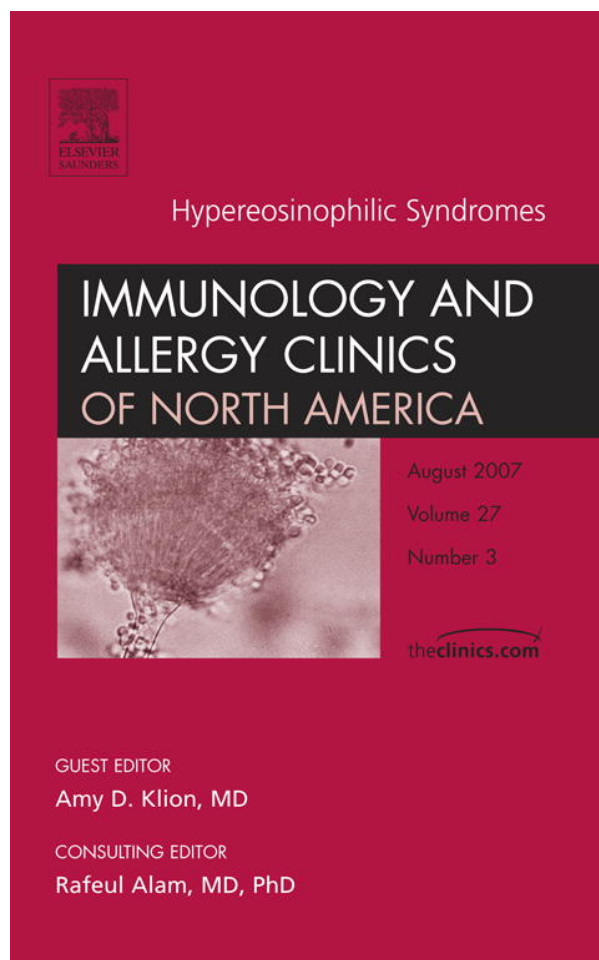




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Mechanisms of Eosinophilia in the Pathogenesis of Hypereosinophilic Disorders

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The paradigm that eosinophils play a significant proinflammatory and tissue-damaging role in the pathogenesis of many eosinophil-associated diseases and hypereosinophilic syndromes continues to be supported by an ever-increasing number of definitive animal model and clinical studies. This support includes recent evidence that eosinophils play a pivotal role in the development of tissue remodeling and fibrosis, in part through their potent elaboration of remodeling and fibrogenic growth factors [1–3]. Studies using two different strains of eosinophil-deficient mice strongly support the concept that the eosinophil contributes to the pathophysiology of allergic diseases such as asthma [4,5]. As well, recent clinical trials using a humanized anti-interleukin (IL)-5 antibody (Mepolizumab) to ablate eosinophils in the bone marrow, blood, and tissues of patients show great promise for reversing eosinophilia and aspects of eosinophil-mediated tissue damage, remodeling, and fibrosis in diseases such as asthma [6,7], eosinophilic esophagitis [8], and the hypereosinophilic syndrome (HES) [9]. This article addresses the current understanding of the mechanisms that regulate eosinophil lineage commitment and differentiation in the bone marrow,

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the development of blood and tissue eosinophilia, and their relationships to the pathogenesis and pathophysiology of hypereosinophilic disorders.

Cytokine regulation of eosinophilopoiesis in the bone marrow

Eosinophils differentiate in the bone marrow from stem cell-derived, CD34⁺, multipotential myeloid progenitors in response to a number of T-cell–derived eosinophilopoietic cytokines and growth factors including IL-3, granulocyte macrophage colony-stimulating factor (GM-CSF), and IL-5. These cytokines affect the eosinophil lineage at three different levels: (1) commitment, proliferation, and differentiation of the hematopoietic progenitors; (2) priming, activation, and survival in the blood and tissues for enhanced functional activities; and (3) recruitment and tissue localization, as discussed later. Activated T cells probably are the primary source for IL-3, IL-5, and GM-CSF pertinent to eosinophil differentiation in the bone marrow and the development of reactive blood and tissue eosinophilia in allergic and parasitic diseases and in some hypereosinophilic syndromes. Other cell types, including mast cells, macrophages, natural killer cells, endothelial cells, epithelial cells, and stromal cells such as fibroblasts, are also producers of these factors (eg, GM-CSF). IL-5, produced primarily by activated T-helper type 2 (Th2) cells [10] and mast cells [11,12], regulates the production of eosinophils both in vitro from purified hematopoietic progenitors and in vivo [13–15]. Both IL-3 and GM-CSF are pluripotent cytokines with activities on other hematopoietic lineages, whereas IL-5 is selective for the eosinophil lineage and plays a crucial role in driving committed eosinophil progenitor-cell proliferation, terminal differentiation, and postmitotic activation [16]. As a late-acting lineage-specific cytokine, IL-5 demonstrates maximum activity on an IL-5-receptor–positive (IL-5R⁺) eosinophil progenitor pool that first is expanded by earlier-acting multipotential cytokines including IL-3 and GM-CSF [16]. IL-5, however, is both necessary and sufficient for eosinophil development to proceed [16,17]. The expression of the high-affinity IL-5R is a prerequisite and very early lineage-specific event in the hematopoietic program for eosinophil development. Overexpression of IL-5 is observed in many eosinophil-associated diseases [18–20], and IL-5–transgenic mice develop profound eosinophilia [21,22], indicating that IL-5 plays important roles in promoting the production and function of eosinophils in vivo. These early observations have been confirmed and expanded in studies of IL-5–deficient (gene knockout) mice [23,24], which nevertheless produce basal levels of normal eosinophils in the bone marrow but, importantly, fail to develop blood and/or tissue eosinophilia or lung damage, airways hyperreactivity, and airways remodeling in murine allergic asthma models [24,25] and do not develop blood or tissue eosinophilic responses to helminth parasites [23]. The finding that IL-5–deficient (knock-out) mice still generate basal levels of eosinophils in the bone marrow is consistent with the current paradigm that basal hematopoiesis occurs

independently of the expression of lineage-specific cytokines such as erythropoietin, granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), and IL-5 (the “inductive/instructive” model) and instead is regulated at the level of gene transcription through the combinatorial activities of hematopoietic-specific transcription factors that act to resolve lineage-promiscuous gene expression patterns in early uncommitted hematopoietic progenitors [26–28].

Transcriptional regulation of eosinophil lineage commitment and differentiation

During the past 15 years studies of the mechanisms that regulate myeloid gene transcription, hematopoietic lineage specification, and differentiation have provided novel insights into the roles of combinatorial networks of transcription factors in determining progenitor cell fate, including eosinophil lineage commitment and terminal differentiation. Current findings from avian, mouse, and human studies suggest that a handful of transcription factors and their functional interactions are critical in influencing eosinophil lineage specification, development and terminal differentiation [29]. The combinatorial activities of GATA-1, C/EBP α (CCAAT enhancer-binding protein α) and the ets factor PU.1 are required for eosinophil development to proceed, with GATA-1 being the pivotal factor that determines whether granulocyte-macrophage progenitors will differentiate into eosinophils (in the presence of GATA-1) or neutrophils or macrophages (in the absence of GATA-1) (Fig. 1). In contrast, friend of GATA-1 (FOG-1), a coactivator of GATA-1 required for erythroid differentiation, functions as a corepressor that antagonizes GATA-1 activity in eosinophil progenitors [30] and therefore must be down regulated for eosinophil development to proceed in the bone marrow [31]. Convincing data from mouse knockout studies have shown that eosinophils do not develop in GATA-1–null mice [32] and that transgenic deletion of a high-affinity palindromic double-GATA site in the hypersensitivity site 2 (HS2) region of the mouse GATA-1 promoter itself results in a lineage-specific block in eosinophil terminal differentiation [33], suggesting that double-GATA sites may regulate eosinophil lineage-specific expression of GATA-1 itself. Consonant with this finding is the presence of similar high-affinity double-GATA–binding sites in the promoters of a number of hallmark eosinophil genes defining this lineage, including those encoding a number of the secondary granule proteins such as major basic protein-1 (MBP1), the IL-5–binding IL-5R α subunit [34], and the eotaxin type 3 CC chemokine receptor (CCR3) [35]. The antagonistic activities of GATA-1 and PU.1 have been defined functionally and mechanistically in erythroid versus myeloid differentiation [36], where they may serve to resolve progenitor-cell lineage promiscuity as part of cell fate specification during hematopoietic development [26–28]. In contrast, however, PU.1 and GATA-1 synergize in the eosinophil

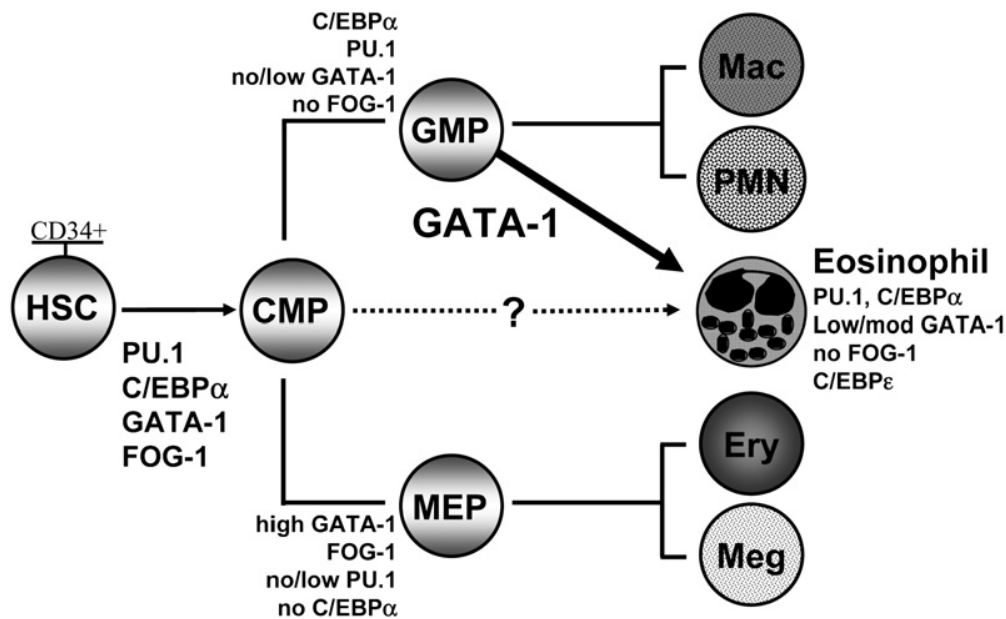


Fig. 1. Combinatorial transcription factor “codes” that specify eosinophil lineage commitment and terminal differentiation. GATA-1 is both necessary and sufficient to drive eosinophil development, and C/EBP ϵ is required for eosinophil terminal differentiation. It remains unclear whether human eosinophils also can develop directly from common myeloid progenitors (CMP). C/EBP α , CCAAT enhancer-binding protein α ; Ery, erythrocyte; FOG-1, friend of GATA-1; GMP, granulocyte-macrophage progenitors; HSC, CD34 $^{+}$ hematopoietic stem cells; Mac, macrophage; Meg, megakaryocyte; MEP, megakaryocyte/erythroid progenitors; PMN, polymorphonuclear leukocyte; PU.1, ets factor (identical to Spi-1 oncogene). (Adapted from McNagny K, Graf T. Making eosinophils through subtle shifts in transcription factor expression. J Exp Med 2002;195(11):F43–7; with permission.)

lineage for transcription of genes such as *MBP1* [34]. The mechanism for PU.1–GATA-1 synergy may involve PU.1 enhancement rather than antagonism of GATA-1 DNA binding to unique double-GATA sites present in a number of key eosinophil genes [37]. As with GATA-1–null mice, knock-out of C/EBP α results in animals that are incapable of producing any granulocytes, including eosinophils [38], and eosinophil terminal differentiation likewise is impaired significantly in PU.1 knockout mice [39,40].

The current consensus regarding the combinatorial transcription factor code that selectively specifies and regulates eosinophilopoiesis as compared with the differentiation of other myeloid lineages is highlighted in Fig. 1. Thus far, the regulation of the relative levels and timing of expression of GATA-1, FOG-1, PU.1, and C/EBP α seems necessary to generate eosinophils, such that the commitment and terminal differentiation of eosinophils from myeloid progenitors requires concomitant expression of C-EBP α , PU.1, a low-to-moderate level of GATA-1, with no expression of FOG-1 [29]. Once myeloid progenitors are committed to the eosinophil lineage, their terminal differentiation and functional maturation in the bone marrow have been shown to require the activity of another member of the C/EBP family of transcription factors, C/EBP ϵ , that is expressed at highest levels during the promyelocyte-to-myelocyte transition. Studies of C/EBP ϵ -null

(knockout) mice have shown that eosinophil (and neutrophil) terminal differentiation requires *C/EBP ϵ* , because these mice lack terminally differentiated, functionally mature granulocytes (both eosinophils and neutrophils) [41,42]. Similarly, patients who have specific granule deficiency have been shown to have a novel mutation in the *C/EBP ϵ* gene that results in loss of function of this transcription factor, the consequence of which is a failure of both neutrophil and eosinophil terminal differentiation and functional maturation, including failed expression of important secondary granule protein genes in both granulocytes [43]. Clearly, greater understanding of the combinatorial and functional interactions of the transcription factors that specify eosinophil lineage commitment and regulate gene transcription and terminal differentiation is needed. Studies in this area ultimately may lead to the identification of novel targets for ablating eosinophil development in general in the bone marrow or selectively knocking down eosinophil expression of key inflammatory mediators, such as the granule cationic proteins, or receptors, such as CCR3, as therapeutic approaches to the treatment of eosinophil-mediated allergic diseases or hypereosinophilic syndromes.

Regulation of eosinophil differentiation by cytokines and exit from the bone marrow

Based on studies with anti-IL-5 antibody, it now is clear that IL-5 is critical for terminal eosinophil differentiation [44,45]. Indeed, one of the key terminal steps in eosinophil hematopoiesis involves surface expression of the IL-5R [46]. Until this point, eosinophils and basophils share maturation pathways. Remnants of these shared differentiation pathways persist even though their divergence is clear when examining their mature circulating counterparts. For example, circulating eosinophils continue to express low levels of the α chain of the high-affinity IgE receptor (Fc ϵ RI) [47,48], whereas basophils express low levels of MBP [49], Charcot-Leyden crystal protein (galectin-10) [50], and eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO) [51]. In the peripheral blood both cell types selectively express the chemokine receptor CCR3 [52], but, at least in mice, its expression on eosinophils in the bone marrow occurs late in differentiation and in conjunction with loss of expression of FIRE (F4/80-related receptor) expressed by mouse eosinophils with an as yet unknown human counterpart [53]. Another terminal differentiation marker is Siglec-8 (and Siglec-F, the latter being its closest functional paralog in the mouse [54,55]), which is expressed more highly on peripheral blood eosinophils than in bone marrow eosinophils and at much higher levels than on basophils [53,56].

IL-5-deficient mice have markedly reduced numbers of bone marrow, tissue, and circulating eosinophils [23]; the opposite is found in IL-5-transgenic mice [21,22]. The exact signals regulating egress from the bone marrow

are not entirely understood, however. Studies in mice suggest that, besides IL-5 [57], CCR3 agonists, such as eotaxin-1 (CCL11), are important for eosinophils to leave the bone marrow [58]. Infusion of a $\beta 2$ integrin–blocking antibody prevented IL-5–mediated marrow release, whereas an antibody to $\alpha 4$ integrin enhanced release. Exactly how this effect occurs is not clear, but it is known that IL-5 and CCR3 agonists alter integrin function in a way that facilitates detachment from various counterligands, as has been observed in vitro in models of endothelial cell adhesion and detachment under flow conditions [59–61]. Separate studies in animals would suggest that eosinophil egress following allergen sensitization and challenge is partially T cell–dependent [62].

Eosinophil trafficking out of the circulation into tissues

Production of IL-5 and/or GM-CSF, as well as administration of these cytokines in humans, results in rapid and sustained peripheral blood eosinophilia [63,64]. Once in the circulation, eosinophils persist there for 18 to 24 hours before migrating to extravascular sites. This circulation time may be even longer in conditions associated with peripheral blood eosinophilia. Diseases associated with eosinophilia, such as hypereosinophilic syndromes, frequently, but not always, are associated with elevations in serum levels of IL-5 or GM-CSF [65–68]. Although the bone marrow is the largest reservoir for eosinophil precursors and their differentiation, their predominant destination beyond the circulation, in normal humans, is the gastrointestinal tract. Based on animal studies, this homing occurs because of constitutive gut epithelial expression of eotaxin-1 (CCL11) [69].

Levels of eosinophils in the circulation undergo diurnal variation, with highest levels in the evening and lowest levels in the early morning, in parallel with diurnal variations of endogenous cortisol levels [70]. Studies with systemically administered adhesion molecule antagonists show that very late antigen-4 (VLA-4, CD49d/CD29), but not leukocyte functional antigen-1 (LFA-1, CD11a/CD18), is involved in constitutive eosinophil trafficking because VLA-4 antibody administration resulted in about a doubling of circulating eosinophil and lymphocyte counts (both express VLA-4), whereas administration of antibodies to LFA-1 had no effect on circulating eosinophil counts [71,72]. The circulating eosinophil half-life under these conditions has not been measured specifically, but the presumption is that VLA-4 blockade interferes with constitutive homing of eosinophils into the gastrointestinal tract and perhaps other sites.

It generally is believed that once eosinophils leave the circulation and migrate into tissue sites, they do not recirculate, although data in animal models suggest that eosinophils exogenously transferred to the lungs may traffic to regional lymph nodes and play a role in antigen presentation [73]. Once in tissues, eosinophil survival depends on local production of cytokines that prevent eosinophil apoptosis. Besides IL-5, other important

locally produced survival factors include GM-CSF and, perhaps to a lesser degree, IL-3, tumor necrosis factor- α (TNF α), interferon- γ , leptin [74], CD40 engagement [75], and others (Fig. 2) [76]. Studies show that, in the right local cytokine milieu, eosinophils and their precursors are capable of differentiating and surviving in tissues for several days [77–79]. In addition, eosinophils that are obtained by bronchoalveolar lavage from asthmatics or following segmental lung allergen challenge of allergic subjects display prolonged survival for several days in culture, even in the absence of added cytokines, whereas peripheral blood counterparts do not survive even 24 hours [80,81]. This finding is consistent with the eosinophils having been exposed to survival-promoting cytokines in the inflamed lung or having been induced to generate endogenous survival factors such as GM-CSF [82].

Based primarily on allergic inflammation models [83], selective recruitment of eosinophils to specific tissue sites is regulated effectively by unique patterns of cytokines that activate endothelial cells and induce tissue-resident cells to produce eosinophil-active chemokines and other chemoattractants to facilitate their preferential migration. In the former category, cytokines such as IL-4 and IL-13 selectively induce endothelial expression of vascular cell adhesion molecule 1 (VCAM-1, CD106), therefore providing ligands for α 4 integrin-mediated recruitment in a pathway that distinguishes eosinophil responses from neutrophil responses, because neutrophils lack α 4 integrins [52,84–86]. Indeed, inhalation of the VLA-4 antagonist IVL745 had modest effects on late-phase sputum eosinophilia in humans but failed to alter the acute or late-phase physiologic response [87]. CCR3-active chemokines, including the eotaxins (CCL11, CCL24, CCL26), RANTES (regulated upon activation, normal T cell expressed and secreted, CCL5), MCP-4 (monocyte chemotactic protein-4, CCL13), and others, derived predominantly from tissue-resident cells such as keratinocytes in the skin and airway epithelium, further facilitate eosinophil adhesion and transendothelial migration [76]. Taking a different approach, it was demonstrated convincingly that lung endothelial G α_{i2} function, apart from its potential role

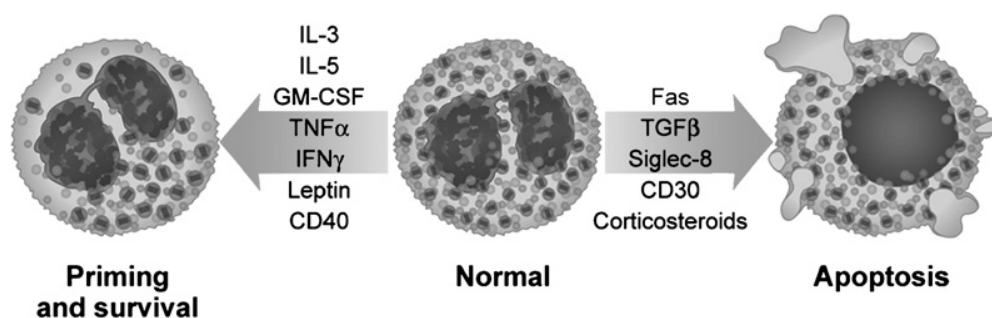


Fig. 2. How eosinophils are influenced to make life-or-death decisions. Stimuli that are known to promote eosinophil priming and survival are displayed and are contrasted with those that facilitate eosinophil apoptosis. Also shown are some of the phenotypic characteristics that accompany the primed state compared with those seen in cells undergoing apoptosis. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN γ , interferon- γ ; TGF β , transforming growth factor- β ; TNF α , tumor necrosis factor- α . (Art by Jacqueline Schaffer.)

involving leukocytes, is required for eosinophil recruitment [88]. Given the eosinophil expression of both VLA-4 and VLA-6 (CD49f/CD29), interactions with specific tissue matrix proteins, especially fibronectin and laminin, also may be important, respectively, in localization of these cells and promoting their autocrine GM-CSF-driven prolonged survival within tissues [89,90]. Both TNF α and IL-1 interact synergistically with IL-4 and IL-13 to augment VCAM-1 expression as well as intercellular adhesion molecule-1 (ICAM-1, CD54) expression [91]. This interaction is consistent with the important role of β 2 integrins in mediating eosinophil recruitment responses [85,92]. Alpha-4 integrins also can contribute to rolling adhesion, but a more important component of eosinophil rolling adhesion probably is mediated through surface PSGL-1 (P-selectin glycoprotein-1, CD162) on the eosinophil interacting with endothelial P-selectin (CD62P) [93–95]. Unlike their important role in neutrophil recruitment, E-selectin (CD62E) and L-selectin play little or no role in eosinophil recruitment responses [84,96,97]. Nevertheless, a pan-selectin antagonist is in clinical trials and, despite a short half-life, was capable of inhibiting allergen-induced late-phase responses in the airways [98,99]. Whether late-phase lung eosinophilia was affected was not determined. Additional chemoattractants implicated in selective eosinophil migration include complement fragments such as C5a, platelet-activating factor, and sulfidopeptide leukotrienes [100]. Priming, a term used to describe the enhanced responsiveness of a cell to normally active stimuli, occurs *in vitro* following exposure to cytokines such as GM-CSF and IL-5. In patients who have eosinophilia, eosinophils have a similar functionally primed phenotype, become “hypodense” on density gradients [101,102], and develop a characteristic microscopic appearance with reduced or condensed granules (see Fig. 2) [103], along with enhanced surface expression of activation markers such as CD44 and CD69 [104]. These cells also demonstrate exaggerated adhesion and migration responses to virtually all the stimuli mentioned previously; these exaggerated responses can be a critical aspect of the enhanced eosinophil trafficking seen in hypereosinophilic conditions [105].

For each of these recruitment pathways, there is some degree of tissue specificity regarding homing patterns. For example, constitutive expression of eotaxin as well as β 7 integrins seems to be critical, if not absolutely necessary, for gut homing [106], whereas eotaxin-3 is markedly overexpressed in the esophagus in patients who have eosinophilic esophagitis [107]. Based on mouse studies, IL-5, IL-13, CD44 (a receptor for hyaluronic acid), and CCR3-active chemokines are implicated in lung homing of eosinophils [108,109], and the injection of, or the appearance following allergen challenge of, eotaxin-1 (CCL11), eotaxin-2 (CCL24), or RANTES (CCL5) is associated with eosinophil accumulation in the skin [110,111]. Blockade of LFA-1 had only modest effects on eosinophil accumulation in the human lung allergen-challenge model [72], whereas an antibody to eotaxin seems to reduce nasal eosinophilia induced by allergen challenge [112,113]. Also of note is that cysteinyl leukotriene receptor antagonists reduce the number

of eosinophils in the airway and blood [114], and mice missing the leukotriene B₄ receptor have a profound decrease in lung eosinophils following allergen sensitization and challenge [115]. Finally, eosinophils, along with basophils, mast cells, and Th2 cells, express chemoattractant receptor-homologous molecule expressed on T helper 2 cells (CRTh2), a high-affinity receptor for prostaglandin D₂ [116,117], whereas both Th2 cells and eosinophils express the H₄ histamine receptor [118]. The relative contribution of these pathways compared with others remains to be delineated when specific antagonists become available.

Activation of eosinophil degranulation and mediator release

For eosinophils to participate in local tissue pathobiology, more must occur than their accumulation; indeed, activation of recruited eosinophils is thought to be a critical aspect of disease pathophysiology. For example, IL-5–transgenic mice have massively increased numbers of eosinophils in the circulation, spleen, and other tissues, but without a second signal these mice are relatively healthy. One of the major pathways by which eosinophils are activated is through cross-linking of surface immunoglobulin receptors, especially those involving IgA and, to a lesser degree, IgG [100]. Although there is general agreement that mouse eosinophils do not express FcεRI, this topic remains controversial for human eosinophils. The preponderance of recent literature suggests that human eosinophils express very low levels, if any, of FcεRI on their surface and that, if present, FcεRI clearly lacks the β chain; therefore, significant, direct activation of eosinophils by means of IgE remains unlikely [119–121]. Stimulation of eosinophils leads to eosinophil granule protein release (eg, ECP, EDN, EPO, MBP), superoxide generation, and synthesis of leukotriene C₄ [100,122], although the last is difficult to induce with traditional eosinophil-activating stimuli. Eosinophils also produce platelet-activating factor and a wide range of cytokines and chemokines, not the least of which are IL-1β and transforming growth factor-β (TGF-β), two key players in the eosinophil-mediated tissue remodeling and fibrosis seen in many eosinophil-associated diseases [3,123–125]. Although the quantities of cytokines and chemokines released per eosinophil versus other cells vary widely, GM-CSF is among the cytokines produced in greatest quantities by eosinophils [100]; as noted previously, it functions in part in an autocrine fashion to prolong eosinophil survival once eosinophils are recruited into tissue inflammatory sites. Eosinophil activation *in vitro* by a number of agonists, including IL-5, interferon-γ, secretory IgA, and others, has been shown to induce secretion of the granule cationic proteins (EPO, MBP1, EDN, ECP) and eosinophil-expressed cytokines (eg, RANTES and IL-4) by a process termed “piecemeal degranulation” [126,127] that involves differential mobilization and vesicular transport of these proteins [114,128,129]. This process is in contrast to secondary granule fusion and classical exocytosis, events rarely observed for eosinophils in

inflammatory foci in tissues [130]. Once secreted, the eosinophil granule cationic proteins have multiple potential proinflammatory activities that have been defined in vitro and in vivo, including membrane-, cell-, and tissue-damaging cytotoxicity [131,132], the ability to activate inflammatory cells (eg, mast cells and basophils) selectively, to release inflammatory mediators (eg, histamine) [133], potent blocking activity for inhibitory M2 muscarinic receptors in the airways in asthma models [134], and the ability to augment TGF- β -primed fibroblast elaboration of the inflammatory and profibrotic IL-6 family of cytokines including IL-6 and IL-11 [135], to name just a few. Thus, eosinophils (1) come fully armed with preformed mediators of inflammation, tissue damage, remodeling, and fibrogenesis that are secreted at sites of eosinophilic inflammation in tissues in eosinophil-associated diseases such as the hypereosinophilic syndromes and (2) have the capacity to generate newly formed protein (cytokines, chemokines) and lipid mediators (leukotriene C₄, platelet-activating factor) of inflammation when primed and further activated during their recruitment from the bone marrow into the tissue in response to allergic and other stimuli [136].

Regulation of tissue eosinophil survival and activation

Once in tissues, if eosinophils do not encounter the appropriate survival milieu, the lack of exposure to such cytokines normally leads to their prompt apoptosis (see Fig. 2). Separate from this process, however, a number of pathways actively and, to varying degrees, selectively induce eosinophil apoptosis. In humans, corticosteroids markedly and rapidly diminish numbers of circulating and tissue eosinophils. The mechanisms responsible for this action are complex and probably involve a combination of altered release from bone marrow, shortened circulation time, redistribution from the circulation into spleen and other organs, induction of apoptosis, and inhibition of cytokines and chemokines needed for eosinophil survival and recruitment [137–141]. Besides steroids, other proapoptotic molecules for eosinophils include lidocaine [142], TGF- β [143], Siglec-8/Siglec-F [144,145], Fas (CD95) [146], and CD30 [147]. Although prior exposure to survival cytokines or priming conditions tends to improve eosinophil resistance to these death pathways, a unique situation is the Siglec-8 pathway, which actually is augmented by priming cytokines [144,148]. Another drug used to reduce eosinophil numbers is hydroxyurea, although this drug is used to cause a more global inhibitory effect on hematopoiesis. Interferon- α and leukotriene synthesis or receptor blockers also reduce circulating eosinophil numbers [149]. Recently, tyrosine kinase inhibitors such as imatinib mesylate (Gleevec) have been shown to have profound effects on eosinophil numbers because a subset of individuals who have hypereosinophilic syndrome have a deletion mutation on chromosome 4 resulting in the fusion of a gene with unknown function, namely *FIP1L1*, with the *PDGFR α* (platelet-derived growth factor receptor- α) gene, resulting in a constitutively

active tyrosine kinase [150]. Thus, patients found to be *FIP1L1-PDGFR α* positive, either by fluorescence in situ hybridization or by reverse transcription polymerase chain reaction, now are treated with imatinib mesylate. Other constitutively active tyrosine kinases, including type-2 fibroblast growth factor receptor-1 and PDGFR β , have been implicated in eosinophilic syndromes [76].

Summary

The heterogeneity of hypereosinophilic syndromes, which ranges from patients who have features of myeloproliferative disorders with cytogenetic abnormalities (eg, *FIP1L1-PDGFR α* -positive chronic eosinophil leukemia, CEL) to patients who have more benign clinical courses (eg, episodic angioedema with eosinophilia), suggests that multiple disease processes are at play that regulate eosinophilopoiesis in the bone marrow, the recruitment of eosinophils to tissues and their survival, the activation and secretion of inflammatory mediators, and pathophysiologic outcomes. Current research aimed at defining the causes of HES and mechanisms that regulate eosinophilia in these diseases and at understanding the development of eosinophil-mediated end-organ damage in eosinophil-associated diseases in general should lead to more selective and improved therapies for hypereosinophilic syndromes. The therapeutic targets for these efforts currently include (1) IL-5 and its high-affinity receptor; (2) underlying T-cell clones (either immunocompetent or occult T-lymphoid malignancies) that elaborate eosinophilopoietins such as IL-5 or GM-CSF [65,68] and tissue- or organ-specific dysfunctional elaboration of eosinophil-active chemoattractant factors such as eosinophil-selective chemokines (eg, eotaxin-3 in the esophagus in eosinophilic esophagitis) [107]; (3) vascular endothelial adhesion molecules (VCAM/VLA-4); and (4) inhibitory receptors such as Siglec-8 [144] and CD300a [151]. The essential absence of end-organ damage in some of the hypereosinophilic syndromes contrasts starkly with the morbidity (and mortality) associated with the development of endomyocardial fibrosis in HES. Because patients who have HES are clearly a heterogeneous group, clinical management based on current knowledge must be tailored specifically to the individual, with the overall goal of controlling the blood and tissue eosinophilia and, in particular, the eosinophil-mediated end-organ damage [152]. Current treatment options permit the control or eradication of eosinophilia and end-organ damage in most patients who have HES [149,152]. The efficacy of imatinib mesylate (Gleevec) in some patients who have HES led to the identification of the *FIP1L1-PDGFR α* gene fusion that encodes a pathogenetically relevant and constitutively active tyrosine kinase [150]. This seminal finding has led to a reclassification of hypereosinophilias into better-defined clinical entities [149,152] and has stimulated new research that ultimately may translate into improved clinical characterization and therapeutic options.

Finally, humanized anti-IL-5 antibody (Mepolizumab) recently has shown clinical efficacy for controlling eosinophilia in HES in clinical trials [9,44,153] and looks highly promising for the treatment of a wide range of patients who have *FIP1L1-PDGFR α* -negative HES and possibly other eosinophilias [149] (eg, eosinophilic gastrointestinal syndromes such as eosinophilic esophagitis) [8]. Future research on HES and CEL should focus on the molecular basis of imatinib responsiveness in both *FIP1L1-PDGFR α* -positive and -negative patients, addressing how the constitutively activated *FIP1L1-PDGFR α* or other fusion or mutant-activated kinases selectively lead to chronic hypereosinophilia and end-organ damage. Studies of the effects of imatinib on the proliferation and terminal differentiation of bone marrow-derived eosinophil progenitors and the survival and intracellular signaling pathways in eosinophils from imatinib-responsive patients may be particularly revealing in terms of the downstream targets of these novel kinases and the roles of eosinophil-active eosinophilopoietins and survival factors such as IL-5 and GM-CSF [154].

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