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Contributions of Eosinophils to Human Health and Disease

Amy D. Klion,[1] Steven J. Ackerman,[2] and Bruce S. Bochner[3]

[1]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA; email: aklion@nih.gov

[2]Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago, Chicago, Illinois 60607, USA; email: sackerma@uic.edu

[3]Department of Medicine, Division of Allergy and Immunology, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611, USA; email: bruce.bochner@northwestern.edu

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Abstract

The human eosinophil has long been thought to favorably influence innate mucosal immunity but at times has also been incriminated in disease pathophysiology. Research into eosinophil biology has uncovered a number of interesting contributions by eosinophils to health and disease. However, it appears that not all eosinophils from all species are created equal. It remains unclear, for example, exactly how having eosinophils benefits the human host when helminth infections in the developed world have become scarce. This review focuses on our current state of knowledge as it relates to human eosinophils. When information is lacking, we discuss lessons learned from mouse studies that may or may not directly apply to human biology and disease. It is an exciting time to be an "eosinophilosopher" because the use of biologic agents that selectively target eosinophils provides an unprecedented opportunity to define the contribution of this cell to eosinophil-associated human diseases.

INTRODUCTION

The eosinophil, one of several cells named by Paul Ehrlich in the late 1800s, is one of the less common blood leukocytes. Its characteristically intense staining with the acidic dye eosin is due to the avidity of this stain for basically charged intracellular granules that are found only within the cytoplasm, imparting this bilobed cell with distinct tinctorial properties. Normal numbers of circulating eosinophils range from 0 to 500 per microliter of human blood, but in certain conditions these values can increase by 20-fold or more. Evolutionarily, the eosinophil, or an eosinophil-like cell, has been maintained in vertebrates, including reptiles and fish, over millions of years, strongly suggesting that this cell contributes important, favorable biology toward the well-being of these species (1, 2). In this regard, a prevailing theory is that the eosinophil participates in innate immunity to parasites, especially helminths. With the availability of constitutive and conditionally eosinophil-deficient mouse strains and other tools, this traditional paradigm is being challenged (3, 4). Now that biologic agents that effectively and selectively deplete eosinophils in people with asthma and other eosinophil-related disorders can be prescribed, we are creating the equivalent of eosinophil-deficient humans with pharmacology (5). These advances place us at the beginning of a new era regarding our understanding of the role of the eosinophil in health and disease (6, 7). What follows is an overview of the role of the human eosinophil in this regard, highlighting gaps in our knowledge while also providing intriguing insights gained from the study of eosinophil biology in mice that may or may not translate to their human counterparts. So, unless otherwise stated, in this article we equate the term eosinophil with the term human eosinophil.

EOSINOPHIL HEMATOPOIESIS AND LINEAGE

Development During Homeostasis

Eosinophils, along with the rest of the myeloid blood cell lineages, develop in the bone marrow microenvironment from multipotent hematopoietic stem cells, which give rise to a population of unique eosinophil-committed progenitors (EoPs) that are capable of terminally differentiating into mature eosinophils in the absence of any lineage-specific growth factors or cytokines, including interleukin (IL)-5 (8). The human EoP (hEoP) is defined by its surface expression of a number of receptors, the most important of which is the high-affinity α subunit of the IL-5 receptor (IL-5Rα). These IL-5Rα$^+$ hEoPs differentiate exclusively into eosinophils, but not basophils or mast cells (**Figure 1**) (9). Under homeostatic conditions in healthy individuals, eosinophilopoiesis is regulated in part by a unique combinatorial program of transcription factors (10), including the requisite expression of GATA-1 (11), which occurs through the use of an eosinophil-lineage specific enhancer in the *GATA1* gene itself (12). Notably, transgenic deletion of a unique high-affinity GATA binding site in the enhancer region of the *GATA1* gene produced an eosinophil-deficient mouse strain (ΔdblGATA) with essentially normal development of other GATA-requiring hematopoietic lineages, including the erythroid lineage (12). In addition to autoregulating eosinophil-specific GATA-1 expression in the mouse, these binding sites are present and functionally important in many hallmark human eosinophil-affiliated genes whose expression defines the eosinophil lineage (13, 14). These genes include those encoding eosinophil granule cationic proteins, such as major basic protein 1 (MBP-1) via its eosinophil-specific P2 promoter, eosinophil peroxidase (EPX), the Charcot–Leyden crystal protein (CLC)/Galectin-10, the eotaxin receptor CCR3, and IL-5Rα (14).

In addition to GATA-1, the eosinophil's baseline combinatorial transcription factor program includes low levels of the ETS factor PU.1; downregulation of FOG-1; and temporally regulated

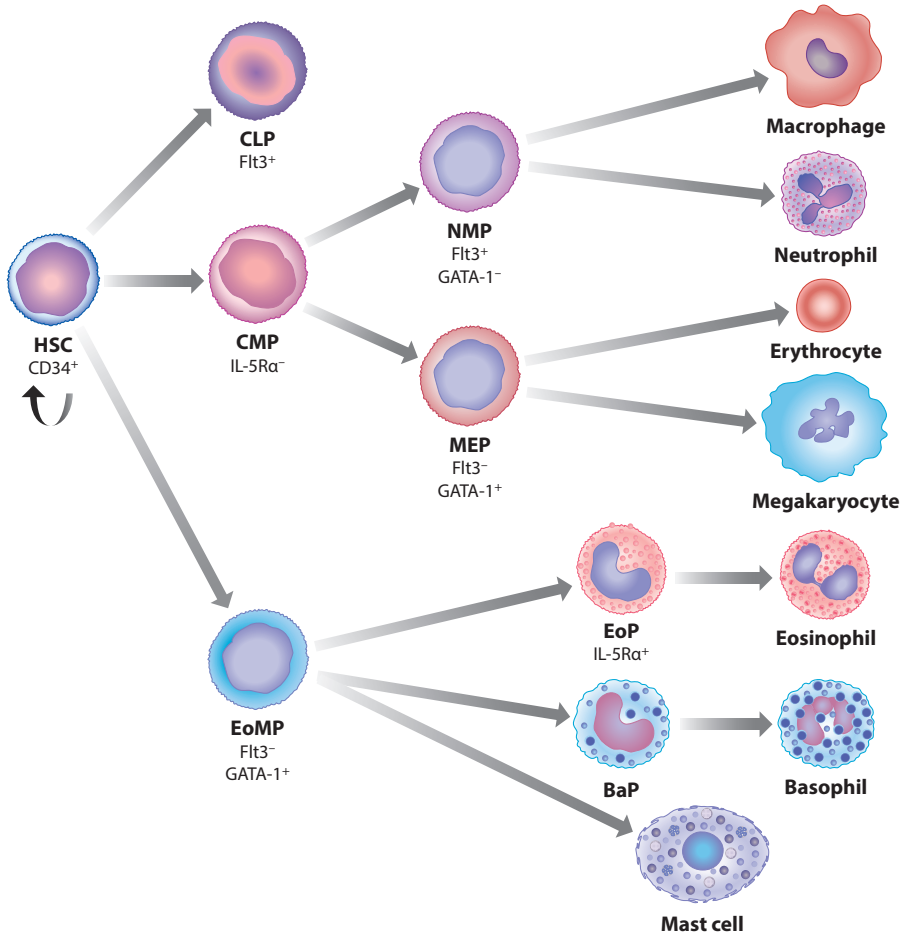


Figure 1

Development of the eosinophil lineage in the context of normal human hematopoiesis. In the current paradigm, hematopoietic stem cells (HSCs) give rise directly to eosinophil/mast cell progenitors (EoMPs), from which IL-5R$^+$ eosinophil progenitors (EoPs) develop and terminally differentiate into mature eosinophils. EoMPs also differentiate into basophil progenitors (BaPs) and mast cells. Expression of GATA-1 versus Flt3 distinguishes between early multipotent progenitors that give rise to EoMPs and megakaryocyte/erythroid progenitors (MEPs) versus common lymphoid progenitors (CLPs) and neutrophil/macrophage progenitors (NMPs, formerly GMPs). Both NMPs and MEPs arise from a common myeloid progenitor (CMP) distinct from the EoMP population. Figure by Jacqueline Schaffer, Medical Illustrator.

expression of members of the CCAAT-enhancer-binding protein (C/EBP) family, C/EBPα and C/EBPε, the latter of which is expressed during eosinophil development as a series of transcriptional activator and repressor isoforms (15) and is required for eosinophil terminal differentiation (16). Finally, baseline eosinophilopoiesis is regulated in part at the level of microRNAs and long noncoding RNAs (17–19) and, epigenetically, by higher-order regulatory mechanisms that are the topic of ongoing research, primarily in mouse models (20).

Changes During Eosinophilia

While basal eosinophilopoiesis requires the hierarchical expression, timing, and levels of specific transcription factors, blood and tissue eosinophilia in allergic reactions, immunity to parasitic infections, and other eosinophil-associated responses is regulated principally by the lineage-specific cytokine IL-5, which amplifies proliferation and terminal differentiation of committed EoPs in the bone marrow. This IL-5 is produced by cells of both the innate and adaptive immune systems, including mast cells, type 2 innate lymphoid cells (ILC2s), and activated T helper 2 (Th2) lymphocytes. Notably, the number of hEoPs increases to represent ~10–20% of the common myeloid progenitor cell population in the bone marrow of patients with blood eosinophilia of various etiologies, indicating that hEoPs participate in expansion of eosinophilopoiesis in eosinophilic disorders. Thus, while the IL-5 knockout mouse is ostensibly eosinophil deficient, it still develops small numbers of blood and tissue eosinophils through the baseline homeostatic transcriptional mechanisms noted above but cannot mount blood or tissue eosinophilia in response to infection with helminths or sensitization and challenge with allergens, because these responses are IL-5 dependent (21). In addition to IL-5, other cytokines and chemokines have been shown, at least in vitro, to drive both murine and human EoPs to terminally differentiate. These include IL-3, granulocyte macrophage colony–stimulating factor (GM-CSF), and the eotaxin family of eosinophil-recruiting chemokines (CCL11, CCL24, and CCL26).

EOSINOPHIL SURFACE PHENOTYPE

The eosinophil's cell surface is richly adorned with cell surface receptors. While many are considered eosinophil selective in their expression [e.g., CCR3, IL-5R, sialic acid–binding, immunoglobulin-like lectin 8 (Siglec-8)], epidermal growth factor–like module-containing mucin-like hormone receptor–like 1 (EMR-1) appears to be absolutely specific for eosinophils, although its function remains unknown (22, 23). Like all leukocytes, eosinophils express many cytokine and chemokine receptors and adhesion molecules (**Figures 2** and **3**) involved in their migration across the vascular endothelium and through the epithelium during recruitment into tissues. Eosinophils express receptors for the three key cytokines required for their differentiation, maturation, priming, activation, and survival in the bone marrow and tissues, including, respectively, the α subunits of the high-affinity receptors for IL-3 (IL-3Rα/CD123), IL-5 (IL-5Rα/CD125), and GM-CSF (GMCSF-Rα/CD116), which heterodimerize with the shared β-common chain (CD131).

Human (and mouse) eosinophils express high levels of the G protein–coupled receptor CCR3, a major receptor involved in eosinophil chemotaxis, migration, recruitment, and degranulation in tissues. As for most chemokine receptors, CCR3 is promiscuous, binding multiple ligands in addition to eotaxin-1, -2, and -3 (CCL11, CCL24, and CCL26). The eotaxins, along with IL-5, are the principal factors accounting for eosinophil maturation, recruitment, and migration within tissues. Under physiologic conditions, CCL11 is the key CCR3 ligand for homeostatic recruitment of eosinophils into the gastrointestinal tract and other organs, a conclusion that is especially clear in mouse models (24). Eotaxin-3 (CCL26) pathologically recruits large numbers of human eosinophils into the esophagus in eosinophilic esophagitis (EoE) and is the most highly upregulated gene transcript in this immune-mediated, food allergy–associated remodeling disease of the esophagus (25). Eosinophils also express the prostaglandin D_2 receptor 2 [PD2R2/chemoattractant receptor–homologous molecule expressed on Th2 cells (CRTh2)] and can migrate in response to prostaglandin D_2.

The eosinophil is endowed with multiple immunoglobulin (Ig) receptors and related family members (**Figure 2**) involved in eosinophil-mediated functional activities, including

Chemokine, complement, and other chemotactic factor receptors

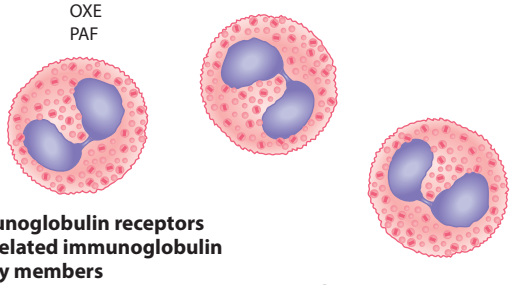
C3a	CysLT2
CCR1	CXCR1
CCR2	CXCR3
CCR3	CXCR4
CCR6	fMLP
CCR8	Histamine (H4)
CD35	LTB_4
CD88	OXE
CRTh2	PAF
CysLT1	

Immunoglobulin receptors and related immunoglobulin family members

CD4	CD66
CD16*	CD84
CD31	CD85
CD32a	CD89
CD32b	CD100
CD47	CD101
CD48	CD112
CD50	HLA class I
CD54*	HLA-DR*
CD58	

Adhesion molecules

αd integrin	CD44
β_7 integrin	CD49d
CD11a	CD49f
CD11b	CD62L
CD11c	CD147
CD15	CD162
CD15s	CD174
CD18	CD321
CD29	Mac-2



Cytokine receptors

GM-CSF	IL-17
IFN-α	IL-23
IFN-γ	IL-33
IL-3	LIF
IL-4	SCF
IL-5	TNFα
IL-6	TNFβ
IL-9	TSLP
IL-13	

Death, signaling, pattern recognition, and other receptors

CD9	CD93	CD298
CD12	CD95	CD300a
CD17	CD97	CD300f
CD24	CD98	CD302
CD28	CD99	CD352
CD30	CD134	EMR1
CD33	CD137	Glucocorticoid
CD37	CD139	KIR2DL3
CD39	CD148	LIR1
CD40	CD151	LIR2
CD43	D153	LIR3
CD52	CD154	LIR7
CD53	CD161	NOD1
CD58	CD165	NOD2
CD60a	CD172a	PIRA
CD63	CD178	PIRB
CD65	CD226	P2X7
CD66	CD244	P2Y2
CD69*	CD253	RAGE
CD71	CD261	Siglec-7
CD80	CD262	Siglec-8
CD81	CD263	Siglec-10
CD82	CD264	TLR1–5, -7, -9
CD86*	CD265	TrkA, -B, -C
CD92	CD295	

Enzymes

CD10	CD45RC	CD59
CD13	CD45RO	CD87
CD45	CD46	CD156a
CD45RB	CD55	PAR-2

Figure 2

Surface molecules expressed on human eosinophils. There is some overlap among categories for some of these proteins. Common names for chemokine (CC and CXC) receptors, TLRs, and others are used here instead of the CD names because of the greater use and familiarity of the former among most readers. Asterisks indicate molecules expressed only on activated eosinophils. Abbreviations: CRTh2, chemoattractant receptor–homologous molecule expressed on Th2 cells; CysLT, cysteinyl leukotriene; EMR-1, epidermal growth factor–like module-containing mucin-like hormone receptor–like 1; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; GM-CSF, granulocyte macrophage colony–stimulating factor; HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; KIR2DL3, killer cell immunoglobulin–like receptor, two immunoglobulin domains and long cytoplasmic tail 3; LIF, leukemia inhibitory factor; LIR, leukocyte immunoglobulin-like receptor; Mac-2, epsilon binding protein; NOD, nucleotide-binding oligomerization domain; OXE, oxoeicosanoid; P2X and P2Y, ATP-gated purinoreceptors; PAF, platelet-activating factor; LTB, leukotriene B; PAR, protease-activated receptor; PIR, paired Ig-like receptor; RAGE, receptor for advanced glycation end products; SCF, stem cell factor; Siglec, sialic acid–binding, immunoglobulin-like lectin; TLR, Toll-like receptor; TNF, tumor necrosis factor; Trk, tropomyosin receptor kinase; TSLP, thymic stromal lymphopoietin. Figure by Jacqueline Schaffer, Medical Illustrator; adapted from Reference 155 with permission.

antibody-mediated cellular cytotoxicity toward helminth parasites and other immune modulatory functions and pathologic activities in eosinophil-associated diseases. For example, eosinophils express FcγRII (CD32), a functional polymeric IgA receptor, and the IgA Fc receptor (CD89) for the IgA secretory component. CD89 is likely the major receptor for IgA-mediated eosinophil activation, for instance, in mucosal tissues of the gastrointestinal tract. Finally, although human eosinophils have been reported to express a number of the component chains of FcεRI, including the α and γ chains, this finding remains controversial, but if present, levels are so low that they likely have negligible functional significance. What is not controversial is that they lack the β chain of FcεRI expressed on basophils and mast cells.

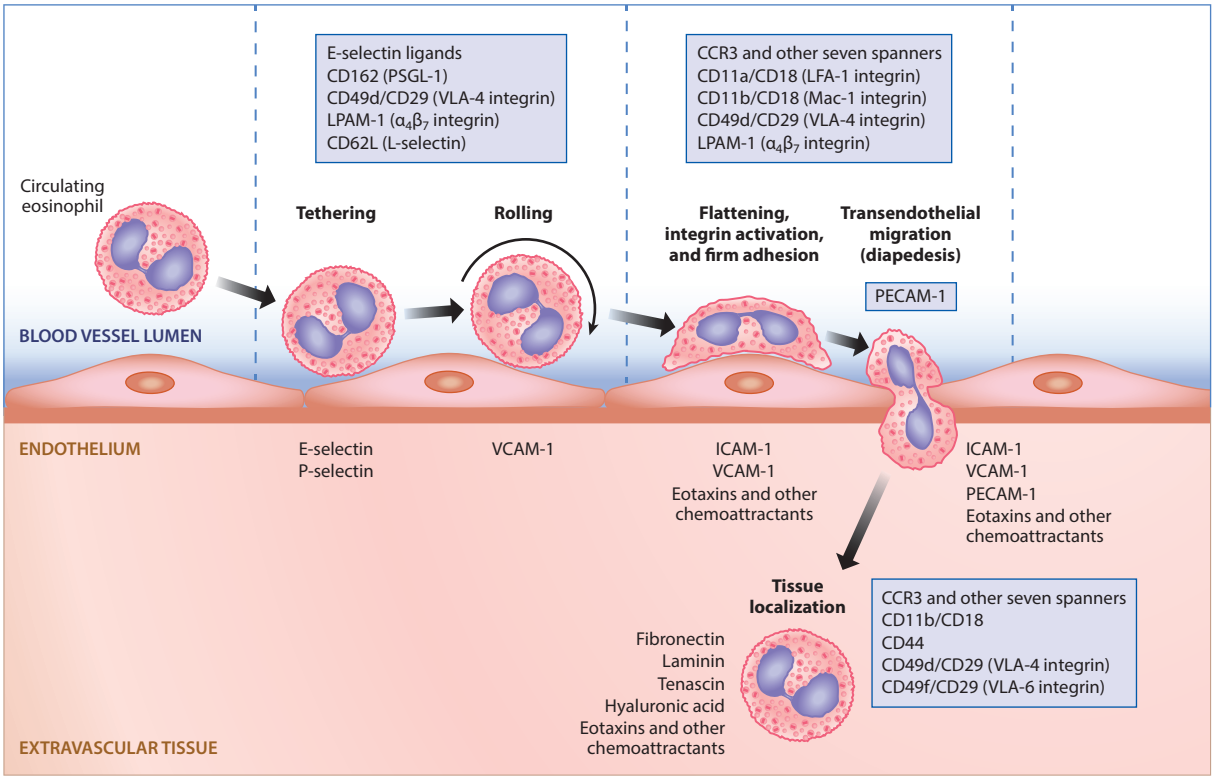


Figure 3

Mechanisms involved in eosinophil extravasation during inflammation. Roles of adhesion molecules, chemoattractants, and other molecules during the process of eosinophil migration from the circulation into tissues. Shown are the contributions of sets of leukocyte, endothelial, and tissue molecules during the steps of tethering, rolling, firm adhesion, transendothelial migration (diapedesis), and localization within tissues. Note that in addition to other adhesion molecules, PECAM-1 on both the leukocyte and the endothelium is uniquely involved in diapedesis. Abbreviations: ICAM, intercellular adhesion molecule; LFA, lymphocyte function-associated antigen; PECAM, platelet/endothelial cell adhesion molecule; VCAM, vascular cell adhesion molecule; VLA, very late antigen. Figure by Jacqueline Schaffer, Medical Illustrator.

One of the largest classes of membrane proteins expressed on the surface of eosinophils includes cell death, signal transduction, and pattern recognition receptors (**Figure 2**). Receptors that function in pattern recognition allow eosinophils to be stimulated directly during host innate immune responses by pathogen-associated molecular patterns and/or damage-associated molecular patterns. These pattern recognition receptors are involved in eosinophil interactions with invading microorganisms (e.g., parasitic helminths, fungi, and certain bacteria) and with its internal tissue microenvironment, where they help regulate eosinophil activation, tissue remodeling responses, survival, and apoptotic cell death. Finally, these eosinophil-expressed sensors of innate immunity also include the proteinase-activated receptors PAR-1 and -2. PAR-2 may play a significant role in eosinophil activation in response to proteases released by aeroallergens such as dust mites, fungi, or pollens.

EVOLUTIONARY ORIGINS AND CONSIDERATIONS

The most recent (and comprehensive) consideration of the evolutionary origins of the eosinophil comes from a scholarly review by McGarry (1). Cells in a variety of invertebrates may represent

evolutionary precursors of modern-day vertebrate eosinophils. Links based on biochemical or genetic similarities are limited but include the expression of the myeloperoxidases, of which EPX is eosinophil specific. Studies suggest that myeloperoxidase (expressed by neutrophils) and EPX diverged some 60–70 million years ago, but they are not sufficiently robust to indicate when the earlier invertebrate-to-vertebrate evolution of the eosinophil lineage occurred.

Vertebrate eosinophils have been identified fairly extensively in representative species, ranging from fish to mammals, at the light/histologic, electron microscopic, and biochemical levels (26). Peroxidase-containing eosinophils have been definitively identified in embryonic and adult zebrafish, which provide a potentially useful vertebrate model that can be genetically manipulated to study eosinophil development and functions (27). Observations in the frog support a role for the eosinophil in tissue remodeling events during metamorphosis (e.g., the shortening of the tadpole gut is accompanied by substantial infiltration of eosinophils), but the specific role of eosinophils in these complex metabolic, physiologic, and anatomical processes remains to be defined. Eosinophils are definitively present in most avian species. In the chicken, transcriptionally regulated differentiation of eosinophil-committed myeloid progenitors to mature eosinophils is remarkably similar to that of human eosinophils (10).

There are numerous published descriptions of mammalian eosinophils. Although these eosinophils are clearly well equipped to kill and/or constrain helminth parasites and their larval stages, their early appearance during evolution and accumulating studies of host immune responses to helminths and other parasites in eosinophil-deficient mouse strains [e.g., PHIL (28), ΔdblGATA (12), MBP-1$^{-/-}$/EPX$^{-/-}$ double knockout (29)] strongly argue against a significant selective advantage in host defense during the evolution of the eosinophil. The absence of significant, life-threatening developmental abnormalities or functional deficiencies in these eosinophil-deficient mouse strains, at least under the specific pathogen-free conditions present in most animal facilities, begs the question of why the eosinophil lineage continues to be ubiquitous in vertebrate species.

HUMAN EOSINOPHILS VERSUS OTHER SPECIES: SIMILARITIES AND DIFFERENCES

Human eosinophils differ to varying degrees from eosinophils of other species, the mouse being of greatest interest. These differences are present at a number of levels, including the origin of their hematopoietic progenitors; polymorphonuclear morphology; ultrastructure of their acidophilic specific granules; expression, types, and amounts of their granule cationic and other major proteins; surface receptors; mechanisms of activation, secretion, and degranulation; and other functionally relevant properties (30). For example, there are differences in the origins of human and mouse EoPs, the human EoP emerging earlier than the common myeloid progenitor population, while the mouse EoP is derived directly from granulocyte/macrophage progenitors (9). That said, some controversy remains, with ongoing revisions to the human and mouse hematopoietic trees based on improved reagents and approaches to identify these cells; their surface phenotypes; and, most recently, transcriptomes at the single-cell level (31).

Human and mouse eosinophils show significant differences in the cationic protein constituents of their specific granules. For example, although EPX and MBP-1 and -2 are well conserved, the human eosinophil contains only two cationic ribonucleases, EDN (RNase2) and ECP (RNase3) (32), whereas mouse eosinophil granules contain upward of seven members of an evolving family of eosinophil-associated ribonucleases that are also expressed by other myeloid cells in the mouse and other rodents (33). Human eosinophils also express large amounts of the cytosolic autocrystallizing CLC/Galectin-10, which represents ~7–10% of total cellular protein and is one of the

earliest and most abundant mRNAs expressed during eosinophil development. In contrast, mouse eosinophils lack a gene encoding CLC/Galectin-10 (32). Although the function or functions of CLC/Galectin-10 in human eosinophil biology remains unclear, it may be required for effective granulogenesis during eosinophil development (29, 32). Additionally, it is readily detected in airway mucus and, when administered to mouse airways, has Th2-like proinflammatory activities (34).

Clearly, functional differences exist between human eosinophils and those of mice and other species. Numerous studies have described the role and specific functions of eosinophils in the development of allergic inflammatory diseases in mice, many of which have been ascribed to secretion of eosinophil-derived cytokines and chemokines, as well as to differences in eosinophil secretory potential among species. Notably, the different pathways for eosinophil activation, degranulation, and secretion of their cationic granule proteins and stored cytokine and chemokine inflammatory mediators, and particularly the mechanisms that regulate piecemeal degranulation (PMD), are based primarily on in vitro and in vivo studies of human eosinophils. These pathways of degranulation in the setting of allergic inflammatory reactions in tissues typically do not occur in most murine models of inflammation (35, 36). Finally, differences in cell surface protein and receptor phenotypes between eosinophils from humans and those from other species are considerable, but they are beyond the scope of this review. Ultimately, the significant differences between human eosinophils and those of other species, particularly in the context of genetically modified mouse models used to assess eosinophil function in homeostasis and eosinophil-associated diseases, argue strongly for the need to confirm these aspects of eosinophil biology using human blood- and tissue-derived eosinophils ex vivo and in vitro, as well as in humanized mouse models.

TISSUE EOSINOPHILIA AND EOSINOPHIL ACTIVATION WITHIN TISSUES

Once eosinophils mature within the bone marrow environment, they exit and circulate for approximately 1 day, as estimated in normal adult humans using nuclear medicine tracer techniques (37). Using similar methods, researchers have estimated that the accumulation of eosinophils into the lung ranges from approximately 30 eosinophils per minute per milliliter of blood in healthy volunteers to rates 10–100 times higher or more in patients with asthma and those with focal eosinophilic lung diseases (38). Under homeostatic conditions, the vast majority of eosinophils are headed for mucosal surfaces of the gastrointestinal tract, sparing the esophagus but including the stomach and small and large intestine. Once there, they are presumed to reside for days. Although their homeostatic life span within these organs is not known, it is almost certainly on the order of days rather than weeks. As is true for all circulating leukocytes, in order for eosinophils to leave the circulation and enter any extravascular compartment, a series of well-orchestrated steps involving leukocyte and endothelial adhesion molecules must occur (**Figure 3**). Initial events are mediated by selectin–sialoglycan interactions that, for eosinophils, are mediated primarily by carbohydrates displayed on P-selectin ligand (CD162) on the eosinophil and P-selectin on activated endothelium (39, 40). Eosinophils also express L-selectin and ligands for E-selectin on their surface, but their roles in eosinophil accumulation are less certain and do not appear to be as important as they are in, for instance, neutrophil or cutaneous T cell recruitment responses (41). Patients with leukocyte adhesion deficiency type 2, whose leukocytes lack fucosylated selectin ligands, have elevated numbers of circulating neutrophils, but not eosinophils. The same phenomenon has been observed in clinical trials of a pan-selectin antagonist (GMI-1070, rivipansel), suggesting that selectin-mediated homeostatic recruitment is likely less important for eosinophils (42, 43).

Subsequent steps that are even more critical for recruitment for eosinophil extravasation beyond tethering and rolling involve integrins and their counterligands on activated endothelium.

These molecules include the β_1 integrin very late antigen 4 ($\alpha_4\beta_1$ integrin, CD49d/CD29), which is not expressed by neutrophils but is found on other leukocytes and recognizes the endothelial ligand vascular cell adhesion molecule 1 (VCAM-1, CD106) and β_2 integrins, especially lymphocyte function–associated antigen 1 ($\alpha_L\beta_2$ integrin, CD11a/CD18) and Mac-1 ($\alpha_M\beta_2$ integrin, CD11b/CD18), which are expressed by eosinophils, neutrophils, and other cells and interact with endothelial intercellular adhesion molecule 1 (ICAM-1, CD54). Eosinophils, like neutrophils, use both ICAM-1 and platelet/endothelial cell adhesion molecule 1 (PECAM-1, CD31) during the process of transendothelial migration (41, 44). Whether eosinophils use CD99 and CD99L2 during this step, as has been described for neutrophils (45), is unknown. There may be an especially critical contribution via the selective interaction of $\alpha_4\beta_1$ integrin with VCAM-1 on the endothelium and the selective induction of VCAM-1 expression caused by IL-4 and/or IL-13 (although IL-1 and tumor necrosis factor α can also influence VCAM-1 expression) (41). Further support for this concept comes from several clinical observations: (*a*) Humans lacking β_2 integrins have a markedly impaired ability to mobilize neutrophils, but not eosinophils or other leukocytes, into tissues during inflammation (46); (*b*) antibody blockade of $\alpha_4\beta_1$ integrin with natalizumab (47), or of the common IL-4Rα chain (shared by both IL-4R and IL-13R) with dupilumab, causes eosinophilia, not neutrophilia (48); and (*c*) blockade of $\alpha_4\beta_7$ integrin (LPAM-1) with vedolizumab has no effect on circulating granulocyte numbers (49).

In addition to the role of adhesion molecules, eosinophils are equipped with a wide range of seven-spanner receptors for chemokines and other chemoattractants (**Figure 2**). Although the recent failure of an oral CCR3 antagonist to alter eosinophil numbers in the sputum of subjects with asthma or eosinophilic bronchitis challenges this paradigm (50), whether the contribution of CCR3 will be more substantial during eosinophil recruitment to other organs in other conditions, or whether newer and potentially more effective CCR3 antagonists (51) will provide additional insight into this dilemma, remains to be determined. Lastly, ongoing clinical trials with bertilimumab (anti-CCL11) in bullous pemphigoid, an eosinophil-rich skin disease, should tell us whether this condition is driven by CCL11 (eotaxin-1).

There is abundant evidence that eosinophil integrins become activated in both blood and tissues in diseases such as asthma. Phenotypic changes occur with eosinophil activation and extravasation, including shedding of some surface proteins (e.g., L-selectin) and de novo expression of others (e.g., CD69), while still others (e.g., Siglec-8) remain unchanged (52, 53). Another consequence of eosinophil activation is platelet adhesion, which not only complicates proteomic analyses of so-called purified eosinophils but also results in platelet-dependent alteration of eosinophil function (54, 55).

Because eosinophils are terminally differentiated cells that can no longer divide, eosinophilic inflammation must be the net result of combinations of enhanced recruitment and production in the bone marrow, along with enhanced survival (9). Regarding the latter, numerous cytokines, such as IL-3, IL-5, and GM-CSF, maintain eosinophil viability for weeks in vitro (**Figure 4**). Anti-IL-5 biologic agents reduce eosinophil numbers in tissues in asthma and eosinophilic esophagitis but not in the normal small intestine, suggesting that this cytokine is not exclusively responsible for prolonging eosinophil survival in vivo. While this effect of anti-IL-5 in vivo may be due, in part, to a partial loss of IL-5R expression on extravasated eosinophils (56), mediators other than IL-5 must be important in maintaining eosinophil longevity at sites of inflammation. Although GM-CSF seemed a likely candidate for this role, trials of anti-GM-CSF in asthma were disappointing (57).

Eosinophil survival can be diminished by a number of pathways (**Figure 4**), as eosinophil death and removal can occur via many different mechanisms, including apoptosis, necrosis, autophagy, necroptosis, antibody-dependent cellular cytotoxicity (ADCC), and phagocytic cell recognition

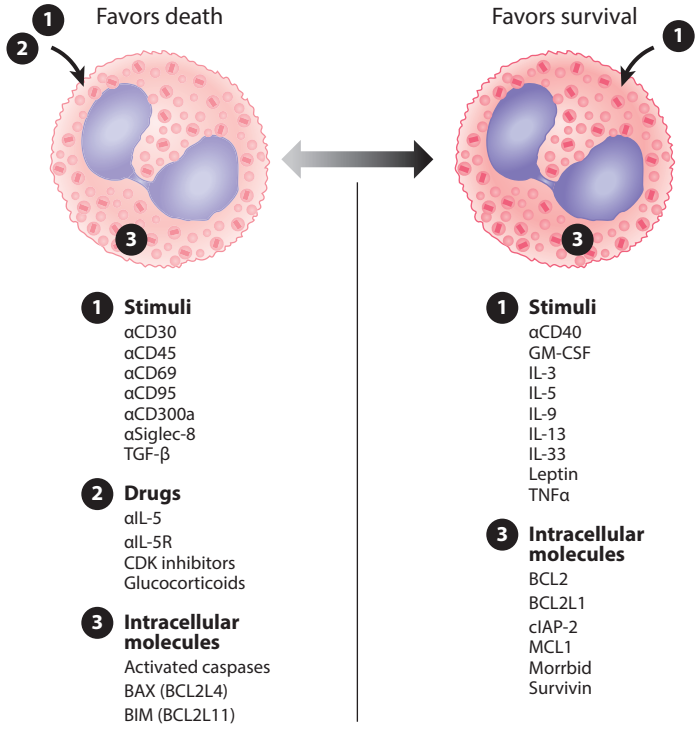


Figure 4

Examples of stimuli, drugs, and intracellular molecules that enhance or reduce eosinophil survival. Abbreviations: BAX, BCL2-like protein 4; BCL2, B cell lymphoma 2; BCL2L, BCL-2-like protein; BIM, BCL2-like protein 11; CDK, cyclin-dependent kinase; cIAP-2, cellular inhibitor of apoptosis 2; GM-CSF, granulocyte macrophage colony–stimulating factor; IFN, interferon; IL, interleukin; MCL1, myeloid cell leukemia 1; Siglec, sialic acid–binding, immunoglobulin-like lectin; TGF, transforming growth factor; TNF, tumor necrosis factor. Figure by Jacqueline Schaffer, Medical Illustrator.

and clearance (efferocytosis) (58). *Morrbid*, a noncoding RNA found in leukocytes, downregulates transcription of the proapoptotic *Bcl2l11* gene (previously called *BIM*) to promote survival in cells including eosinophils. Indeed, levels of *Morrbid*, as well as of cellular inhibitor of apoptosis 2 and survivin, all of which are antiapoptotic, are abnormally overexpressed in eosinophils from subjects with hypereosinophilic syndrome (HES) compared with normal individuals (59). Therapeutically, anti-IL-5 antibodies such as mepolizumab and reslizumab reduce eosinophil hematopoiesis and induce eosinophil apoptosis, while non-fucosylated IgG1 monoclonal antibodies to IL-5R (benralizumab) and Siglec-8 (AK002) actively deplete eosinophils via ADCC (60–62).

Separate from recruitment pathways and the competition between prosurvival and prodeath signals that eosinophils encounter in situ while in tissues, additional forms of activation result in secretion of a host of mediators ranging from preformed granule proteins to lipid mediators, cytokines, chemokines, enzymes, growth factors, and other substances. Structures involved in secretion, such as vesicle-associated membrane proteins including CD63, are found on the granule membranes themselves. During the process of degranulation, a key phenomenon by which eosinophils contribute to host defense and disease, preformed contents are released via at least three different pathways: (*a*) typical exocytosis, where granules fuse with the outer plasma membrane; (*b*) PMD involving intracellular vesicle formation associated with loss of granule integrity,

followed by movement and fusion with the outer plasma membrane; and (*c*) ETosis, or cytolytic degranulation associated with plasma membrane rupture and release of free granules along with extracellular traps (4, 63, 64). Stimulated eosinophils can rapidly release other substances besides granule proteins, as has been observed when so-called traps containing mitochondrial DNA and granule proteins combine to form structures that then bind and kill bacteria (65).

While the exact mechanisms for various types of eosinophil degranulation in vivo in humans remain poorly characterized, in vitro studies have shown that engagement of FcαR by secretory IgA is particularly effective, while exposure to combinations of cytokines, chemokines, and other chemoattractants can also elicit secretion in an integrin/adhesion-dependent manner. Finally, compared with other cells, eosinophils release relatively small numbers of cytokines, chemokines, and growth factors, and determining their relative contribution in comparison to other cells is difficult to do. In contrast, eosinophils make appreciable quantities of lipid mediators, especially leukotriene C_4 (LTC_4) via LTC_4 synthetase, located in the lipid bodies, and the 5-lipoxygenase product 5-hydroxyeicosatetraenoic acid, along with cyclooxygenase products such as thromboxane B_2 and prostaglandins E_1 and E_2.

ROLES FOR EOSINOPHILS IN HEALTH

The association between eosinophilia and helminth infection was noted soon after Ehrlich first described eosinophils. This association, coupled with studies demonstrating eosinophil killing of helminth larvae in vitro, led to the hypothesis that the primary role of eosinophils was in antipathogen responses, specifically those involving helminths. As helminth infection has become less common and eosinophils have persisted, the role of eosinophils in host defense against external pathogens has become less clear, and other homeostatic functions of eosinophils have been described (**Figure 5**) (2).

Parasitic Infections

Peripheral eosinophilia is commonly, but not always, associated with a wide variety of helminth infections, particularly those that involve migration of parasites through tissues, in ectoparasite infestations, and rarely in the setting of protozoan infection (i.e., *Sarcocystis* myositis and *Cystoisospora* infection). Human and mouse eosinophils can adhere to and kill infective helminth larvae through ADCC, and eosinophil granule protein deposition around dead and dying parasites has been demonstrated in tissue biopsies from infected patients (66). That said, the role that eosinophils play in protection remains uncertain. In murine models, eosinophils are sometimes protective (e.g., they prevent secondary infection by *Trichuris muris* and *Trichinella spiralis*), sometimes of no consequence (e.g., they do not affect granuloma formation in schistosomiasis), and sometimes required for parasite survival (e.g., they maintain *Trichinella* larvae encysted in muscle through their effects on nurse cells) (67). Data supporting a role for human eosinophils in protection against helminth infection in vivo are scarce, with the exception of schistosomiasis, where post-praziquantel eosinophil levels have been correlated with resistance to reinfection in many different epidemiologic settings (68).

Fungal Infections

Many fungal infections are characterized by blood and/or tissue eosinophilia. Whereas data from most mouse models suggest that the presence of eosinophils is protective in fungal infection, eosinophilia appears to be associated with disseminated or more severe human fungal disease (69). The exception is allergic fungal disorders, including allergic bronchopulmonary aspergillosis and

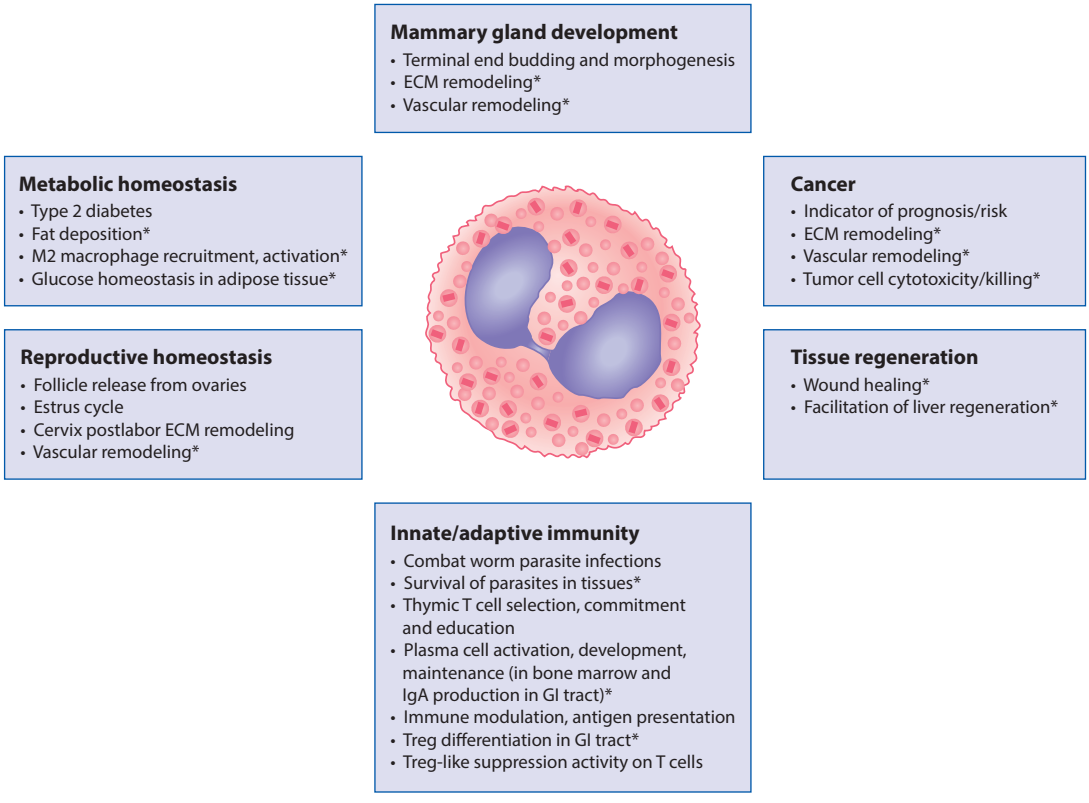


Figure 5

Roles of eosinophils in normal tissue and metabolic homeostasis in health. Major functions of the eosinophilic leukocyte include the maintenance of tissue microenvironments during normal organismal development and the establishment and regulation of host innate and adaptive immune responses. Findings from mouse models that have yet to be confirmed in humans are denoted with an asterisk. Abbreviations: ECM, extracellular matrix protein; GI, gastrointestinal; Ig, immunoglobulin; M2 macrophage, an alternatively activated macrophage that arises in response to exposure to Th2-type cytokines; Treg, T regulatory cell. Figure by Jacqueline Schaffer, Medical Illustrator.

allergic fungal sinusitis, in which eosinophilia can be dramatic and fungal elements are scarce. Eosinophil extracellular DNA traps appear to be involved in destroying fungal organisms (70).

Viral Infections and Others

Viral infections are typically associated with a decrease in circulating eosinophils in the blood. The most notable exception is human immunodeficiency virus infection (71). Tissue eosinophilia in the absence of blood eosinophilia has been described in a variety of viral infections, including viral myocarditis and respiratory syncytial virus (RSV) pneumonia. Whether eosinophils play a role in antiviral defense, are responsible for tissue destruction, or are simply recruited to sites of tissue damage is unknown. Although data from experimental murine infection with RSV and influenza A support the hypothesis that eosinophils play a predominantly protective role (72, 73), human studies have produced conflicting results, with (*a*) comparable prevalence rates of respiratory viral infection but increased clinical severity in asthma patients with >3% sputum eosinophils (74); (*b*) similar airway inflammatory responses following experimental rhinovirus infection in asthma patients and healthy controls, despite increased eosinophils in the former; and (*c*) effects

of mepolizumab (which substantially reduces blood and sputum eosinophils) on macrophage, B cell, and neutrophil responses without effect on infection severity following rhinovirus challenge (75). Finally, whereas eosinopenia is the rule in acute bacterial infection, eosinophils may play a role in the host response to some chronic bacterial infections, including mycobacterial infection (76) and *Clostridium difficile* colitis (77).

Antitumor Responses

Our understanding of the role and contribution of eosinophils to human tumor biology and immunology is still evolving. Some of the more intriguing recent information on this topic comes from analyses of tumor biopsies and correlations between prognosis and numbers of eosinophils in the tumor microenvironment, detected histologically or based on the presence of eosinophil-specific gene signatures (78, 79). These approaches suggest that the presence of eosinophils within the tumor microenvironment can be good (e.g., breast cancer, melanoma), bad (e.g., lung cancer, Hodgkin lymphoma), or of unclear prognostic significance (e.g., brain cancer). The obvious disadvantage of this approach is that it provides no insight into the actual contribution of the eosinophil itself to tumor progression or remission, as tissue eosinophilia may simply be a biomarker of type 2 inflammation. Note that neither mice nor humans lacking eosinophils appear to be at increased risk of developing cancers. Ultimately, long-term safety data with biologic agents that selectively deplete eosinophils may be the best way to directly answer this question.

Eosinophil Deficiency in Humans

Despite multiple murine models demonstrating the viability and reproductive capability of mice lacking eosinophils (12, 28), congenital eosinophil deficiency has not been described in humans to date. Although this may be due to underreporting in the absence of characteristic clinical features, an analysis of blood smears from 24,300 patients at the University of Pittsburgh found no cases of unexplained eosinopenia (<1 eosinophil per 1,000 cells counted) (80). Rare cases of acquired eosinophil deficiency have been reported, most commonly in patients with thymoma and agammaglobulinemia (Good's syndrome) (81), and do not appear to be associated with specific clinical features (82).

Hereditary abnormalities involving eosinophil granule proteins are also uncommon. Specific granule deficiency (SGD) is a rare primary immunodeficiency in which mutations in *CEBPE*, the gene encoding C/EBPε, or *SMARCD2*, which encodes a factor that interacts with C/EBPε, lead to impaired transcription of granule components in neutrophils and eosinophils (83). Patients with SGD present with recurrent bacterial and fungal infections attributed to impaired neutrophil differentiation and function. Eosinophils from patients with SGD are deficient in three major components of eosinophil secondary granules (ECP, MBP-1, and EDN) but do contain EPX and respond to stimulation with GM-CSF (84). The clinical consequences of the eosinophil abnormalities in SGD are unknown. Abnormal eosinophil granule morphology without apparent clinical manifestations is also characteristic of the nearly 100 reported cases of hereditary EPX deficiency (85).

FURTHER INSIGHTS INTO EOSINOPHIL BIOLOGY FROM EXPERIMENTAL MODELS

Regulation of Tissue Remodeling and Fibrosis

While it is clear that most eosinophil-associated disorders involve some sort of pathologic tissue remodeling and fibrosis, whether the eosinophil is directly contributory or is guilty by association

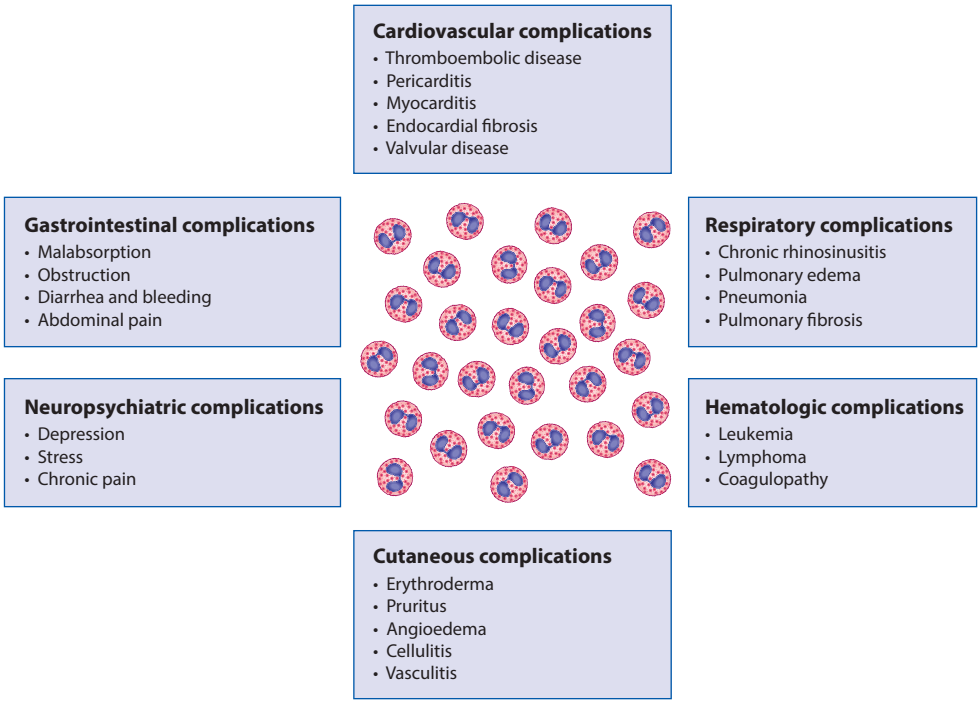


Figure 6

Roles of eosinophils in disease pathogenesis. Contributions of eosinophils to complications of various diseases as separated by organ involvement that can occur independently of underlying disease pathogenesis. Figure by Jacqueline Schaffer, Medical Illustrator.

is less clear. The most compelling data come from studies employing eosinophil-deficient and other genetically modified mouse strains in which roles in tissue remodeling associated with both normal physiologic processes and disease pathogenesis have been observed (**Figures 5** and **6**) (2). Eosinophils appear to be a major source of the profibrotic cytokine transforming growth factor β in the allergic inflamed lung and in EoE (86–88). In addition, eosinophil granule cationic proteins have profibrogenic activities both in vitro (on fibroblasts and epithelial cells) and in vivo in mouse models (89). Eosinophil granule proteins induce production of IL-6 and related fibrogenic cytokines from fibroblasts, fibroblast proliferation and transdifferentiation to myofibroblasts, fibroblast-mediated collagen gel contraction, and expression of various matrix metalloproteinases involved in fibrogenesis (89, 90).

Regulation of Metabolism, Adipose Tissue, and Glucose Homeostasis

A rather unexpected role for mouse eosinophils and their production of IL-4 emerged from a series of publications demonstrating their important role in adipose tissue and obesity by sustaining alternatively activated M2 macrophages, glucose homeostasis, and the development of beige fat (91–94). Notably, this collaboration between eosinophils and macrophages is regulated in part by ILC2s, which sustain both the adipose eosinophils and the alternatively activated M2 macrophages (95). A recent study demonstrated that cross talk between the inhibitory receptor CD300f and IL-5 functionally modifies eosinophil regulation of metabolism (96). Whereas an intriguing role has emerged for mouse eosinophils in regulating metabolic functions and

adiposity, this is by no means incontrovertible (97), and whether these findings will translate to human eosinophils requires further investigation.

Regulation of Other Immune Responses

Eosinophils in mouse bone marrow have been reported to secrete a number of survival factors, including the plasma cell proliferation–inducing ligands APRIL and IL-6, which promote maintenance of the plasma cell niche (98). Subsequent studies showed that eosinophils promoted class switching toward secretory IgA and were required for the development and maintenance of IgA-producing plasma cells (99, 100). Eosinophil deficiency was also associated with altered composition of gastrointestinal microbiota, altered development of Peyer's patches, and decreased mucus production in the small intestine (101). However, two subsequent studies using ΔdblGATA eosinophil-deficient mice found that eosinophils were dispensable for the survival of plasma cells in the bone marrow and did not contribute to IgA antibody production or autoantibody-mediated disease (102, 103).

A potential role for eosinophil regulation of B cells has been proposed on the basis of in vitro data showing that there is an IL-5-independent and cell-cell contact–independent but eosinophil-dependent enhancement of B cell proliferation and survival, as well as a modest correlation between the number of circulating eosinophils and B cells in patients with HES (104). The demonstration of major histocompatibility complex class II expression on both mouse and human eosinophils and their ability to present antigen to T cells suggest that eosinophils may also play a role in antigen presentation (105–107). Despite these data, the relative contribution of human eosinophils to each of these processes has been difficult to delineate.

ROLES FOR EOSINOPHILS IN DISEASE

Definitions

Peripheral blood eosinophilia is generally defined as an absolute eosinophil count (AEC) $\geq$500 per microliter, although normal levels may vary depending on the patient population and method of quantification. An AEC $\geq$1,500 per microliter is considered marked peripheral eosinophilia (or hypereosinophilia). Tissue eosinophilia and hypereosinophilia are much more difficult to define because consensus guidelines have not been established for most tissues, and eosinophils themselves may be absent despite marked tissue deposition of eosinophil granule proteins consistent with eosinophilic inflammation. For the purposes of this review, we define HES according to a consensus definition developed by a multispecialty group of experts as (*a*) an AEC $\geq$1,500 per microliter and clinical manifestations attributable to the eosinophilia or (*b*) tissue hypereosinophilia with blood eosinophilia (an AEC above the upper limit of normal for the reference laboratory) (108). Notably, this definition does not distinguish eosinophilia that is idiopathic from eosinophilia that is secondary to a known cause.

The definition of eosinophil-related diseases that do not meet the criteria for HES has evolved over the past decade (109, 110). These tend to be disorders in which increased numbers of eosinophils in blood and/or tissues are thought to cause pathology, but often the eosinophilia or eosinophilic inflammation does not occur in isolation. Examples include common and uncommon human disorders ranging from allergic conditions such as asthma and atopic dermatitis to eosinophilic gastrointestinal disorders (EGID), eosinophilic granulomatosis with polyangiitis (EGPA), bullous pemphigoid (**Figure 7**), and others. Due to the availability of eosinophil-targeted therapies, defining the role of eosinophils in eosinophil-related disorders may finally be feasible.

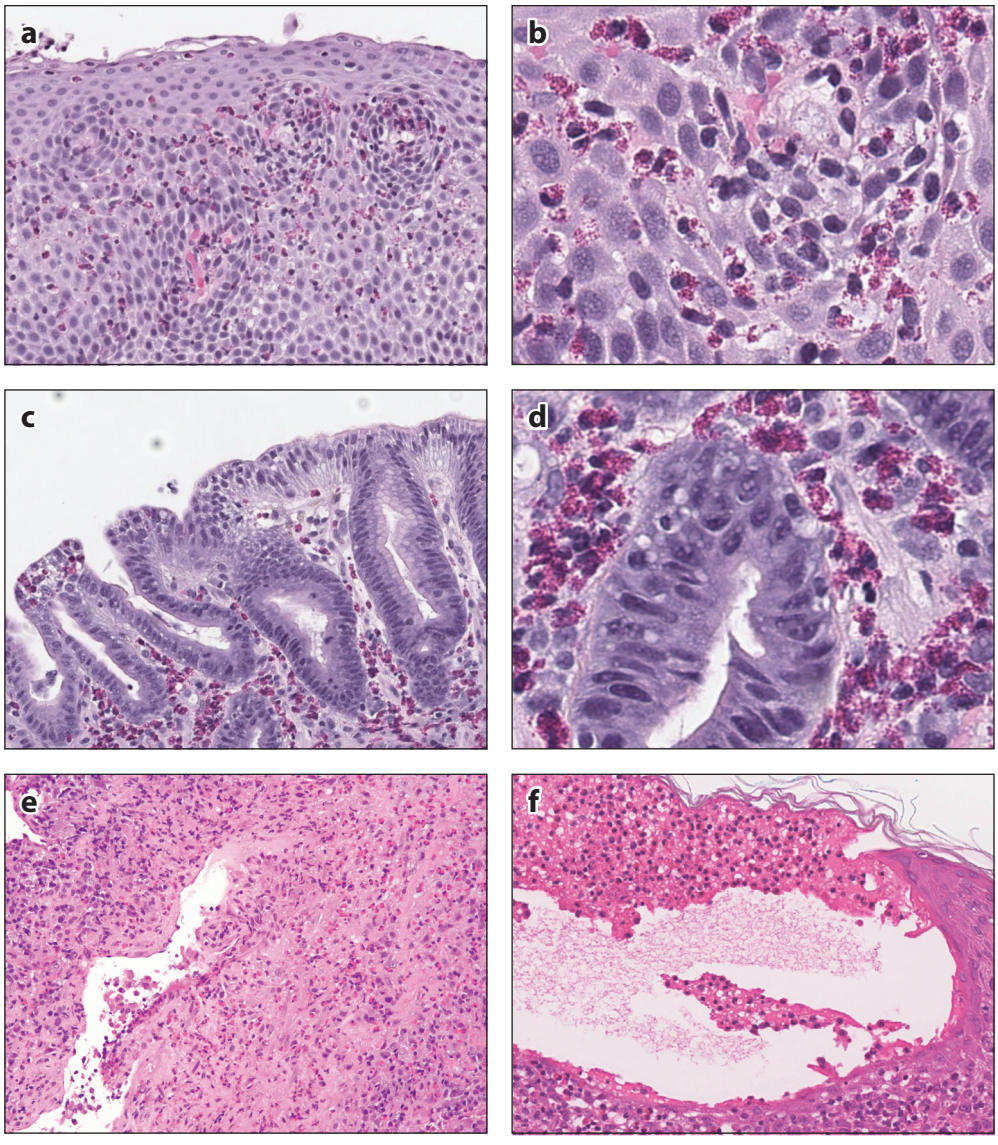


Figure 7

Histologic findings in eosinophilic gastrointestinal disorders (EGID), eosinophilic granulomatosis with polyangiitis (EGPA), and bullous pemphigoid. (*a*,*b*) Hematoxylin and eosin–stained section of a biopsy from a patient with eosinophilic esophagitis, at both lower- and higher-power magnification, respectively, showing increased intraepithelial eosinophils, epithelial spongiosis, and basal cell hyperplasia. (*c*,*d*) Hematoxylin and eosin–stained section of a biopsy from a patient with eosinophilic gastritis, at both lower- and higher-power magnification, showing increased eosinophils in the gastric lamina propria. (*e*) Hematoxylin and eosin–stained section of a lung biopsy from a patient with EGPA showing a dense interstitial infiltrate rich in eosinophils, lymphocytes, and plasma cells involving a vessel wall with focal fibrinous changes. (*f*) Hematoxylin and eosin–stained section of a skin biopsy from a patient with bullous pemphigoid showing a subepidermal blister with numerous eosinophils aligned along the cutaneous basement membrane zone. Also present is significant epidermal edema (spongiosis) with a few intraepithelial eosinophils. Photomicrographs provided by (*a*–*d*) Dr. Guang-Yu Yang (Northwestern University Feinberg School of Medicine), (*e*) Dr. Stefania Pittaluga (Laboratory of Pathology, National Cancer Institute, National Institutes of Health), and (*f*) Dr. Kyle Amber (College of Medicine, University of Illinois).

Clinical Subtypes of Hypereosinophilic Syndrome

As defined above, HES comprises a diverse group of disorders related only by the presence of markedly increased numbers of blood and/or tissue eosinophils and evidence of eosinophil-mediated pathology. In an attempt to address this issue, investigators have described a number of clinical subtypes on the basis of likely etiology and approach to management (111, 112).

Myeloid hypereosinophilic syndrome. Approximately 15–20% of patients who present with HES have definitive or presumptive evidence of a primary myeloid neoplasm. Of these, the vast majority (≥80% in most series) have an interstitial deletion in chromosome 4 giving rise to the fusion gene *FIP1L1-PDGFRA*. Prior to the availability of imatinib, these patients had a very poor prognosis, with a 5-year mortality rate of 30–50% due primarily to the development of endomyocardial fibrosis and thromboembolic events (113). Imatinib response rates approach 100%, and recent data suggest that a significant proportion of patients with this fusion gene may be cured after prolonged molecular remission (114). Other genetic abnormalities that can give rise to myeloid HES include *PFGFRB* and *FGFR1* fusion genes as well as point mutations and translocations involving *JAK2*. The spectrum of myeloid HES also includes "chronic eosinophilic leukemia, not otherwise specified," and patients without an identifiable mutation who have clinical and bone marrow characteristics suggestive of a myeloid neoplasm, including eosinophil dysplasia (**Figure 8**), involvement of other lineages, elevated serum vitamin B_{12} and/or tryptase levels, and splenomegaly (115). Myeloid HES involving abnormalities in *PDGFR* is observed almost exclusively in males, whereas other molecular phenotypes and idiopathic myeloid HES do not appear to have a gender preference.

Lymphocytic hypereosinophilic syndrome. Lymphocytic HES refers to HES accompanied by the presence of a clonal and/or phenotypically aberrant T cell clone that secretes IL-5 or other cytokines that drive the eosinophilia (116). The most common abnormal T cell phenotype is $CD3^{-}CD4^{+}$. Similar to myeloid HES, this clinical subtype is a spectrum, ranging from an indolent lymphoproliferative syndrome to frank lymphoma. Skin manifestations appear to be most common, although any organ can be involved. Serum levels of IgE and the chemokine TARC (CCL17) are usually elevated. Up to 30% of patients with lymphocytic HES and no evidence of malignancy will ultimately develop lymphoma. This may be preceded by a change in the peripheral clonal population (increase or decrease) or a new cytogenetic abnormality.

Episodic angioedema with eosinophilia (Gleich syndrome) is an unusual HES variant characterized by the monthly occurrence of eosinophilia, neutrophilia, lymphocytosis, angioedema, urticaria, and systemic symptoms that resolves spontaneously between episodes (117). Although $CD3^{-}CD4^{+}$ T cell clones are also detectable in the majority of patients with this syndrome and serum IL-5 levels (as well as a number of other soluble mediators) cycle up and down with the cyclic hypereosinophilia in a synchronized fashion, the role of the aberrant T cells in this multilineage disorder is unclear.

Overlap hypereosinophilic syndrome. The term overlap HES is used to denote single-organ eosinophilic disorders, including EGID and eosinophilic fasciitis, and recognized multisystem eosinophilic syndromes with characteristic clinical features, such as EGPA. These disorders are distinguished from other forms of HES because of the collection of existing data, including specific approaches to treatment and prognostic factors. Conversely, they are included under the broad umbrella of HES because eosinophils are believed to play a primary role in disease pathogenesis. Moreover, the clinical presentation may be difficult to distinguish from that of other forms of HES.

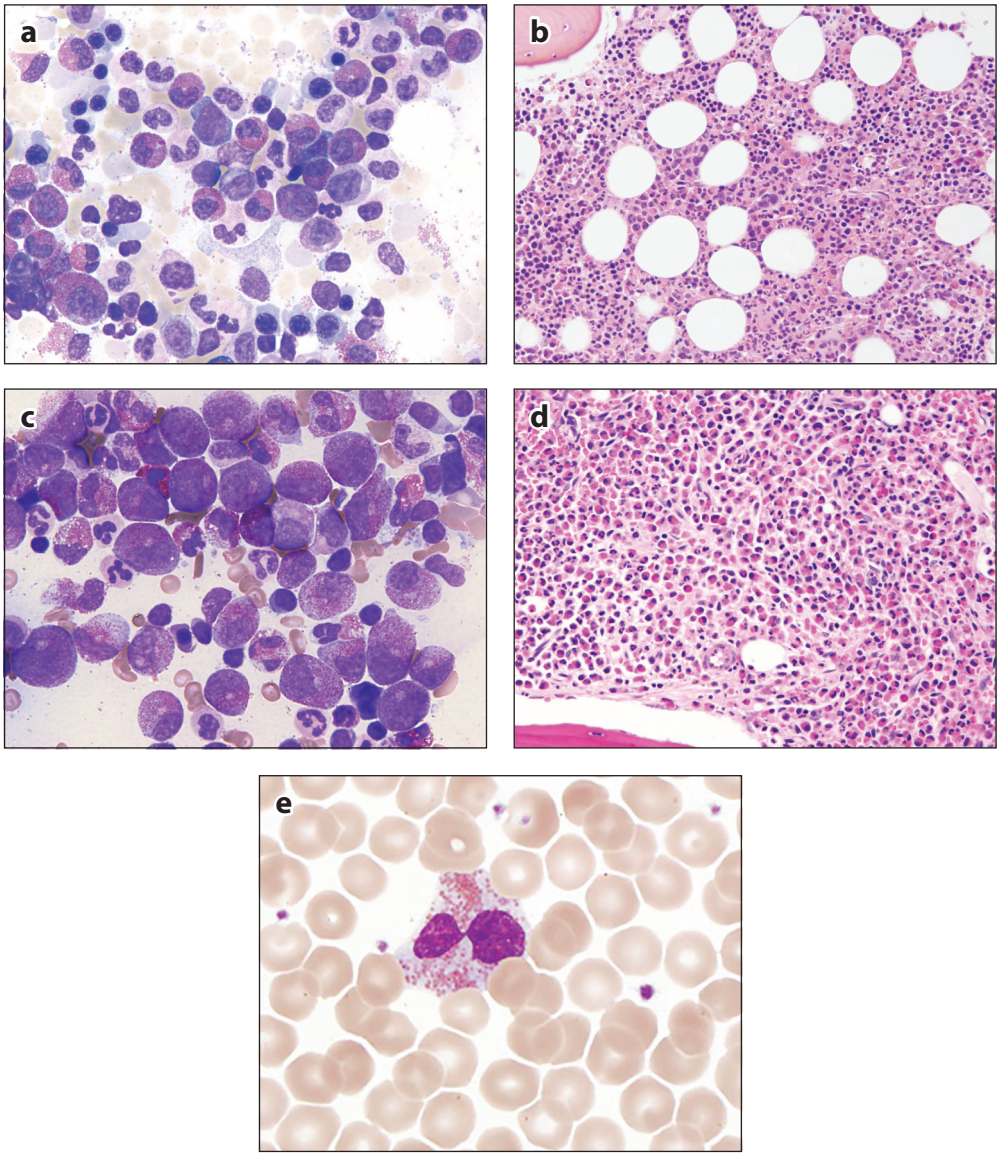


Figure 8

Bone marrow and cytopathologic findings in hypereosinophilic syndrome (HES). (*a*) Giemsa-stained bone marrow aspirate and (*b*) hematoxylin and eosin–stained bone marrow biopsy from a patient with idiopathic HES. (*c*) Giemsa-stained bone marrow aspirate and (*d*) hematoxylin and eosin–stained bone marrow biopsy from a patient with a *FIP1L1-PDGFRA*-positive myeloid neoplasm. (*e*) An example of a dysplastic eosinophil seen on a peripheral blood smear from a patient with HES that was accidentally misidentified as a neutrophil in an electronic differential blood count. Photomicrographs provided by (*a–d*) Dr. Irina Maric (Department of Laboratory Medicine, Clinical Center, National Institutes of Health) and (*e*) Dr. Yi-Hua Chen (Northwestern University Feinberg School of Medicine).

Associated hypereosinophilic syndrome. Associated HES refers to HES in the setting of a defined cause for which treatment is not directed at the underlying eosinophilia, including parasitic infections, drug hypersensitivity, solid tumors, and primary immunodeficiency syndromes. Although the broad list of secondary causes of HES is beyond the scope of this review, we note that the clinical manifestations of hypereosinophilia can be identical irrespective of the cause. Secondary treatable causes of HES need to be considered and excluded in all patients presenting with AEC $\geq$1,500 per microliter.

Familial hypereosinophilia. Although familial clustering has been reported in EGID and EGPA (118, 119), clearly defined genetic transmission of hypereosinophilia has been described in only a handful of families. The best described is a large multigenerational cohort with autosomal dominant transmission mapped to a region on chromosome 5q31–33 that contains the IL-5 cytokine cluster. Despite hypereosinophilia from birth, most affected family members have remained completely asymptomatic. Although the genetic abnormality remains obscure, a recent study suggests that selective overexpression of IL-5 is responsible for driving the eosinophilia in this family (120).

Idiopathic hypereosinophilic syndrome. As diagnostic methods and our understanding of the mechanisms driving eosinophilia improve, the proportion of patients who cannot be classified into one of the above categories (i.e., with idiopathic HES) continues to decrease. That said, these patients represent a heterogeneous mix, with clinical manifestations ranging from relatively mild to life-threatening. Any organ can be affected, although skin, gastrointestinal tract, and pulmonary involvement are most common.

Therapeutic Considerations in Hypereosinophilic Syndrome

Corticosteroids remain the first-line therapy for most eosinophil-associated disorders, including HES, although long-term use is associated with significant toxicity and some patients do not respond (121). The exceptions are patients with myeloid HES who have targetable mutations or rearrangements, including *FIP1L1-PDGFRA* and translocations in *PDGFRB*. Conventional second-line therapies include hydroxyurea, interferon-α, imatinib (for patients with suspected myeloid HES), and methotrexate (122). The choice of second-line agent typically depends on clinical subtype, concomitant medical issues, cost, and patient and physician preference. Response rates to second-line therapies vary, and discontinuation of therapy is common due to lack of efficacy and side effects. Novel targeted agents with improved efficacy and toxicity profiles are desperately needed. Several such agents have been approved by the US Food and Drug Administration (FDA) and/or are in clinical development for the treatment of eosinophilic disorders (**Table 1**).

The first randomized, placebo-controlled, double-blind clinical trial of a therapy for HES was conducted more than a decade ago with mepolizumab [750 mg intravenously (iv) monthly]. This study demonstrated that blocking IL-5 was well tolerated and effective as an oral steroid-sparing agent in the treatment of steroid-responsive, *PDGFRA*-negative HES (123). Although this trial did not lead to FDA approval of mepolizumab for HES, it provided a proof of principle. Subsequent trials confirmed the efficacy of mepolizumab in the treatment of eosinophilic asthma [at 100 mg subcutaneously (sc) monthly] and EGPA (at 300 mg sc monthly) and resulted in FDA approval for these indications. Approval of reslizumab (3 mg/kg iv monthly) and benralizumab (30 mg sc monthly for 3 months, followed by 30 mg sc every 2 months) for the treatment of eosinophilic asthma followed shortly thereafter.

As with other therapies, there appears to be considerable variability in the response to agents targeting IL-5 in patients with HES. For example, despite an 85% response rate in patients with

Table 1 Eosinophil-targeted therapies approved or in clinical development[a]

	Mepolizumab	Reslizumab	Benralizumab	AK002	Dexpramipexole
Target	IL-5	IL-5	IL-5Rα	Siglec-8	Unknown
Antibody (parent)	Humanized IgG1κ (murine 2B6)	Humanized IgG4κ (rat 39D10)	Humanized afucosylated IgG1κ	Humanized non-fucosylated IgG1	Not applicable
Maximum dose in clinical trials	10 mg/kg iv 300 mg sc	3 mg/kg iv	3 mg/kg iv 200 mg sc	3 mg/kg iv	300 mg orally daily
Approved indications	Severe eosinophilic asthma (100 mg sc monthly) EGPA (300 mg sc monthly)	Severe eosinophilic asthma (3 mg/kg iv monthly)	Severe eosinophilic asthma (30 mg sc monthly for 3 months and then every 2 months)	None	None
Pediatric approval	>12 years of age	No	>12 years of age	No	No
Studies in multisystem HES	Phase 2 completed, phase 3 ongoing	Phase 2 completed	Phase 2 completed, Phase 3 planned	None	Phase 2 completed, Phase 3 planned
Studies in EGID	Phase 2 in EoE completed	Phase 2 in EoE completed	Phase 2 in eosinophilic gastritis ongoing	Phase 2 in eosinophilic gastritis and gastroenteritis ongoing	None
Studies in EGPA	Phase 3 completed	Phase 2 ongoing	Phase 2 ongoing	None	None
In vivo effects on target cells					
Peripheral eosinophils	Profound reduction	Profound reduction	Complete depletion	Complete depletion	Complete depletion
Tissue eosinophils	Partial depletion	Partial depletion	Complete depletion	NA	Complete depletion
Eosinophil precursors	Maturational arrest	NA	Complete depletion	NA	Maturational arrest
Basophils	NA	NA	Reduction	NA	Reduction
Mast cells	No effect	No effect	No effect	NA	No effect

[a]This table does not include therapies that target mutations associated with eosinophilic myeloid neoplasms, including the tyrosine kinase inhibitor imatinib.

Abbreviations: EGID, eosinophilic gastrointestinal disorders; EGPA, eosinophilic granulomatosis with polyangiitis; EoE, eosinophilic esophagitis; HES, hypereosinophilic syndrome; Ig, immunoglobulin; IL, interleukin; iv, intravenously; NA, published data not available; sc, subcutaneously.

systemic HES, mepolizumab has shown limited efficacy in the treatment of eosinophilic esophagitis. A similar lack of efficacy has been observed with reslizumab (124). Whether this is due to the lack of complete eosinophil depletion in tissue; involvement of other cells, such as mast cells, in the pathology; or issues with trial design (length of therapy, outcome measures) is unknown. Recent data examining high-dose mepolizumab treatment of patients with life-threatening HES on a compassionate-use protocol suggest that clinical subtype is an important factor in response

to anti-IL-5 therapy (125). A Phase 3 study of mepolizumab (300 mg sc monthly) is currently under way. Other biologic agents that target eosinophils currently in clinical development for the treatment of HES include benralizumab (126) and AK002 [a novel antibody targeting Siglec-8, a receptor on the surface of eosinophils and mast cells (127)] in eosinophilic gastritis.

Whereas biologic agents account for the overwhelming majority of eosinophil-targeted agents in clinical development, safe, well-tolerated, and effective oral agents for the treatment of HES are highly desirable. Dexpramipexole, an oral agent developed for the treatment of amyotrophic lateral sclerosis and repurposed for the treatment of HES, shows promise in this regard. In a recent open-label Phase 2 trial, 4 of 10 subjects with corticosteroid-responsive HES were able to taper their corticosteroid dose by ≥50% while on dexpramipexole (128). Dramatic reductions of both blood and tissue eosinophilia were observed in responders, concomitant with evidence of maturation arrest of eosinophil lineage development in the bone marrow. Only mild and transient treatment-related side effects were observed. Interestingly, the same drug was also tested in patients with chronic rhinosinusitis with nasal polyposis, a disorder associated with prominent tissue eosinophilia. Despite a 97% reduction in eosinophil numbers in the tissue while on the drug, no change in polyp size was observed. This brings into question the role of eosinophils in this condition (129), reminiscent of the inconsistent effects seen in a small trial with mepolizumab (130) and in stark contrast to the benefits observed with dupilumab (131).

The availability of novel therapies that dramatically reduce blood and tissue eosinophilia has provided a unique opportunity to examine the side effects of acquired eosinopenia in humans. To date, there have been no reports of adverse consequences of eosinophil depletion in patients treated with therapies that specifically target eosinophils, including mepolizumab, reslizumab, and benralizumab, despite the availability of some of these agents for almost two decades. Side effects have generally been mild; rare cases of anaphylaxis have been reported. Although two cases of shingles occurred in patients receiving mepolizumab in pivotal clinical trials versus none in patients receiving placebo, and although herpes zoster vaccination is recommended in the package insert for this agent, the lack of an association between shingles and either reslizumab or benralizumab therapy suggests that eosinophil depletion is not the underlying mechanism.

In contrast to murine models, few human studies have directly examined homeostatic mechanisms affected by eosinophil depletion. These include a study of recall responses to immunization with tetravalent influenza vaccine in 103 patients enrolled in a placebo-controlled study of benralizumab (132) and an assessment of B cell responses following rhinovirus challenge in 28 patients with eosinophilic asthma enrolled in a placebo-controlled trial of mepolizumab (75). In neither instance was eosinophil depletion detrimental. In fact, mepolizumab appeared to enhance B cell function and secretory IgA production in response to rhinovirus challenge (75).

UNMET NEEDS AND OPPORTUNITIES FOR EXPANDED UNDERSTANDING

Biomarkers for Diagnosis and Prognosis

As pointed out by expert panels, the need for biomarkers in the assessment of diagnosis, prognosis, choice of treatment, and disease severity and activity remains a hugely important unmet need in eosinophil-related diseases (109, 110). Fortunately, there are a few examples of highly useful diagnostic tests for the diagnosis of these disorders, such as detection of the *FIP1L1-PDGFRA* fusion gene in blood or bone marrow cells in a subset of patients with HES; serum antineutrophil cytoplasmic antibody positivity in a minor subset of patients with EGPA; observations of elevated serum levels of vitamin B_{12} and tryptase and dysplastic eosinophils in the myeloid variant of HES; and the finding, by flow cytometric immunophenotyping of whole blood, of aberrant

T cell clones in the lymphocytic variant of HES (111). With the exception of loss of detectable *FIP1L1-PDGFRA* during remission following treatment with imatinib (114, 133), what is urgently needed are tests that assess disease activity or predict treatment responsiveness to a given agent. This deficit is not due to lack of trying, as there are plenty of examples of failed efforts to find such biomarkers. For instance, measurements of eosinophil activation markers, both on the cell surface and in the serum of soluble proteins originating from the cell surface, such as IL-5Rα and Siglec-8, have so far not proven to be clinically useful (61, 134). Levels of chemokines such as eotaxin-3 (CCL26) may be associated with mucosal inflammation in chronic eosinophilic rhinosinusitis (135), but in EGPA, their utility as a biomarker of disease remains controversial. To date, attempts to find serum biomarkers for eosinophilic esophagitis and gastritis, including measures of sizable panels of cytokines and chemokines, have been disappointing, even though eosinophilic gastritis is much more frequently associated with peripheral blood eosinophilia. More promising is the use of a gene panel for analysis of biopsy material in eosinophilic esophagitis, which so far has been highly accurate in distinguishing disease from controls and thus might be useful for following disease activity over time (136, 137). Newer approaches such as single-cell RNA sequencing of esophageal biopsies has demonstrated, among several interesting findings, that $CD4^+$ T cells are the source of Th2 cytokines in this disorder. This type of approach might eventually offer additional diagnostic options while expanding our understanding of disease pathogenesis (138).

Regarding eosinophil-related disorders that especially affect the skin, CCL17 (TARC) is more commonly elevated in patients with the lymphocytic variant of HES (139). In bullous pemphigoid (**Figure 7**), serum levels of antihemidesmosomal protein antibodies, cytokines, chemokines, and other substances may be somewhat useful as biomarkers to assess disease severity or risk of relapse, but they are far from optimal (140). Clearly, biomarkers beyond tracking the AEC, including those that predict disease relapse and organ specificity of disease involvement and activity, are needed.

Less/Minimally Invasive Biomarkers of Disease Activity and Remission

One might expect that eosinophil-derived proteins, such as the granule cationic proteins (EPX, MBP-1, EDN, and ECP) or CLC/Galectin-10, would serve as excellent peripheral biomarkers of eosinophil activation and secretion locally at tissue sites of allergic eosinophil-dominant inflammation, including host responses to helminth infestations. However, quantitative measurement of these proteins in blood has generally failed to be sufficiently sensitive and specific to be clinically useful for disease diagnosis or monitoring patient responses to treatment. This is likely because most of these granule cationic proteins bind strongly to negatively charged tissue elements with long half-lives and thus fail to enter the peripheral circulation (141, 142). In fact, the peripheral blood AEC correlates better with tissue eosinophilia in a number of biomarker studies assessing the utility of serum granule protein levels (143–145).

In asthma, although the eosinophilic phenotype can be identified through invasive bronchoalveolar lavage, quantitation of eosinophils in induced sputum currently serves this role, with ≥2% sputum eosinophils being considered diagnostic for eosinophilic asthma (146). However, counting sputum eosinophils is both laborious and fraught with considerable lab-to-lab and patient-to-patient variability. Fortunately, efforts to develop rapid immunoassays, such as measurement of EPX or CLC/Galectin-10 in induced sputum extracts (147, 148), are showing considerable clinical promise and utility for identifying patients with eosinophilic asthma for targeted therapy with antieosinophilic agents.

To date, no single peripheral blood biomarker, or panel of biomarkers, has been identified that can reliably distinguish patients with active EoE from those with inactive (or successfully treated) EoE, patients with gastroesophageal reflux disease from those with EoE, or even patients with

EoE from healthy controls. Consequently, EoE patients are currently diagnosed and monitored with repeat endoscopy with biopsies. With the goal of developing a minimally invasive method for following mucosal eosinophilic inflammation in EoE, investigators have created a novel capsule-based technology, the Esophageal String Test™ (EST), that captures a liquid biopsy containing esophageal luminal secretions and inflammatory and epithelial cells from the entire length of the esophagus, with quantitative measurement of eosinophil-associated protein biomarkers, CLC/Galectin-10 (149), and eotaxin-3 (150). The overnight EST showed considerable sensitivity and specificity comparable to those of histologic eosinophil counts in biopsies and the same biomarkers measured in biopsy extracts, and a clinically convenient 1-h EST is currently being evaluated in a Phase 2 clinical validation study (150). Similar minimally invasive capsule-based devices, such as the Cytosponge™, have also shown promise in EoE, but their use may be restricted to adults due to the size of the capsule and swallowing difficulties for pediatric patients (151, 152).

Comparisons Among Available Biologic Agents

There are no data directly comparing the efficacy or safety of approved biologic agents that target eosinophils in patients with eosinophil-related disorders. A meta-analysis of the clinical trial data from five studies of the use of mepolizumab and reslizumab for the treatment of eosinophilic asthma found no differences in efficacy or safety by indirect comparison (153). A more recent indirect analysis of 11 published studies compared the clinically significant impact on asthma exacerbations of mepolizumab, reslizumab, and benralizumab and concluded that mepolizumab was more effective than either of the other two therapies (154). Theoretical differences between the three biologic agents include mode of administration (benralizumab and mepolizumab are approved as subcutaneous injections, whereas reslizumab is administered intravenously), dosing (fixed dosing for benralizumab and mepolizumab versus weight-based dosing for reslizumab), and degree of depletion of tissue eosinophils (partial for mepolizumab and reslizumab versus more complete for benralizumab). Direct comparisons of these three biologic agents in patients with the various eosinophil-related disorders are clearly needed to sort out these issues.

Long-Term Safety of Targeting Eosinophils

Despite the lack of worrisome safety signals to date, the effects of long-term depletion of eosinophils remain unknown. Whereas pharmacovigilance is clearly needed because these drugs are used in larger and more diverse populations (including populations in countries where helminth infection is endemic), carefully designed clinical studies to assess the impact of eosinophil depletion on homeostatic mechanisms, including immune responses, tumor surveillance, metabolic pathways, and tissue remodeling, are required.

CLOSING REMARKS

In the roughly 150 years since the discovery of the eosinophil, our knowledge of its role in health and disease has evolved tremendously. Within the last decade alone, major developments in mouse models, especially those in which eosinophils are congenitally or conditionally absent, have shed light on both expected and unexpected roles for these cells in health. On the clinical side, the ability to selectively target eosinophils using the precision of approved biological therapies has helped to cement the long-suspected role of the eosinophil in human asthma pathogenesis, especially asthma exacerbations, as well as in EGPA. Ongoing clinical studies offer the potential to expand this list to include other eosinophil-associated skin diseases, including chronic rhinosinusitis with or without nasal polyps, EGID, HES, and others. At the same time, there remains a need for novel

eosinophil-targeted agents, including those that have the potential to be disease modifying. Biomarkers other than AEC that will assist the physician in more confidently assessing the diagnosis, prognosis, choice of best treatment, disease severity, disease activity, and risk of relapse would be a welcome addition to clinical practice. Also needed are direct comparisons of antieosinophil therapies in patients suffering from various eosinophil-related disorders, with the goal of optimizing care for each condition. Finally, continued monitoring for the emergence of any safety signals associated with long-term reductions of eosinophils remains important and may advance our understanding of the unique contribution of the eosinophil to human health.

DISCLOSURE STATEMENT

S.J.A. is a cofounder, chief scientific officer, board member, and consultant of and holds equity in EnteroTrack, LLC, and is entitled to a share of royalties from the University of Illinois at Chicago/University of Colorado in conjunction with licensing of intellectual property to EnteroTrack, LLC. B.S.B. is a cofounder, scientific advisory board member, and stockholder of Allakos, Inc., and is entitled to a share of royalties from Johns Hopkins University in conjunction with the licensing of intellectual property to Allakos, Inc. He also receives royalties for his role as an editor for UpToDate™ and Elsevier. A.D.K. is not aware of any memberships, affiliations, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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