Eosinophils: Old Players in a New Game

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Abstract

Eosinophils are terminal polymorphonuclear cells with a high number of cytoplasmic granules that originate in bone marrow. Some are exosomes, which contain multiple molecules, such as specific eosinophilic proteins, cytokines, chemokines, enzymes, and lipid mediators that contribute to the effector role of these cells. Moreover, exosomes present a large number of receptors that allow them to interact with multiple cell types. Eosinophils play an important role in defense against infestations and are a key element in asthma and allergic diseases. Eosinophils are recruited to the inflamed area in response to stimuli, modulating the immune response through the release to the extracellular medium of their granule-derived content. Various mechanisms of degranulation have been identified. Polymorphonuclear leukocytes contain multivesicular bodies that generate exosomes that are secreted into the extracellular environment. Eosinophilic exosomes participate in multiple processes and mechanisms. Eosinophils participate actively in asthma and are hallmarks of the disease. The cells migrate to the inflammatory focus and contribute to epithelial damage and airway remodeling. Given their relevance in this pathology, new therapeutic tools have been developed that target mainly eosinophils and their receptors.

In this manuscript, we provide a global, updated vision of the biology of eosinophils and the role of eosinophils in respiratory diseases, particularly asthma. We also summarize asthma treatments linked to eosinophils and new therapeutic strategies based on biological products in which eosinophils and their receptors are the main targets.

Key words: Eosinophils. Exosomes. Asthma. Biological treatments.

Resumen

Los eosinófilos son células polimorfonucleares terminales originadas en la médula ósea con un número importante de gránulos citoplasmáticos, algunos de los cuales son exosomas, que contienen múltiples moléculas como proteínas eosinofílicas específicas, citocinas, quimiocinas, enzimas y mediadores lipídicos que contribuyen al papel efector de estas células. Además, presentan una gran cantidad de receptores que les permiten interactuar con múltiples tipos celulares. Los eosinófilos desempeñan un papel importante en la defensa contra las infestaciones y son un elemento clave en el asma y las enfermedades alérgicas. Los eosinófilos se reclutan hacia el área de inflamación en respuesta a varios estímulos, modulando la respuesta inmune a través de la liberación al medio extracelular del contenido derivado de sus gránulos, existiendo diferentes mecanismos de degranulación. Estos leucocitos polimorfonucleares contienen cuerpos multivesiculares que generan exosomas que se secretan al ambiente extracelular. Estos exosomas eosinofílicos participan en múltiples procesos y mecanismos. En relación con la enfermedad asmática, los eosinófilos participan activamente en los elementos distintivos de esta patología. Estas células migran al foco inflamatorio y contribuyen al daño del epitelio y a la remodelación de las vías respiratorias. Debido a su relevancia en esta patología, se han desarrollado nuevas herramientas terapéuticas, siendo los eosinófilos y sus receptores sus objetivos principales.

En este manuscrito, proporcionamos una visión global y actualizada sobre la biología de los eosinófilos, su papel en las enfermedades respiratorias, centrando nuestra atención en la patología asmática, así como un resumen de los tratamientos y nuevas estrategias terapéuticas basadas en tratamientos biológicos en los que los eosinófilos y sus receptores son los principales objetivos.

Palabras clave: Eosinófilos. Exosomas. Asma. Tratamientos biológicos.

Introduction

Eosinophils were first described by Paul Ehrlich in 1879. Since their presence in pathogenic processes was identified in 1922 [1], many studies have attempted to understand their unique biology. Based on this growing body of knowledge, eosinophils have ceased to be understood as passive cells and are now known as powerful effector cells. Eosinophils are involved in host defense, immune and adaptive responses, tissue damage, and airway remodelling [2].

Eosinophil Differentiation

In humans, eosinophil progenitors are $CD34^+$ hematopoietic stem cells (common myeloid progenitor) [3]. Under normal conditions, eosinophils are produced from $CD34^+$ progenitor cells located in the bone marrow under the effect of granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin (IL) 3, and IL-5 [4]. IL-5 is an essential cytokine in eosinophil development, as it promotes terminal differentiation, growth, and survival, as well as the activation of eosinophils [5]. The role of IL-5 in eosinophil differentiation has been widely described, and several studies have shown that IL-5–deficient mice do not develop peripheral blood or tissue eosinophilia under asthmatic conditions or in helminth infection [6,7]. Several authors recently demonstrated the eosinophilopoietic potential of IL-33 and thymic stromal lymphopoietin (TSLP) [8,9]. Since these $CD34^+$ cells can coexpress IL-5 receptor alpha subunit (IL-5Rα) on their surface [10], $CD34^+$/IL5-Rα^+ cells may be the earliest progenitors committed to the eosinophil lineage [3]. The IL-5 receptor is composed of an α subunit that only binds IL-5 and the ß chain, which is shared between the receptors for IL-3 and GM-CSF [11]. Furthermore, a high number of these cells express the eotaxin receptor CCR3 ($CD34^+$/$CCR3^+$). It has been shown that $CD34^+$ cells are increased in bone marrow and peripheral blood, a feature of allergic diseases, including asthma [12]. In this context, allergen exposure augments the number of $CD34^+$ cells in asthmatic airways [13].

The fate of cells, including lineage commitment, is guided by the action of both lineage-determining and secondary transcription factors that orchestrate gene expression, cell development, and cell differentiation. In eosinophil development, common myeloid progenitors must express CCAAT/enhancer binding protein α (C/EBPα), CCAAT/enhancer binding protein ε (C/EBPε), PU.1, and interferon regulatory factor 8 (IRF8) [14].

During the process by which common myeloid progenitors mature into eosinophil progenitors, there occurs a decrease in the expression of friend of GATA-1 (FOG-1) and an increase in the expression and activity of GATA-1 transcription factor [15], as FOG-1 inhibits eosinophil differentiation [16]. GATA are a family of transcription factors that are essential for eosinophil lineage commitment, and GATA-1 and GATA-2 are specifically observed in eosinophil and mast cell differentiation [17,18].

Normally, all these processes occur in the bone marrow; however, in inflamed tissues of allergic patients, local differentiation of $CD34^+$/IL-5Rα^+ cells can occur, in addition to eosinophil recruitment from bone marrow, thus promoting higher levels of eosinophilia [19]. This fact suggests that the control of $CD34^+$ cells released from bone marrow and local differentiation of $CD34^+$/IL-5Rα^+ can control the eosinophilic inflammation associated with eosinophil development.

Morphology and Characteristics of Eosinophils

Eosinophils are terminal polymorphonuclear leukocytes with a bilobed nucleus and an 8-µm diameter. After differentiation and maturation in the bone marrow, they are released into the bloodstream, where they make up about 1%-3% of white blood cells. Eosinophils have a limited life span; they remain in circulation for 8 to 18 hours and in tissues for 3 to 4 days [20]. Normally, these cells are resting; during the inflammatory response, however, eosinophils can be activated and release their content owing to the actions of several molecules, including cytokines, lipid mediators, and proinflammatory agents.

Eosinophils are supplied with a preformed battery of cationic granule proteins, cytokines, chemokines, growth factors, lipid mediators, and immunomodulatory molecules, as well as diverse transmembrane proteins (integrins) and surface receptors (Figure 1). Most molecules are accumulated within intracellular granules and can be released in response to certain stimuli.

Eosinophil-Derived Granule Proteins

Eosinophils play an important role in allergic and inflammatory processes, including asthma [21], and are implicated in host resistance against helminths. Moreover, they exhibit antimicrobial activity toward viral and bacterial pathogens. These functions develop through granule proteins, principally cationic granule proteins, which are necessary to resolve infections and infestations, although they are toxic to human cells [22]. Cationic granule proteins are present in the specific or secondary granules of eosinophils and include the following: major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN).

MBP is abundant in the electron-dense crystalloid core of secondary granules. With its high toxicity, it can harm helminths, microbes, and mammalian cells by disrupting the cell membrane or altering enzymatic activity [23]. In the context of asthma, this protein induces bronchoconstriction and has been implicated in epithelial tissue damage [24]. MBP has 2 homologs: MBP-1 and MBP-2, the former being more potent than the latter in activities such as cell destruction, histamine induction, and leukotriene C4 release from basophils [25].

EPO is the most abundant cationic protein of the matrix of secondary granules, and one that generates potent oxidizing species [26]. It not only produces proinflammatory oxidants, but also plays a cytotoxic role as a cationic toxin against both parasites and mammalian cells [27]. Panagopoulos et al [28] recently discovered a new role for EPO and other peroxidase enzymes as drivers of angiogenesis.

EDN and ECP belong to the ribonuclease A superfamily and are located in the matrix of the secondary granules of

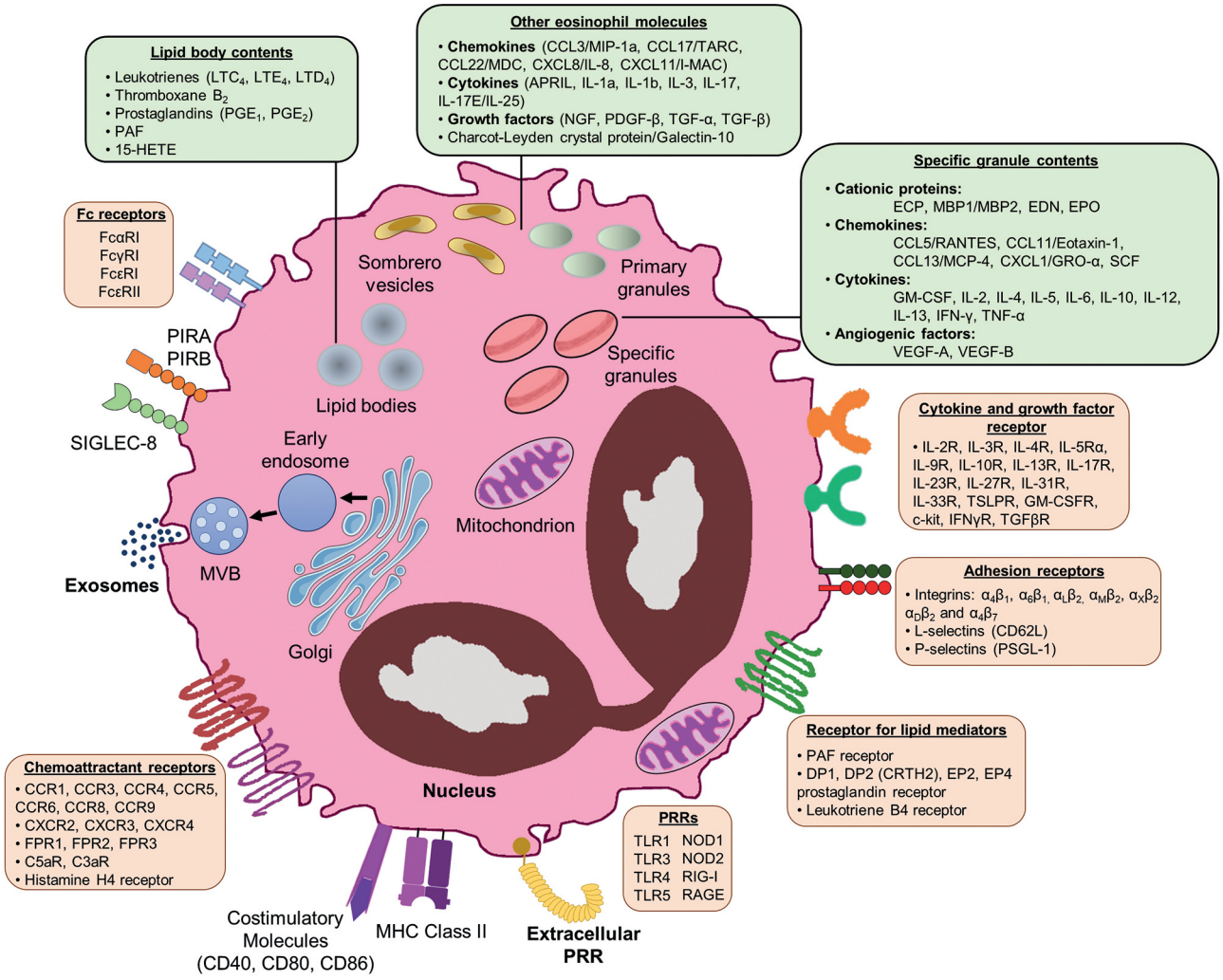

Figure 1. Molecular characteristics of eosinophils. Human eosinophils contain a high number of proteins, receptors, and enzymes that allow them to interact with the microenvironment. Some of them are released. Eosinophils are a source of cationic proteins, chemokines, cytokines, lipid mediators, and growth factors, which have a broad spectrum of action in health and disease. Primary granules store the Charcot-Leyden crystal protein/galectin-10, a characteristic eosinophil protein implicated in asthma and parasitic infections. Specific granules contain 4 principal cationic proteins: MBP, ECP, EPO, and EDN. Lipid bodies are responsible for synthetizing and releasing prostaglandins, thromboxane, and leukotrienes, which take part in allergic inflammation, fibrosis, and thrombosis. Some of the granule contents are released via membrane-bound vesicles called sombrero vesicles. Eosinophils also contain MVB, which fuse with the plasma membrane and release exosomes. Eosinophils express a broad variety of receptors implicated in activation, growth, survival, adhesion, migration, and pattern recognition. 15-HETE indicates 15-hydroxyeicosatetraenoic acid; APRIL, a proliferation-inducing ligand; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; FcR, receptor of Fc for immunoglobulin; FPR, formyl peptide receptor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFNγ, interferon gamma; LT, leukotriene; MBP, major basic protein; MCH, major histocompatibility complex; MCP-4, monocyte chemoattractant protein-4; MVB, multivesicular body; NGF, nerve growth factor; NOD, nucleotide-binding oligomerization domain protein; PAF, platelet-activating factor; PDGF-β, platelet-derived growth factor; PG, prostaglandin; PIR, paired immunoglobulin-like receptor; PRR, pattern-recognition receptor; PSGL-1, P-selectin glycoprotein ligand 1; RAGE, receptor for advanced glycation end-products; RIG-I, retinoic acid-inducible gene I; SCF, stem cell factor; SIGLEC-8, sialic acid-binding immunoglobulin-like lectin 8; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumour necrosis factor; TSLPR, thymic stromal lymphopoietin receptor; VEGF, vascular endothelial growth factor.

eosinophils [29,30]. EDN has cytotoxic, neurotoxic, and antiviral (single-stranded RNA viruses) activity [31,32]; however, it shows poor toxicity for parasites and mammalian cells [31]. ECP has marked toxicity for a wide variety of helminths, bacteria, single-stranded RNA viruses, and host tissues [33].

In addition, Charcot-Leyden crystal protein/galectin-10 is another important protein located in the primary granule of eosinophils. This hydrophobic, self-crystallizing, noncationic protein accounts for about 7% and 10% of all eosinophil proteins and has been considered a potential biomarker of eosinophilic airway inflammation [34].

Surface Markers, Receptors, Cytokines, Chemokines, and Other Mediators

Eosinophils express a wide variety of surface receptors, molecular surface markers, cytokines, lipid mediators, and other major molecules. These are stored in numerous eosinophil granules, can be released in response to specific stimuli, and affect the microenvironment and various cell functions. Figure 1 shows the principal molecules produced by these cells.

IL-5, the chemoattractant CCL11 (eotaxin-1), and GM-CSF can exert several effects on eosinophils themselves, regulating critical functions such as eosinophil differentiation, survival, and chemotaxis [35]. In addition, eosinophils can release immunomodulatory cytokines such as IL-4, IL-13, and IL-25 [36,37], promoting T_H2 responses and participating in allergic processes and immune responses against parasitic infestations [38]. Moreover, eosinophils can produce and secrete IL-6, tumor necrosis factor alpha (TNF-α), and interferon-gamma (IFN-γ), which are proinflammatory cytokines that can cause tissue damage [39]. Transforming growth factor beta (TGF-β), which is also produced by eosinophils, improves proliferation and plays a critical role in fibrotic processes and tissue remodelling in chronic inflammatory diseases, including asthma [40]. In addition, eosinophils produce a large quantity of lipid mediators, such as prostaglandins, leukotrienes, and platelet-activating factor (PAF) [39].

Eosinophils have a broad variety of surface receptors, which allow them to interact with the microenvironment and respond to several stimuli. The main receptors expressed by eosinophils include IL-5Rα, CCR3, sialic acid-binding immunoglobulin-like lectin 8, PAF-receptor, prostaglandins (CRTH2), leukotriene B4 receptors (IL-4R, IL-5R, IL-33R, IFN-γR, TGF-βR, CCR1, CCR3, CCR4, and TSLPR), crystallizable fragment of immunoglobulin (Fc) α receptor (FcαR), FcγR, and pattern-recognition receptors [41].

Eosinophils can also express receptors involved in T_H2 immunity, such as major histocompatibility complex class II (MHCII) and costimulatory molecules such as CD40, CD80, and CD86, which are major players in eosinophil antigen presentation to stimulate T-cell proliferation, thereby initiating T_H2 immune responses [42,43].

Rolling along endothelium and transmigration towards tissues are regulated by coordinated action between chemokine and cytokine signaling, which augments expression of eosinophil adhesion molecules. Eosinophils express several integrins, including α4β1 (CD49d/CD29), α6β1 (CD49f/CD29), αLβ2 (CD11a/CD18), αMβ2 (CD11b/CD18), αXβ2 (CD11c/CD18), αDβ2, and α4β7, which interact with their ligands including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), laminin, fibronectin, and periostin located in other cells or in the extracellular matrix (reviewed in Johansson, 2014) [44]. Several studies have demonstrated the implication of these adhesion molecules in the anchoring and transmigration of eosinophils from the bloodstream to inflamed tissue [45,46]. VLA-4– and ICAM-1–deficient mice prevent eosinophil migration to allergic airways.

Eosinophils also express selectins, such as L-selectin (CD62L) and P-selectin glycoprotein ligand-1 (PSGL-1, CD162), which are highly and constitutively expressed in blood eosinophils [47,48].

Eosinophil Subtypes

Eosinophils have classically been described as terminally differentiated cells with uniform phenotype and function. However, this view of the eosinophil is changing. Nowadays, eosinophils are classified into subtypes based on their state of maturity, organ location, or the morphogenetic activity of tissues [49,50]. Thus, the local environment induces changes in eosinophil phenotype for tissue-specific functions. In addition, eosinophils in homeostasis recruited under inflammatory conditions are heterogeneous as much in function as in their surface markers. For example, eosinophils residing in the lung at baseline can play a regulatory role, whereas eosinophils recruited into the lung following allergen challenge are

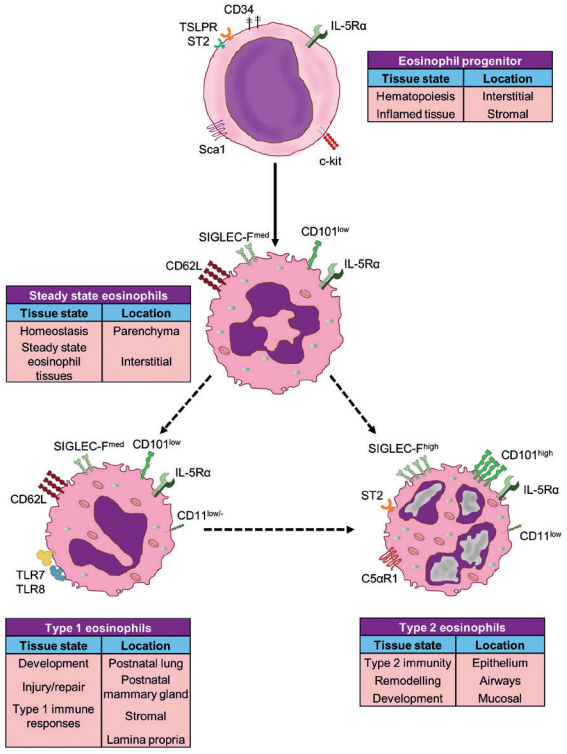

Figure 2. Tissue-based murine eosinophil subphenotypes. Murine eosinophils are divided into 4 tissue-based categories, according to shape, morphology, and tissue contexts. Eosinophil progenitor: immature eosinophils recruited as committed precursors. Steady state: resident eosinophils in true steady state found in morphogenetically quiescent tissues and characterized by their nonsegmented "donut-shape" nucleus. Type 1: usually resident in interstitial (stromal in general) in acute inflammatory, innate defense and transient morphogenetic contexts, featuring a segmented nuclear shape but without vacuolization. Type 2: eosinophils associated with a Type 2 immune response, usually found in epithelial environments, such as murine asthmatic lungs; type 2 eosinophils have a different morphology with an extremely segmented nucleus and the presence of vacuoles. C5αR1 indicated complement component 5a receptor 1; IL-5Rα, IL-5 receptor alpha subunit; Sca-1, stem cell antigen-1; SIGLEC-F, sialic acid binding Ig-like lectins F; ST2, interleukin 33 receptor; TLR, toll-like receptor; TSLPR, thymic stromal lymphopoietin receptor.

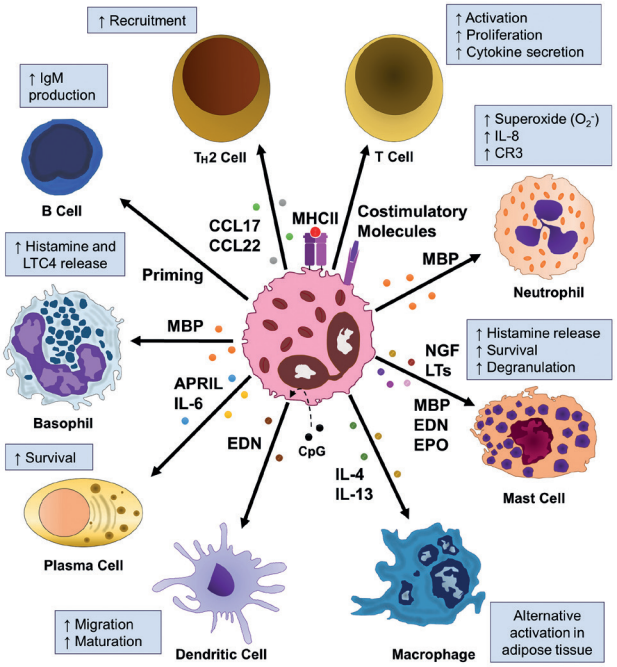

Figure 3. Modulation of leukocyte function by eosinophils. Eosinophils are able to modulate the functions of various immune cells. Eosinophils express MHCII and costimulatory molecules, process antigens, and promote proliferation and cytokine production on T cells in an antigen-specific manner. They also release CCL17 and CCL22, regulating the recruitment of T_H2 cells. Eosinophils produce and release APRIL and IL-6, sustaining long-lived plasma cells in bone marrow, and priming B cells, which induces IgM secretion. Eosinophils stimulated by CpG DNA promote maturation and activation of dendritic cells, and EDN induces dendritic cell recruitment. Eosinophil-derived MBP stimulates neutrophils and basophils, causing them to secrete superoxide and IL-8 and increase CR3 expression and histamine and LT4 production, respectively. Moreover, eosinophils maintain alternatively activated macrophages in adipose tissue by IL-4 and IL-13. In mast cells, eosinophils can induce histamine release and prolong their survival by secreting MBP, EPO, EDN, NGF, and LTs. APRIL indicates a proliferation-inducing ligand; CCL, CC-chemokine ligand; CR3, cell-surface integrin receptor 3; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; IL, interleukin; LTs, leukotrienes; MBP, major basic protein; MHCII, major histocompatibility complex class II; NGF, nerve growth factor.

proinflammatory. Abdala-Valencia et al [51] propose 4 phenotypes (Figure 2): EoP, or immature or precursor of eosinophils; steady state, or tissues residing in quiescent tissues; Type 1, referring to interstitial eosinophils in acute inflammatory, innate defense, and transient morphogenetic contexts; and Type 2, eosinophils associated with a type 2 immune response.

Future studies are needed to clarify the diversity of eosinophils and their therapeutic modulation.

Interaction Between Eosinophils and Other Cells

Eosinophils are multifunctional cells implicated in multiple processes and, as such, can interact with a variety of cell types. They can interact with other leukocytes and modulate their functions (Figure 3); for example, they can induce antigen-specific IgM production by priming B cells and sustain long-lived plasma cells in the bone marrow by secreting a proliferation-inducing ligand (APRIL) and IL-6 [52].

It has been widely observed that eosinophils respond to diverse signals from T cells (such as IL-5), although T cells can also respond to signals provided by eosinophils [53]. Eosinophils can process antigens and act as antigen-presenting cells [54]. Moreover, eosinophils can contribute to the adaptive immune response by producing chemoattractants such as EDN, CCL17, CCL22, CXCCL9, and CXCL10, which recruit dendritic cells and T_H2 cells [55,56], and CpG-DNA-stimulated eosinophils bring about dendritic cell maturation [57].

Historically, eosinophils and mast cells have been considered the principal cells in allergic inflammation, and both can interact via soluble mediators and physical contact [58-61]. Furthermore, eosinophils can indirectly activate mast cells [62], neutrophils [63], basophils [25], and macrophages in adipose tissue [64].

Eosinophil Degranulation

In response to various stimuli, eosinophils are recruited from peripheral blood to the area of inflammation, where their effects take place and where they modulate the inflammatory response through the release of granule-derived content [65,66].

There are 3 known types of degranulation in eosinophils: exocytosis, piecemeal degranulation, and cytolytic degranulation. In the first case, intracellular granules fuse with the plasma membrane and release the total granule content (classical exocytosis). In the variant of classical exocytosis called compound exocytosis, individual granules fuse with each other before fusing with the plasma membrane and releasing granules into the extracellular space [67]. Harmful stimuli, the presence of parasitic helminths [68], and specific environmental conditions such as presence of fungi [69] generate this pattern of secretion. However, this type of degranulation is not very common in other scenarios where eosinophils act.

Piecemeal degranulation is associated with cytoplasmic vesiculation [70]. The mechanism is based on packaging of the contents of cytoplasmic granules in small secretory vesicles, named sombrero vesicles, which are transported into the cellular membrane through tubulovesicular structures that act as vesicular carriers to fuse with the cellular membrane, thus releasing their contents into the extracellular space [71]. This is a carefully regulated mechanism, and the most common physiological process [72-74]. Piecemeal degranulation is induced by cytokines and chemokines such as IFN-γ [75], CCL-11 (eotaxin-1), and TNF-α [76]. Discharge of granule content does not imply the death of the eosinophils, which remain fully functional and able to respond to other stimuli after piecemeal degranulation. This mechanism is not exclusive of eosinophils, as has also been established in mast cells and basophils [77].

In cytolytic degranulation, intact intracellular granules are released through specific eosinophil cell death that

is morphologically different from both apoptosis and necrosis [78]. This type of eosinophilic cell death known as ETosis ("extracellular trap cell death"), involves the disintegration of the nuclear membrane and DNA de-condensing into the surrounding cytoplasm. The secreted granules are fully competent.

Although piecemeal degranulation is the most common type of eosinophil degranulation, cytolysis takes place in a smaller proportion [79] and is typically associated with augmented inflammatory effects of eosinophils.

Despite the fact that eosinophils exert their effects through degranulation, this process does not take place when eosinophils are in blood circulation. As a result, in several conditions mediated by eosinophils, they do not release their granules until they have reached the inflamed tissue [80,81].

Eosinophilic Exosomes

There is considerable vesicular traffic within eosinophils, and some of these granules may be endosomes and multivesicular bodies (MVB), which are characteristic of exosome biogenesis.

Exosomes are small vesicles that contain bioactive lipids, nucleic acid, and proteins, which are delivered to different locations in the body. Intercellular communication appears to be one of the most important functions of exosomes. They also have specific molecules related to their biogenesis, enabling them to be characterized (exocarta.org/exosome_markers). Exosomes have been defined by their size, density, and expression of specific biomarkers (eg, tetraspanins). Exosomes are secreted, constitutively and upon stimulation, by different types of cells; consequently, their composition differs depending on their cellular origin.

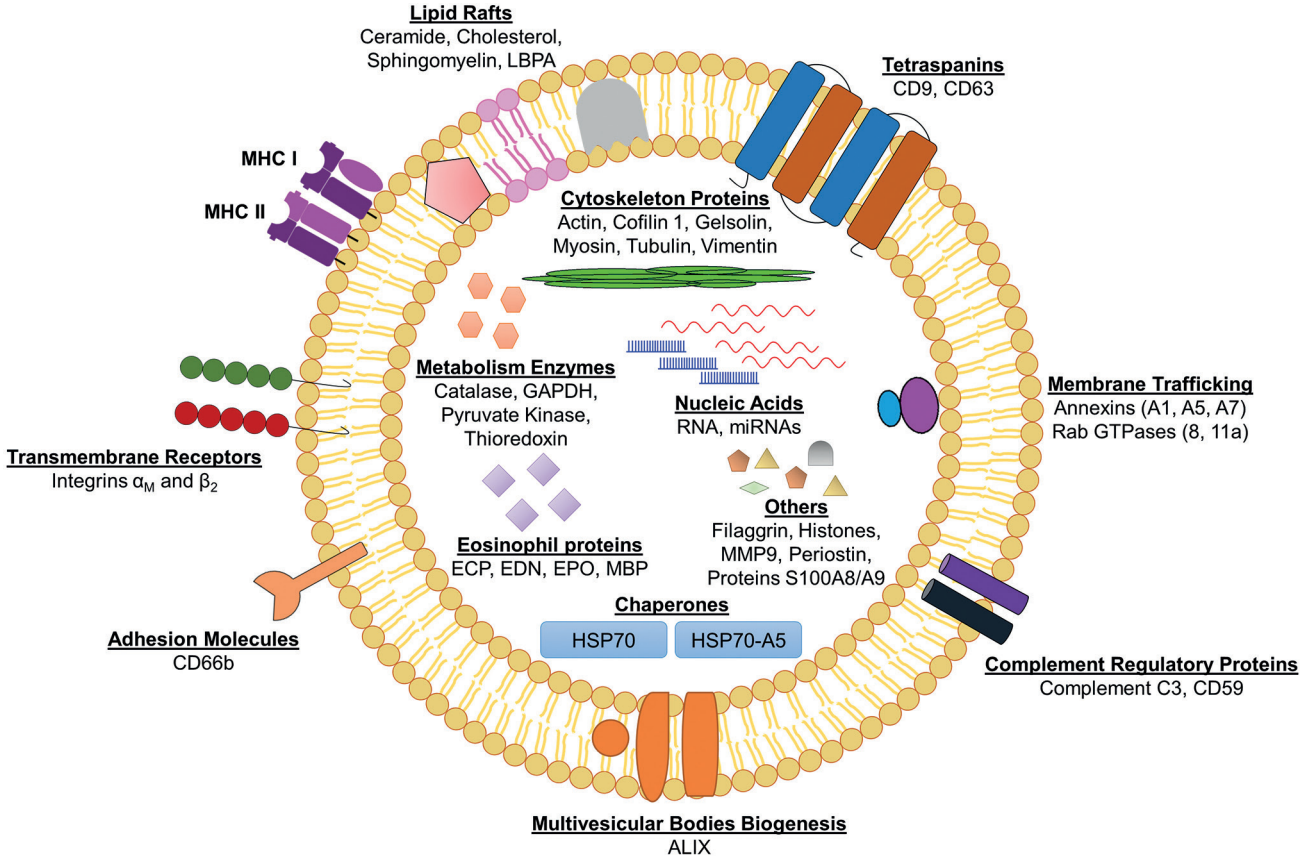

Figure 4. Typical structure and molecular composition of an eosinophil-derived exosome. Exosomes contain a wide range of molecules, including proteins, lipids, mRNAs, and miRNAs. The contents of exosomes can be transferred from their cells of origin to target cells involved in intercellular communication. The exosomes released by eosinophils carry out specific functions in asthma as a result of their protein composition. These exosomes carry several proteins involved in antigen presentation (MHC I and II), proteins involved in targeting and adhesion (tetraspanins, integrins, adhesion molecules), cytoskeleton proteins (actin, tubulin, gelsolin, myosin), MVB biogenesis and secretion-associated proteins (ALIX, annexins, Rab GTPases), chaperone proteins (HSP70, HSP70-A5), metabolic enzymes (catalase, pyruvate kinase, GAPDH), eosinophil-cationic proteins (MBP, EPO, EDN and ECP), and other proteins (periostin, filaggrin, histones and S100 proteins). They also contain lipid rafts composed by ceramide, cholesterol, sphingomyelin and LBPA. ECP indicates eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HSP, heat shock protein; LBPA, lysobisphosphatidic acid; MBP, major basic protein; MHC I, major histocompatibility complex class I; MHC II, major histocompatibility complex class II; miRNAs, microRNAs; MMP9, matrix metalloproteinase 9.

Eosinophils contain MVBs (exosome precursors) and are capable of secreting exosomes into the extracellular environment [82]. Exosome eosinophils carry characteristic eosinophil proteins such as major basic protein (MBP) and eosinophil peroxidase (EPO), tetraspanins (CD9, CD63), MVB biogenesis proteins (ALIX), and metabolomic enzymes (catalase, pyruvate kinase, GAPDH) [82] (Figure 4). Proteome profiling of exosomes from eosinophils established almost 100 different proteins [83] linked to multiple process and mechanisms such as immune response, inflammation, migration, cell signaling, and specific eosinophil granule proteins.

This finding supports the hypothesis that exosomes can act as independent functional units, perpetuating the inflammatory damage generated by eosinophils even after these cells are not present. We demonstrated that exosomes from eosinophils act on structural lung cells to produce epithelial damage, increase smooth muscle proliferation, and modify the expression of several proinflammatory cytokines and signaling factors [84].

Eosinophil Involvement in Asthma and Allergy

Eosinophil Recruitment and Survival: Key Steps in the Pathophysiology of Allergic Asthma

Alterations in eosinophil function and homeostasis have been linked to the pathogenesis of asthma since 1988, when an elevation in eosinophil number and in the levels of eosinophilic MBP was described in the bronchoalveolar lavage fluid of mild asthmatics [85].

Since this discovery, allergic asthma has been characterized by airway obstruction, bronchial hyperresponsiveness, and inflammation of the airway, where eosinophils play a key role in damaging the epithelium and orchestrating an immune response [86]. In eosinophilic asthma, also described as T_H2 asthma, the rise in the number of eosinophils in the airways starts when the airway epithelium is exposed to an allergen or antigen, thus causing the activation of an immunological cascade that drives eosinophils to the airways by T_H2 cytokines and chemoattractants [87]. When helper T cells are activated by an allergen, they skew to the T_H2 phenotype and start to secrete IL-4, IL-5, and IL-13 [88,89]. IL-5 and RANTES have been reported to be the most potent inducers of eosinophil migration into the asthmatic lung [90]. The airway epithelium is also involved in the secretion and production of these T_H2 cytokines by secreting another set of cytokines that promote the T_H2 immune response, such as IL-33, TSLP, and IL-25, which are secreted in the event of epithelial insult of any type [91-93]. These epithelial cytokines also activate type 2 innate lymphoid cells from the innate immune system, which also secrete and produce IL-4, IL-13, and IL-5 [94]. These may be the first cells involved in eosinophil recruitment, as recently described by Chen et al [94], since they were the first to experience an increase in the sputum of asthmatic patients 24 hours after allergen exposure and their levels correlated with eosinophil levels at all time points [94].

All secreted T_H2 cytokines are involved in eosinophil recruitment, migration, or survival and are responsible for the increased eosinophil numbers in bronchoalveolar lavage fluid as described in 1988 [85]. IL-4 promotes eosinophil migration by inducing epithelial expression of VCAM-1 and eotaxin and induces the isotype switch to IgE in B lymphocytes [95]. Specifically, eosinophils bind to vascular endothelial VCAM-1 by its binding molecule, VLA-4 [96]. Both IL-5 and eotaxin promote the release of eosinophils from the bone marrow; specifically, IL-5 promotes survival and migration, and the eotaxin enhances migration by binding to CCR3 expressed on the eosinophil surface [97,98]. Within the eotaxin group, we should differentiate between eotaxin-1 (CCL11), which binds to CCR3, as described above, and eotaxin-3 (CCL26), which plays a key role in eosinophil recruitment in eosinophilic esophagitis, where it is highly upregulated in the esophagus [99]. Expression of CCR3 is expressed in eosinophils, but not on the surface of neutrophils, which is one of the reasons behind eosinophilic recruitment when eotaxin-1 is overexpressed in T_H2-type diseases [100]. Alongside IL-5, IL-3 and GM-CSF are known mediators of eosinophil survival in the airways and perform a key role in their differentiation and migration processes [101].

Lipids such as leukotrienes and prostaglandins are also important mediators of inflammation. Eosinophils are attracted to the airways by leukotrienes, such as leukotriene E4, and PAF [102-104]. In addition, prostaglandin D2 has been described as a chemoattractant of eosinophils, as it binds to the receptor CRTH2 in eosinophils, also known as DP2, thus inducing its recruitment and activation [105].

Several studies have determined the number of eosinophils in the airways as a biomarker for asthma, even showing a correlation with the level of airway hyperresponsiveness and disease severity, as measured in samples such as sputum or peripheral blood [106,107]. The recruitment phase is followed by the effector phase. When activated, eosinophils can promote their own survival capacity in the tissues by autocrine secretion of IL-5, which inhibits apoptosis, and by secreting GM-CSF and IL-3 when eosinophils adhere to fibronectin, thus revealing why eosinophils survive at inflammatory sites [20,108]. Therefore, adding the effect of both cytokines released through autocrine secretion by eosinophils allows them to remain in the tissue and exert their effect for long periods.

Activated Eosinophils Contribute to Asthma Hallmarks

Asthma phenotype is biologically explained by a dysfunction in airway homeostasis. This is provoked by epithelial damage, mucus secretion, and muscle hypertrophy, all leading to a state of airway hyperresponsiveness triggered by exposure to an antigen. Eosinophils are one of the most highly implicated cells in the development of these events. First, they damage the airway epithelium by releasing hazardous molecules. Previously described enzymes secreted by eosinophils such as MBP, EPO, and ECP can damage epithelial cells in vitro when administered in doses similar to those found in asthmatic sputum [109,110]. Upon activation by TNF-α, an upregulated molecule found at the sites of inflammation, eosinophils secrete matrix metalloproteinase-9, an enzyme implicated in airway remodelling and a hallmark of asthma [111]. Airway remodelling is a process that can

occur physiologically, but also pathologically, as in asthma, with epithelial barrier disruption, goblet cell hyperplasia, and membrane thickening and fibrosis, accompanied by airway smooth muscle hyperplasia or hypertrophy and increased formation of new blood vessels (angiogenesis). Enzymes secreted by eosinophils have been reported to contribute to tissue disruption, although some studies have shown that eosinophils also induce muscle cell proliferation and collagen deposition in mice, whereas an eosinophil-depleted model did not [21]. Another molecule that is secreted by eosinophils and regulates airway remodelling is TGF-β, which, according to biopsy samples from patients with severe asthma, is upregulated in mRNA and correlates with the thickness of the basement membranes [112].

Inorganic chemicals are also released by eosinophils, and these have various effects on receptor tissue. Nitric oxide (NO) is a mediator of inflammation and has been described as another recruiter of eosinophils when synthesized by the inducible nitric oxide synthase enzyme in vascular endothelial cells and epithelial cells and is correlated with levels of exhaled nitric oxide, a biomarker of asthma [113,114]. Besides structural cells such as endothelial or epithelial cells, one of the major producers of NO are eosinophils, which also secrete reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals, and hydrogen peroxide, as previously described, which are implicated in the pathogenesis of asthma by contributing to airway injury and inflammation [115,116].

Lipid mediators, in addition to promoting eosinophil migration, are molecules that are released by eosinophils and include lipoxin A4, thromboxane B2, prostaglandin E2, and cysteinyl leukotrienes, and can thus modulate the immune response by adding these molecules to the refinement of

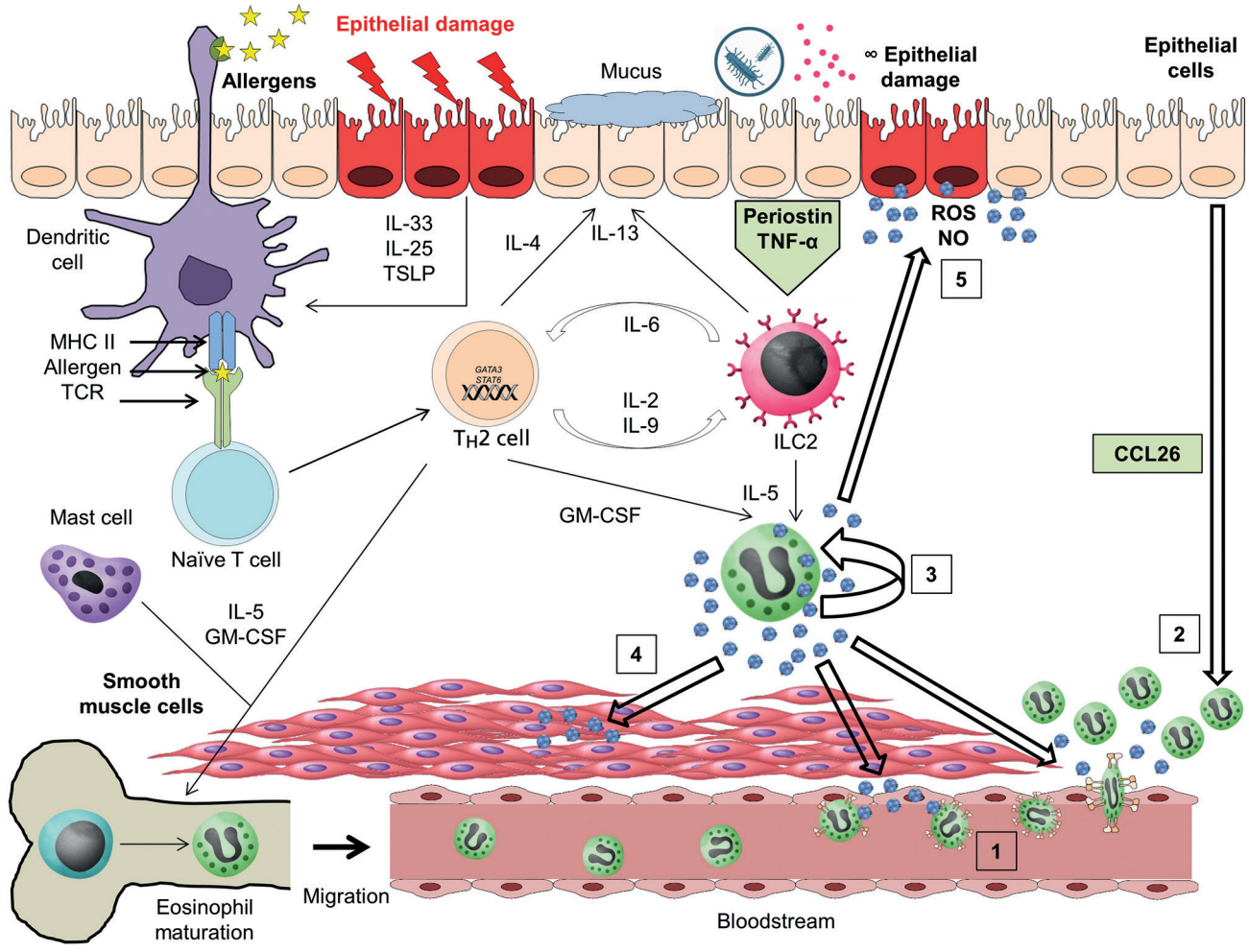

Figure 5. Eosinophil involvement in asthma and allergy. Eosinophils perform their effector functions when an immune response is orchestrated. Allergens activate the epithelium of the airways that secrete cytokines such as IL-33, IL25, or TSLP, which will activate ILC2 to secrete pro-eosinophilic recruitment molecules such as IL4-IL5 and IL-13. Dendritic cells process the allergen and activate T_H2 CD4+ cells that promote eosinophil recruitment through the production of IL-4, IL-5, GM-CSF, RANTES, and IL-13. The recruitment of eosinophils is also promoted directly in the epithelium by endothelial cells, epithelial cells, and fibroblasts, as well as by secreting molecules such as periostin and eotaxin. Eosinophil survival and activation is promoted by IL-5 produced by immune cells, but also through autocrine secretion by eosinophils. Once recruited and activated, eosinophils perform their immune functions inappropriately, targeting the airways, secreting the toxic and enzymatic content of their granules (ECP, MPB, NO, ROS, MMP9), causing damage, and secreting exosomes that impair wound healing by increasing apoptosis. Airway remodeling is associated with muscle cell hyperplasia, which is activated by TGF-β and exosomes secreted by eosinophils. All these features are behind the role of eosinophils in T_H2 immune disease.

immunologic reactions [117]. Within the group of lipids that promote restoration of cell function after inflammation, resolvins have been the most widely studied in asthma. Eosinophils secrete resolvin E3, an anti-inflammatory mediator that inhibits neutrophil chemotaxis both in vivo and in vitro [118].

In summary, eosinophils secrete exosomes that can act both in an autocrine fashion on themselves, thus increasing their capacity for producing NO and ROS species, and in an exocrine fashion, by releasing the exosomes onto the airway epithelium. In asthma, they reduce the repair capacity of the epithelium by elevation of apoptosis rates and increasing the proliferation of muscle cells, indicating that eosinophils also contribute to airway remodelling by exosome release [83,84].

Pathophysiology of Eosinophils in Allergic Diseases and Other T_H2 Disorders

As mentioned above, eosinophil migration is dependent on various molecules such as cytokines released by T_H2 cells (IL-4, IL-5, IL-13, RANTES), which are mainly released when T-cells interact with antigen-presenting cells. Eosinophil migration targets the tissue site where the immune reaction takes place. Eosinophils are also attracted to the esophagus in eosinophilic esophagitis, where eotaxin-3 (CCL26) principally recruits eosinophils, which damage the epithelium and activate it, as occurs in the pathogenesis of asthma [119]. The esophageal epithelium then induces gene expression of molecules that regulate its own barrier function, such as filaggrin and periostin. These molecules, and in particular periostin, have also been studied in asthma, and it has been shown that periostin is upregulated both in asthmatic and in eosinophilic esophagitis patients and that filaggrin is downregulated in eosinophilic esophagitis biopsies [120,121]. Periostin has been studied as a key regulator for the infiltration of eosinophils into the lungs and esophagus of allergic mice, as described in a null model for the periostin gene [122]. Atopic dermatitis behaves in a similar fashion. Atopy is defined as an allergic predisposition that is inheritable; it is responsible for less than half of all asthma cases [123]. In atopic dermatitis, the allergen is exposed principally to the skin, at which point fibroblasts secrete periostin, which in turn activates keratinocytes, which secrete cytokines such as TSLP and others, skewing to a T_H2 response and recruiting eosinophils into the epithelium [124].

Other kinds of allergic and asthmatic diseases that are more allergen- or trigger-specific are also characterized by eosinophil recruitment and involvement. Infection with *Aspergillus fumigatus* may be followed by a type of allergy known as allergic bronchopulmonary aspergillosis, where sensitization to *Aspergillus* species can induce an allergic response that is also mediated by eosinophils [125]. In aspirin-related disease, hypersensitivity to nonsteroidal anti-inflammatory drugs is developed after deregulation in arachidonic acid metabolism that occurs under the effect of aspirin, thus triggering a dramatic

Table. Examples of Agents Targeting Eosinophils

Target	Mechanism of action	Examples/Drugs	Ref
Blockade of eosinophil recruitment			
CCR3	Antagonist/Inhibition	GW766994, Ki19003	147, 148
CCL11	Blocks eotaxin-1	Bertilimumab	149
CD49d	Blocks CD49D specific mAb or small-molecule VLA4 antagonists	Natalizumab, TR14035	150-152
CRTH2	CRTH2 Antagonist	OC000459	153, 154
H4R	H4R Antagonists	INCB38579, UR-63325	155, 156
IL-4	Blocks IL-4	Altrakincept	157
IL-13	Blocks IL-13	Lebrikizumab, anrukinzumab	158-160
IL-4Rα/IL-13Rα	Inhibits binding of IL-4 and/or IL-13 to IL-4Rα	Pitrakinra, AMG317, dupilumab	160-165
Inhibition of eosinophil survival			
IL-5	Blocks IL-5	Mepolizumab and reslizumab	166-168
IL-5Rα	Inhibits binding of IL-5 to IL-5R		
ADCC against eosinophils		Benralizumab	169
Siglec-8	Agonism	Anti-Siglec-8 mAb	170, 171
IgE	Binds free IgE	Omalizumab	172-174
EMR1		Afucosylated anti-EMR1 mAb	175
TSLP	Antagonism	Tezepelumab	176
Inhibition of eosinophil activation			
Notch	Inhibition	Semagacestat	177
CD52		Alemtuzumab	178

rise in eosinophils and the onset of asthma [126]. Finally, occupational asthma is another kind of asthma disease, where exposure to a specific compound occurs repeatedly over a prolonged period of time, normally on a daily basis, as in the workplace. It has been reported that 90% of occupational asthma cases are characterized by eosinophil infiltration and high IgE levels [127].

A visual representation of the role of eosinophils and their exosomes in asthma and allergy is shown in Figure 5.

Eosinophilic Disorders

Eosinophils accumulate for reasons other than allergen exposure. In acute idiopathic eosinophilic pneumonia, eosinophils accumulate and damage the airways by means of a currently unclear mechanism that may be related to clonal T cells [128]. Chronic eosinophilic pneumonia has also been described, although compared with the acute disorder, the chronic form is often associated with previous asthma or atopy [88,129]. Pulmonary fibrosis is also caused by the accumulation of eosinophils, although in this case it is accompanied by neutrophils, and its pathophysiology is caused by interstitial collagen deposition, which leads to fibrosis [88,130]. Eosinophilia has also been associated with hematologic malignancies and is less common with solid tumors [131]. Finally, hypereosinophilic syndromes are a heterogeneous group of diseases with an elevated number of eosinophils in blood or other tissues in which the manifestations vary depending on where the eosinophilia are found [132].

Targeting Eosinophils

Targeting eosinophilia may help to control several diseases associated with eosinophils (eg, asthma, allergy, eosinophilic esophagitis, hypereosinophilic syndromes). Eosinophils provide an ideal target for biological treatment owing to expression of specific surface receptors on their membranes and because evidence from eosinophil-deficient mouse models and case reports in humans without eosinophils suggest that eosinophil depletion has little effect on immunity to infection and other essential host defense mechanisms.

Historically and nowadays, corticosteroids are one of the most effective drugs used to reduce eosinophil count and, consequently, eosinophil-mediated damage. Similar to the pleiotropic effects on other leukocytes, these drugs cause eosinophil apoptosis [133], although long-term toxicity and persistent eosinophilia, even after treatment with oral corticosteroids, limit their therapeutic use. Clearly, effective alternative therapies are needed. Therefore, current therapies are moving forward to control eosinophils by means of approaches based on blocking the recruitment and migration of eosinophils into tissues, impairment of the survival of mature eosinophils, blocking of eosinophil production in bone marrow, and inhibition of eosinophil activation (Table).

As the development of eosinophilic inflammation is dependent on the activity of IL-5, biologicals targeting IL-5 are an obvious therapeutic option (Figure 6). Two different humanized IL-5–specific antibodies (ie, mepolizumab and reslizumab) have been developed and tested in clinical trials for asthma and other eosinophilic disorders [134-136]. Early trials using anti-IL-5 (mepolizumab and reslizumab) were largely unsuccessful until researchers recognized the need to quantify eosinophils in asthma patients in order to identify those in whom these medications would be more effective. Recent trials reported efficacy in eosinophilic asthma, and both drugs have received market authorization [137-142]. Similarly, another attractive therapeutic approximation includes the IL-5 receptor (IL-5R), which is composed of α and β subunits and expressed on the surface of eosinophils, eosinophil progenitors ($CD34^+$), mast cells, and basophils [143]. Benralizumab is an anti-IL-5Rα antibody that leads to the interruption of IL-5R–mediated signal transduction and is afucosylated, thus improving antibody-dependent, cell-mediated cytotoxicity [144]. It is effective in eliminating eosinophils in both serum and tissue [145,146].

Future challenges should include determining which eosinophil-reducing treatment is more effective and safe for patients with disorders associated with eosinophils.

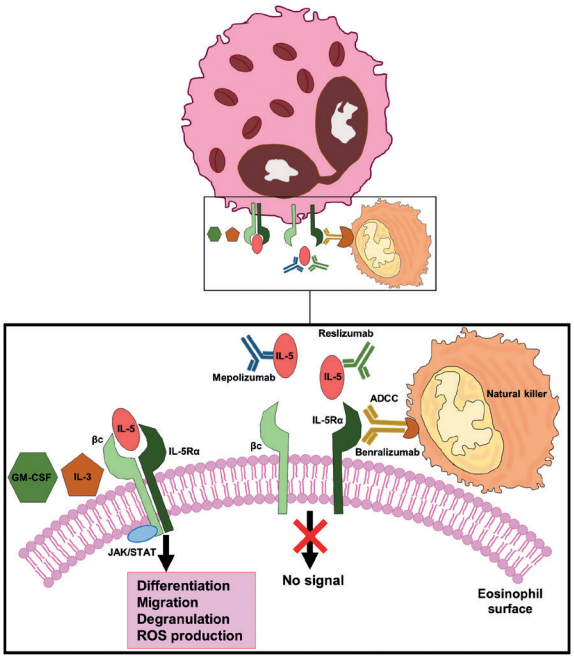

Figure 6. Antibody-based treatment blocking eosinophil functions. IL-5 plays a critical role in eosinophil development and differentiation, maturation in bone marrow, and migration at inflammatory sites. The interaction between IL-5 and its receptor (IL-5R) induces eosinophil degranulation and release of granule proteins and increases the respiratory burst, thus generating reactive oxygen species (ROS). IL-3 and GM-CSF only interact with the βc subunit of IL-5R. Anti-IL-5 therapies based on monoclonal antibodies (mepolizumab and reslizumab) block binding of IL-5 to IL-5Rα located on the eosinophil surface. Benralizumab is a humanized monoclonal antibody that binds to IL-5Rα, thus enhancing ADCC. Natural killer cells bind to anti-IL-5Rα antibody through FcR and improve eosinophil-depleting activity. ADCC indicates antibody-directed cell cytotoxicity; βc, beta chain subunit; FcR, receptor of Fc for immunoglobulin; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; IL-5R, IL-5 receptor; IL-5Rα, IL-5 receptor alpha subunit.

Conclusion

Eosinophils perform a critical role in asthma, allergic reactions, and atopic dermatitis, where they are attracted to the affected site and carry out their toxic functions. The increasing interest in eosinophils owing to the development of new biological treatments is generating new challenges in research in this field.

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Conflicts of Interest

VdP has been a consultant/speaker for AstraZeneca. The remaining authors declare that they have no conflicts of interest.

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