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Roles and Regulation of Gastrointestinal Eosinophils in Immunity and Disease

YunJae Jung*† and Marc E. Rothenberg*

Eosinophils have historically been considered to be destructive end-stage effector cells that have a role in parasitic infections and allergic reactions by the release of their granule-derived cytotoxic proteins. However, an increasing number of experimental observations indicate that eosinophils also are multifunctional leukocytes involved in diverse inflammatory and physiologic immune responses. Under homeostatic conditions, eosinophils are particularly abundant in the lamina propria of the gastrointestinal tract, where their involvement in various biological processes within the gastrointestinal tract has been posited. In this review, we summarize the molecular steps involved in eosinophil development and describe eosinophil trafficking to the gastrointestinal tract. We synthesize the current findings on the phenotypic and functional properties of gastrointestinal eosinophils and the accumulating evidence that they have a contributory role in gastrointestinal disorders, with a focus on primary eosinophilic gastrointestinal disorders. Finally, we discuss the potential role of eosinophils as modulators of the intestinal immune system.


Eosinophils are multifunctional proinflammatory leukocytes involved in the pathogenesis of allergic disorders and are implicated in the protection against helminth infections (1, 2). Eosinophils are generally thought of as proinflammatory cells because they release pleotropic cytokines, chemokines, and lipid mediators, as well as toxic cytoplasmic granule constituents, including major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin (EDN) (3, 4). Although eosinophils are recognized as circulating cells, composing 1–5% of peripheral blood leukocytes, they are primarily resident in the lamina propria of the small intestine, where they compose a substantial fraction (e.g., 20–30%) of the cellular population (2, 5). Recently, a standard protocol for the isolation of murine eosinophils from the intestinal lamina propria using eosinophil-specific surface markers was established (5). Additionally, the development of eosinophil-deficient mouse strains has expanded the understanding of the role of intestinal eosinophils from dogmatic antiparasitic effector cells to immune modulatory cells. In this article, we discuss emerging advances in the understanding of intestinal eosinophils at baseline and during inflammatory gastrointestinal disorders, including primary eosinophilic gastrointestinal disorders (EGIDs), such as eosinophilic esophagitis (EoE) (6).

Developmental properties of eosinophils

Eosinophils develop in the bone marrow from pluripotent stem cells that become eosinophil progenitors marked by CD34+CD125* expression. Eosinophil lineage specification is determined by the interplay of several transcription factors, including the zinc finger transcription factor GATA-binding protein 1 (GATA-1), the E26 transformation-specific family member PU.1, IFN consensus sequence binding protein, and C/EBP family members (2, 7, 8), as well as by regulation by microRNAs, including miR-21 and miR-223 (9, 10). Of the transcription factors, GATA-1 is the most important; targeted deletion of the high-affinity double palindromic GATA-1 binding site in the Gata1 promoter results in eosinophil-depleted mice, referred to as ΔdblGATA mice (11). ΔdblGATA mice, along with another eosinophil-deficient strain generated using a promoter of the eosinophil peroxidase gene to drive expression of cytoidal diptheria toxin A (referred to as PHIL mice) (12), are being used to uncover the function of eosinophils (13). Eosinophil development is guided by signals from the aforementioned transcription factors; subsequently, permissive proliferation and differentiation are regulated primarily by IL-5, although IL-3 and GM-CSF can also contribute (2). Of these cytokines, IL-5 is the most specific for selective differentiation of eosinophils, stimulating their migration from the bone marrow to the circulation (2, 14). Eosinophils released into the blood migrate into the thymus, mammary gland, uterus, and the gastrointestinal tract, with the last having the highest eosinophil levels under homeostatic conditions (2). Levels of gastrointestinal
eosinophils were estimated to be $\geq 10$-fold higher than in the circulation (2, 3). Examination of the entire gastrointestinal tract reveals that only the esophagus is devoid of baseline eosinophils and that eosinophil levels progressively increase from the stomach to the colon, where they can be fairly high (i.e., as many as 50 eosinophils/high-power microscopic field [400×]) (15).

**Unique characteristics of intestinal eosinophils**

Until recently, no detailed phenotypic analysis of intestinal eosinophils had been performed because of the difficulties inherent in identifying and/or isolating sufficient numbers of these cells from the gastrointestinal tract. However, phenotypic characterization of murine eosinophils in the intestinal lamina propria was reported recently by several groups (5, 16, 17). For detection of eosinophils in mice, there are several available markers, including CCR3, sialic acid–binding Ig-like lectin (Siglec)-F (homolog of siglec-8 in humans), and CD125, which encodes for IL-5Rα (18–20). Although these markers are primarily expressed on eosinophils, CCR3 also is expressed on mast cells and Th2 cells (21). Siglec-F is detected on alveolar macrophages (22), and IL-5Rα is also expressed on peritoneal B-1 cells (20). Therefore, none of these markers can be considered to represent a definitive specificity for eosinophils. However, because eosinophils contain a dense concentration of cytoplasmic granules, their side scatter patterns under flow cytometry are readily distinguishable from those of other lineages. Thus, a combination of the relative expression of CCR3, Siglec-F, and/or IL-5Rα with their side scatter patterns can be used to delineate eosinophil subsets in the intestine (Fig. 1A) (5, 16, 17). In addition to these markers, intestinal eosinophils of mice express higher levels of myeloid marker CD11b than do their blood counterparts and are positive for CD11c, a surface marker used to identify intestinal dendritic cells (Fig. 1A) (5). Meanwhile, small intestinal eosinophils isolated from mice are negative for other markers associated with intestinal dendritic cells, such as MHC class II, CD80, CD103, and CD205 (DEC-205) (5). Considering that CD11c expression also occurs on murine eosinophils in the thymus and uterus, but not in the blood (5), it seems plausible that it is affected by the local microenvironment rather than being indicative of their Ag-presenting capacity.

Along with CD11c, Siglec-F expression also is observed at higher levels in eosinophils of the small intestine (5). It remains to be determined whether the relative expressions of CD11c and Siglec-F in murine eosinophils from different sources correlate with their functional differences in specific tissues. CD22, a B cell–specific Siglec belonging to the inhibitory receptor family, is highly expressed on the surface of murine, small intestinal eosinophils but is undetectable on eosinophils from the blood (Fig. 1A) (17). Because the small intestine is a milieu rich in substances that stimulate eosinophils (23–25), CD22’s abundant expression on intestinal eosinophils might prevent overactivation of intestinal eosinophils under homeostatic conditions. Notably, CD22 expression on small intestinal eosinophils is decreased under various inflammatory conditions, such as bacterial colonization, systemic IL-5 overexpression, and OVA-induced gastrointestinal inflammation (17). Therefore, it can be posited that small intestinal eosinophils receive negative-feedback signals from highly expressed inhibitory CD22 under the steady-state but are ready for conversion to proinflammatory cells via downregulation of CD22.

Under homeostatic conditions, most eosinophils produced in the bone marrow migrate to the small intestine; this process is regulated by eosinophil expression of CCR3 and α4β7 integrin because their cognate ligands (eotaxin and mucosal vascular addressin molecule 1, respectively) are constitutively expressed in the intestine (15, 26). B7 integrin appears to be particularly important in the large intestine, whereas eotaxins mediate eosinophil homing in the large and small intestine of mice (26, 27). Gastrointestinal eosinophils are postmitotic and have limited survival in the absence of survival-promoting cytokine signals (28); conversely, cytokine signaling through the common γ-chain increases the lifespan of murine small intestinal eosinophils (Fig. 1A) (5). Therefore, in combination with a specialized influx mechanism, the prolonged survival of eosinophils (≥ 14 d) contributes to their predominance in the gastrointestinal tract (5). In addition, signal regulatory protein α (SIRP-α), highly expressed in small intestinal eosinophils, inhibits degranulation of eosinophils by interacting with their ligand CD47, thus promoting eosinophil survival (Fig. 1A) (16). Also, type 2 innate lymphoid cells (ILC2s) have been identified as a specialized cell population supporting the maintenance of murine intestinal eosinophils by secreting IL-5 and IL-13, which promote eosinophil survival and recruitment to the small intestine, respectively, via upregulation of eotaxin (29). Notably, the coexpression of IL-5 and IL-13 by ILC2s is enhanced after caloric intake and regulated by vasoactive intestinal peptide, which stimulates ILC2s through vasoactive intestinal peptide receptor type 2 to release IL-5 via a circadian rhythm (Fig. 1A) (29). However, under nutrient deprivation, ILC2s and ILC2–derived IL-5 and IL-13 are also increased in the gut (30), thus suggesting complex interactions between eosinophil survival and intestinal nutritional conditions.

**Migratory properties of intestinal eosinophils**

Most eosinophils generated in the bone marrow migrate to all segments of the gastrointestinal tract, with the exception of the esophagus, coming to reside in the intestinal lamina propria under baseline conditions (2). Prenatal mice have comparable numbers of eosinophils in the gastrointestinal tract as do adult mice; thus, at least relative to other leukocytes, eosinophil homing to the gastrointestinal tract seems to be independent of the enteric flora (15), likely mediated by constitutive ILC2 and eotaxin-1 (29). The recruitment of murine eosinophils to the gastrointestinal tract under the steady-state is regulated primarily by eotaxin-1, which is localized in Ly6c<sup>high</sup>CCR2<sup>+</sup>F4/80<sup>CD11b</sup> cells that are under the regulation of calprotectin (S100a8/S100a9) (31, 32). The importance of eotaxin-1 in regulating the baseline level of eosinophils in the gastrointestinal tract is underscored by the severe deficiency in eosinophils in the intestinal mucosa, without any significant decrease in bone marrow and peripheral blood eosinophils, in eotaxin-1–deficient mice (15, 33). By genomic analyses, two additional chemokines, designated eotaxin-2 and eotaxin-3, were identified on the basis of their eosinophil-selective chemotactic activity (2). The specific activity of the eotaxin subclass of chemokines is mediated by the G protein–coupled receptor CCR3, which is primarily expressed on eosinophils (Fig. 1A) (34, 35). Accordingly, deficiency in gastrointestinal
eosinophils in mice, with the targeted deletion of CCR3, supports the critical role of eotaxin-1 in the maintenance of intestinal eosinophils under homeostatic conditions (36). Although eotaxin-1 is the major chemokine required for the baseline level of eosinophils in the gastrointestinal tract, eotaxin-1 alone is unlikely to be sufficient, as it is abundantly expressed in the upper gastrointestinal segments (e.g., tongue, esophagus), and eosinophils are not normally present in these locations (15). Paired Ig-like receptor (PIR)-B, which is highly expressed in eosinophils and can suppress eotaxin/CCR3-mediated eosinophil migration to the small intestine, may be an inhibitory checkpoint for esophageal eosinophil trafficking (Fig. 1A) (37). Although it inhibits eosinophil migration, PIR-B supports IL-5–induced expansion of murine eosinophils by suppressing PIR-A–induced apoptosis of bone marrow eosinophils (38).

Eosinophils also express a number of adhesion molecules involved in cell trafficking, including integrin α4β7, integrin α4β1 (VLA-4), and the β2-integrin family (the CD18 family) (2, 26). Integrin α4β1 interacts with the endothelium via VCAM-1 and fibronectin, the CD18 family of molecules binds to ICAM-1, and α4β7 integrin interacts with the mucosal vascular addressin cell adhesion molecule 1 that is expressed by the vascular endothelium in the intestinal tract (2, 26). These integrins have prominent roles in eosinophil trafficking during inflammation rather than during homeostasis, as demonstrated by the reduced number of small intestinal eosinophils after oral allergen stimulation in β7 gene–targeted mice compared with nondeficient mice (26). Furthermore, in β7-deficient mice, eosinophil accumulation in the small intestine after *Trichinella spiralis* infection is delayed compared with nondeficient mice (39). The trafficking of eosinophils to inflammatory sites also involves a number of cytokines, particularly those of the Th2 type, such as IL-4, IL-5, and IL-13, of which only IL-5 is implicated in selective tissue distribution (2). Under baseline conditions, eosinophils in the Peyer’s patch are barely detected in mice; however, after IL-5 overexpression, they substantially localize to the interfollicular regions via eotaxin-1– and IL-5–dependent mechanisms (40).

Functional characteristics of intestinal eosinophils

Eosinophils have long been considered effector cells that have a protective role against parasitic helminth infection. However, eosinophils also are associated with numerous gastrointestinal disorders, such as EoE, eosinophilic gastritis, eosinophilic enteritis, and eosinophilic colitis, which are collectively referred to as EGIDs, as well as inflammatory bowel diseases.
Beneficial role of eosinophils

The antiparasitic functionality of eosinophils is based primarily on their increase in the circulation and affected tissues during helminth infections (41), as well as their ability to mediate in vitro Ab-dependent cellular toxicity against helminthes (42, 43). Murine models of parasitic infection demonstrated eosinophil recruitment to infected tissues and parasite death mediated by the release of toxic cytoplasmic granules, such as major basic protein (Fig. 1B) (44). Indeed, impaired resistance against secondary infection with intestinal Nippostrongylus brasiliensis is observed in ΔdblGATA mice (45). However, Schistosoma mansoni–infected ΔdblGATA and PHIL mice have normal disease progression, despite the recruitment of large numbers of eosinophils in the affected liver (46). In Trichinella spiralis–infected ΔdblGATA mice, the marked death of T. spiralis muscle larvae by inducible NO synthase–producing neutrophils and macrophages is observed, and excessive host inflammatory responses are linked to pathologic changes in infected muscle (47). Because transfer of eosinophils into ΔdblGATA mice restores larvae survival, eosinophils have been paradoxically implicated in parasite survival, specifically through the promotion of Th2 cell recruitment and the prevention of macrophage- and neutrophil-induced parasite death (47). Collectively, these data suggest that, under some conditions, there may be a symbiotic association between gastrointestinal eosinophils and parasites that could contribute to the maintenance of tissue homeostasis by allowing parasites to reside in the host tissues with limited consequences of such infection. Accordingly, we conclude that the eosinophil response to parasitic infection may vary by both helminth species and the specific tissue infected. Although murine models provide an opportunity to delineate the role of the eosinophil in parasite infection, it is important to point out that murine eosinophils are less effective than rat eosinophils in killing schistosomes (48) and do not bind IgE, which may be an effector mechanism for human eosinophils against parasites (49). Additionally, experimental infection with parasites in mice is unlikely to adequately mimic natural infections in human. Therefore, cautious interpretation of the murine results is warranted. Eosinophils express a broad range of pattern-recognition receptors, which supports their potential role in responses against viral and bacterial infections (50). RNA viruses, such as respiratory syncytial virus, are susceptible to the antiviral activities of eosinophils, particularly those mediated by eosinophil granule ribonucleases (e.g., eosinophil cationic protein and EDN) (51). IL-5–transgenic mice have improved clearance of Pseudomonas aeruginosa (52); however, it is possible that IL-5 mediates antibacterial effects independently of eosinophils (53). Even so, a previously unrecognized antibacterial function of eosinophils was demonstrated that involves eosinophils releasing their mitochondrial DNA in response to LPS from Gram-negative bacteria (Fig. 1B) (54). Together with granule proteins, the secreted eosinophil-derived mitochondrial DNA binds and kills bacteria in the extracellular matrix of the mice intestine (54). Considering the abundant numbers of eosinophils in the intestinal lamina propria, trapping bacteria with the DNA complexes of eosinophils (mitochondrial DNA nets) could be a highly effective mechanism for protecting the gastrointestinal tract against pathogenic bacterial invasion.

Nonbeneficial role of eosinophils in gastrointestinal disorders: primary EGID and IBD

The most common primary EGID, EoE, is a worldwide emerging disease representing the second most common cause of chronic esophagitis (55, 56). Although infiltration of eosinophils into the esophageal mucosa is the hallmark of EoE, accumulation of activated immune cells, such as mast cells, B cells, and T cells, as well as APCs, is also observed in active EoE (56, 57). Along with inflammatory cell infiltration, hyperplasia of esophageal epithelial cells is a general histologic characteristic of EoE (58). Although not fully understood, immune sensitization to a variety of foods and Th2-polarized allergic inflammation in the esophageal mucosa have been posited as the critical immunologic aspects of EoE development (57). In the absence of eosinophils, disease features, such as tissue remodeling (e.g., epithelial hyperplasia), collagen accumulation, and gastric motility, are attenuated, implicating eosinophils as key effector cells, at least in these animal models (59, 60). Eosinophil-derived TGF-β is implicated in tissue remodeling of EoE and induces expression of periostin, an extracellular matrix protein that increases eosinophil infiltration in the mucosal layer in patient biopsies, thus further promoting disease pathogenesis (56, 61). Genetic susceptibility in humans is linked to sequence variants at genetic locus 5q22 (encoding thymic stromal lymphopoietin), CRLF2 (the thymic stromal lymphopoietin receptor), FLG (filaggrin), and CCL26 (eotaxin-3), consistent with the complex interplay of epithelial cell gene products and Th2 immunity (62–64). Indeed, the Th2 cytokines IL-4, IL-5, and IL-13 are elevated in the esophageal mucosa, with IL-13 having a particularly important role in EoE pathogenesis (Fig. 1B) (56). IL-13 drives marked upregulation of eotaxin-3, thus promoting the chemotraction of CCR3+ eosinophils; further, IL-13 induces an EoE-like transcriptome in primary esophageal epithelial cell cultures (65) and triggers production of periostin in primary esophageal cultures (61). Impaired barrier function of the esophageal epithelium also was indicated as a potential pathophysiological mechanism, as verified, at least in part, by the decrease in desmosomal cadherin desmoglein 1, an ICAM, in active EoE (66, 67). In fact, downregulation of desmoglein 1 by IL-13 not only induces impaired barrier function of the esophageal epithelium, it also initiates a proallergic transcriptional response, including POSTN (periostin) expression (67). Considering that periostin can directly enhance eosinophil adhesion, the decreased expression of desmoglein 1 may further potentiate the inflammatory response of EoE by increasing migration of eosinophils.

IBDs are characterized by chronic inflammation of the intestine, and elevated levels of eosinophils have been observed in IBDs that correlate with disease severity (68, 69). Murine models of IBD have provided important insights about the role of eosinophils in their pathogenesis. Increased numbers and degranulation of eosinophils are observed in chemical-induced
models of IBD, and they are attenuated in eosinophil-deficient mice and eotaxin-1–deficient mice, which exhibit reduced clinical scores and pathology (70, 71). Progression of colonic inflammation is also attenuated with depletion of eosinophils by administration of anti–IL-5 or CCR3 Abs (72, 73). The tissue immune microenvironment is suggested to influence the downstream immune consequences mediated by eosinophils, leading either to exacerbation of local inflammatory responses or maintenance of tissue homeostasis (74).

Eosinophils as modulators of intestinal immune responses: interaction with T cells

Accumulating evidence suggests a role for eosinophils as modulators of T cell–mediated immune responses. Several studies found that eosinophils can express MHC class II and costimulatory molecules, which suggests a capacity to function as APCs. Despite the fact that blood eosinophils do not express MHC class II in the steady-state, human blood eosinophils stimulated with IL-3, IL-4, GM-CSF, and IFN-γ express MHC class II molecules (75, 76). Although the Ag-presenting capabilities of small intestinal eosinophils have not been examined in depth, intestinal eosinophils isolated from mice were found to express only relatively low levels of CD86 and MHC class II, which implies an inability to present Ags to naive CD4+ T cells under homeostatic conditions (77). Notably, eosinophils secrete an array of cytokines, such as IL-2, IL-4, IL-6, IL-10, and IL-12, along with TNF-α, which are capable of activating dendritic cells (2). Moreover, it was reported that EDN can activate dendritic cells by stimulating the TLR2-signaling pathway of those cells and by inducing their Th2-polarization capacity in mice (78). On the basis of these observations, eosinophils may constitute a portion of nonconventional APCs that promote the activity of dendritic cells, at least in the murine gastrointestinal tract (Fig. 1B).

Eosinophils as modulators of intestinal immune responses: role in mucosal IgA class switching

The gastrointestinal tract, exposed to potentially harmful commensals and airborne and ingested pathogens, protects itself via production of IgA, the most abundant Ab isotype in the human body for neutralization of microbes in a noninflammatory manner (79). Murine eosinophils in the bone marrow are known to support the survival of plasma cells by secreting a proliferation-inducing ligand (APRIL) (80). Together with B cell–activating factor, APRIL is known to induce IgA class switching in a T cell–independent manner (81). Therefore, small intestinal eosinophils may have a role in the gastrointestinal tract’s T cell–independent IgA production, specifically by producing APRIL. Although the involvement of intestinal eosinophils in IgA class switching has not been examined directly, the impaired IgA production reported in CD47-deficient mice suggests a potential role in IgA synthesis (82). As noted above, small intestinal eosinophils highly express SIRP-α, a cognate receptor for CD47, and SIRP-α/CD47 signaling contributes to the prolonged survival of murine intestinal eosinophils by regulation of their degradation (16). The impaired production of IgA in CD47-deficient mice may correlate with reduced viability of small intestinal eosinophils. The constitutive presence of eosinophils in the intestinal tissues suggests their potential role in IgA class switching in this location (Fig. 1B). In the healthy state, eosinophils are barely present in the Peyer’s patch or mesenteric lymph nodes (40), where T cell–dependent IgA class switching takes place. Therefore, it is more likely that eosinophils contribute to T cell–independent IgA class switching, which mainly occurs in the small intestinal lamina propria, where abundant numbers of eosinophils reside. However, eosinophils produce TGF-β, a cytokine critical for the Ab class switching toward IgA in response to T cell–dependent Ags (2, 83). Therefore, in terms of TGF-β secretion, eosinophils can be posited to play a supportive role in the induction of IgA class switching in organized lymphoid tissue. Notably, we observed an IgA deficiency in the intestinal lavage fluid and serum of genetically modified eosinophil-deficient mice (Y. J. Jung and M. E. Rothenberg, unpublished observations), which implicates gastrointestinal eosinophils as a regulator of IgA class switching in the intestine.

Eosinophils as modulators of intestinal immune responses: role in adipose tissue metabolism

The incidence of obesity has increased rapidly worldwide, and obesity-associated diseases, including insulin resistance, type 2 diabetes, fatty liver disease, atherosclerosis, and stroke, constitute major health problems (84). Inflammation is a key feature of obesity, and infiltration of proinflammatory macrophages, neutrophils, CD8+ T cells, CD4+ T cells, and mast cells is observed in visceral adipose tissue with obesity (85). Meanwhile, in nonobese, normal visceral tissue of mice, eosinophils and alternatively activated macrophages are observed, suggesting a role for eosinophils in adipose tissue metabolism (86). Alternatively activated macrophages in adipose tissue improve insulin sensitivity (glucose homeostasis), and eosinophils are necessary for that specific subset of macrophages (86, 87). Furthermore, the absence of eosinophils leads directly to adiposity and systemic insulin resistance in mice (88), implying a protective role for eosinophils against the development of type 2 diabetes (Fig. 1B). Maintenance of eosinophils in visceral adipose tissue is dependent on IL-5 and IL-13, and ILC2s expressing both of these cytokines promote the accumulation of eosinophils in visceral adipose tissue (88). Therefore, interaction between ILC2s and eosinophils not only supports survival of intestinal eosinophils as previously noted, it also seems to be implicated in metabolic homeostasis.

Conclusions

Eosinophils have been considered to be end-stage effector cells that are involved in host protection against parasitic infection and the development of inflammatory disorders. However, accumulating evidence now indicates that eosinophils are multifunctional leukocytes that have a broader role in host responses against a wide range of infections (bacteria and viruses) and are potentially key modulators of the intestinal immune system. Likely, they may intimately interact and/or regulate commensal intestinal microflora, especially in view of the antibacterial effect of their granule proteins (7) and ability to modify innate immunity. The recent recognition of EGIDs, which are increasing rapidly in prevalence, calls attention to the potential importance of translating the molecular and cellular immunological knowledge focused on gastrointestinal eosinophils into clinical treatment strategies. Indeed, the development of anti–eotaxin 1 (bertilimumab), anti–IL-5

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