The Eosinophil

Marc E. Rothenberg and Simon P. Hogan

Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229; email: Rothenberg@cchmc.org, simon.hogan@cchmc.org

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Abstract

Eosinophils have been considered end-stage cells involved in host protection against parasites. However, numerous lines of evidence have now changed this perspective by showing that eosinophils are pleiotropic multifunctional leukocytes involved in initiation and propagation of diverse inflammatory responses, as well as modulators of innate and adaptive immunity. In this review, we summarize the biology of eosinophils, focusing on the growing properties of eosinophil-derived products, including the constituents of their granules as well as the mechanisms by which they release their pleiotropic mediators. We examine new views on the role of eosinophils in homeostatic function, including developmental biology and innate and adaptive immunity (as well as interaction with mast cells and T cells). The molecular steps involved in eosinophil development and trafficking are described, with special attention to the important role of the transcription factor GATA-1, the eosinophil-selective cytokine IL-5, and the eotaxin subfamily of chemokines. We also review the role of eosinophils in disease processes, including infections, asthma, and gastrointestinal disorders, and new data concerning genetically engineered eosinophil-deficient mice. Finally, strategies for targeted therapeutic intervention in eosinophil-mediated mucosal diseases are conceptualized.

INTRODUCTION

Eotaxin family of chemokines: the group of related eosinophil-selective chemoattractant proteins eotaxin-1, eotaxin-2, and eotaxin-3

Major basic protein (MBP): a major protein in eosinophil granules

EPO: eosinophil peroxidase

Eosinophils are multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes, including parasitic helminth infections and allergic diseases (1–3). In response to diverse stimuli, eosinophils are recruited from the circulation into inflammatory foci, where they modulate immune responses through an array of mechanisms. Triggering of eosinophils by engagement of receptors for cytokines, immunoglobulins, and complement can lead to the secretion of an array of proinflammatory cytokines [IL-2, IL-4, IL-5, IL-10, IL-12, IL-13, IL-16, IL-18, and TGF (transforming growth factor)-α/β], chemokines (RANTES and eotaxin-1), and lipid mediators [platelet-activating factor and leukotriene C4 (LTC4)] (4) (**Figure 1**). These molecules have proinflammatory effects, including upregulation of adhesion systems, modulation of cellular trafficking, and activation and regulation of vascular permeability, mucus secretion, and smooth muscle constriction. Eosinophils can initiate antigen-specific immune responses by acting as antigen-presenting cells (APCs). Furthermore, eosinophils can serve as major effector cells inducing tissue damage and dysfunction by releasing toxic granule proteins and lipid mediators (5).

In this review, we summarize the biology of eosinophils, focusing on the growing properties of eosinophil-derived products, including the constituents of their granules as well as the mechanisms by which they release their pleiotropic mediators. We examine new views on the role of eosinophils in homeostatic function, including developmental biology and innate and adaptive immunity (including interaction with mast cells and T cells). The molecular steps involved in eosinophil development and trafficking are described, with special attention to the important role of the transcription factor GATA-1 and the eosinophil-selective cytokine IL-5 and the eotaxin subfamily of chemokines. Furthermore, we review the role of eosinophils in disease processes, including infections, asthma, and gastrointestinal disorders. We also review new data concerning genetically engineered eosinophil-deficient mice. Finally, strategies for targeted therapeutic intervention in eosinophil-mediated diseases are conceptualized.

EOSINOPHIL GRANULE PROTEINS

Eosinophils secrete an array of cytotoxic granule cationic proteins [major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN)] that are capable of inducing tissue damage and dysfunction (5). Eosinophil granules contain a crystalloid core composed of MBP-1 (and MBP-2) and a matrix composed of ECP, EDN, and EPO (5). MBP, EPO, and ECP are toxic to a variety of tissues, including heart, brain, and bronchial epithelium (6–9). ECP and EDN are ribonucleases and have been shown to possess antiviral activity, and ECP causes voltage-insensitive, ion-selective toxic pores in the membranes of target cells, possibly facilitating the entry of other cytotoxic molecules (10–13). ECP also has a number of additional noncytotoxic activities, including suppression of T cell proliferative responses and immunoglobulin synthesis by B cells, induction of mast cell degranulation, and stimulation of airway mucus secretion and glycosaminoglycan production by human fibroblasts (14). MBP directly alters smooth muscle contraction responses by dysregulating vagal muscarinic M2 and M3 receptor function and by inducing mast cell and basophil degranulation (15–17). MBP has recently been implicated in regulating peripheral nerve plasticity (18). EPO, which constitutes ~25% of the total protein mass of specific granules, catalyzes the oxidation of pseudohalides [thyiocyanate (SCN^-)], halides [chloride (Cl^-), bromide (Br^-), iodide (I^-)], and nitric oxide (nitrite) to form highly reactive oxygen species

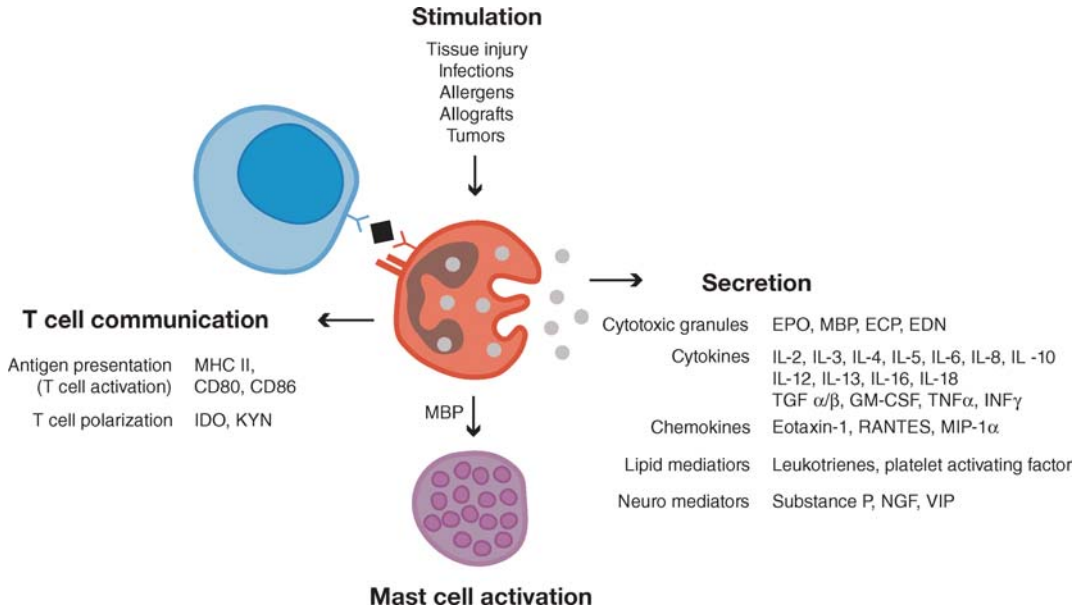


Figure 1

Schematic diagram of an eosinophil and its multifunctional effects. Eosinophils are bilobed granulocytes with eosinophilic staining secondary granules. The secondary granules contain four primary cationic proteins, designated eosinophil peroxidase (EPO), major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN). All four proteins are cytotoxic molecules; in addition, ECP and EDN are ribonucleases. Eosinophils respond to diverse stimuli, including nonspecific tissue injury, infections, allografts, allergens, and tumors. In addition to releasing their preformed cationic proteins, eosinophils can also release a variety of cytokines, chemokines, lipid mediators, and neuromodulators. Eosinophils directly communicate with T cells and mast cells in a bidirectional manner. Eosinophils activate T cells by serving as APCs, and eosinophil-derived MBP is a mast cell secretagogue. Eosinophils can also regulate T cell polarization through synthesis of indoleamine 2,3-dioxygenase (IDO), an enzyme involved in oxidative metabolism of tryptophan, catalyzing the conversion of tryptophan to kynurenines (KYN), a regulator of Th1/Th2 balance.

(hypohalous acids) and reactive nitrogen metabolites (perioxynitrate). These molecules oxidize nucleophilic targets on proteins, promoting oxidative stress and subsequent cell death by apoptosis and necrosis (19–21).

Eosinophils predominantly secrete their granule protein by regulated exocytosis and degranulation (22). In a process of piecemeal degranulation, eosinophils selectively release components of their specific granules (23). For example, activation of human eosinophils by IFN-γ promotes the mobilization of granule-derived RANTES to the cell periphery without inducing cationic protein release (24, 25). Regulated exocytosis occurs by the formation of a docking complex composed of soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptors (SNAREs) located on the vesicle (v-SNAREs) and the target membrane (t-SNAREs). SNAREs are classified into two categories based on the presence of a conserved amino acid (arginine [R] or glutamine [Q]). Human eosinophils express the Q-SNAREs SNAP-23 and syntaxin-4, which are predominantly localized to the plasma membrane (26), and the R-SNARE VAMP (vesicle-associated membrane protein)-2, which is localized to cytoplasmic secretory vesicles. It is postulated that receptor-coupled activation of eosinophils leads to rapid mobilization of cytoplasmic vesicles to the plasma membrane, leading to

SNARE: soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor

the formation of a SNARE complex (VAMP-2/SNAP-23/syntaxin-4) and subsequent mediator release (22).

EOSINOPHILS AND HOMEOSTATIC FUNCTION

Early clinical investigations have demonstrated an association between eosinophils and parasitic infections, leading investigators to hypothesize that eosinophils were classical end-stage effector cells involved in host defense (27). However, in recent years, eosinophils have been shown to be involved in numerous biological processes, including postpubertal mammary gland development (28), estrus cycling (29, 30), organ transplantation (31), viral infection (13), allergic inflammatory responses, and neoplasia (32).

Eosinophils and Reproduction

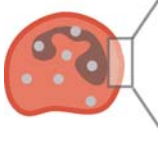
Eosinophils are a prevalent cell population in the female reproductive tract, with numbers reaching maximum levels at estrus. Eosinophils are predominantly localized to the endometrial stroma subadjacent to the luminal and glandular epithelium and at the endometrial-myometrial junction (28). Eosinophil recruitment into the uterus is regulated by IL-5; however, while uterine eosinophil numbers are depleted in IL-5-deficient mice, a residual population of eosinophils is still present, and their localization in the subepithelial stroma is comparable to wild-type mice, suggesting that IL-5-independent mechanisms regulate the tissue-specific recruitment of eosinophils into the uterus (30). Consistent with this notion, in response to ovarian steroid hormones, the expression of the eosinophil-active chemokines eotaxin-1, RANTES, and MIP-1α is upregulated, paralleling eosinophil infiltration into the uterus (29, 33, 34). Indeed, eotaxin-1-deficient mice not only have a deficiency of uterine eosinophils, but also have a two-week delay in the onset of estrus, along with a delay in the first age of parturition, suggesting a role for eosinophils in preparing the mature uterus for pregnancy (35). Furthermore, eosinophils infiltrate the endometrium following copulation (36), and investigators have postulated that this cell may have a role in blastocyst implantation and protection against infection; however, this has yet to be proven (37, 38). Interestingly, eosinophil MBP is ectopically expressed by the uterus during pregnancy, but this is not directly related to eosinophils (39).

Eosinophils have also been implicated in postnatal mammary gland development (40). Eosinophils reside in the postnatal developing mammary gland and are predominantly localized around the head of the terminal end buds. The expression level of eotaxin-1 mRNA is low between zero and four weeks of age; however, it is significantly increased in the mammary gland at five weeks of age. Notably, increased expression of eotaxin-1 at this time coincides with eosinophil infiltration into the head of the terminal end bud (40). Depletion of eosinophils from the postnatal mammary gland by deletion of the eotaxin-1 gene results in reduction in terminal end bud formation and reduced branching complexity of the ductal tree (40). It is likely that eosinophils regulate mammary gland ductal outgrowth through local secretion of eosinophil-derived TGF-β (40).

Thymic Eosinophils

Eosinophils migrate into the thymus during the neonatal period, localizing to the corticomedullary region and reaching maximum levels by two weeks of age. Interestingly, their absolute levels are approximately equivalent to that of thymic dendritic cells (41). In mice, a second influx of eosinophils is observed at 16 weeks of age, corresponding to the commencement of thymic involution. Eosinophils localize to the medullary region.

Thymic eosinophils express high levels of MHC class II molecules and moderate levels of MHC class I and the costimulatory molecules CD86 (B7.2) and CD30L (CD153) (**Figure 2**). Furthermore, thymic eosinophils



Immunoglobulin receptors and members of the immunoglobulin superfamily

CD4	CD47	CD58	CD101
CD16	CD48	CD66	HLA class 1
CD32	CD50	CD89	HLA-DR
CD33	CD54	CD100	FcεR1

Cytokine receptors

CD25	CD120	CD131	TGFβR
CD116	CD123	CD213	
CD117	CD124	IL-9R	
CD119	CD125	IL-13Rα1	

Adhesion molecules

CD11a	CD18	CD49f	CD162
CD11b	CD29	CD62L	CD174
CD11c	CD44	CD156	αd integrin
CD15	CD49d	CD162	β7 integrin

Chemokine, complement, and other chemotactic factors

CD35	CD191	PAFR	CystLT2R
CD88	CD192	LTB_4R	fMLPR
CD182	CD193	C3aR	CRTH2
CD183	CD196	CystLT1R	Histamine 4R

Enzymes

CD13	CD46	PAR-2
CD45	CD55	
CD45RB	CD59	
CD45RO	CD87	

Apoptosis, signaling, and others

CD9	CD52	CD82	CD139	Siglec-10
CD17	CD53	CD86	CD148	LIR1
CD24	CD63	CD92	CD149	LIR2
CD28	CD65	CD95	CD151	LIR3
CD37	CD69	CD97	CD153	LIR7
CD39	CD71	CD98	CD161	TLR7
CD43	CD76	CD99	CD165	TLR8
CD48	CD81	CD137	Siglec-8	

Figure 2

Eosinophil surface markers. This schematic diagram lists surface molecules expressed by human eosinophils. Molecules have been listed generally based on convincing evidence for their expression as assessed by flow cytometry or inferred by cellular responsiveness to specific stimuli. Cluster designation (CD) for particular molecules is indicated based on the most recent classification (**www.ncbi.nlm.nih.gov/prow/**).

are CD11b/CD11c double positive and appear to be activated as they lose expression of GL-1 and CD62L and upregulate CD25 and CD69 surface expression. Analysis of thymic eosinophil cytokine production reveals that eosinophils express mRNA for the proinflammatory cytokines TNF-α, TGF-β, IL-1α, and IL-6 and the Th2-cytokines IL-4 and IL-13 (41). Notably, the recruitment of eosinophils into the thymus is regulated by eotaxin-1, which is constitutively expressed in the thymus (42).

It has been postulated that eosinophils are associated with MHC class I–restricted thymocyte deletion. Consistent with this notion, the biphasic recruitment of eosinophils and their anatomical localization within discrete compartments of the thymus coincide with negative selection of double-positive thymocytes (41). Employing an experimental model

of acute negative selection, researchers have demonstrated increased thymic eosinophil levels in MHC class I–restricted male (H-Y) antigen T cell receptor (TCR) transgenic mice following cognate peptide injection. In addition, eosinophils are associated with clusters of apoptotic bodies, suggesting eosinophil-mediated MHC class I–restricted thymocyte deletion. Thymic eosinophils have the capacity to promote thymocyte apoptosis as they express costimulatory molecules that are involved in clonal deletions, such as CD30 ligand (CD153) and CD66 (41). Additionally, eosinophils may induce thymocyte apoptosis through free radicals, as thymic eosinophils express high levels of NADPH oxidase activity; notably, developing thymocytes have increased sensitivity to free radicals owing to the downregulation of Cu^{2+}/Zn^{2+} superoxide dismutase.

EOSINOPHILS AND IMMUNE REGULATION

In recent years, investigators have shown that eosinophils can perform numerous immune functions, including antigen presentation (43, 44) and exacerbation of inflammatory responses through their capacity to release a range of largely preformed cytokines and lipid mediators (2, 5).

Antigen Presentation

Recent clinical and experimental investigations have shown that eosinophils can function as APCs (**Figure 1**). Eosinophils can process and present a variety of microbial, viral, and parasitic antigens. (45). In addition, granulocyte-macrophage colony stimulating factor (GM-CSF)-treated eosinophils promote T cell proliferation in response to staphylococcal superantigen (*Staphylococcus* enterotoxins A, B, and E) stimulation (46). Furthermore, eosinophils incubated with human rhinovirus-16 promote rhinovirus-16-specific T cell proliferation and IFN-γ secretion (47). Eosinophils can also effectively present soluble antigens to $CD4^+$ T cells, thereby promoting T cell proliferation and polarization. Adoptive transfer of antigen-pulsed eosinophils results in eosinophil-dependent T cell proliferation (44). Furthermore, addition of antigen to eosinophil and T cell cocultures promotes heightened T cell proliferative responses (43). The capacity of eosinophils to present antigen has been debated in some publications. It is interesting to note that the failure of eosinophils to present antigen may be related to the methods used for isolating eosinophils. For example, lysis of erythrocytes with ammonium chloride, an inhibitor of lysosome acidification (needed for antigen presentation), negatively correlates with eosinophil antigen presentation activity (43, 48).

Eosinophils secrete an array of cytokines (IL-2, IL-4, IL-6, IL-10, IL-12) capable of promoting T cell proliferation, activation, and Th1/Th2 polarization (4, 43, 44, 49) (**Figure 1**). Recent attention has been drawn to the ability of murine eosinophils to produce IL-4. Employing mice with enhanced green fluorescent protein (GFP) in the IL-4 gene locus (4get mice), investigators have demonstrated that eosinophils are a primary source of GFP following parasitic infection or anti-IgD treatment (a strong Th2 stimulator). Notably, although the IL-4 gene locus is transcriptionally active in eosinophils, the amount of IL-4 protein production appears to be lower than in T cells and basophils (50–52). Furthermore, murine eosinophils promote IL-4, IL-5, and IL-13 secretion by $CD4^+$ T cells (44). Eosinophils can also regulate T cell polarization through their synthesis of indoleamine 2,3-dioxygenase (IDO), an enzyme involved in oxidative metabolism of tryptophan, converting tryptophan to kynurenines (KYN). KYN regulates Th1 and Th2 imbalance by promoting Th1 cell apoptosis (53). The eosinophil-mediated T cell proliferative and cytokine secretion responses are dependent on costimulation. Indeed, blockade of CD80, CD86, and CTLA-4 by neutralizing antibodies inhibits

eosinophil-elicited T cell proliferation and cytokine secretion (45).

Fluorescent labeling studies revealed that eosinophils instilled into the trachea of mice traffic into the draining peritracheal lymph nodes and localize to the T cell–rich paracortical regions (B cell zones) within 24 h (43). Employing models of allergic airway disease and gastrointestinal allergy, investigators have demonstrated that inhalation of antigen promotes eosinophil homing to the draining endotracheal lymph nodes and Peyer's patches (44, 54–56).

Interestingly, a recent investigation suggests that eosinophils can only promote proliferation of effector T cells but not naive T cells (48). Moreover, eosinophils pulsed with OVA peptide and cocultured with OVA-specific TCR transgenic T cells (D011.10 T cells) induced effector T cell proliferation; however, when cocultured with naive $CD4^+$ T cells, no T cell proliferation was observed. It is tempting to speculate that eosinophils traffic to draining lymph nodes to recruit activated effector T cells and promote proliferation of effector T cells.

Mast Cell Regulation

A substantial body of literature has emerged demonstrating that eosinophils have the capacity to regulate mast cell function (**Figure 1**). Notably, human umbilical cord blood–derived mast cells can be activated by MBP to release histamine, PGD-2, GM-CSF, TNF-α, and IL-8 (57). The activation of mast cells by MBP elicits not only exocytosis, but also eicosanoid generation and cytokine production, both of which are prominent responses following FcεRI-dependent activation of mast cells (57). Incubation of rat peritoneal mast cells with native MBP, EPO, and ECP (but not EDN) results in concentration-dependent histamine release (15). Several studies have shown that MBP induces mast cell activation via a pathway similar to that observed with other polybasic compounds such as substance P, compound 48/80, and bradykinin (16). Freshly isolated human lung mast cells are resistant to IgE-independent activation; however, highly purified lung mast cells cocultured with human lung fibroblasts are sensitive to IgE-independent activation by MBP (57). Interestingly, activation of eosinophils with the mast cell protease chymase promotes production of eosinophil-derived stem cell factor, a critical mast cell growth factor. Eosinophils also produce nerve growth factor (NGF) (58), a cytokine not only involved in survival and functional maintenance of sympathetic neurons but also in immune regulation. For example, NGF promotes mast cell survival and activation (59, 60). NGF is preformed in eosinophils and acts in an autocrine fashion by activating release of EPO (58). EPO activates rat peritoneal muscles to release histamine, suggesting a role for eosinophil-derived NGF in mast cell–eosinophil interactions. Thus, eosinophils and mast cells communicate in a bidirectional fashion.

EOSINOPHIL DEVELOPMENT

Eosinophils are produced in the bone marrow from pluripotential stem cells, which first differentiate into a hybrid precursor with shared properties of basophils and eosinophils and then into a separate eosinophil lineage (61). Eosinophil lineage specification is dictated by the interplay of at least three classes of transcription factors, including GATA-1 (a zinc family finger member), PU.1 (an ETS family member), and C/EBP members (CCAAT/enhancer-binding protein family) (62–64) (**Figure 3**). Although these transcription factors are expressed in a variety of hematopoietic lineages, their mechanism of action in eosinophils is unique. In particular, graded expression of PU.1 specifies distinct cell lineage fates, with low levels specifying lymphocytic and high levels myeloid differentiation (65–67). Although GATA-1 and PU.1 antagonize each other's function in most cell types, they have synergistic activity in regulating eosinophil lineage specification (and

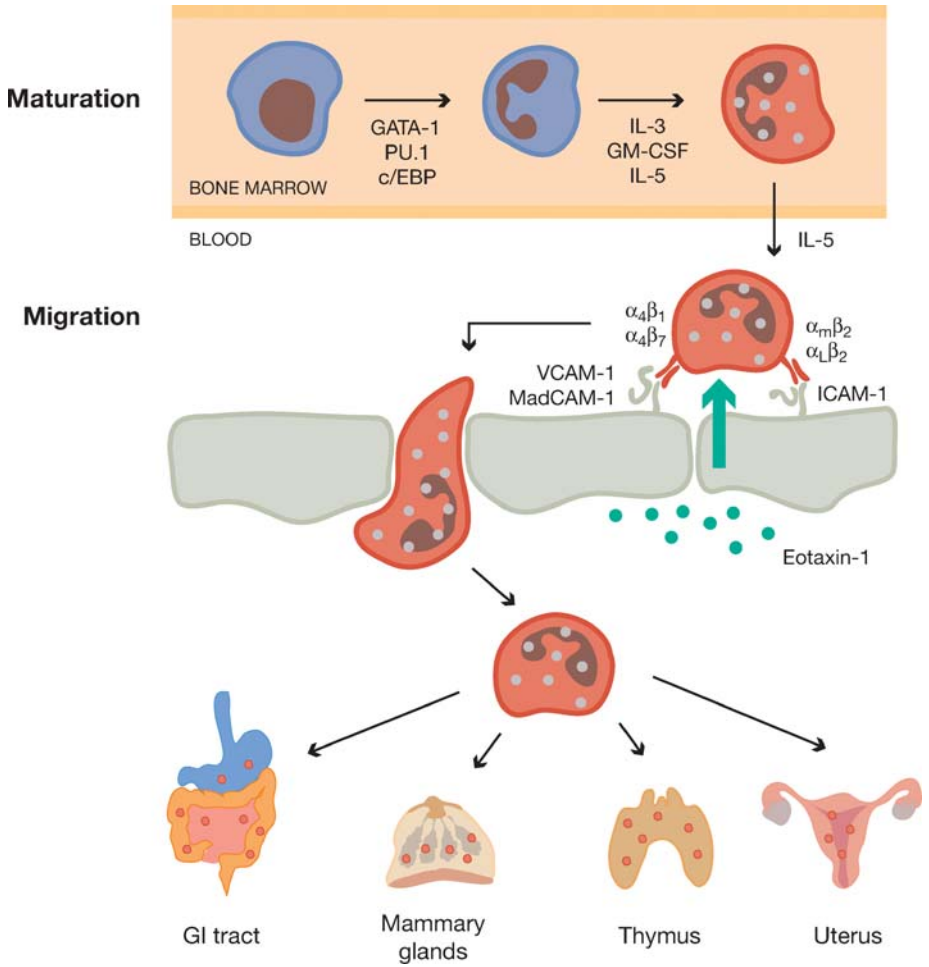


Figure 3

Schematic representation of eosinophil trafficking. Eosinophils develop in the bone marrow, where they differentiate from hematopoietic progenitor cells into mature eosinophils under the control of critical transcription factors, especially GATA-1. The eosinophilopoietins IL-3, IL-5, and GM-CSF regulate eosinophil expansion, especially in conditions of hypereosinophilia. Eosinophil migration out of the bone marrow into the circulation is primarily regulated by IL-5. Circulating eosinophils subsequently interact with the endothelium by processes involving rolling, adhesion, and diapedesis. Depending on the target organ, eosinophils cross the endothelium into tissues by a regulated process involving the coordinated interaction between networks involving the chemokine eotaxin-1, eosinophil adhesion molecules ($\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_m\beta_2$, $\alpha_L\beta_2$), and adhesion receptors on the endothelium (MAdCAM-1, VCAM-1, and ICAM-1). Under homeostatic conditions, eosinophils traffic into the thymus, mammary gland, uterus, and most prominently into the gastrointestinal tract.

eosinophil granule protein transcription) (67). The specificity of these factors for eosinophils is conserved across species, as C/EBP factors and GATA-1 drive differentiation of chicken progenitor cells into eosinophils (62). Of these transcription factors, GATA-1 is clearly the most important for eosinophil lineage specification, as revealed by the loss of the eosinophil lineage in mice harboring a targeted deletion of the high-affinity GATA-binding site in

Figure 4

Double GATA site. Sequence alignment of the palindromic double GATA high-affinity binding site identified in a hypersensitivity region of the murine GATA-1 regulatory locus with the dual GATA sites found in the promoters of three human eosinophil lineage-selective genes, including the human IL-5Rα gene promoter 1, human MBP promoter 2, and the human CCR3 regulatory exon-1. This figure was kindly provided by Drs. J. Du and S. Ackerman, University of Illinois, Chicago.

the GATA-1 promoter (68) and by eosinophil differentiation experiments in vitro (69). In particular, the specific activity of GATA-1 in eosinophils but not other GATA-1$^+$ lineages (mast cells, megakaryocytes, and erythroid cells) appears to be mediated by a high-affinity palindromic (or double) GATA site (67). This double GATA site is present in the downstream GATA-1 promoter and also in the regulatory regions of eosinophil-specific genes, including the eotaxin receptor CC chemokine receptor-3 (CCR3), MBP, and the IL-5 receptor alpha (IL-5Rα) gene (**Figure 4**), and it accounts for eosinophil-specific gene expression (67, 68, 70). For example, the tandem double GATA site in the human MBP-P2 promoter is required for both promoter activity in human eosinophil cell lines and for the synergistic transactivation by GATA-1 and PU.1 (67).

Three cytokines, IL-3, IL-5, and GM-CSF, are particularly important in regulating eosinophil development (71–74) (**Figure 3**). These eosinophilopoietins likely provide permissive proliferative and differentiation signals following the instructive signals specified by the transcription factors GATA-1, PU.1, and C/EBPs. These cytokines are encoded by closely linked genes on chromosome 5q31. They bind to receptors that share a common beta chain and have unique alpha chains (75). Of these three cytokines, IL-5 is the most specific to the eosinophil lineage and is responsible for selective differentiation of eosinophils (76). IL-5 also stimulates the release of eosinophils from the bone marrow into the peripheral circulation (77). The critical role of IL-5 in the production of eosinophils is best demonstrated by genetic manipulation of mice. Overproduction of IL-5 in transgenic mice results in profound eosinophilia (78–81), and deletion of the IL-5 gene causes a marked reduction of eosinophils in the blood and lungs after allergen challenge (82, 83). The overproduction of one or a combination of these three cytokines occurs in humans with eosinophilia, and diseases with selective eosinophilia are often accompanied by overproduction of IL-5 (84). The critical role of IL-5 in regulating eosinophils in humans has been demonstrated by several clinical trials with humanized anti-IL-5 antibody; this currently unapproved drug dramatically lowers eosinophil levels in the blood and to a lesser extent in the inflamed lung (85–87).

Double GATA site: a high-affinity palindromic (or double) GATA site located in the regulatory region of eosinophil-specific genes

CCR3: CC chemokine receptor-3, the major eosinophil chemokine receptor that binds the eotaxin family of chemokines

Eosinophilopoietins: IL-3, IL-5, and GM-CSF

EOSINOPHIL TRAFFICKING

Under baseline conditions, most eosinophils traffic into the gastrointestinal tract where they normally reside within the lamina propria of all segments except the esophagus (88) (**Figure 3**). The gastrointestinal eosinophil is the predominant population of eosinophils. Under baseline conditions, eosinophil levels in the gastrointestinal tract occur independently of lymphocytes and enteric flora, indicating unique regulation compared with other leukocytes (88). Indeed, the recruitment of gastrointestinal eosinophils is regulated by the constitutive expression of eotaxin-1,

as demonstrated by the marked decrease of this population of eosinophils in eotaxin-1-deficient mice. The importance of eotaxin-1 in regulating the baseline level of eosinophils is reinforced by the observation that mice with the targeted deletion of CCR3 (but not eotaxin-2-deficient mice) also have a deficiency in gastrointestinal eosinophils (89, 90). In addition to trafficking into the gastrointestinal tract, under homeostatic conditions, eosinophils home into the thymus, mammary gland, and uterus, also under the regulation of eotaxin-1 (40, 91) (**Figure 3**). Of note, trafficking into the uterus is regulated by estrogen, as eosinophil and eotaxin-1 levels cycle along with estrus (29).

The trafficking of eosinophils into inflammatory sites involves a number of cytokines (in particular, Th2 and endothelial cell products IL-4, IL-5, and IL-13) (92–94), adhesion molecules (e.g., β1-, β2-, and β7-integrins) (95), chemokines (e.g., RANTES and the eotaxins) (96), and other recently identified molecules (e.g., acidic mammalian chitinase) (97). Tissue eosinophils likely can survive for at least two weeks based on in vitro observations (92). Of the cytokines implicated in modulating leukocyte recruitment, only IL-5 and the eotaxins selectively regulate eosinophil trafficking (98). IL-5 regulates growth, differentiation, activation, and survival of eosinophils and provides an essential signal for the expansion and mobilization of eosinophils from the bone marrow into the lung following allergen exposure (77). However, antigen-induced tissue eosinophilia can occur independently of IL-5, as demonstrated by residual tissue eosinophils in trials using anti-IL-5 in patients with asthma (86) and using IL-5-deficient mice (82, 99). Recent studies have demonstrated an important role for the eotaxin subfamily of chemokines in eosinophil recruitment to the lung (96).

Eotaxin was initially discovered using a biological assay in guinea pigs designed to identify the molecules responsible for allergen-induced eosinophil accumulation in the lungs (98, 100, 101). Subsequently, using genomic analyses, two additional chemokines were identified in the human genome that encode for CC chemokines with eosinophil-selective chemoattractant activity and have thus been designated eotaxin-2 and eotaxin-3 (96). Eotaxin-2 and eotaxin-3 are only distantly related to eotaxin-1 because they are only ~30% identical in sequence and are located in a different chromosomal position (102, 103). The specific activity of all eotaxins is mediated by the selective expression of the seven-transmembrane spanning, G protein–coupled receptor CCR3, primarily expressed on eosinophils (104–106). Notably, the eotaxin chemokines cooperate with IL-5 in the induction of tissue eosinophilia. IL-5 increases the pool of eotaxin-responsive cells and primes eosinophils to respond to CCR3 ligands (96). Furthermore, when given exogenously, eotaxins cooperate with IL-5 to induce substantial production of IL-13 in the lung (96). The finding that IL-4 and IL-13 are potent inducers of the eotaxin chemokines by a STAT6-dependent pathway provides an integrated mechanism to explain the eosinophilia associated with Th2 responses (96). Recent studies have identified that eosinophil recruitment to the lung is dependent on STAT6 and a bone marrow–derived lung tissue resident non-T or non-B cell (51); in particular, eotaxin-2 production by airway macrophages likely accounts for this (90, 107). Of further interest, recently CCR3 has been shown also to deliver a powerful negative signal in eosinophils, depending on the ligand engaged. For example, pretreatment with the chemokine Mig inhibits eosinophil responses by a CCR3- and Rac2-dependent mechanism (108).

Using eotaxin-1 and eotaxin-2 single- and double-gene-deficient mice or neutralizing antibodies, investigators have shown that both chemokines have nonoverlapping roles in regulating the temporal and regional distribution of eosinophils in an allergic inflammatory site (90, 109, 110). In a standard experimental asthma model induced by systemic sensitization with OVA/alum followed

by respiratory OVA challenge, only a modest reduction in lung eosinophils was found in CCR3-deficient mice (89). However, when the same CCR3-deficient mouse line was subjected to experimental asthma induction by epicutaneous OVA sensitization, there was a marked deficiency of lung and bronchoalveolar lavage eosinophils (111). It was proposed that these apparently conflicting results may be related to the sensitization protocol (111), but the reason for this apparent discrepancy remains unclear. Notably, another CCR3-deficient mouse strain has recently been shown to have a profound reduction in eosinophil recruitment to the lung in the standard OVA/alum systemic sensitization model (107).

Substantial preclinical evidence now supports a role for the eotaxin chemokines in human allergic disease (96). Experimental induction of cutaneous and pulmonary late-phase responses in humans has revealed that the eotaxin chemokines are produced by tissue resident cells (e.g., respiratory epithelial cells and skin fibroblasts) and allergen-induced infiltrative cells (e.g., macrophages and eosinophils). Following allergen challenge in the human lung, eotaxin-1 is induced early (6 h) and correlates with early eosinophil recruitment; in contrast, eotaxin-2 correlates with eosinophil accumulation at 24 h (96). In another study, eotaxin-1 and eotaxin-2 mRNA was increased in patients with asthma compared with normal controls; however, there was no further increase following allergen challenge (96). In contrast, eotaxin-3 mRNA was dramatically enhanced 24 h after allergen challenge (96). The chemoattractant activity of the bronchoalveolar lavage fluid from patients with asthma is inhibited by antibodies against RANTES, MCP (monocyte chemoattractant protein)-3, MCP-4, and eotaxin-1 (96). Further support for an important role of eotaxin-1 in human asthma is derived from analysis of a single nucleotide polymorphism (SNP) in the eotaxin-1 gene. A naturally occurring mutation encoding for a change in the last amino acid in the signal peptide (alanine→threonine) results in less effective cellular secretion of eotaxin-1 in vitro and in vivo (112). Notably, this SNP is associated with reduced levels of circulating eotaxin-1 and eosinophils and improved lung function (e.g., FEV1) (112). Furthermore, a SNP in the eotaxin-3 gene is associated with atopy in a Korean population (113). Recently, the activity of eotaxin-1 and eotaxin-2 in humans has been investigated by injection of these chemokines into the skin of humans; both eotaxin-1 and eotaxin-2 induce an immediate wheal and flare response associated with mast cell degranulation and subsequent infiltrations by eosinophils, basophils, and neutrophils (114). The infiltration by neutrophils is likely to be mediated indirectly by the mast cell degranulation. These results provide substantial evidence that the biological activities attributed to eotaxins in animals are conserved in humans.

Eosinophils express numerous adhesion molecules, and most attention has focused on their highly expressed integrins, including $\alpha_4\beta_7$, the CD18 family of molecules (β2-integrins), and the very late antigen (VLA)-4 molecules (β1-integrins) (95) (**Figure 1**). The CD18 family of molecules includes lymphocyte function antigen (LFA)-1 and Mac-1 that interact with endothelial cells via intercellular adhesion molecule (ICAM)-1. VLA-4 interacts with endothelium via vascular cell adhesion molecule (VCAM)-1, as well as fibronectin. The $\alpha_4\beta_7$ integrin interacts with the mucosal addressin cell adhesion molecule (MAdCAM)-1 expressed by vascular endothelium in the intestinal tract. These integrins have variable roles in eosinophil trafficking during inflammation, but the role of specific adhesion molecules in the baseline homing of eosinophils into the gastrointestinal tract has yet to be elucidated. For example, in β7 gene-targeted mice, there is a delay and reduced magnitude in the development of intestinal eosinophilia following *Trichinella spiralis* infection (115) and when the eotaxin-1 intestine transgene is expressed, but no changes in the baseline level of small intestine eosinophils

(81). Analysis of anti-β1-treated mice or VLA-4-deficient mice has shown the critical participation of this family of molecules in regulating eosinophil homing to the allergic lung (116–118). Indeed, eotaxin-1-stimulated eosinophils have increased expression and avidity of VLA-4 (119). It has also become clear that engagement of eosinophil adhesion molecules with their ligands not only induces a proadhesive pathway, but also activates expression of a series of proinflammatory genes within eosinophils, including GM-CSF, that then propagate eosinophil survival by a paracrine pathway.

Numerous other pathways for regulating eosinophil accumulation and trafficking are operational in various inflammatory models. However, recently several lines of evidence have focused attention on the importance of arachidonic acid metabolites, especially leukotriene B4 (LTB4), the cysteinyl leukotrienes (LTC4, LTCD4, and LTE4), and prostaglandin (PG) D2. Notably, cysteinyl leukotriene type 1 receptor antagonists (now approved for asthma therapy) reduce blood and lung eosinophilia. Mice with the targeted deletion of the LTB4 receptor also have markedly reduced allergen-induced lung eosinophilia (120). Furthermore, eosinophils express high levels of a high-affinity PGD2 type 2 receptor. Interestingly, this receptor is also expressed by basophils and Th2 cells [and is now designated chemoattractant receptor Th2 cells (CRTH2)] and appears to co-mediate Th2 cell and eosinophil/basophil recruitment (121). Eosinophils have recently also been shown to express high levels of the histamine receptor 4 (H4) that mediates eosinophil chemoattraction and activation in vitro (122).

ROLE OF EOSINOPHILS IN DISEASE

Infections

The beneficial function of eosinophils has been primarily attributed to their ability to defend the host against parasitic helminths. This is based on several lines of evidence, including (*a*) the ability of eosinophils to mediate antibody- (or complement-) dependent cellular toxicity against helminths in vitro (27), (*b*) the observation that eosinophil levels increase during helminthic infections and that eosinophils aggregate and degranulate in the local vicinity of damaged parasites in vivo, and (*c*) the results in experimental parasite infected mice that have been depleted of eosinophils by IL-5 neutralization and/or gene targeting (123). Murine studies are particularly problematic because mice are not the natural hosts of many of the experimental parasites; nevertheless, in some primary infection models, a role for IL-5 in protective immunity has been suggested following infection with *Strongyloides venezuelensis*, *Strongyloides ratti*, *Nippostrongyloides brasiliensis*, and *Heligmosomoides polygyrus* (123, 124). These in vivo studies need to be interpreted with caution because IL-5 neutralization may have effects on other IL-5 receptor bearing cells (including murine B cells, human basophils, and possibly human respiratory smooth muscle cells) (76, 125–127). Other approaches, including analysis of CCR3- and eotaxin-1-deficient mice, have recently demonstrated a role for eosinophils in the encystment of larvae in *Trichinella spiralis* and in controlling the *Brugia malayi microfilariae*, respectively (128, 129). Perhaps analysis of the recently generated eosinophil-deficient mice following experimental parasitic infection will provide further compelling evidence that eosinophils participate in host defense against parasites. Thus, although the debate continues, it seems likely that eosinophils participate in the protective immunity against selected helminths.

Evidence is emerging that eosinophils may also have a protective role in other infections, especially against RNA viruses such as respiratory syncytial virus (RSV) and the related natural rodent pathogen, pneumonia virus of mice (PVM), in vivo (13, 130). Notably, eosinophil granule proteins include abundant

ribonucleases [such as human ECP and EDN, and at least 11 eosinophil-associated ribonuclease (EAR) orthologs in mice] that degrade single-stranded RNA containing viruses (13). In fact, ECP and EDN are the most divergent coding sequences in the entire human genome (compared with other primates) (13). Despite their divergence, they have conserved ribonuclease activity across species, strongly implicating evolutionary pressure to preserve this critical enzymatic activity.

Asthma

Elevated levels of eosinophil granule proteins (e.g., MBP) have been found in bronchoalveolar lavage fluid from patients with asthma, and importantly these concentrations are sufficient to induce cytotoxicity of a variety of host tissue, including respiratory epithelial cells in vitro (3). Direct degranulation of mast cells and basophils, triggered by MBP, is thought also to be involved in disease pathogenesis (3). In addition to being cytotoxic, MBP directly increases smooth muscle reactivity by causing dysfunction of vagal muscarinic M2 receptors, which is thought to contribute to the development of airway hyperreactivity (AHR), a cardinal feature of asthma (131). Additionally, eosinophils generate large amounts of the cysteinyl leukotrienes (132). Of note, eosinophil granule proteins contain all the biochemical machinery necessary to synthesize cysteinyl leukotrienes (132). These mediators lead to increased vascular permeability and mucus secretion and are potent smooth muscle constrictors. Indeed, inhibitors of cysteinyl leukotrienes are effective therapeutic agents for the treatment of allergic airway disease.

Multiple studies employing experimental models of asthma (primarily in mice, guinea pigs, and monkeys) have demonstrated that neutralization of IL-5 can block various aspects of asthma (82, 133). Although extensive investigations have implicated the eosinophil as a central effector cell in asthma and an important clinical target for the resolution of this disease, the role of this granulocyte in the development and exacerbation of asthma pathogenesis has been controversial. This controversy stems in part from distinctions between human asthma and experimental murine models of asthma. For example, in contrast to human asthma, mice with eosinophil lung disease triggered by allergens or helminthic infection have variable levels of eosinophil degranulation (50, 134). In experimental models, inhibition of the actions of IL-5 consistently suppresses pulmonary eosinophilia in response to antigen inhalation; however, this effect does not always correlate with a reduction of AHR (135). This dichotomy is highlighted by findings in allergic IL-5-deficient mice of the C57BL/6 strain (82) that do not develop antigen-induced AHR, whereas IL-5-deficient BALB/c mice develop enhanced reactivity independent of this factor (136). Although eosinophil trafficking to the allergic lung is profoundly attenuated in IL-5-deficient mice or in those treated with anti-IL-5 antibodies in comparison to wild-type responses (137–139), a marked residual tissue eosinophilia can persist in these mice after allergen inhalation (82, 140, 141). Furthermore, the degree of residual tissue eosinophilia is under genetic regulation, as lung eosinophilia is 10- to 100-fold greater in the BALB/c strain, where AHR persists, compared with the C57BL/6 strain, where AHR is abolished in the absence of IL-5 (82, 99, 138).

Studies with transgenic mice overexpressing IL-5 (in T cells, lung epithelial cells, or enterocytes) have demonstrated that overexpression of IL-5 is sufficient for the development of eosinophilia (78–81, 142); however, elevated levels of eosinophils are not universally associated with the development of asthma-like changes in the lung. Indeed, clinical studies in patients have shown that AHR correlates with mast cell localization near pulmonary nerves, whereas pulmonary eosinophilia relates more strongly with chronic cough (143). However, depletion of murine eosinophils (by administration

Airway hyperresponsiveness or hyperreactivity (AHR): increased constriction of the airways to various stimuli such as methacholine

Airway remodeling: microscopic changes (e.g., goblet cell metaplasia, collagen deposition, smooth muscle hyperplasia) in the lungs associated with functional alterations in lung function

PHIL mice: genetically engineered eosinophil-deficient mice produced by insertion of the diphtheria toxin A chain into the EPO gene locus

Δdbl-GATA-1 mice: genetically engineered eosinophil-deficient mice produced by deleting the high-affinity double GATA site in the GATA-1 promoter

of complement-fixing antibodies against CCR3) has demonstrated an important role for eosinophils in the development of asthma-associated AHR (144); a role for other $CCR3^+$ cells was not ruled out, but there was no evidence for CCR3 expression by non-eosinophils (144). Accordingly, a humanized antibody against IL-5 has recently been tested for asthma (85). In the early studies with this reagent, patients with mild to moderate asthma were shown to have a drop in their circulating and sputum eosinophil levels (85); however, no clinical benefit (e.g., improvement in FEV1) was demonstrated. This result prompted some investigators to conclude that eosinophils were not effector cells in human asthma (85); however, the anti-IL-5 study was not properly designed to address the efficacy of this drug (145). In support of these preclinical studies, a very recent study has demonstrated that anti-IL-5 in humans blocks lung eosinophil recruitment by only 55% (146), providing evidence that accessory molecules (in addition to IL-5) regulate lung eosinophilia. Thus, anti-IL-5 treatment does not completely resolve tissue eosinophilia in the allergic lung, and therefore this cell may still contribute to disease pathogenesis even in the presence of IL-5 neutralization. With the discovery of the eotaxins, and the finding that IL-5 cooperates with eotaxins in regulating eosinophil tissue recruitment, it became critical to determine if the lack of efficacy of anti-IL-5 in humans was related to the inability of this drug to block eosinophil tissue recruitment or to the noneffector role of eosinophils. One possibility is that local chemokine systems (eotaxins) can operate independently of IL-5 to recruit eosinophils into the allergic lung. Studies with eotaxin-1 gene-targeted mice, IL-5 gene-targeted mice, and eotaxin-1/IL-5 double-gene-targeted mice have revealed an independent and synergistic role for both of these molecules in regulating the tissue level of eosinophils in the asthmatic lung and in the induction of AHR (139). Although early studies with anti-IL-5 in human asthma have continued to find no improvement in airflow measurements (FEV1), pathological markers of chronic airway remodeling (e.g., deposition of tenascin, procollagen III, and lumican) are improved by anti-IL-5 (146). Decreased levels of TGF-β in the bronchoalveolar lavage fluid following anti-IL-5 treatment have been found, suggesting that eosinophil-derived TGF-β regulates lung remodeling. In support for a role of eosinophils in the pathogenesis of human asthma, a very recent study has demonstrated improved clinical outcome when asthma treatment decisions are based on monitoring sputum eosinophil counts rather than conventional guidelines from the British Thoracic Society (147).

Recently, two different lines of eosinophil-deficient mice were developed (see **Table 1** and Eosinophil-Deficient Mice). Lee et al. (148) targeted the depletion of eosinophils by using an eosinophil-specific promoter to drive expression of a cytocidal protein, diphtheria toxin A chain. The eosinophil-deficient character of these mice (called PHIL mice) was assessed by examination of peripheral blood and by immunohistochemistry of tissues with abundant resident populations (e.g., bone marrow, uterus, small intestine, and thymus) using antibodies specific for eosinophil

Table 1 Effect of eosinophil depletion on experimental asthma parameters[a]

Mouse line	PHIL	Δdbl-GATA
Asthma parameter		
BALF eosinophils	+	+
Lung tissue eosinophils	+	+
BALF mononuclear cells	NE	NE
AHR	+	NE
Mucus production	+	NE
Collagen deposition	ND	+
Th2 antibody production	ND	NE
Th2 cytokines	+	NE

[a]If the genetic manipulation of the mouse resulted in protection from or reduction in severity of the asthma parameter, the parameter is labeled with a "+". If there was no change in the asthma parameter between the genetically modified mouse and wild-type control mice, the parameter is labeled with "NE" for no effect. If the asthma parameter was not measured, the parameter is labeled with "ND" for not determined.

granule proteins. In comparison, Yu et al. (68) developed mice harboring a deletion of a high-affinity GATA-binding site in the GATA-1 promoter (ΔdblGATA) which led to the specific ablation of the eosinophil lineage. RT-PCR analysis of gene expression in the bone marrow of the ΔdblGATA mice revealed no expression of EPO, but expression of MBP was only partially reduced and CCR3 expression remained unchanged. Nevertheless, eosinophil deficiency in these mice was verified by morphological observation of cells from the blood, bone marrow, and spleen. Using both lines of eosinophil-deficient mice, eosinophils were shown to have an integral role in experimental allergic asthma. However, their specific contribution toward allergen-induced AHR and mucus cell metaplasia was different (**Table 1**). Perhaps ΔdblGATA mice have residual eosinophils or unappreciated hematological abnormalities, or alternatively, diptheria toxin treatment of PHIL mice may induce toxic effects on noneosinophils; these and other explanations for the distinct results will hopefully be uncovered soon. It should be noted that ΔdblGATA mice had impaired development of lung remodeling in a chronic model of asthma, consistent with the results of anti-IL-5 in patients with asthma. Taken together, compelling evidence now exists that eosinophils are prominent effector cells in eliciting multiple parameters of experimental asthma.

Eosinophil-Deficient Mice

Two different lines of eosinophil-deficient mice have recently been developed. One group targeted the depletion of eosinophils using an eosinophil-specific promoter (the EPO gene) to drive expression of a cytocidal protein diphtheria toxin A (13). These mice (called PHIL) are protected from the development of AHR in a model of experimental asthma. Another group developed mice harboring a deletion of the high-affinity GATA-binding site in the GATA-1 promoter (ΔdblGATA); this led to the specific ablation of the eosinophil lineage even when these mice were crossed with IL-5 transgenic mice (48). The ΔdblGATA mice are protected from features of airway remodeling but not AHR in an experimental model of asthma. It is anticipated that these newly generated eosinophil-deficient mouse lines will transform eosinophil research over the next decade, especially because the ΔdblGATA mice are now commercially available from Jackson Laboratories, Inc.

Gastrointestinal Disorders

The accumulation of eosinophils in the gastrointestinal tract is a common feature of numerous disorders, such as drug reactions, helminth infections, hypereosinophilic syndromes, eosinophilic gastroenteritis, allergic colitis, inflammatory bowel disease, and gastroesophageal reflux disease (150). A subset of these diseases, referred to as primary eosinophil-associated gastrointestinal disorders (EGID), includes eosinophilic esophagitis (EE), eosinophilic gastritis, and eosinophilic gastroenteritis. These are hypersensitivity disorders that lie in the middle of a spectrum ranging from anaphylaxis to Celiac disease (150). EGID usually occurs independently of peripheral blood eosinophilia, indicating the significance of gastrointestinal-specific mechanisms for regulating eosinophil levels. Indeed, in murine models of EGID, a definitive role for eosinophils and eotaxin-1 has been demonstrated. Notably, eosinophils are frequently associated near damaged enteric nerves, and indeed eotaxin-1-deficient mice are protected from this feature of disease. EE is distinguished from gastroesophageal reflux disease by several important differences, including the relatively higher prevalence of atopy, dysphagia, male gender, familial inheritance, degree of proximal esophagitis, and intensity of esophageal pathology [e.g., epithelial hyperplasia and eosinophil density (generally >24 eosinophils/high power field)] (150). Consistent with the high rate of atopic respiratory disease in patients with EE, experimental EE develops in mice following respiratory allergen exposure or following intratracheal IL-13 delivery (151). These

Hypereosinophilic syndrome: a group of disorders characterized by severely elevated blood eosinophil levels and end-organ damage

EGID: eosinophil-associated gastrointestinal disorders

EE: eosinophilic esophagitis

***FIP1L1-PDGFRA*:** activated tyrosine kinase fusion gene product that occurs in hypereosinophilic syndrome owing to an 800-kb interstitial deletion in chromosome 4

results establish an intimate immunological connection between the lung and esophagus. The epithelial hyperplasia associated with EE and the level of esophageal eosinophils is attenuated in IL-5-deficient mice (152), providing strong evidence that eosinophils are effector cells in this gastrointestinal disease. Indeed, a recent preliminary evaluation of humanized anti-IL-5 in patients with EE demonstrates lowering of esophageal eosinophil levels. Supporting a connection between allergic responses in the lung and gastrointestinal tract, eotaxin-1 intestine transgenic mice not only develop intestinal eosinophilia but also AHR by an IL-13-dependent mechanism (153). Thus, increased expression of eotaxin-1 in the gastrointestinal compartment can lead to increased $CD4^+$ T cell–derived Th2 lymphocyte-cytokine production that drives aberrant immunophysiological responses in distant noninflamed mucosal tissue (the lung). These results provide a possible explanation for the altered lung function seen in some patients with inflammatory gastrointestinal disorders.

ANTI-EOSINOPHIL THERAPEUTICS

Numerous drugs inhibit eosinophil production or eosinophil-derived products. They include glucocorticoids, myelosuppressive drugs, leukotriene synthesis or receptor antagonists, tyrosine kinase inhibitors, IFN-α, and humanized anti-IL-5 antibodies. The etiology of the primary disease often specifies the best therapeutic strategy. For example, a subset of patients with hypereosinophilic syndrome have an 800-kb interstitial deletion on chromosome 4 (4q12) that results in the fusion of an unknown gene *FIP1L1* with the platelet-derived growth factor receptor-α (*PDGFRA*) gene (154, 155). This fusion gene produces a constitutively active tyrosine kinase (PDGFRA) that is exquisitely sensitive to the inhibitor imatinib mesylate, which is now approved for the treatment of several malignancies (Gleevec™). Although PDGFRA is not normally active in hematopoietic cells, the activated kinase renders cells growth factor independent, perhaps by activating STAT5 signal transduction. Thus, eosinophilic patients with *FIP1L1-PDGFRA*$^+$ disease are now treated with Gleevec™ as first-line therapy (156). In addition, a variety of other activated tyrosine kinases have just been associated with hypereosinophilic syndromes, including PDGFRB, Janus kinase-2, and fibroblast growth factor receptor-1.

In most other individuals, glucocorticoids are the most effective agents for reducing eosinophilia (3). They suppress the transcription of a number of genes for inflammatory mediators, including the genes for IL-3, IL-4, IL-5, GM-CSF, and various chemokines including the eotaxins. Recently, the main action of glucocorticoids on eosinophil-active cytokines has been shown to involve mRNA destabilization, thus reducing the half-life of cytokines such as eotaxins (157). In addition, glucocorticoids inhibit the cytokine-dependent survival of eosinophils (158). Systemic or topical (inhaled or intranasal) glucocorticoid treatment typically causes a rapid reduction in eosinophils, but some patients are glucocorticoid resistant and maintain eosinophilia despite high doses (159). The mechanism of glucocorticoid resistance is unclear, but a reduced level of glucocorticoid receptors and alterations in transcription factor activator protein (AP)-1 appear to be at least partially responsible (159).

Glucocorticoid-resistant patients sometimes require other therapy such as myelosuppressive drugs (hydroxyurea, vincristine) or IFN-α (3). IFN-α can be especially helpful because it inhibits eosinophil degranulation and effector function (160). Notably, patients with myeloproliferative variants of hypereosinophilic syndrome can often go into remission with IFN-α therapy. Cyclophilins (e.g., cyclosporine A) have also been used because they block the transcription of numerous eosinophil-active cytokines (e.g.,

IL-5, GM-CSF) (3). Recently, lidocaine has been shown to shorten eosinophil survival, and its effects mimic those of glucocorticoids and are noncytotoxic (161). Indeed, an early clinical trial has shown that nebulized lidocaine is safe and effective in subjects with asthma (162).

Drugs that interfere with eosinophil chemotactic signals include recently approved leukotriene antagonists and inhibitors. 5-lipoxygenase inhibition (e.g., zileuton) blocks the rate-limiting step in leukotriene synthesis and inhibits the generation of the eosinophil chemoattractant, LTB4, and the cysteinyl leukotrienes (163). Cysteinyl leukotriene receptor antagonists block the muscle contraction and increased vascular permeability mediated by leukocyte-derived leukotrienes (164). Some of the third generation antihistamines inhibit the vacuolization (165) and accumulation (166) of eosinophils after allergen challenge and directly inhibit eosinophils in vitro (165, 167). Cromoglycate and nedocromil inhibit the effector function of eosinophils, such as antibody-dependent cellular cytotoxicity (167).

The identification of molecules that specifically regulate eosinophil function and/or production offers new therapeutic strategies in the pipeline. Agents that interrupt eosinophil adhesion to the endothelium through the interaction of CD18/ICAM-1 (168) or VLA-4 /VCAM-1 may be useful (169, 170). Indeed, antibodies that block these pathways have recently been approved for other diseases, but their anti-eosinophil activity has yet to be determined (171). Antibodies against IL-5, now humanized by two different pharmaceutical companies, are under active clinical investigation (172, 173). Although their utility for asthma may be limited owing to redundant pathways, anti-IL-5 is particularly promising for hypereosinophilic syndromes. Numerous inhibitors of the eotaxin/CCR3 pathway, including small molecule inhibitors of CCR3 and a human anti-eotaxin-1 antibody, are being developed (96). Early results with a phase I trial of human anti-eotaxin-1 antibody in patients with allergic rhinitis have demonstrated the ability of this apparently safe drug to lower levels of nasal eosinophils and to improve nasal patency (96). Anti-human IL-13 antibody is now in preclinical trials (174) and looks promising for lowering tissue eosinophil levels. Finally, a recently identified eosinophil surface molecule Siglec-8 may offer a therapeutic opportunity (175). Siglec-8 is a member of the sialic acid–binding lectin family and contains ITIMs (immunoreceptor tyrosine-based inhibitory motifs) that can induce efficient eosinophil apoptosis when engaged by anti-Siglec-8 crosslinking antibodies. Siglec-8 as well as CCR3 and CRTH2 are coexpressed by other cells involved in Th2 responses, including Th2 cells, mast cells, and basophils. Thus, agents that block these receptors may be particularly useful for allergic disorders.

PERSPECTIVE

Historically, eosinophils have been considered end-stage cells involved in host protection against parasites. However, numerous lines of evidence have now changed this perspective by showing that eosinophils are pleiotropic multifunctional leukocytes involved in initiation and propagation of diverse inflammatory responses, as well as modulators of adaptive immunity by directly activating T cells. As normal constituents of the mucosal immune system, particularly in the gastrointestinal tract, eosinophils are likely to have a physiological function. Indeed, eosinophils have been implicated in innate immunity by being an early and possibly instrumental source of cytokines (e.g., IL-4) and have a role in developmental processes such as mammary gland development. Analysis of recently generated genetically engineered eosinophil-deficient mice will soon answer critical questions concerning the true involvement of this cell type in a variety of processes. Breakthroughs in identifying key eosinophil regulatory cytokines such as IL-5 and the eotaxin subfamily of chemokines

have uncovered mechanisms that selectively regulate eosinophil production and localization at baseline and during inflammatory responses. In particular, an integrated mechanism involving Th2 cell–derived IL-5 regulating eosinophil expansion in the bone marrow and blood and Th2 cell–derived IL-13 regulating eotaxin production now explains the means by which T cells regulate eosinophils. Based on these findings, targeted therapy against key eosinophil regulators (e.g., humanized anti-IL-5 and CCR3 antagonists) will likely transform medical management of eosinophilic patients.

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