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EOSINOPHILS: MULTIFUNCTIONAL AND DISTINCTIVE PROPERTIES

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1. Introduction

Eosinophils are capable of producing a wide variety of pro-inflammatory mediators and immunoregulatory molecules. Eosinophils are normally resident in mucosal tissues and, during Th2-type immune responses, they are recruited from bone marrow and blood to the sites of inflammation. Previously, eosinophils have been considered pro-inflammatory cells involved in host protection against parasite infection and immunopathology of allergic diseases. Recent findings suggest that eosinophils may also be involved in tissue homeostasis, modulation of adaptive immune responses, and innate immunity to certain microbes. Therefore, it would be reasonable to consider the eosinophil a multifunctional leukocyte that contributes to a wide variety of physiological and pathological processes depending on their location and activation status. This article summarizes the perspective concerning the multifaceted immunobiology of eosinophils and discusses the distinct biological features of eosinophils.

2. Eosinophils at baseline condition

The eosinophil is primarily a tissue-dwelling cell [1]. In healthy individuals, most eosinophils are found in the gut, mammary gland, uterus, thymus, bone marrow and adipose tissues [2]. Eosinophil localization to these tissues is likely mediated by constitutively expressed eotaxin-1. Newly identified type 2 innate lymphoid cells (ILC2s) also likely play a role [3]. Eosinophils in these tissues may be involved in tissue morphogenesis and maintenance of homeostasis. For example, eosinophils may prepare the mature uterus for pregnancy and blastocyst implantation [4], and may be involved in postnatal mammary gland development [5]. In addition, eosinophils may maintain epithelial barrier integrity [6] and/or innate host defense in the gastrointestinal tract [7]. Eosinophils in white adipose tissues produce interleukin 4 (IL-4) and support alternatively activated macrophages, resulting in maintenance of metabolic homeostasis and enhanced glucose tolerance [8].

Another potential role for eosinophils under baseline conditions may be immune homeostasis. In the thymus, eosinophils are suggested to be involved in major histocompatibility complex (MHC) class I-restricted thymocytes deletion [9]. Eosinophils

were associated with apoptotic bodies in MHC class I-restricted male antigen T cell receptor (TCR) transgenic mice injected with a cognate antigen. In bone marrow, eosinophils localize together with plasma cells, and they support survival of plasma cells by secreting a proliferation-inducing ligand (APRIL) and IL-6 [10]. Depletion of eosinophils induced apoptosis in long-lived bone marrow plasma cells. Altogether, this evidence suggests that steady-state presence of eosinophils under baseline conditions may play roles in tissue and immune homeostasis in various organs. Further studies will be necessary to determine whether these observations in mice apply to humans.

3. Regulation of immune response and tissue condition by eosinophils

3.1. Eosinophil production of immunoregulatory molecules

Eosinophils can perform various immune regulatory functions likely through two main mechanisms: production of a range of immunomodulatory molecules; and presentation of antigens [11, 12]. Eosinophils produce cytokines, which are able to act on eosinophils themselves, the so-called “autocrine” cytokines, including IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [13]. Other eosinophil-derived cytokines can influence tissue cells and other inflammatory cells. For example, eosinophil-derived transforming growth factor- β (TGF- β) enhances proliferation and collagen synthesis of lung and dermal fibroblasts [14]. TGF- α produced by cytokine-activated eosinophils increases mucin production by airway epithelial cells [15]. Other tissue growth factors, such as osteopontin, vascular endothelial cell growth factor (VEGF) and nerve growth factor (NGF) are also produced by eosinophils [16]. IL-4 protein has been localized to eosinophils in airway [17] and skin [18] tissue specimens from patients with allergic diseases. When stimulated with eotaxin-1 (CCL11) or RANTES (CCL5), human eosinophils rapidly release stored IL-4 by vesicular transport to the local milieu [19]. Several other cytokines and chemokines are also transcribed and/or produced by eosinophils, including IL-6, IL-10, tumor necrosis factor (TNF)- α and a variety of chemokines [12].

Eosinophils and their products can affect the functions of immune cells independent of cytokines. Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catalyzes the oxidative catabolism of tryptophan to kynurenines; kynurenines inhibit proliferation and promote preferential apoptosis of Th1 cells [20]. Human eosinophils from allergic donors constitutively express IDO [21]. Eosinophil-derived neurotoxin (EDN), an eosinophil granule protein, has potent immunomodulatory capacity. EDN is a chemoattractant [22] and activator [23] of dendritic cells (DCs). As a consequence, EDN enhances Th2 responses through a Toll-like receptor 2 (TLR2)-dependent mechanism [24]. Finally, eosinophils possess the ability to internalize, process and present antigenic peptides within the context of MHC class II. They also have the capacity to provide co-stimulatory signals to T cells through surface expression of molecules, such as CD80, CD86 and CD40 [25]. Indeed, antigen-pulsed eosinophils instilled intratracheally into recipient mice were sufficient for the expansion of Th2 cells within draining lymph nodes in vivo.

3.2. Tissue remodeling and immunoregulatory functions of eosinophils in vivo

The immunoregulatory functions of eosinophils are clearly demonstrated in murine models of allergen sensitization and challenge, exposure to natural allergens, and helminth infection. An early wave of eosinophil influx into inflammation sites precedes that of lymphocytes [26, 27] and it occurs even in mice deficient in adaptive immunity [28, 29]. This 'innate' eosinophil infiltration is likely mediated by ILC2s that have the capacity to produce a large quantity of type 2 cytokines [27, 30]. Interestingly, in IL-5/eotaxin double-knockout mice, in which eosinophil numbers in both blood and tissues are severely decreased, IL-13 production by Th2 cells in response to antigen challenge is attenuated [31], suggesting regulation of the adaptive Th2-type immune response by eosinophils.

Eosinophil-deficient animals that were generated by genetic manipulation show comparable findings. A strategy to deplete eosinophils used an eosinophil-specific promoter that drives expression of a cytotoxic protein, diphtheria toxin A chain (PHIL mice) [32]. Another strategy deleted a high-affinity GATA-binding site in the GATA-1 promoter (dbl-GATA), resulting in specific ablation of the eosinophil lineage [33]. When these mice were sensitized and challenged with ovalbumin antigen, Th2-type airway inflammation and asthma-like pathology (e.g. airway hyperreactivity and mucus production) were attenuated, and these responses were restored by reconstitution of eosinophils alone [34] or a combination of eosinophils and antigen-specific T cells [35]. The deficiency in eosinophils resulted in the defect of airway chemokine responses [34, 35], suggesting that eosinophils may be involved in priming of the tissue environment for effective mobilization of Th2 cells. Human clinical studies confirmed some aspects of these observations. For example, an improvement in airway remodeling was observed in asthma patients treated with anti-IL-5 [36]. Overall, eosinophils can provide a wide variety of growth factors, cytokines and chemokines, suggesting that eosinophils are potentially involved in diverse biological responses, from tissue remodeling to activation of immune cells.

4. Eosinophil proinflammatory and cytotoxic functions

4.1. Eosinophil granule proteins

Traditionally, eosinophils are considered effector cells with pro-inflammatory and destructive capabilities. Human eosinophils contain several highly basic and cytotoxic granule proteins, including major basic protein (MBP), MBP2, eosinophil cationic protein (ECP), eosinophil peroxidase (EPX) and EDN. Human MBP is a highly basic and amphipathic protein rich in arginine [1]. Because human MBP binds to and disrupts the membrane of *Schistosoma*, it directly damages *S. mansoni* [1]. MBP is also cytotoxic against other helminths and certain bacteria, such as *Staphylococcus aureus* and *Escherichia coli*. Human MBP is toxic to mammalian cells by disrupting the integrity of lipid bilayers [37]. Human ECP is another basic protein with an isoelectric point (pI) of 10.8 [1]. ECP possesses ribonuclease (RNase) activity and this RNase activity is required for the neurotoxic properties of the protein. Human EDN is another RNase and is a powerful neurotoxin that can severely damage myelinated neurons [1]. While EDN shows marked amino acid sequence homology (67%) with ECP, EDN has about 100 times more RNase activity than ECP. EDN shows significant toxicity against newborn larvae of *Trichinella*

spiralis. Importantly, EDN as well as ECP have antiviral activities and decrease the infectivity of respiratory syncytial virus (RSV) dependent on their RNase activities [38]. In guinea pigs infected with parainfluenza, pretreatment with anti-IL-5 and reduction of eosinophils markedly increased the viral content in the airways [39]. EPX is a member of a mammalian peroxidase family. EPX is a central participant in generating reactive oxidants and radical species by activated eosinophils [1]. EPX plus H₂O₂ and halide (Cl⁻, Br⁻, or I⁻) kills a variety of microorganisms.

4.2. Mechanisms of eosinophil activation

Extensive studies have been performed to elucidate the mechanisms by which eosinophils release these cytotoxic granule proteins and other inflammatory mediators. IL-3, IL-5 and GM-CSF, besides being important growth and maturation factors for eosinophils, stimulate effector functions of mature human eosinophils [40]. Other Th2 cytokines, such as IL-4 and IL-13, also activate eosinophils [41]. A prototypic Th1 cytokine, interferon (IFN)- γ , activates eosinophils, but does so in a delayed manner [42]. More recently, the cytokines derived from the epithelium, such as IL-33 and thymic stromal lymphopoietin (TSLP), have been implicated in promoting Th2-type immune response [43]. Both TSLP [44] and IL-33 [45] activate eosinophil effector functions such as adhesion to matrix proteins, cytokine production and degranulation. Interestingly, IL-33 does not affect neutrophil functions [45], suggesting that IL-33 may regulate functions of eosinophils specifically in airway mucosa. Human eosinophils recognize a wide variety of products that are expressed or produced by microbes, resulting in cellular activation and release of inflammatory mediators. For example, human eosinophils constitutively express TLR1, TLR4, TLR7, TLR9 and TLR10 messenger RNAs, and the TLR7 ligand, R-848, can activate eosinophils [46].

4.3. Distinct mechanisms of eosinophil activation

Although cellular activation induced by exposure to cytokines and TLR ligands are commonly observed in various types of inflammatory cells, eosinophils appear to have several unique, although perhaps not specific, properties. Proteolytic enzymes, which are produced by microbes and allergens, potentially induce activation and non-cytotoxic degranulation of eosinophils. Proteases, especially serine proteases, activate hematologic and interstitial cells through a family of G-protein-coupled protease-activated receptors (PAR) and induce production of several pro-inflammatory mediators [47, 48]. Authentic proteases, such as trypsin and papain, potentially induce human eosinophils to degranulate and produce superoxide anion [48]. Importantly, eosinophils are activated by a natural cysteine protease from mite allergens, *Derf 1* [48]. Eosinophils also recognize the aspartate protease activity and cysteine protease activity that are produced by the fungus *Alternaria alternate* [49] and cockroaches [50], respectively, through PAR-2. Thus, human eosinophils are equipped with machineries that recognize and respond to proteases, such as those found in microbes and at allergic response sites, resulting in active release of pro-inflammatory mediators.

An association between fungal exposure and asthma has been widely recognized [51], and the products of fungi may contribute to the development and exacerbation of allergic airway diseases. Indeed, extracts of the fungus *Alternaria* strongly activate eosinophils, resulting in

degranulation, increases in intracellular calcium concentration, cell surface expression of CD63 and CD11b, and production of IL-8 [52]. Interestingly, *Alternaria* does not induce neutrophil activation, suggesting specificity of the responding cell type. In addition, when human eosinophils are exposed to live *Alternaria alternata* fungus, eosinophils release their cytotoxic granule proteins into the extracellular milieu and onto the surface of fungal organisms, killing the fungus in a contact-dependent manner [53]. Eosinophils use their versatile $\beta 2$ integrin molecule, CD11b or Mac-1, to recognize and adhere to a major fungal cell wall component, β -glucan.

Eosinophil activation can be induced by other biological molecules. During tissue injury, endogenous molecules are released by stressed or damaged tissues, including uric acid, adenosine triphosphate (ATP), high mobility group box (HMGB)-1 and S100 calcium-binding protein family members [54]. Eosinophils are attracted to uric acid crystals, and produce a large quantity and various kinds of cytokines and chemokines [55]. The endogenous ATP produced by eosinophils themselves likely provides a positive feedback signal to eosinophils through a P2Y purinoreceptor, P2Y2. Similarly, HMGB-1 attracts eosinophils and enhances their survival in vitro [56]. Thus, eosinophils may recognize damaged tissues/cells, and thereby may contribute to attaining tissue homeostasis or modulating the inflammatory and immunological processes.

4.3. Roles for cellular adhesion

One of the unique and important biological characteristics of eosinophils is their ability to recognize organisms with a large surface. For example, initial studies of eosinophil degranulation in vitro used parasites as a model. Eosinophils incubated with antiserum-coated schistosomula of *S. mansoni* degranulate and release MBP [1]. Furthermore, 'artificial parasites', namely Sepharose beads coated with immunoglobulin G (IgG), IgA and secretory IgA (sIgA), stimulated eosinophil degranulation [57]; sIgA was the most effective of these immunoglobulins. Eosinophils possess a binding site(s) for the secretory component of secretory IgA [58], and the interaction with the secretory component greatly enhanced the pro-inflammatory functions of eosinophils, but not of neutrophils [58].

Notably, an integrin adhesion molecule, Mac-1 (CD11b/CD18, $\alpha M\beta 2$), likely plays pivotal roles in regulating eosinophil degranulation; this molecule is not only important for eosinophil migration but also crucial for eosinophil effector functions. Historically, as described above, receptor ligands immobilized to relatively large surfaces, such as sIgA-coated Sepharose beads, and parasites are potent and effective stimuli for eosinophil degranulation [57]. Later, it was found that $\beta 2$ integrins, especially Mac-1, play a crucial role in the activation of eosinophils stimulated by IgG when IgG is immobilized onto tissue culture plates or Sepharose beads [59]. Similarly, the eosinophil functional response to PAF or GM-CSF is greatly influenced by the availability of cellular adhesion [60, 61]. When eosinophils are stimulated with these soluble mediators, they adhere to the tissue culture wells through $\beta 2$ integrins, and this adhesion process provides critical signals to amplify cellular activation and degranulation [62]. Conversely, when eosinophils are activated with sIgA but kept in suspension (i.e. without Sepharose beads), they produce cytokines such as GM-CSF and IL-8 but do not release granule proteins. Mac-1 is also used to recognize β -

glucan on the surface of fungi and to release granule proteins [53]. Indeed, eosinophil granule release can be induced by the direct ligation of integrins by antibodies or ligands to Mac-1 [62] or VLA-4 [63] in the absence of additional stimuli. While the molecular mechanism to explain pronounced activation of eosinophils by cellular adhesion through $\beta 2$ integrin is not fully understood, it likely involves other cell surface molecules that are physically associated with the integrin, such as a GPI-anchored protein, CD66b, and signaling molecules localized in lipid rafts [64]. Thus, $\beta 2$ integrins, in particular CD11b/CD18, may play a pivotal role in determining the functional outcomes of human eosinophils that are exposed to immunological and inflammatory stimuli. The ability of human eosinophils to effectively engage the integrins and to respond to microbes/molecules with a large surface likely makes this leukocyte distinct from other granulocytes.

5. Summary and future directions

Eosinophils have been considered end-stage cells involved in host protection against parasite infection and immunopathology in Th2-type inflammation. However, recent studies have changed this perspective and eosinophils are now considered multifunctional leukocytes involved in tissue homeostasis, modulation of adaptive immune responses and innate immunity to certain microbes. However, such new information does not preclude that eosinophils, in particular human eosinophils, are also effector cells with pro-inflammatory and destructive capabilities. The damaging characteristics of eosinophil lipid mediators and granule proteins have been well established in 1990s. Eosinophils with activation phenotypes are observed in biological specimens from patients with disease, and deposition of eosinophil products is readily seen in the affected tissues from these patients. Therefore, it would be reasonable to consider the eosinophil a multifaceted leukocyte that contributes to various physiological and pathological processes depending on its location and activation status (Figure 1).

Two major challenges in the field of eosinophil biology are identified. First, mice have been used extensively and successfully to study the mechanisms of Th2-type inflammation in asthma and parasite infection and the roles of eosinophils in these disease models. However, significant differences exist between mice and humans in immune system development and activation [65], and unfortunately, eosinophils are not exempt from these challenges. For example, mouse eosinophil-associated RNases are remarkably divergent (>50%) orthologs of human EDN and ECP [66]. In addition, among various molecules involved in activation and effector functions of human eosinophils, as discussed above, Fc α RI (IgA receptor), Fc γ RIIA and C (activating IgG receptor), and CD66b cannot be identified in the mouse genome. Indeed, mouse eosinophils shows a distinct response to cytokines and other agonists and have a limited propensity to degranulate in vitro [67, 68]. Therefore, we run the risk of overlooking aspects of human eosinophils that do not occur, or cannot be reproducibly modeled, in mouse models of human diseases.

The second challenge is that some functions of eosinophils most likely overlap with the effector functions of other immune cells (e.g. cytokine production by CD4⁺ T cells), and the role of eosinophils alone may be difficult to demonstrate in physiological settings. Furthermore, inflammation is a complex event involving both tissue damage and tissue

repair/remodeling [69]. It would be difficult to discern the role of eosinophils if eosinophils are involved in both the damaging and repair processes. It would be impossible to categorize eosinophils simply as a 'good guy' or 'bad guy'. Therefore, further studies will be necessary to answer critical questions concerning the true involvement of eosinophils in health and disease processes. This goal can be accomplished by integrated and multidisciplinary approaches including better characterization of the immunobiology of eosinophils and their products in vitro, careful analyses of animal models in vivo, and more in-depth investigation of specimens from patients. Although this is a challenging task, future studies have the promise of revealing the true importance of eosinophils in human health and disease.

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Status	Resting	Regulatory	Inflammatory
Stimuli	None	Cytokines Soluble Ig Danger molecules	Microbes Immobilized Ig Strong adhesion
Eosinophil products	Growth factors?	Cytokines Growth factors	Granule proteins Inflammatory mediators
Outcomes	Immune homeostasis Tissue development	Immune regulation Tissue repair/remodeling	Microbial killing Tissue damage

Figure 1.

Multifaceted functions of eosinophils. Functional properties of eosinophil can be divided into at least three stages including resting, regulatory and inflammatory. Different types of immunological stimuli (or absence of stimuli) trigger production and release of different molecules by eosinophils. Accordingly, eosinophils may affect tissue development, homeostasis and repair, regulate the immune response and exert pro-inflammatory effector functions.