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Mechanisms of eosinophilia in the pathogenesis of hypereosinophilic disorders

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Synopsis

Increased numbers of activated eosinophils in the blood and tissues that typically accompany hypereosinophilic disorders result from a variety of mechanisms. At one end of the process is augmented bone marrow production and egress, while at the other end of the process is enhanced selective tissue recruitment and survival. Accumulation alone is insufficient to lead to eosinophil-associated pathology, hence the importance of additional means for activation and release of eosinophil-derived mediators. Common themes in these events include participation of subsets of transcription factors, cytokines, chemokines, adhesion molecules and survival regulatory pathways. Besides an enhanced understanding of the contributions of these and other molecules to hypereosinophilic disorders achieved from mouse models and molecular strategies, exciting advancements translating these discoveries to the clinic have led to a flurry of new therapeutics specifically designed to target eosinophil-associated diseases. So far, this form of hypothesis testing in humans in vivo via pharmacology has generally supported the paradigms generated in vitro and in animal models, raising hopes and expectations that a spectrum of novel therapies may soon become available to help those with eosinophil-associated diseases.

Keywords

eosinophil; hematopoiesis; survival; apoptosis; adhesion; migration

Introduction

The paradigm that eosinophils play a significant pro-inflammatory 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 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

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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-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 allergic diseases such as asthma [6, 7] and eosinophilic esophagitis (EE) [8], and the hypereosinophilic syndrome (HES) [9]. This review addresses our current understanding of the mechanisms that regulate eosinophil lineage commitment and differentiation in the bone marrow, 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, GM-CSF and IL-5. These cytokines affect the eosinophil lineage at three different levels including: (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 (see below). Activated T cells are likely 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 some hypereosinophilic syndromes, but 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, e.g. GM-CSF. IL-5, produced primarily by activated Th2 cells [10] and mast cells [11, 12], regulates the production of eosinophils both *in vitro* from purified hematopoieitic 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 post-mitotic activation [16]. As a late-acting lineage-specific cytokine, IL-5 demonstrates maximum activity on an IL-5R+ eosinophil progenitor pool that is first expanded by earlier-acting multipotential cytokines including IL-3 and GM-CSF [16]. However, IL-5 is both necessary and sufficient for eosinophil development to proceed [16, 17]. The expression of the high-affinity receptor for IL-5 (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 (knockout) 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 (EPO), G-CSF, M-CSF, and IL-5 (the “inductive/instructive” model), and is instead 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] (see below).

Transcriptional regulation of eosinophil lineage commitment and differentiation

Studies over the past 15 years 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/EBPα (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 (requires GATA-1) or neutrophils or macrophages (absence of GATA-1) (Figure 1). In contrast, FOG-1 (friend of GATA-1), a co-activator of GATA-1 required for erythroid differentiation, functions as a co-repressor that antagonizes GATA-1 activity in eosinophil progenitors [30], and must therefore 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 HS-2 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 MBP1 and the IL-5-binding IL-5Rα subunit [34] and the eotaxin receptor CCR3 [35]. The antagonistic activities of GATA-1 and PU.1 have been functionally and mechanistically defined 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 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 (Du J and Ackerman SJ, manuscript submitted). As with GATA-1 null mice, knockout of C/EBPα results in animals that are incapable of producing any granulocytes including eosinophils [37], and eosinophil terminal differentiation is likewise significantly impaired in PU.1 knockout mice [38](Du J and Ackerman SJ, manuscript in preparation).

The current consensus regarding the combinatorial transcription factor code that selectively specifies and regulates eosinophilopoiesis as compared to the differentiation of other myeloid lineages is highlighted in Figure 1. Thus far, it appears that regulation of the relative levels and timing of expression of GATA-1, FOG-1, PU.1 and C/EBPα are necessary to generate eosinophils, such that the commitment and terminal differentiation of eosinophils from myeloid progenitors requires concomitant expression of C-EBPα, 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 has been shown to require the activity of another member of the C/EBP family of transcription factors, C/EBPε, that is expressed at highest levels during the promyelocyte to myelocyte transition. Studies of C/EBPε null (knockout) mice have shown that eosinophil (and neutrophil) terminal differentiation requires C/EBPε, as these mice lack terminally differentiated functionally mature granulocytes (both eosinophils and neutrophils) [39, 40]. Similarly, patients with specific granule deficiency (SGD) have been shown to have a novel mutation in the C/EBPε 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 [41]. 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 may ultimately 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 is now clear that IL-5 is critical for terminal eosinophil differentiation [42, 43]. Indeed, one of the key terminal steps in eosinophil hematopoiesis involves surface expression of the IL-5 receptor (CD125/CD131) [44]. Until this point, both 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 (FcεRI) [45, 46] while basophils express low levels of major basic protein (MBP) [47], Charcot-Leyden crystal (CLC) protein (galectin-10) [48], and eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO) [49]. Both cell types in the peripheral blood selectively express the chemokine receptor CCR3 [50], 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, an F4/80-related receptor expressed by mouse eosinophils with an as yet unknown human counterpart [51]. Another terminal differentiation marker is Siglec-8 (and Siglec-F, the latter being its closest functional paralog in the mouse [52, 53]), which is expressed more highly on peripheral blood eosinophils than in bone marrow eosinophils, and at much higher levels than on basophils [51, 54].

IL-5 deficient mice have markedly reduced numbers of bone marrow, tissue and circulating eosinophils [23], while the opposite is found in IL-5 transgenic mice [21, 22]. However, the exact signals regulating egress from the bone marrow are not entirely understood. Studies in mice suggest that besides IL-5 [55], CCR3 agonists, such as eotaxin-1 (CCL11), are important for eosinophils to leave the bone marrow [56]. Infusion of a β2 integrin-blocking antibody prevented IL-5-mediated marrow release, while an antibody to α4 integrin enhanced release. Exactly how this 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 counter-ligands as has been observed *in vitro* in models of endothelial cell adhesion and detachment under flow conditions [57-59]. Separate studies in animals would suggest that eosinophil egress following allergen sensitization and challenge is partially T cell-dependent [60].

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 [61, 62]. Once in the circulation, eosinophils persist there for 18-24 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, are frequently, but not always, associated with elevations in serum levels of IL-5 or GM-CSF [63-66]. Whereas 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 eotaxin-1 (CCL11) [67].

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 [68]. Studies with systemically administered adhesion molecule antagonists show that VLA-4 (CD49d/CD29), but not 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 [69, 70]. The circulating eosinophil half-life under these conditions has not been specifically measured, but the presumption is that VLA-4 blockade interferes with constitutive homing of eosinophils into the gastrointestinal tract and perhaps other sites.

It is generally believed that once eosinophils leave the circulation and migrate into tissue sites, they do not recirculate, although data in animal models would suggest that eosinophils exogenously transferred to the lungs may traffic to regional lymph nodes and play a role in antigen presentation [71]. Once in tissues, survival is dependent 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, TNF-α, IFN-γ, leptin [72], CD40 engagement [73] and others [74] (Figure 2). Studies show that in the right local cytokine milieu, eosinophils and their precursors are capable of differentiating and surviving in tissues for several days [75-77]. 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 even survive 24 hours [78, 79]. This is consistent with their having been exposed to survival-promoting cytokines in the inflamed lung, or having been induced to generate endogenous survival factors such as GM-CSF [80].

Based primarily on allergic inflammation models [81], selective recruitment of eosinophils to specific tissue sites is effectively regulated 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 category of the former, cytokines such as IL-4 and IL-13 selectively induce endothelial expression of VCAM-1 (CD106), therefore providing ligands for α4 integrin-mediated recruitment in a pathway that distinguishes eosinophil responses from neutrophil responses, since neutrophils lack α4 integrins [50, 82-84]. 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 [85]. CCR3-active chemokines including the eotaxins (CCL11, CCL24, CCL26), RANTES (CCL5), MCP-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 [74]. Taking a different approach, it was convincingly demonstrated that lung endothelial $G\alpha_{i2}$ function, separate from its potential role on leukocytes, is required for eosinophil recruitment [86]. Given the eosinophil expression of both VLA-4 and VLA-6 (CD49f/CD29), interactions with specific tissue matrix proteins, especially fibronectin and laminin respectively, may also be important in localization of these cells and promoting their autocrine GM-CSF-driven prolonged survival within tissues [87, 88]. Both TNFα and IL-1 synergistically interact with IL-4 and IL-13 to augment VCAM-1 expression as well as ICAM-1 (CD54) expression [89]. This is consistent with the important role of β2 integrins in mediating eosinophil recruitment responses [83, 90]. Alpha-4 integrins can also contribute to rolling adhesion, but a more important component of eosinophil rolling adhesion is likely mediated through surface PSGL-1 (CD162) on the eosinophil interacting with endothelial P-selectin (CD62P) [91-93]. Unlike its important role in neutrophil recruitment, E-selectin

(CD62E) and L-selectin play little or no role in eosinophil recruitment responses [82, 94, 95]. 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 [96, 97]. 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, as well as sulfidopeptide leukotrienes [98]. Priming, a term used to describe the enhanced responsiveness of a cell to normally active stimuli, occurs *in vitro* following exposure to cytokines like GM-CSF and IL-5. In patients with eosinophilia, their eosinophils have a similar functionally primed phenotype, become "hypodense" on density gradients [99, 100], and develop a characteristic microscopic appearance with reduced or condensed granules (Figure 2) [101], along with enhanced surface expression of activation markers such as CD44 and CD69 [102]. These cells also demonstrate exaggerated adhesion and migration responses to virtually all of the stimuli mentioned above which can be a critical aspect of enhanced eosinophil trafficking seen in hypereosinophilic conditions [103].

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 β7 integrins appear to be critical, if not absolutely necessary, for gut homing [104], while eotaxin-3 is markedly overexpressed in the esophagus in patients with eosinophilic esophagitis [105]. Based on mouse studies, IL-5, IL-13, CD44 (a receptor for hylauronic acid) and CCR3-active chemokines are implicated in lung homing of eosinophils [106, 107], and injections of, or appearance following allergen challenge of, eotaxin-1 (CCL11), eotaxin-2 (CCL24), or RANTES (CCL5) is associated with eosinophil accumulation in the skin [108, 109]. Blockade of LFA-1 had only modest effects on eosinophil accumulation in the human lung allergen challenge model [70], while an antibody to eotaxin appears to reduce nasal eosinophilia induced by allergen challenge [110, 111]. Also of note is that cysteinyl leukotriene receptor antagonists reduce numbers of eosinophils in the airway and blood [112], and mice missing the LTB4 receptor have a profound decrease in lung eosinophils following allergen sensitization and challenge [113]. Finally, eosinophils, along with basophils, mast cells and Th2 cells, express CRTh2, a high affinity receptor for prostaglandin D2 [114, 115], while both Th2 cells and eosinophils express the H4 histamine receptor [116]. The relative contribution of these pathways compared to others remains to be delineated when specific antagonists become available.

Activation of eosinophil degranulation and mediator release

In order for eosinophils to participate in local tissue pathobiology, more must occur besides their accumulation; indeed, activation of recruited eosinophils is felt 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, yet, 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 [98]. Whereas there is general agreement that mouse eosinophils do not express FcεRI, this topic remains controversial for human eosinophils. The bulk of recent literature would suggest that human eosinophils express very low levels, if any, FcεRI on their surface, and if present clearly lack the β chain; so significant, direct activation of eosinophils via IgE remains unlikely [117-119]. Stimulation of eosinophils leads to eosinophil granule protein release (e.g., ECP, EDN, EPO, MBP), superoxide generation and synthesis of leukotriene C4 [98, 120], although the latter 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 include IL-1β and TGF-β, two key players in the eosinophil-mediated tissue remodeling and fibrosis seen in many eosinophil associated diseases [3, 121-123]. While 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 [98], and as noted above, it functions in part in an autocrine fashion to prolong eosinophil survival once they are recruited into tissue inflammatory sites. Eosinophil activation *in vitro* by a number of agonists including IL-5, IFN-γ, sIgA and others has been shown to induce secretion of the granule cationic proteins (EPO, MBP1, EDN, ECP) and eosinophil-expressed cytokines (e.g. RANTES and IL-4) by a process termed piecemeal degranulation [124, 125] that involves differential mobilization and vesicular transport of these proteins [112, 126, 127]. This is in contrast to secondary granule fusion and classical exocytosis, events rarely observed for eosinophils in inflammatory foci in tissues [128]. Once secreted, the eosinophil granule cationic proteins have multiple potential pro-inflammatory activities that have been defined *in vitro* and *in vivo* including membrane, cell and tissue-damaging cytotoxicity [129, 130], the ability to selectively activate inflammatory cells, such as mast cells and basophils, to release inflammatory mediators (e.g. histamine) [131], potent blocking activity for inhibitory M2 muscarinic receptors in the airways in asthma models [132], as well as 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 [133], to name just a few. Thus, eosinophils: (1) come fully armed with pre-formed 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 (LTC4, PAF) of inflammation when primed and further activated during their recruitment from the bone marrow into the tissue in response to allergic and other stimuli [134].

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 (Figure 2). Separate from this, however, are a number of pathways that actively, and to varying degrees, selectively induce eosinophil apoptosis. In humans, corticosteroids markedly and rapidly diminish numbers of circulating and tissue eosinophils, although the mechanisms responsible for this 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 [135-139]. Besides steroids, other pro-apoptotic molecules for eosinophils include lidocaine [140], TGF-β [141], Siglec-8/Siglec-F [142, 143], Fas (CD95) [144] and CD30 [145]. While prior exposure to survival cytokines or priming conditions tends to improve resistance of the eosinophil to these death pathways, a unique situation is the Siglec-8 pathway, which is actually augmented by priming cytokines [142, 146]. Other drugs used to reduce eosinophil numbers include 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 [147]. Recently, tyrosine kinase inhibitors such as imatinib mesylate (Gleevec™) have been shown to have profound effects on eosinophil numbers because a subset of individuals with hypereosinophilic syndrome have a deletion mutation on chromosome 4 resulting in the fusion of a gene with unknown function, namely FIP1L1, with the PDGFRα gene, resulting in a constitutively active tyrosine kinase [148]. Thus, patients found to be FIP1L1-PDGFRα positive, either by fluorescence *in situ* hybridization or RT-PCR, are now treated with imatinib mesylate. Other constitutively active tyrosine kinases have been implicated in eosinophilic syndromes, including Type-2 fibroblast growth factor receptor-1 and PDGFRB [74].

Conclusions

The heterogeneity of hypereosinophilic syndromes, which ranges from patients with features of myeloproliferative disorders with cytogenetic abnormalities (e.g. FIP1L1-PDGFRα-

positive chronic eosinophil leukemia, CEL), to patients with more benign clinical courses, such as 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, their survival, 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 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 Tlymphoid malignancies) that elaborate eosinophilopoietins such as IL-5 or GM-CSF [63, 66], tissue or organ-specific dysfunctional elaboration of eosinophil-active chemoattractant factors such as eosinophil-selective chemokines (e.g. eotaxin-3 in the esophagus in eosinophilic esophagitis, EE) [105], 3) vascular endothelial adhesion molecules (VCAM/VLA-4), and 4) inhibitory receptors such as Siglec-8 [142] and CD300a [149]. 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 HES patients are clearly a heterogeneous group, clinical management based on current knowledge must be specifically tailored to the individual, with the overall goal of controlling the blood and tissue eosinophilia, and in particular, the eosinophil-mediated end organ damage [150]. Current treatment options permit the control or eradication of eosinophilia and end organ damage in most HES patients [150, 151]. The efficacy of imatinib mesylate (Gleevec™) in some patients with HES led to the identification of the FIP1L1-PDGFRα gene fusion that encodes a pathogenetically relevant and constitutively active tyrosine kinase [148]. This seminal finding has lead to a reclassification of hypereosinophilias into better-defined clinical entities [150, 151], and has stimulated new research that may ultimately translate into improved clinical characterization and therapeutic options.

Finally, humanized anti-IL-5 antibody (Mepolizumab™) has recently shown clinical efficacy for controlling eosinophilia in HES in clinical trials [9, 42, 152], and looks highly promising for the treatment of a wide range of patients with FIP1L1-PDGFRA negative HES and possibly other eosinophilias [151], for example, eosinophilic gastrointestinal syndromes such as eosinophilic esophagitis, EE [8]. Future research on HES and CEL should focus on the molecular basis of imatinib responsiveness in both FIP1L1-PDGFRα-positive and -negative patients, addressing how the constitutively activated FIP1L1-PDGFRα 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 [153].

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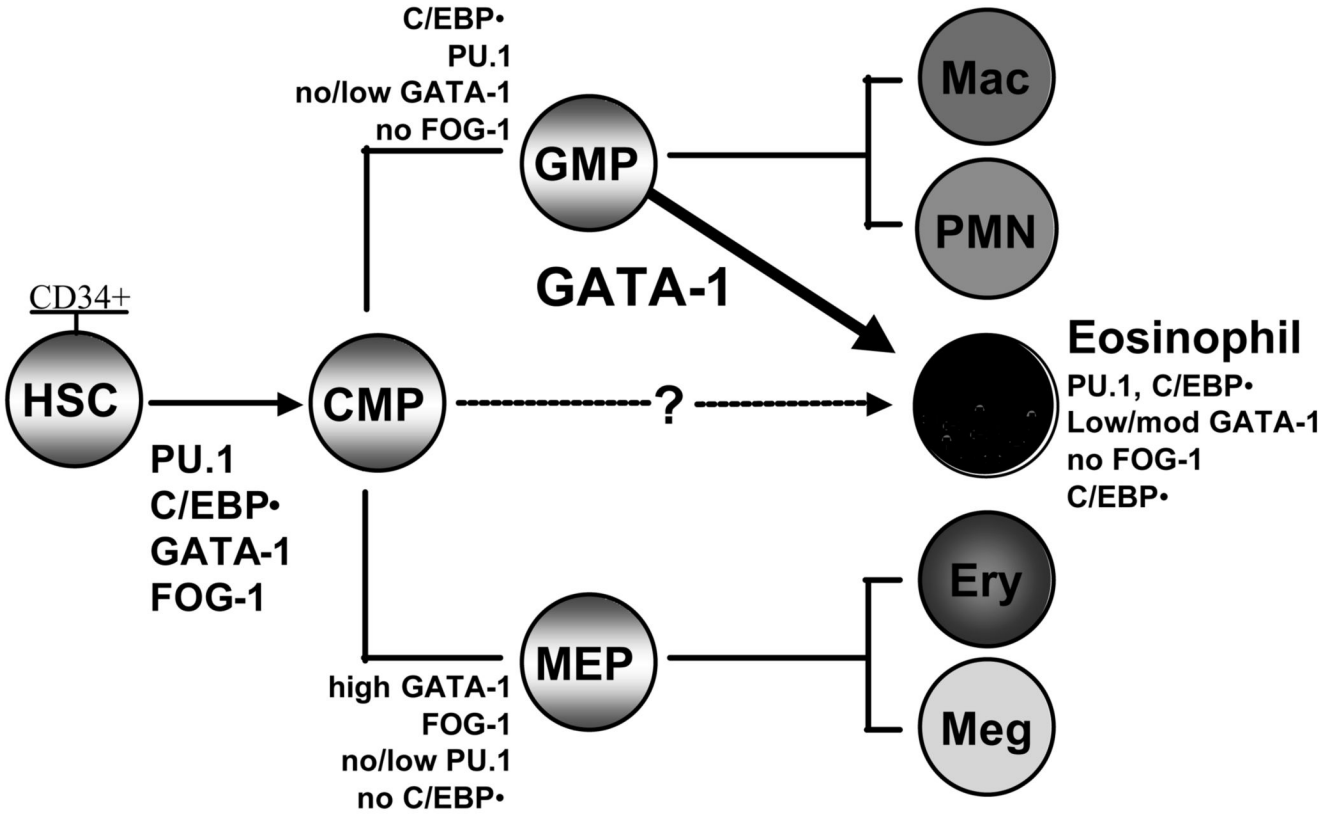

Figure 1. Combinatorial transcription factor "codes" that specify eosinophil lineage commitment and terminal differentiation

HSC, CD34+ hematopoietic stem cells; CMP, common myeloid progenitors; GMP granulocyte-macrophage progenitors; MEP, megakaryocyte/erythroid progenitors. GATA-1 is both necessary and sufficient to drive eosinophil development, and C/EBPε is required for eosinophil terminal differentiation. It remains unclear whether human eosinophils can also develop directly from CMP *(Modified and updated from reference* [29]).

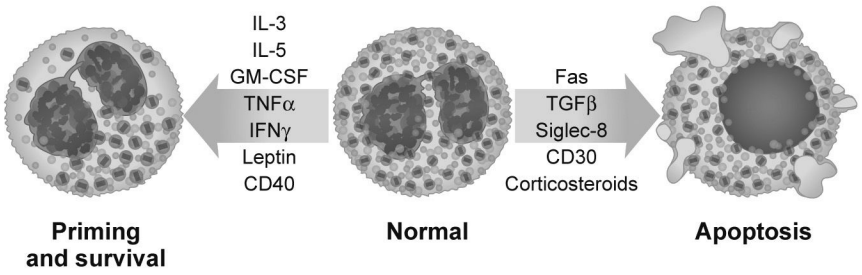

Figure 2. How eosinophils are influenced to make life or death decisions

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 to those seen in cells undergoing apoptosis. Art by Jacqueline Schaffer.