The Emerging Role of Eosinophils as Multifunctional Leukocytes in Health and Disease

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ABSTRACT

Eosinophils are terminally differentiated cytotoxic effector cells that have a role in parasitic infections and allergy by releasing their granule-derived cytotoxic proteins. However, an increasing number of recent observations indicate that eosinophils are not only associated with the pathogenesis of a wide range of diseases, but also contribute to the maintenance of homeostatic responses in previously underappreciated diverse tissues, such as the gastrointestinal (GI) tract and adipose tissue. In this review, we describe biological characteristics of eosinophils, as their developmental properties, permissive proliferation and survival, degranulation activity, and migration properties enable them to distribute to both homeostatic and inflamed tissues. We describe pathologic aspects of eosinophils with a role in asthma and in various GI diseases, including eosinophilic GI disorders, inflammatory bowel disease, and radiation-induced enteropathy. Finally, we discuss the beneficial role of eosinophils, which contribute to the resolution of pathogenic conditions and to the modulation of homeostatic biologic responses.

Keywords: Eosinophils; Eosinophil granule proteins; Hypersensitivity; Eosinophilic gastrointestinal disorders; Homeostasis

INTRODUCTION

Eosinophils are terminally differentiated leukocytes that make up approximately 1%–5% of peripheral blood leukocytes in healthy individuals (1,2). Eosinophils are produced in the bone marrow, released into the peripheral blood, and finally migrate to inflammatory sites, as well as peripheral tissues in homeostasis, in response to stimulating signals, primarily IL-5 and eotaxin-1 (CCL11) (3,4). During homeostasis, most eosinophils reside in tissues, including the thymus, uterus, adipose tissue, and lamina propria of the gastrointestinal (GI) tract, indicating the various physiological roles of these cells (5,6). Mature eosinophils possess cytotoxic granules containing major basic protein (MBP), eosinophil-derived neurotoxin, eosinophil peroxidase, and eosinophil cationic protein (ECP) (3,7). In addition to releasing these cytotoxic cationic proteins, eosinophils release a variety of cytokines, chemokines, lipid mediators, and neuromodulators that are associated with multiple biological responses in the specific locations where eosinophils are distributed (5,6). Although eosinophils are...
perceived as effector cells implicated in the pathogenesis of type-II immune responses (i.e., helminth infection and allergic asthma) (2,5), eosinophils are increasingly recognized as multifunctional leukocytes, based on their wide tissue distribution and production of an array of immune mediators (5,8). In this article, we discuss recent advances in understanding the roles of tissue-distributed eosinophils in homeostasis and various inflammatory states.

BIOLOGICAL CHARACTERISTICS OF EOSINOPHILS

Development of eosinophils
Eosinophils develop in the bone marrow and lineage specification is determined by the interplay of GATA-binding protein 1 (GATA-1), the E26 transformation-specific family member PU.1, IFN consensus sequence binding protein, and CCAAT-enhancer-binding protein family members (Fig. 1) (3). GATA-1 is the most important transcription factor for the development of eosinophils, as demonstrated by loss of eosinophils in mice with targeted deletion of the high-affinity GATA-1 binding site in the Gata1 promoter (9). Differentiation and proliferation of eosinophils are further regulated by IL-5, IL-3 and GM-CSF, which share a common β-chain receptor (Fig. 1) (10). Although IL-3 and GM-CSF stimulate multi-lineage hematopoietic cells, IL-5 is the most specific cytokine secreted for inducing the selective differentiation and mobilization of eosinophils from the bone marrow during allergic inflammation, as well as for the homing of eosinophils into various tissues in the steady state (11). In addition to T H2 cells, group 2 innate lymphoid cells (ILC2s) are a major non-T cell source of IL-5 (Fig. 1) (12). ILC2-derived IL-5 supports the maintenance of small intestinal eosinophils (13) and metabolic homeostasis by promoting eosinophil accumulation in visceral adipose tissue (14). GM-CSF is constitutively secreted by intestinal epithelial cells and supports the survival and homeostatic functions of small intestinal eosinophils (15,16). IL-25, IL-33, and thymic stromal lymphopoietin are produced by epithelial cells during inflammation as well as homeostasis, and they also function as important regulators of eosinophils by inducing the secretion of T H2-type cytokines (17-19).

Eosinophils also express inhibitory receptors, such as sialic acid-binding Ig-like lectin 8 (Siglec-8), paired Ig-like receptor B (PIR-B), and CD300a (Table 1) (20). Activation of Siglec-8 inhibits survival of eosinophils by inducing apoptosis and the generation of reactive oxygen species (20). PIR-B and CD300a inhibit survival signals for eosinophils induced by IL-5 (21,22). Therefore, it is plausible to suggest that the proliferation and tissue accumulation of eosinophils is modulated by a complex interaction between pro-survival signaling factors, such as IL-3, IL-5, and GM-CSF, and eosinophil-expressed inhibitory receptor signaling.

Migration of eosinophils
Although IL-5 promotes the activation and survival of eosinophil in the periphery, tissue distribution of eosinophils can be independent of IL-5, as demonstrated by a presence of residual tissue eosinophils in IL-5-deficient mice (11). Recent studies have demonstrated an important role for the eotaxin subfamily of chemokines in eosinophil recruitment to tissue (5). Eotaxin includes 3 family members, eotaxin-1 (CCL11), eotaxin-2 (CCL24), and eotaxin-3 (CCL26), expressed within stromal cells and tissue epithelial cells (Fig. 1). Eotaxin-induced extravasation and migration of eosinophils into tissues is primarily dependent on the expression of C-C chemokine receptor 3 (CCR3), the 7-transmembrane spanning, G protein-coupled receptor expressed on eosinophils (Fig. 1 and Table 1) (23,24). Therefore, eotaxin
promotes selective recruitment of eosinophils into eotaxin-expressing tissues cooperatively with IL-5 via an IL-5-independent manner.

Eosinophils also express numerous adhesion molecules, including α4β7 and α4β1 integrin (Fig. 1 and Table 1) (3). α4β1 mediates the interaction of eosinophils with endothelium via vascular cell adhesion molecule 1 (VCAM-1). Eosinophil recognition of VCAM-1 by α4β1 is an important mechanism for the selective infiltration of eosinophils over neutrophils, as there is little or no expression of α4β1 detected on purified human neutrophils (25). α4β7 interacts with mucosal addressin cell adhesion molecule 1 (MAdCAM-1), expressed by vascular endothelium in the intestinal tract (3). Along with CCR3, α4β7 in eosinophils has a role in the homeostatic recruitment of eosinophils into the GI tract, due to the constitutive expression of MAdCAM-1 in the intestine (26). However, α4β7-mediated eosinophil trafficking to the...
small intestines is more prominent during inflammation than homeostasis, as demonstrated by the reduced number of eosinophils in β7-deficient mice after oral allergen challenge compared to non-β7-deficient mice (27). Eosinophil trafficking to inflammatory tissues also involves a number of cytokines and chemokines, particularly the T1,2 cytokines, such as IL-4, IL-5, and IL-13.

**Tissue distribution of eosinophils**

Eosinophils are enriched in the thymus, adipose tissue, and lamina propria of the GI tract during homeostatic conditions, although recruitment and accumulation of tissue eosinophils has largely been studied in T1,2 cell-mediated inflammatory conditions (Fig. 1) (28). The GI tract harbors the largest number of tissue-resident eosinophils in the body (e.g., 20%–30% of the total number of leukocytes), whereas eosinophils in the adipose tissue and the lung constitute only ≤4% of the stromal/vascular fraction and ≤1% of total leukocytes, respectively (29,30). Eosinophils are recruited to the GI tract and thymus independent of the microbiota, as demonstrated by detectable level of eosinophils in prenatal mice (26,31), while resident eosinophils in the lung are speculated to be influenced by the microbiota given their postnatal emergence (12). Unlike thymus eosinophils, which decrease after peaking around 2 wk after birth (31), eosinophils in the GI tract are sustained at high levels throughout life (26).

Mature eosinophils are postmitotic and have a limited life-span in the absence of survival-promoting signals (1). The half-life of eosinophils in the lung and blood is relatively shorter than eosinophils in the thymus, uterus, and small intestine (32). Cytokine signaling through the common γ-chain increases eosinophil survival; this receptor is expressed on intestinal and uterine eosinophils but is absent from lung resident eosinophils (28,32). The influence of the tissue microenvironment on eosinophil survival implies the capacity of these cells to respond to and integrate tissue-tropic signals.

**Degranulation of eosinophils**

Eosinophils may secrete the entire content of granules by classical exocytosis, whereby intracellular granules fuse with the plasma membrane and release granule contents (33). Classical exocytosis of eosinophils has rarely been observed in vivo, although it is observed upon interaction of eosinophils with metazoan parasites in vitro (34). In most other
physiologically relevant conditions, tissue eosinophils secrete their granules secondary to
cytolysis (cytolytic degranulation) or through a vesicle-dependent process termed piecemeal
degranulation (33).

Activated eosinophils exhibit a non-apoptotic extracellular trap cell death (ETosis), which
is characterized by nuclear-derived DNA traps released by lytic eosinophils (35). ETotic
eosinophils show extracellular release of intact membrane-bound granules, as opposed to
the membrane-free granule-derived proteins observed in neutrophil ETosis (36). As intact
eosinophil granules released by ETosis do not have phosphatidylserine exposed on the
external leaflet of the membrane, they can escape from macrophage phagocytic clearance
(35). In addition, competent cytokine, chemokine, and eicosanoid receptors remain on
eosinophil granules released by ETotic degranulation (36,37). Therefore, ETotic eosinophils
and released granules not only induce long-lasting inflammation, but may also have
immunoregulatory roles in distributed tissues.

Piecemeal degranulation represents the release of bits of granule contents by transporting them
to the cell surface in membrane-bound cytoplasmic vesicles, while eosinophils are viable (4).
Therefore, piecemeal degranulation is the most physiological form of eosinophils degranulation,
which occurs in response to a cytokine or chemokine stimulus present in the tissue (4).

EOSINOPHILS AS PRO-INFLAMMATORY DESTRUCTIVE
CELLS IN DISEASE

Eosinophils in asthma
Asthma is a chronic inflammatory disease that is characterized by airway hyperreactivity and
obstruction in response to various allergens (38). Eosinophil infiltration is a common feature
of asthma, as they are recruited from the bloodstream to the airways by cytokines that are
primarily released from activated T_{h}2 cells and by an array of cytokines (39). Activated airway
eosinophils contribute to mucus hypersecretion, airway fibrosis, and dysregulation of airway
nerves, indicating a critical role of eosinophils in the pathogenesis of airway inflammation
(40). Extracellular DNA traps released by lytic eosinophils contribute to the sticky secretions
and subsequent plugging of mucus (35,41). Furthermore, fibrogenic mediators, such as
TGF-β, matrix metalloproteinase-9, VEGF, and granule proteins released by eosinophils
induce airway remodeling by promoting fibroblast proliferation and the production of
collagen and glycosaminoglycans (40).

Airways are densely innervated by sensory and parasympathetic nerves that regulate
airway tone (42). Therefore, changes in either the sensory or parasympathetic tone
induce bronchoconstriction triggered by a variety of stimuli, such as histamine, cold air,
and allergens (42). Eosinophils are recruited to airway nerves in asthma, as eotaxin-1 is
constitutively expressed by sensory and parasympathetic nerves and its expression is
increased with allergen challenge (43). Eosinophils that infiltrate into the airway induce
bronchoconstriction by increasing parasympathetic activation, as eosinophil granules,
especially MBP, allosterically inhibit M2 muscarinic receptors, which reduce acetylcholine-
induced bronchoconstriction (44). In addition, eosinophils induce sensory nerve-mediated
bronchoconstriction, as demonstrated by increased airway sensory innervation in transgenic
mice with airway eosiophilia derived by over-expression of IL-5 from airway epithelium (42).
Eosinophils influence the structure and function of sensory nerves by increasing expression
of neuropeptides, such as substance P (Table 1) (42), and by altering electrical stimulation (45), respectively.

**Eosinophils in various GI diseases**

In spite of the abundant number of eosinophils in a homeostatic GI tract, eosinophils also are associated with primary eosinophilic GI disorders, such as eosinophilic esophagitis (EoE), eosinophilic gastritis, eosinophilic enteritis, eosinophilic colitis, and with inflammatory bowel disease (IBD) (1). Although eosinophils are not normally found in the esophagus, infiltration of eosinophils (≥15 eosinophils per high power field) in the esophageal epithelium with associated symptoms, such as dysphagia and food impaction, is the hallmark of EoE (3,46). Dysregulated eosinophilia, induced by an immune sensitization to a variety of foods, and subsequent T_hi-polarized allergic inflammation has been posited as critical to the development of EoE pathogenesis (3,4). A whole-genome messenger RNA esophageal expression analysis identified a unique EoE transcriptome (47), which derived a diagnostic panel consisting of 96 genes, reflecting the immune pathogenesis of EoE (48). Eosinophilic gastritis is the second most common form of eosinophilic GI disorders, but consensus recommendations for either clinical or pathological diagnosis do not exist (49).

Although the role of eosinophils in IBD development has not been fully elucidated, eosinophils are one of the many inflammatory cells involved in the pathogenesis of IBD, as demonstrated by disease severity correlated with eosinophilia (50). Recent observations suggest that neuronal hyperexcitability induced by eosinophils might account for the neuronal alteration of IBD patients, either by promoting secretion of neuropeptides and inflammatory mediators (51,52), or by inducing neuronal damage through degranulation (53). Considering that the protective role for anti-inflammatory lipid mediators produced by eosinophils has been reported in a mouse model of acute colitis (54), eosinophils in the early stages of intestinal inflammation may not necessarily be an indicator of disease, but rather a homeostatic response promoting mucosal healing and subsequent intestinal health.

Although radiation therapy plays a curative role in many cancers, radiation enteropathy, which includes mucosal inflammation, atrophy, and intestinal wall fibrosis, is frequently observed in patients who receive pelvic or abdominal radiation therapy, with a significant impact on patients’ quality of life (55,56). A recent study demonstrated a bi-directional interaction of eosinophils with α-smooth muscle actin-positive (α-SMA+) stromal cells for inducing radiation-induced intestinal fibrosis in a T_hi2 cell independent manner (57). α-SMA+ cells, stimulated by dead crypt cell-derived adenosine triphosphate, express upregulated eotaxin-1, thus attracting eosinophils into the submucosa (57). α-SMA+ cells also support survival of eosinophils by producing GM-CSF; TGF-β from GM-CSF-stimulated eosinophils promotes collagen expression in α-SMA+ cells (57). Therefore, blocking either adenosine triphosphate signaling of α-SMA+ cells or eotaxin-1/CCR3-dependent eosinophil chemotaxis can be a promising approach for treating radiation-induced intestinal fibrosis.

**EOSINOPHILS AS BENEFICIAL REGULATORS IN HOST DEFENSE AND MODULATORS OF TISSUE HOMEOSTASIS**

**Pathogen recognition and clearance of pathogens**

Eosinophils express a broad range of pattern-recognition receptors, including TLRs, nucleotide-binding oligomerization domain-like receptors, retinoic acid-inducible gene-I-
like receptors, C-type lectin receptors and a receptor for advanced glycation end products (Table 1), which supports their potential role in responses against pathogen-associated molecular patterns induced by viral, bacterial, and fungal infections (58).

RNA viruses, such as respiratory syncytial virus, are susceptible to the antiviral activities of eosinophils, particularly those mediated by TLR7-induced production of eosinophil-associated ribonucleases (59,60). In this context, eosinophils cooperate with macrophages to limit the progression of respiratory syncytial virus infection from uninfected epithelial cells, suggesting a positive role of eosinophils in clearing viral infections through TLRs (6,61). However, eosinophil-mediated suppression of IFNs in human rhinovirus-infected epithelial cells and the subsequent increase of viral load indicate the complexity of eosinophil function in viral infections (62).

The role of eosinophil granule proteins is highlighted in bacterial infections, as demonstrated by the antibacterial effect of the binding of ECP to bacterial LPS and peptidoglycans (63). Together with granule proteins, the secreted eosinophil-derived mitochondrial DNA binds and kills bacteria in the extracellular space of the intestine in a reactive oxygen species-dependent and eosinophil death independent manner (64). Considering the abundant numbers of eosinophils in the GI tract, trapping bacteria using eosinophil mitochondrial DNA nets could be a highly effective mechanism for protecting the GI tract against pathogenic bacterial invasion.

Supporting regeneration of injured tissue

Damaged epithelial cells stimulate eosinophils to produce growth factors, such as TGF-β, VEGF, and fibroblast growth factor that induce remodeling of allergic inflammatory tissues by increasing extracellular matrix proteins, smooth muscle mass, and angiogenesis (6,65). Eosinophil-derived growth factors also have beneficial roles in tissue repair, and eosinophil-mediated repair of injured tissue has been demonstrated by recent studies showing that eosinophil IL-4 production is necessary during muscle repair and hepatocyte regeneration (66,67). Eosinophils are rapidly recruited to damaged muscle and secrete IL-4 to activate the regenerative action of muscle resident fibro/adipocyte progenitors to support myogenesis while inhibiting adipocyte differentiation (67). Injured liver also induces recruitment of eosinophils by upregulating eotaxin-1, and IL-4 secreted by infiltrated eosinophils promotes the proliferation of quiescent hepatocytes (66). These observations indicate that eosinophil-derived IL-4 contributes to tissue repair in highly regenerative organs by stimulating resident progenitors within the respective organs. Meanwhile, IL-5-overexpressing transgenic mice show delayed wound healing with an increase of eosinophils and CD4+ cells (68), implying that tissue regenerating properties of eosinophils can be modulated by immune responses within the local microenvironment.

Modulation of the anti-tumor microenvironment

Tumor infiltrating eosinophils are frequently observed in several types of solid tumors, including gastric, colorectal, esophageal, head and neck squamous carcinoma, breast, ovary, and prostate cancer, and have been associated with favorable prognosis (40,69). However, the role of eosinophils in cancer is controversial since tumor-associated tissue eosinophils and peripheral blood eosinophilia have been associated with both favorable and poor prognoses (70). An array of receptors and mediators expressed by eosinophils have been suggested to function in their anti-tumor activities in GI and head and neck cancers, as demonstrated by the anti-tumorigenic activity of eosinophils in colorectal cancer through increased
recruitment, prolonged survival, and degranulation-mediated cytotoxicity (71). Indeed, the tumor-derived alarmin, IL-33, induces eotaxin-1-mediated eosinophil recruitment around hepatocellular carcinoma and breast cancer, and the subsequent degranulation of eosinophils leads to tumor cell cytotoxicity in a T cell-independent manner (72).

Tumor-associated eosinophils are also associated with a poor prognosis, as observed in Hodgkin lymphoma, which indicates a strong correlation between neoplastic proliferation and tissue eosinophilia (73). Eosinophils can promote tissue healing and repair, as these cells store and release multiple growth factors and cytokines (3,5), that can promote fibroblast proliferation and tumor angiogenesis. In addition, eosinophils store Th2 cytokines, such as IL-4 and IL-13, which can direct macrophage polarization to the immunosuppressive tumor-associated M2 state (29,74).

The divergent prognostic outcomes associated with tumor-associated eosinophils may indicate the functional heterogeneity of eosinophils influenced by tumor microenvironments, as demonstrated by the opposing functions of macrophages, neutrophils, and dendritic cells affected by activation state (70).

**Modulation of IgA class switching**

Ab-secreting plasma cells that differentiate from activated B cells account for long-term humoral immunity in an Ag and T cell independent way (75). The differentiation and survival of long-lived plasma cells in bone marrow is promoted by cytokines, including a proliferation-inducing ligand (APRIL), IL-6, IL-10, and TNF; murine eosinophils in the bone marrow are a major source of APRIL and IL-6 (Table 1) (76). There are a decreased number of plasma cells in the bone marrow of eosinophil-deficient mice, both in the homeostatic and immunization states (76). Unlike the dominant role of IgG Abs in systemic humoral immune responses, humoral immunity at mucosal tissue is predominantly mediated by IgA (5).

The GI tract, exposed to potentially harmful commensals, as well as airborne and ingested pathogens, produces the most IgA in the human body, which is secreted by plasma cells and exerts a front-line defense with neutralization of microbes in a non-inflammatory manner (77). Intestinal eosinophils support IgA class switching, as demonstrated by decreases of IgA and IgA-secreting lamina propria B cells in mice in which eosinophils were either depleted or genetically ablated (5,78,79). The defective production of IgA in eosinophil-deficient mice is associated with impaired mucus secretion, intestinal microbial composition and Peyer’s patch development (79), thus indicating the profound function of eosinophils in the maintenance of homeostatic intestinal immune responses.

**Modulation of adipose tissue homeostasis**

Adipose tissue macrophages represent the most abundant leukocytes in adipose tissue, and are involved in inflammatory responses and regulation of insulin sensitivity (80). The majority of macrophages in lean adipose tissue are immunosuppressive alternatively activated M2 macrophages, while obese adipose tissue is populated with M1 macrophages that secrete inflammatory cytokines and promote insulin resistance associated with low-grade inflammation (80). The Th2 cytokines IL-4, IL-13, and IL-10 drive polarization of macrophages toward an M2 phenotype; adipose tissue eosinophils are closely associated with M2 polarization, due to their constitutive production of IL-4 (29,81). Accordingly, eosinophil-deficient mice fed a high-fat diet show glucose tolerance and insulin resistance (29,82). Conversely, weight gain induced by a high-fat diet can be inhibited with a sufficient number of eosinophils, as shown in eosinophilic IL-5 transgenic or parasite-infected mice.
These results imply a protective role for eosinophils against obesity-associated development of metabolic abnormalities. IL-4 production by adipose tissue eosinophils also promotes remodeling of subcutaneous white adipose tissue into thermogenic beige fat in a M2 macrophage and myeloid cell-derived catecholamine-dependent manner (83). Maintenance of eosinophils in adipose tissue is dependent on IL-5 and IL-13; ILC2s expressing both cytokines promote the accumulation of eosinophils in adipose tissues (14). Collectively, it is plausible to suggest that the coordinated interaction among eosinophils, M2 macrophages and ILC2s in adipose tissue contributes to metabolic homeostasis.

CONCLUSION

With recent technology advances, the understanding of eosinophils has changed from the view of inflammatory effector cells that are involved in parasitic infections and allergic inflammation to multifunctional leukocytes involved in a wide range of inflammatory, as well as homeostatic responses. Early studies on the roles of eosinophils as end-stage allergic effector cells focused on the effects of eosinophil degranulation, with release of cationic proteins that are uniquely packaged within eosinophil granules. However, eosinophils are equipped with an array of cytokines, chemokines, enzyme, and lipid mediators and express a wide range of receptors that potentially support interactions with the signals provided by the microenvironment. An intricate, eosinophil-centered, signaling network comprising T_{h2} cells, B cells, and locally-residing cells is involved in pathogen clearance, tissue regeneration, modulation of anti-tumor responses, and homeostatic intestinal and metabolic responses. Meanwhile, similar responses mediated by dysregulated eosinophils accounts for the development of tissue damage in response to parasite infection, allergic inflammation, and autoimmune disease. Considering that eosinophils are primarily tissue-resident cells, delineating the local stimuli that specifically modulate eosinophils will contribute to characterization of heterogeneous diseases associated with dysregulated eosinophils, and to a wide range of responses regulated by homeostatic eosinophils.

ACKNOWLEDGEMENTS

This work was supported by funding from the National Research Foundation of Korea (2020R1A2C1003351), funded by the Ministry of Science and ICT of the Korean government.

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