 (CCL-11), eotaxin-2 (CCL-24), and eotaxin-3 (CCL-26). These bind to CCR3 receptors on the cell membranes of eosinophils and induce chemotaxis. 5-oxo 6, 8, 11, 14-eicosatetraenoic acid (5-oxo-EETE) is another eosinophil chemoattractant.

In vitro, the presence of prostaglandin-D2 (PGD2) has been shown to significantly enhance the chemoattractant effects of eotaxin-1 and 5-oxo-EETE on eosinophils and—unlike eotaxin-1 or 5-oxo-EETE—PGD2 retains its chemoattractant effect in the presence of blood or plasma (35). It is therefore proposed that

PGD2 acts as the initial chemoattractant, triggering the migration of circulating eosinophils to specific tissues, where eotaxins and 5-oxo-EETE then predominate. PGD2 is released from activated mast cells (36) and acts *via* CRTh2 (chemoattractant receptor-homologous molecule expressed on T_H2 cells).

In allergic inflammation and asthma, circulating eosinophils adhere to the vascular endothelium and roll along it, before extravasating to the lung tissue. Initial tethering to the endothelium occurs as a result of the eosinophil cell membrane adhesion receptor P-selectin binding to P-selectin glycoprotein ligand-1 on the endothelium (37). Binding of the integrin VLA-4 to vascular cell adhesion molecule-1 promotes eosinophil activation and extravasation (37). IL-13 results in increased eosinophil expression of P-selectin and increased P-selectin mediated adhesion to endothelial cells (38).

The eosinophil's ability to store several preformed cytotoxic mediators ready for rapid release upon appropriate stimulation facilitates a much quicker reaction to pro-inflammatory stimuli, compared to other cells, whose responses depend on upregulating the transcription of genes coding for such proteins.

The bronchial epithelium produces the cytokines IL-25, IL-33, and thymic stromal lymphopoietin, collectively known as the alarmins, in response to irritants such as allergens, pollutants, and pathogens. These cytokines trigger an inflammatory cascade involving, among others, T helper-2 (T_H2) cells and type-2 innate lymphoid cells (ILC2s), resulting in increased production of numerous cytokines including IL-4, IL-5, and IL-13, therefore prompting eosinophil activation (1, 39).

High mobility group box 1 protein, acting *via* receptors TLR-2, TLR-4, and RAGE, also promotes eosinophilia, although less is known regarding its mechanism of action (2).

Specific granule contents may be released *via* three different degranulation processes. Conventional exocytosis entails the specific granules fusing with the eosinophil cell membrane, resulting in the release of the entire contents of the specific granule. Alternatively, the eosinophil may be lysed (cytolysis), releasing all the cell contents, including the intact specific granules. These extracellular granules can be found in tissues affected by eosinophil-mediated disease and may subsequently release their contents in response to pro-inflammatory stimuli (40). However, the most common mechanism of eosinophil granulation is termed piecemeal degranulation (PMD). In this process, vesicles (both round and tubular) are released from specific granules and travel to the cell membrane to discharge their contents to the extracellular domain (41). The tubular vesicles tend to curl into a hoop-like morphology, giving rise to the term “eosinophil sombrero vesicles” (42). Vesicles with particular contents may be selectively released in response to particular cytokines, allowing eosinophils to supply a specific combination of cytotoxic mediators on demand (42, 43).

The activation of TLRs on eosinophils has been shown to promote adhesion and the release of certain cytokines and superoxides (44). Activation of TLR-2 and TLR-9 triggers eosinophil degranulation (44, 45). *In vitro*, eosinophils from atopic subjects have been shown to produce more IL-8 and EDN in response to stimulation of TLR-7 and TLR-9, compared to healthy controls (45).

Eosinophil survival is promoted by IL-3, IL-5, GM-CSF, and eotaxin (37). Activation of TLR-7 (the most abundant TLR subtype expressed by eosinophils) also enhances eosinophil survival (45).

IL-3, IL-5, AND GM-CSF

Among the type-2 cytokines, IL-3, IL-5, and GM-CSF are particularly important for the initiation and perpetuation of eosinophilic airway inflammation. These three cytokines are closely linked, in that the genes for all three are all located on chromosome 5, and their receptors also share a common β -subunit (β c).

Monoclonal antibodies against IL-5 have been developed in order to treat eosinophil-mediated diseases such as eosinophilic asthma. Although inhibition of IL-5 activity in this manner (using mepolizumab) results in significant depletion of circulating eosinophils, the effect on bronchial tissue eosinophilia is less marked, with a median reduction of 55% (46). The residual tissue eosinophilia may reflect ongoing effects mediated by IL-3 and GM-CSF.

In a mouse model of allergic airways inflammation, allergen-induced lung tissue eosinophilia was abolished in mice bred to lack the common β -subunit, therefore incapable of responding to IL-3, IL-5, and GM-CSF (47). In the same study, lung tissue from β c-deficient mice was found to contain fewer myeloid dendritic cells, and the local T_H2 cells showed a reduced ability to proliferate and produce type-2 cytokines (47). These findings suggest a multifactorial role for the common β -subunit in the regulation of allergic airway inflammation.

THE ROLE OF THE EOSINOPHIL IN HEALTH

In comparison to the roles that eosinophils play in diseases and infections, relatively little is known about their purpose in health. However, an increasing number of homeostatic mechanisms have been attributed to—or at least associated with—eosinophils in recent years. This has prompted a call for a fundamental change of the perception of eosinophils purely as cytotoxic effector cells (48, 49).

In health, eosinophils are found in the thymus, spleen, lymph nodes, and gastrointestinal (GI) tract (50). The number of eosinophils in the thymus declines with age (51). Eosinophils may have a role in T cell selection. In a mouse model of MHC I-restricted acute negative selection, eosinophil recruitment to the corticomedullary region of the thymus and association with apoptotic bodies has been demonstrated (52). Eosinophils also enhance the ability of macrophages to phagocytose apoptotic thymic cells (53).

Eosinophils migrate to the GI tract during embryonic development, i.e., prior to the development of any viable gut flora (54). In health, they are present throughout the GI tract—with the notable exception of the esophagus. Eosinophils contribute to the immune defense against gut microorganisms, due to multiple antimicrobial properties. (The antimicrobial properties of eosinophils are discussed in detail further on, with specific

relation to respiratory pathogens.) Other potential homeostatic roles for eosinophils within the gut are not currently well defined but may relate to their ability to interact with the enteric neuronal system and increase smooth muscle reactivity (*via* release of MBP) (55).

In murine white adipose tissue, a positive correlation was identified between eosinophil counts and the numbers of arginase-1-expressing macrophages (56). Macrophages expressing arginase-1 are thought to contribute to glucose homeostasis, although macrophage classification is contentious (57). In a more recent study involving more than 9,000 human participants, the peripheral blood eosinophil percentage was found to be inversely associated with the risk of type-2 diabetes mellitus and insulin resistance (58).

Eosinophils have also been implicated in the regeneration of liver tissue (59) and skeletal muscle (60). The increased presence of eosinophils in preovulatory ovarian follicles (61) and in endometrium (62) has prompted speculation that they may have a role in tissue remodeling related to ovulation and menstruation.

Eosinophils also perform several important immunomodulatory functions, discussed in the following section.

THE EOSINOPHIL'S ROLE IN ASTHMA PATHOPHYSIOLOGY

Asthma pathophysiology is complex, and the relative contributions of the various cytokine networks involved vary between patients. Core features include airway hyperresponsiveness (AHR), mucus hypersecretion, tissue damage, and airway remodeling. See **Figure 2** for an overview of the eosinophil's role in asthma pathophysiology.

It has long been observed that eosinophil counts in peripheral blood and bronchoalveolar lavage (BAL) fluid are higher in asthmatics compared to healthy controls (63). Analysis of BAL fluid obtained from patients with atopic asthma reveals increased expression of T_H2 cytokines (64), including IL-5, which are strongly associated with eosinophilic inflammation (65). In general, the degree of eosinophilia correlates with disease severity and exacerbation frequency (63, 66). However, non-eosinophilic asthma phenotypes are also recognized (67). Peripheral blood eosinophilia may also occur in numerous other conditions (see **Box 1**).

AHR and Mucus Hypersecretion

Eosinophils may be prompted to release a number of different mediators with the capacity to cause AHR. Human MBP is known to result in AHR when administered to primates (70) and rats (71). In the former study, administration of EPO also caused AHR, although ECP and EDN did not (70). Data from the latter study suggested the mechanism of action involved the stimulation of bradykinin production (71). MBP can also trigger mast cells and basophils to release histamine, a potent mediator of bronchial hyperreactivity (72, 73).

Eosinophils are a source of several cytokines including IL-13, which causes AHR, and also promotes mucus hypersecretion *via* enhanced differentiation of goblet cells (74). IL-13 is also

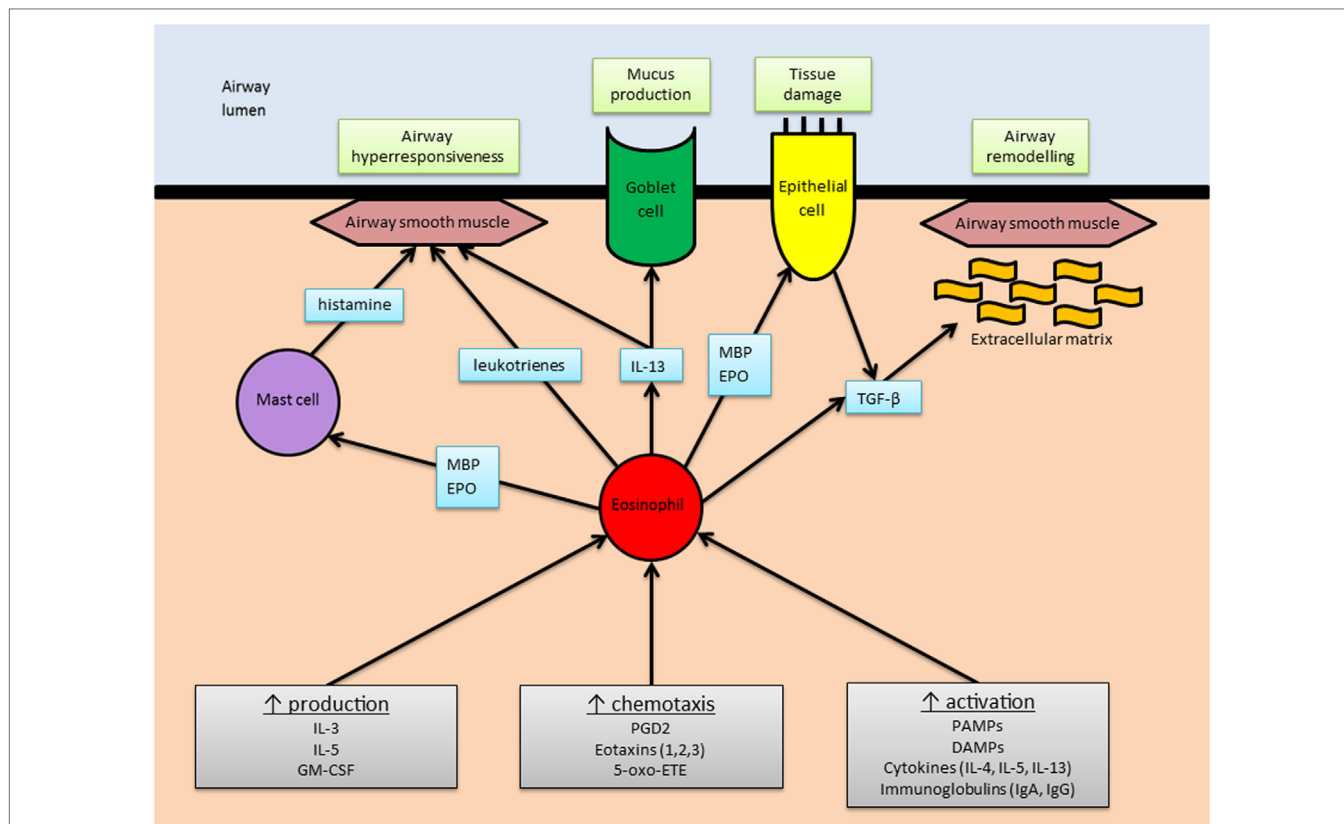


FIGURE 2 | The role of eosinophils in asthma. An overview of the main stimuli for eosinophilic airway inflammation (gray boxes) and the means by which eosinophils elicit the main pathophysiological changes associated with asthma (green boxes). Abbreviations: MBP, major basic protein; EPO, eosinophil peroxidase; IL, interleukin; TGF-β, transforming growth factor-β; GM-CSF, granulocyte-macrophage colony-stimulating factor; PGD2, prostaglandin-D2; 5-oxo-EETE, 5-oxo 6, 8, 11, 14-eicosatetraenoic acid; PAMPs, pathogen associated molecular patterns; DAMPs, damage associated molecular patterns; Ig, immunoglobulin.

BOX 1 | Alternative (i.e., non-asthma) causes of peripheral eosinophilia (68, 69).

Respiratory

- Eosinophilic granulomatosis with polyangiitis (EGPA)
- Allergic bronchopulmonary aspergillosis
- Sarcoidosis

Hematological and neoplastic

- Myeloproliferative hypereosinophilic syndrome
- Lymphocytic-variant hypereosinophilic syndrome
- Certain leukemias and lymphomas
- Systemic mastocytosis
- Solid tumors—adenocarcinomas, squamous cell carcinomas, large cell lung carcinomas, transitional cell carcinoma of the bladder

Infective

- Parasitic infection, in particular helminths
- Human immunodeficiency virus

Dermatological

- Eczema
- Scabies infestation

Iatrogenic

- Certain drug hypersensitivity reactions
- Graft vs host disease

produced by T_H2 cells and ILC2s. Lipid mediators such as leukotrienes, which are produced in eosinophil lipid bodies (and mast cells), also cause AHR and mucus hypersecretion (75).

Studies involving two different strains of eosinophil-deficient mice have attempted to clarify the role of eosinophils in an ovalbumin model of asthma but yielded seemingly contradictory results. In one study, performed by Lee et al., eosinophil-deficient mice were protected from AHR and mucus hypersecretion (76). However, another study, led by Humbles, found that eosinophil deficiency was not protective in this regard (77). Several theories have been put forward to explain the conflicting results, including evidence of residual lung eosinophils in the Humbles study and differences between the underlying mouse strains (78).

In terms of practical application, the existence in humans of eosinophilic bronchitis, a condition characterized by marked airway eosinophilia in the absence of AHR, calls into question the concept that eosinophils—acting alone—have a clinically significant impact on AHR. In patients with mild asthma, administration of a monoclonal antibody to IL-5 has been shown to reduce blood and sputum eosinophilia but had no effect on AHR (79). This may reflect the cellular redundancy of AHR pathophysiology, which involves several cell types including T_H2 cells, ILC2s,

and mast cells. In addition, the current evidence relating to AHR does not specifically study the pathophysiology of asthma exacerbations, during which it is possible that eosinophil degranulation may contribute to worsening AHR.

Tissue Damage and Airway Remodeling

Postulation that eosinophils are major effectors of lung tissue damage in asthma is well founded, given their propensity to release highly charged basic proteins, which exert multiple cytotoxic effects. MBP is toxic to respiratory epithelial cells *in vitro* and has been identified in postmortem lung tissue specimens of patients who have died of asthma, in association with epithelial damage (80). ECP and EDN share 67% amino acid sequence homology and tend to be grouped together as eosinophil-associated RNases, although ECP's RNase activity is much less potent (81). ECP binds to cell membranes and alters their permeability (82). EDN, as its name suggests, is neurotoxic. It was first identified following the observation that, in rabbits, the intracerebral administration of eosinophils resulted in the destruction of cerebellar Purkinje cells and neurological features named "the Gordon phenomenon" (83, 84). EPO catalyzes the oxidation of halides and thiocyanate, resulting in cytotoxic reactive oxidant species (85).

Cell damage triggers the activation of repair pathways which, if excessive, may contribute to structural changes referred to as airway remodeling. The underlying pathological processes include hyperplasia of fibroblasts, airway smooth muscle (ASM) and goblet cells, deposition of ECM proteins, and angiogenesis (86). Airway remodeling is associated with the severity of asthma (87). It has been hypothesized that airway remodeling is responsible for the accelerated decline in lung function and development of fixed airway obstruction observed in some asthmatic patients. However, bronchial biopsies of children with difficult asthma have been shown to display reticular basement membrane thickening to a similar degree compared with adult asthmatics (88). Furthermore, there is evidence that some pathological features of airway remodeling can become evident within 24 h of allergen exposure (89).

Eosinophils release multiple growth factors and fibrogenic mediators that promote airway remodeling (see **Table 1**). For example, eosinophils are known to produce TGF- β in disease states involving the skin (atopy) (90), nose (nasal polyposis) (91), and blood (idiopathic hypereosinophilic syndrome) (92). Eosinophils are the main source of TGF- β in bronchial biopsies taken from asthmatic patients (93) and can also stimulate epithelial cells to produce a number of mediators, including TGF- β (94). TGF- β is implicated in tissue remodeling *via* fibroblast proliferation and increased production of collagen and glycosaminoglycans (95–97).

Eosinophils isolated from asthmatics, when cocultured with ASM cells, promote enhanced ASM proliferation, which is inhibited by the addition of the leukotriene antagonist montelukast (117). It appears that eosinophils and ASM enjoy a reciprocal relationship, as ASM cells are also known to produce pro-eosinophil cytokines (118). Mouse studies lend further support to the assertion that eosinophils have an important role in airway remodeling, as eosinophil-deficient mice are protected against airway deposition of collagen and smooth muscle (77).

Treatment with the anti-IL-5 monoclonal antibody mepolizumab has been shown to reduce bronchial tissue eosinophilia, in association with decreased TGF- β 1 in BAL specimens, and reduced reticular basement membrane procollagen III, tenascin, and lumican (119).

Asthma Exacerbations

Airway eosinophilia is an early feature of asthma exacerbations. In a study of steroid-dependent asthmatic patients, whose prednisolone dose was gradually reduced to below their maintenance requirement, the sputum eosinophil count started to rise 4 weeks before the blood eosinophil count and 6 weeks prior to spirometric and symptomatic deterioration (120). In fact, the adoption of an asthma treatment strategy based on sputum eosinophilia rather than traditional markers of disease activity (such as symptoms and spirometry) was found to reduce the frequency of exacerbations, with no overall increase in the average daily corticosteroid dose (121).

The primary action of anti-IL-5 therapies appears to be a reduction in exacerbation frequency. Administration of mepolizumab to selected patient groups reduced exacerbation rates by approximately 50% (122–124). A similar reduction in exacerbation rates was seen with reslizumab, another anti-IL-5 monoclonal antibody (125). Mepolizumab has also been found to have a moderate glucocorticoid-sparing effect in a phase III clinical trial (126).

Benralizumab is a monoclonal antibody targeted against the alpha subunit of the IL-5 receptor (IL-5R α). As well as blocking the interaction between IL-5 and its receptor, benralizumab causes eosinophil cell death *via* antibody-dependent cell-mediated cytotoxicity (127), resulting in striking (95%) airway eosinophil depletion (128). Phase III clinical trials have demonstrated reductions in exacerbation rates (129, 130).

Immunomodulation

In addition to the direct effects of eosinophils on asthma pathophysiology, they have an important role in immunomodulation (2). MBP, released from eosinophil-specific granules, stimulates inflammatory responses from neutrophils (increased production of superoxide and IL-8) (131) and mast cells (increased histamine release) (72). Nerve growth factor (also released from specific granules) has also been shown to prolong the survival of neutrophils (132) and mast cells (133). EDN promotes the activation of dendritic cells (134), which in turn trigger the proliferation of T cells (both helper and cytotoxic) and B cells *via* antigen presentation. Eosinophils themselves can also present antigens to T cells (135, 136).

The cytokines released from eosinophil-specific granules have various immunomodulatory effects. For example, IL-4 and IL-13 simulate the proliferation of B cells and IgE production (137, 138), and IL-6 enhances survival of plasma cells (139, 140). Eosinophil-specific granules are also capable of releasing several chemokines. Depending on the stimulation they receive, these include CCL-17 and CCL-22, which attract T_H2 cells, and CXCL-9 and CXCL-10, which are T_H1 chemokines (141). In addition, eosinophils express indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes the production of kynurenine,

TABLE 1 | Factors produced by eosinophils that are associated with airway remodeling.

Factor	Mechanism(s) and evidence
TGF- β	<ul style="list-style-type: none"> • Epithelial/submucosal expression of TGF-β correlates with basement membrane thickness and fibroblast numbers (98). • In allergen-challenged human atopic skin, eosinophils expressing TGF-β1 are associated with myofibroblast formation and deposition of tenascin and procollagen-1 (90). • TGF-β induces hypertrophy and increased contractility of ASM <i>in vitro</i> (99). • Administration of anti-TGF-β antibody to mice with established eosinophilic airway inflammation significantly reduces airway remodelling (100).
MMP-9 and TIMP-1	<ul style="list-style-type: none"> • MMP-9 breaks down ECM proteins; TIMP-1 inhibits MMP-9. • Sputum MMP-9 and TIMP-1 concentrations are higher in asthmatics compared to controls; The MMP-9/TIMP-1 ratio is lower in patients with asthma and chronic bronchitis, and positively correlates with FEV1 (101). • MMP-9 is required for angiogenesis in mice (102).
VEGF, bFGF, and angiogenin	<ul style="list-style-type: none"> • VEGF, bFGF, and angiogenin promote angiogenesis. • Bronchial biopsies of asthmatics exhibit greater immunoreactivity to VEGF, bFGF, and angiogenin; Immunoreactivity to these factors positively correlates with vascular area (103).
Specific granule proteins	<ul style="list-style-type: none"> • MBP and ECP are toxic to airway epithelial cells. • Damaged airway epithelium produces TGF-β (104). • ECP induces fibroblast migration (105) and inhibits fibroblast-mediated proteoglycan degradation (106).
IL-17	<ul style="list-style-type: none"> • Fibroblasts isolated from bronchial biopsies produce more IL-6 and IL-11 (profibrotic cytokines) when stimulated by IL-17 (107). • In a mouse model of asthma, administration of IL-17A results in increased vascular remodelling; <i>in vitro</i>, IL-17A accelerates EPC migration (108).
IL-13	<ul style="list-style-type: none"> • Mice bred to overexpress IL-13 exhibit eosinophilic airway inflammation, epithelial cell hypertrophy, mucus metaplasia, and subepithelial fibrosis (109). • <i>In vitro</i>, IL-13 induces human bronchial epithelial cells to release TGF-β (110).
HB-EGF	<ul style="list-style-type: none"> • Recombinant HB-EGF promotes migration of ASM cells <i>in vitro</i> and accelerates the thickening of the ASM layer in a mouse model of asthma (111).
NGF	<ul style="list-style-type: none"> • NGF causes migration of vascular smooth muscle cells and fibroblasts, and proliferation of epithelial cells and ASM cells (112). • In mice with chronic allergen-induced airway inflammation, anti-NGF antibodies reduce airway collagen deposition (113).
Cysteinyl leukotrienes	<ul style="list-style-type: none"> • In a mouse model of allergen-induced airway remodelling, administration of montelukast (a CysLT1 receptor blocker) reverses established ASM layer thickening and subepithelial fibrosis (114).
SCF	<ul style="list-style-type: none"> • SCF promotes mast cell proliferation and activation. • Mast cells produce TNF-α, which can damage bronchial epithelial cells (115) and stimulate fibroblasts to produce TGF-β (116).

TGF, transforming growth factor; MMP, matrix metalloproteinase; ECM, extracellular matrix; TIMP, tissue inhibitor of metalloproteinase; FEV1, forced expiratory volume in 1 s; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; MBP, major basic protein; ECP, eosinophil cationic protein; IL, interleukin; EPC, endothelial progenitor cell; HB-EGF, heparin-binding epidermal growth factor-like growth factor; ASM, airway smooth muscle; NGF, nerve growth factor; CysLT1, cysteinyl leukotriene 1; SCF, stem cell factor; TNF, tumor necrosis factor.

suppressing T_H1 activity and promoting a type-2 inflammatory milieu (142, 143).

See **Figure 3** for an overview of the eosinophil's immunomodulatory roles in asthma.

INTERACTIONS BETWEEN EOSINOPHILS AND RESPIRATORY PATHOGENS

Eosinophils have traditionally been regarded as end-stage effector cells, responding to infections directly, i.e., by releasing substances that are toxic to pathogens (in particular, helminths) and resulting in the unwanted secondary effect of human tissue damage. However, research performed over the last 30 years has revealed additional roles fulfilled by the eosinophil, involving links with both the innate and adaptive immune systems. These roles include antigen presentation and interaction with other parts of the immune system, such as the complement pathway (144).

Pattern recognition receptors on the cell membranes of eosinophils allow them to detect the presence of PAMPs such as lipopolysaccharide (LPS) and beta-glucans, cell wall components

of bacteria and fungi, respectively (144). The cysteine and serine proteases produced by mites and fungi activate eosinophils *via* protease-activated receptors (5). TLR-7, the most common TLR expressed by eosinophils, is activated by viral single-stranded RNA (2).

The contents of eosinophil-specific granules are directly cytotoxic to pathogens. MBP causes disruption of cell membranes due to its highly basic nature (145). ECP has antiviral activity (146) and can also agglutinate Gram-negative bacteria by binding to LPS and peptidoglycans (147). EDN is only mildly toxic to helminths, compared to MBP and ECP (148). However, EDN significantly reduces the infectivity of respiratory syncytial virus group B, indicating a role in the immune response to viruses (149). EPO facilitates the generation of toxic reactive oxygen species (150).

In addition to releasing cytotoxic proteins, eosinophils have been shown to phagocytose bacteria (albeit less efficiently than neutrophils) (151). More recently, the “catapult-like” extrusion of “traps” consisting of mitochondrial DNA and eosinophil granule contents, in response to Gram-negative bacteria, has been observed (152).

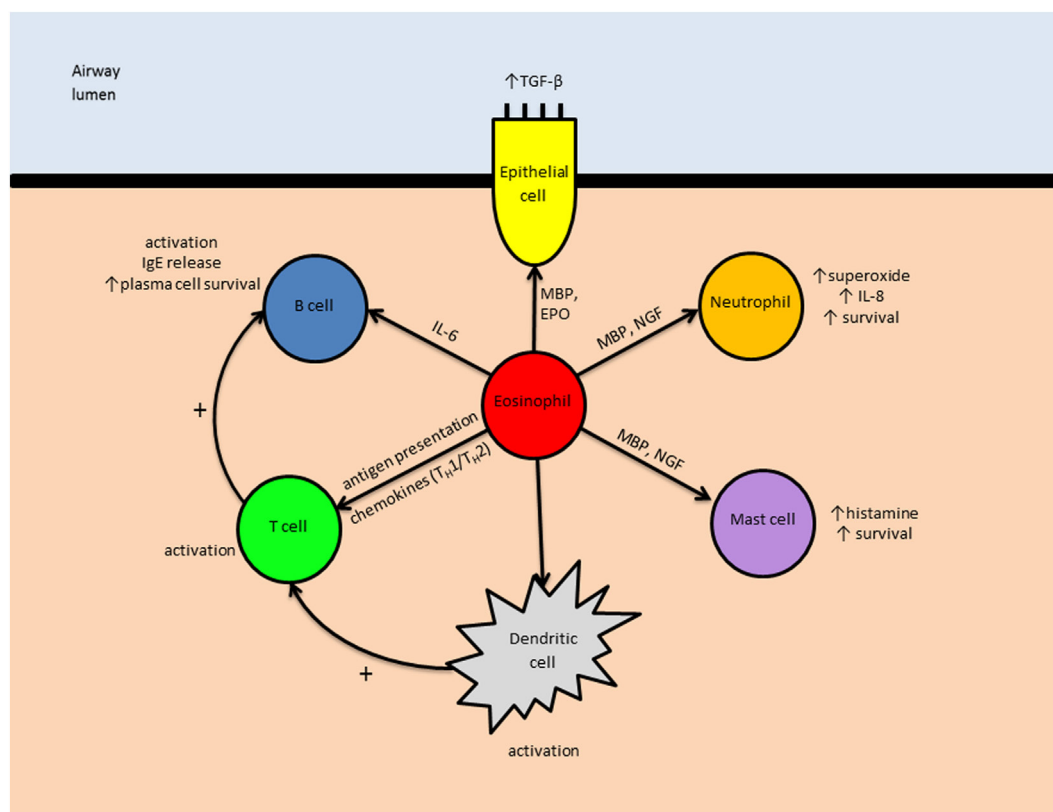


FIGURE 3 | The immunomodulatory role of eosinophils in asthma. Eosinophils may influence other leukocytes both directly (e.g., IL-6-induced B cell activation) and indirectly (e.g., by enhancing antigen presentation by dendritic cells). Abbreviations: TGF- β , transforming growth factor- β ; IgE, immunoglobulin E; IL, interleukin; MBP, major basic protein; EPO, eosinophil peroxidase; NGF, nerve growth factor; T_H1 , type 1 T helper cell; T_H2 , type 2 T helper cell.

Eosinophils express receptors for various complement proteins, including C3a and C5a, which are known to promote eosinophil recruitment, extravasation, and activation (153, 154). Complement is thought to facilitate eosinophil adherence to, and damage of, nematode larvae (155), although the development of secondary immunity is unaffected in complement-deficient mice (156).

As fungi are known to trigger the production of T_H2 -associated cytokines (i.e., type-2 cytokines) and eosinophilia, it has been hypothesized that subclinical fungal infection/colonization of the airways may play a role in the genesis of diseases characterized by eosinophilia. Such diseases include severe eosinophilic asthma, as well as related conditions (e.g., chronic rhinosinusitis). One study of patients undergoing sinus surgery found that 74% of those with T_H2 -associated conditions had evidence of airway surface mycosis, compared to just 16% of controls (157). However, potential confounding factors such as inhaled and/or systemic corticosteroid usage must be considered.

The increased susceptibility to respiratory viral infections observed in patients with asthma has been linked to reduced production of type I and type III interferons (158, 159). Eosinophils may contribute to this impairment by producing TGF- β , which has been shown to diminish the ability of bronchial epithelial cells to produce interferons in response to human rhinovirus *in vitro* (160).

The lung is known to harbor communities of bacteria, known as the lung microbiome, during health, which are deranged in disease states including asthma (161, 162). Data have recently been published suggesting a possible link between the level of eosinophilia and microbiome community structure in asthma (163). Further dedicated studies, examining subject groups matched for baseline characteristics, are required.

CONCLUSION

Although eosinophils have been associated with asthma since their initial discovery, our understanding of their roles in health and disease has evolved significantly over time. The eosinophil's status as a cytotoxic effector cell appears to be justified, due its capacity to release potent destructive basic proteins, capable of antimicrobial effects as well as host tissue damage. However, its ability to modulate the innate and adaptive immune systems may be just as important.

An appreciation of the numerous receptors expressed by eosinophils offers some insight into the many different interactions this versatile cell is capable of. Not only is the eosinophil recruited to the lungs in the context of pro-inflammatory type-2 cytokines but it is also a promoter of the type-2 inflammatory milieu, taking on roles such as antigen presentation and cytokine-mediated modulation of local lymphocytes.

There is strong evidence that eosinophils contribute to airway remodeling in asthma. Mechanisms also exist by which eosinophils could promote AHR and mucus hypersecretion.

The development of new anti-eosinophilic drugs, capable of selective depletion of eosinophils, offers great potential to explore further questions relating to the role of eosinophils in asthma and the consequences of their eradication. Research into variation in eosinophil-related gene expression between individuals may provide further insights regarding the relative contributions of eosinophils in different asthma phenotypes and the potential application of personalized medicine to this field.

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CM performed the initial literature review and drafted the article. AM-G provided critical review and additional content.

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- Conflict of Interest Statement:** CM has attended an international conference with Boehringer Ingelheim. AMG has attended advisory boards for Glaxo SmithKline, Novartis, Astra Zeneca, Boehringer Ingelheim and Teva. He has received speaker fees from Novartis, Astra Zeneca, Vectura, Boehringer Ingelheim and Teva. He has participated in research for which his institution has been remunerated with Hoffman La Roche, Glaxo SmithKline and Boehringer Ingelheim. He has attended international conferences with Napp and Astra Zeneca and has consultancy agreements with Astra Zeneca and Vectura.

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