Immunobiology of Intestinal Eosinophils - A Dogma in the Changing?

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Immunobiology of intestinal eosinophils – a dogma in the changing?

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Abstract

Infiltration of eosinophils into the intestinal mucosa is a typical hallmark of anti-parasite immune responses and inflammatory disorders of the intestinal tract, and eosinophils are thought to contribute to anti-parasite responses and tissue damage associated with the inflammatory disorders by release of their cytotoxic granule content. However, utilizing novel tools to study eosinophils, it has been recognized that eosinophils are present in the gastrointestinal tract constitutively. In addition as the dogmatic anti-parasite function of eosinophils has proven difficult to document experimentally, it has become increasingly clear that eosinophils are likely to have a more complex role than previously appreciated. Thus, the prevailing dogma of eosinophils merely as anti-parasitic effector cells is in the changing. Instead, it has been suggested that eosinophils can contribute also to several other processes in the intestinal mucosa, e.g. local tissue homeostasis and adaptive immune responses.

This review describes the current knowledge regarding characteristics and functions of intestinal eosinophils, and the regulation of eosinophil trafficking to the intestinal mucosa during steady state and inflammation. Finally, potential additional and novel roles of intestinal eosinophils in the intestinal mucosal immune system are discussed.

Introduction

Eosinophils are cells of the innate immune system, belonging to the family of granulocytes together with basophils and neutrophils, and named after the characteristic red-appearing cytoplasmic granules that result from treatment of eosinophils with the acidic dye eosin. The dogmatic view of eosinophil function states that eosinophils are primarily effector cells that localize to infected tissue and participate in anti-parasite defense. In addition, eosinophils are suggested to contribute to tissue injury during several inflammatory disorders of the intestine. Eosinophil cytoplasmic vesicles contain a variety of substances, ranging from the eosinophil-specific major basic protein (MBP), eosinophil-peroxidase (EPO), eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) (although these are not entirely eosinophil-specific, as MBP and EPO can also be produced by basophils, and neutrophils produce EDN and ECP), to more generic soluble mediators such as a wide variety of cytokines, chemokines and proinflammatory substances. Recently, several new tools to study eosinophils have emerged, including eosinophil-specific surface markers and eosinophil-deficient mouse strains. Work in eosinophil-deficient mice has made it increasingly clear that the dogmatic view of eosinophils is too narrow and that eosinophils are likely to have a broader range of functions. In addition it is now evident that eosinophils not only localize to tissues upon inflammation, but that they are normal constituents of the cellular pool in several organs at steady state, including the gastrointestinal tract. Thanks to recent developments of novel tools with which to study eosinophils, we are better situated to examine in depth the substantial population of eosinophils in the murine intestinal tract, to understand the function of these cells. The current review aims to describe and discuss

the recent development within the field of intestinal eosinophils, with a particular focus on findings in the mouse as this is currently the most feasible "model system" in which to study eosinophils.

Intestinal eosinophils have unique phenotypic and functional characteristics

Until recently, detailed phenotypic analysis of eosinophils has been difficult to perform, due to their low frequency and lack of eosinophil-specific cell surface markers. In the mouse there are currently two markers commonly used to identify eosinophils, CCR3 and Siglec-F. Expression of the chemokine receptor CCR3 is induced during eosinophil development in the bone marrow, and CCR3 is uniformly expressed by all peripheral eosinophils [1]. In addition to eosinophils, CCR3 can also be expressed by a subset of Th2-type CD4 cells [2]. Siglec-F belongs to the family of sialic acid-binding immunoglobulin-like lectins, the Siglecs. Siglecs are negatively regulating receptors that induce apoptosis upon ligation by their natural ligands. Siglec-F is considered to be largely eosinophil-specific [3], however some reports suggest that subsets of activated T cells and a population of alveolar macrophages during respiratory inflammation can also express Siglec-F [4, 5]. Although not entirely exclusive to eosinophils, the combination of CCR3 and Siglec-F, or either of them in combination with the granular appearance of eosinophils or additional surface markers such as those described below, make them very useful and potent in the study of eosinophils.

One of the caveats in characterizing intestinal eosinophils has been the lack of protocols for isolation of these cells. Carlens *et al* utilized a standard protocol for isolation of intestinal lamina propria cells by enzymatic digestion of intestinal tissue [6].

The resulting population of small intestinal lamina propria cells contained approximately 25-30% live $CD45^+$ granular cells, 95% of which were demonstrated to be eosinophils based on expression of CCR3 and Siglec-F, together with their cytospin appearance. The high frequency of eosinophils in the intestinal lamina propria is likely not a direct reflection of their actual prevalence, as eosinophils appear far from this abundant by *in situ* histological analysis. Instead it appears that the isolation methods favor the survival of eosinophils above other lamina propria populations, leading to a net enrichment of eosinophils in the resulting lamina propria cell suspension [6]. Further characterization of small intestinal eosinophils demonstrated that they were homogenously positive for the myeloid marker CD11b, expressed intermediate levels of the neutrophil marker Gr-1 and, surprisingly, expressed low levels of CD11c that is more commonly associated with dendritic cells. In contrast the small intestinal eosinophils were negative for other lineage markers, including CD3, c-kit and F4/80. Small intestinal eosinophils were also negative for MHC-II and CD80 involved in antigen-presentation to T cells, and CD14, CD103 and DEC-205 that are expressed by subsets of antigen-presenting cells. Other reports however demonstrate F4/80 and CD80 expression by small intestinal eosinophils [Uematsu NI2008]; the difference between these reports is not known. Using a similar approach, we and others have examined the phenotype of large intestinal eosinophils, demonstrating expression of CCR3, Siglec-F, CD11b, and low but detectable levels of F4/80 (author's unpublished results, [7]). In contrast we did not detect expression of TLR2, TLR4 and CD62L (author's unpublished results). Expression of CD11c and variation in the expression levels of Siglec-F and CD62L can be utilized to differ between the eosinophils populations in various tissues. Similar to the intestinal eosinophils, eosinophils in the

thymus, uterus and MLN are $CD11c^+$ Siglec-F^{hi}, whereas lung and blood eosinophils are $CD11c^-$ Siglec-F^{low} (although other studies report that lung eosinophils express high levels of Siglec-F [8]) [6, 9]. Furthermore while MLN and large intestinal eosinophils are $CD62L^{low/-}$, blood eosinophils express high levels of CD62L [9]. In summary intestinal eosinophils are granular, $CD45^+$ Siglec-F^+ $CCR3^+$ $CD11b^+$ $CD11c^+$ Gr-1^{int} $CD3^-$ c-kit^- cells; in addition, while large intestinal eosinophils express F4/80, this remains to be clarified for small intestinal eosinophils.

In addition to a phenotypic difference between eosinophils from different tissues they also display differences in lifespan, a characteristic that appeared to correlate with the expression of CD11c (although the functional relevance of this association is unknown) [6]. Thus, while $CD11c^-$ eosinophils from the blood and lung rapidly turn over (half-life <36h), consistent with the dogmatic view of eosinophils as short-lived effector cells, eosinophils in the uterus, thymus and intestine were considerably more long-lived (>7d for the intestinal eosinophils) [6]. The longevity of intestinal eosinophils correlated with high expression of the γc-chain cytokine receptor utilized for signaling by several cytokine ligands (IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21), and γc-chain deficient mice had a selective reduction in intestinal eosinophils [6]. The γc-chain did not appear to influence the half-life of eosinophils however, as this remained unchanged in γc-chain-deficient mice. Instead, the reduced number of intestinal eosinophils in γc-chain-deficient mice appeared to correlate with decreased production of the known eosinophil hematopoietin GM-CSF and the eosinophil-attractant CCL11 (eotaxin-1) in the intestinal tissue [6] (see below for further discussion of these molecules), implicating a potential role of these in the observed effect. One possible mechanism of increased eosinophil

survival may be via autocrine production of GM-CSF, as it has been demonstrated *in vitro* that eosinophils can interact with fibronectin (one component of the intestinal lamina propria stroma) via the integrin $\alpha 4\beta 1$ and that this interaction induces GM-CSF production by the eosinophil itself and promotes enhanced survival [10]. In summary intestinal eosinophils display enhanced survival compared to eosinophils from other tissues by a currently unknown mechanism.

Eosinophils are constitutively present in the intestinal mucosa and accumulate during inflammatory conditions

Although it's well-documented that eosinophils accumulate in the intestinal mucosa during infections with gastrointestinal parasites and certain intestinal inflammatory disorders, eosinophils have been demonstrated to make up a substantial population of cells of the intestinal mucosal cells also at steady state [6, 11, 12]. Eosinophils were detected throughout the entire gastrointestinal tract except the tongue and esophagus at steady state [12], with comparably more eosinophils residing in the duodenum [6, 12]. Eosinophils were detected preferentially in the submucosa and lamina propria of the tissue, although duodenal eosinophils also localized to the villi [12]. The requirements for the steady state localization of eosinophils to the intestinal mucosa have been well characterized [6, 11, 12]. Eosinophils are present in the fetal intestine at embryonic day 19 at concentrations comparable to those of adult mice. Consistently, eosinophils do not require the bacterial flora to populate the intestine as this occurs with normal efficiency in germ-free mice [12]. Finally, lymphocytes do not appear to play a major role in the accumulation of eosinophils in the intestine at steady state, as the

number of eosinophils in the intestinal mucosa are only mildly affected by the absence of T cells (in athymic mice) and lymphocytes (RAG-1 or RAG-2 deficient mice) [6, 12].

Chemokine/chemokine receptor usage during eosinophil accumulation in the intestine at steady state and during inflammation

Tissue-selective leukocyte migration is mediated by expression of combinations of cell lineage or subset-specific receptors (e.g. integrins, chemokine receptors) on the surface of the migrating leukocyte and the ligands for these receptors (e.g. cell adhesion molecules (CAMs), chemokines), displayed in a tissue-selective manner on the surface of endothelial vessels. In addition, chemokines and integrins may also be important for the retention of cells in various tissues. The chemokine receptor CCR3 is expressed on all eosinophils and bind to several chemokine ligands in the mouse, including CCL11 and CCL24 (eotaxin-2; humans also have CCL26 (eotaxin-3) that is a pseudogene in mice), CCL5 (RANTES), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP-4) and CCL20 (MIP-3α). Of these, CCL11 appears to play a dominant role in regulation of eosinophil accumulation in the intestine under steady state conditions. In contrast to the other chemokines, CCL11 is constitutively and abundantly expressed by mononuclear lamina propria cells throughout the entire intestinal tract [11, 12]. In mice deficient for CCL11, steady state eosinophil accumulation in the intestinal mucosa is severely impaired, while eosinophil levels remains unperturbed in bone marrow and blood [11]. Finally, mice deficient in CCR3 also have severely reduced numbers of intestinal eosinophils [13]. Together these observations demonstrate the critical importance of CCL11/CCR3 in migration/retention of eosinophils in the intestinal mucosa at steady state (Figure 1).

During intestinal inflammation, the expression of CCL11 in the intestinal tissue increases further [14, 15]. It is unknown what regulates CCL11 expression in the small and large intestine, however in the inflamed esophagus CCL11 is upregulated following treatment with the Th2-associated cytokine IL-13 [16]. The role of CCL11 in localization of eosinophils to the gastrointestinal tract during intestinal inflammation has been studied in a variety of models. In an experimental antigen (OVA)-induced eosinophilic gastrointestinal allergy model, infiltration of eosinophils into the small intestinal mucosa following challenge OVA-administration occurred in a CCL11-dependent manner [17]. Furthermore CCL11, but not CCL24, expressed by a population of $F4/80^{+}$ $CD11b^{+}$ lamina propria cells was required for eosinophil accumulation in the colonic mucosa in the Dextran sodium sulphate (DSS) colitis model of large intestinal inflammation [14]. Finally, CCL11-deficient mice infected with the small and large intestinal nematode parasites *Trichinella spiralis* and *Trichuris muris*, respectively, also displayed significantly reduced, although not completely abrogated, small and large intestinal eosinophilia [18]. Other reports demonstrate a role also for CCR3 during intestinal inflammation. Thus, consistent with a role of CCL11/CCR3 in eosinophil migration/retention in the small intestinal mucosa, CCR3-deficient mice infected with *T. spiralis* had reduced numbers of jejunal eosinophils compared to wild-type littermates [19]. Further, in an experimental model of eosinophilic esophagitis, the number of esophageal eosinophils was severely reduced in CCR3-deficient mice [15]. Thus, CCL11/CCR3 plays a dominant role in regulation of eosinophil migration/retention in the intestinal mucosa under steady state and inflammatory conditions (Figure 1).

Role of integrins during eosinophil migration/retention in the intestinal mucosa

Comparably little is known regarding the involvement of integrins during steady state migration and retention of eosinophils in the intestinal mucosa. At steady state, eosinophils in the periphery express α4β7 integrin [20], the signature integrin for leukocyte migration to the intestinal mucosa, via interaction with its ligand MAdCAM-1 that is selectively expressed by postcapillary venules of the intestinal mucosa. However, β7 integrin-deficient mice have normal numbers of SI eosinophils at steady state [21], suggesting that α4β7 is not required for eosinophil migration/retention in the small intestinal mucosa at steady state. Apart from α4β7, peripheral eosinophils also express β2 integrins at steady state, including αLβ2 (LFA-1) and αMβ2 (Mac-1) [9, 22], and localization of eosinophils to the colonic mucosa at steady state is reduced in mice treated with antibodies towards the β2 integrin ligand ICAM-1 [22] (Figure 1).

β7 integrin appears to play a more prominent role in regulating eosinophil accumulation in the small intestinal mucosa under inflammatory conditions. In the experimental oral allergy model previously described, eosinophil numbers in the small intestinal mucosa was reduced in β7 integrin-deficient mice [20]. Similarly, following infection with *T. spiralis*, small intestinal eosinophilia was reduced and delayed [23]. Finally, small intestinal eosinophilia resulting from overexpression of CCL11 under control of the promoter for the small intestinal epithelium-specific gene intestinal fatty acid binding protein (Fabpi), was demonstrated to depend on β7 integrin [21]. In contrast, conflicting results have been reported regarding the role of β7 integrin in regulating large intestinal eosinophilia following inflammation, possible due to variation in the models used to induce large intestinal inflammation. Thus while eosinophil migration/retention in

the large intestinal mucosa was impaired in β7 integrin-deficient mice in the experimental oral allergy model [20], β7 integrin was dispensable in other models. In the DSS colitis model large intestinal eosinophilia occurred independently of β7 and α4 integrins and L-selectin, but instead eosinophil accumulation in the injured large intestinal tissue was dependent on the β2 integrin ligand ICAM-1 [22]. Consistent with a potential role in regulating migration to the large intestine, splenic and large intestinal eosinophils expressed the ICAM-1 ligands αLβ2 and αMβ2 integrins [22]. Finally, caecal eosinophilia during infection with *T. muris* was β7/MAdCAM-1-independent [23, 24], but α4 integrin-dependent, implicating a role of α4β1 integrin in migration/retention in the large intestinal mucosa in the absence of α4β7 [24]. Thus while accumulation of eosinophils in the inflamed small intestinal mucosa relies largely on β7 integrins, redundant mechanisms, including α4, β2 and β7 integrins and ICAM-1, appear to regulate large intestinal eosinophilia depending on the model utilized (Figure 1).

Other factors involved in eosinophil accumulation in the gastrointestinal tract

The key cytokines in eosinophil development, survival and function, IL-5, IL-3 and GM-CSF have also been examined for a potential role in regulating eosinophil accumulation in the intestinal mucosa. However, most of the current evidence indicates that although these molecules affect intestinal eosinophils, this appears to preferentially be due to secondary rather than direct effects. IL-5 is critical for the development of eosinophils in the bone marrow, and the release of mature eosinophils from the bone marrow into the blood. Furthermore, IL-5 together with IL-3 and GM-CSF is also important for post-mitotic events, such as survival and activation. IL-5-deficient mice had

approximately 50% reduced level of intestinal eosinophils compared to control mice [12]. Similarly, mice deficient in the βc cytokine receptor chain common for IL-5, IL-3 and GM-CSF had an 80% reduction in intestinal eosinophils. However the reduction in intestinal eosinophils in both models was accompanied by a similar reduction of eosinophils in the periphery [12]. Finally, mice overexpressing IL-5 have increased levels of eosinophils in several tissues including the intestinal tract, however accumulation of eosinophils in the intestine remains strictly dependent on expression of CCL11 [12]. In summary, it appears that although IL-5 and other cytokines can affect eosinophil availability by regulating the level of eosinophil output and the pool of circulating eosinophils, they are not sufficient to directly regulate eosinophil accumulation in the intestinal tract.

Importantly, the increased number of eosinophils observed in the gastrointestinal tract in association with inflammatory processes may not be regulated solely on the level of eosinophil influx into the intestinal tissue. Recently it was demonstrated that increased level of peritoneal eosinophils in response to infection with the nematode *Nippostrongylus brasiliensis* was highly dependent on increased survival of eosinophils rather than increased output from the bone marrow into the blood stream and entry into the peritoneum [25]. Indeed as mentioned previously, intestinal eosinophils also display an increased life-span compared to eosinophil populations from other organs; this is however evident already at steady state and it is currently not clear if their life span is further prolonged during inflammatory processes [6].

Eosinophils accumulate in intestinal-associated lymphoid tissues during intestinal inflammation

Eosinophils can also be detected in the gut-associated lymphoid tissues (GALT), the Peyer's patches (PP) of the small intestine and the intestine-draining mesenteric lymph nodes (MLN). Eosinophils are very rare in PP and MLNs at steady state in wild-type mice [9, 26, 27], although they are detected in both organs of IL-5 transgenic (IL-5-Tg) mice [27, 28]. While being infrequent at steady state, eosinophils accumulated in MLNs in response to infection with the helminthic parasites *T. muris* [9] and *T. spiralis* [26]. Eosinophil accumulation in MLNs occurred relatively early during *T. muris* infection [9], while peak eosinophilia coincided with the height of infection and worm expulsion in both models [9, 26]. Within the MLN eosinophils were predominantly present in the medullary region, but they were also detected in the subcapsular sinus and the paracortical T cell area. The route via which eosinophils migrate to the MLN, i.e. if eosinophils migrate from the intestinal mucosa via lymphatic vessels or if eosinophils enter from the blood via high endothelial venules, and the mechanism that regulates this process remains to be determined (Figure 1). Eosinophil accumulation in the PP of wild-type mice was examined in a model of experimental oral allergy [27]. Under these conditions, eosinophils localized preferentially to the cortical and paracortical area of the PP [27], at least partly under regulation of CCL11 [27].

Documented functions of intestinal eosinophils

Prior to the recent development of eosinophil-deficient mouse strains, the role of eosinophils were studied by depletion of eosinophils by administration of anti-eosinophil

serum or monoclonal anti-IL-5 antibodies, or alternatively in genetic models such as IL-5-deficient and -overexpressing mice, CCR3- or CCL11-deficient, or IL-5/ CCL11 double-deficient mice. Although there is a documented reduction in eosinophils (or increase, in IL-5-Tg mice), to a variable degree, in these models, neither of them cause complete eosinophil deficiency. Furthermore the molecules targeted in these models are not entirely eosinophil-specific, and the observed effects may therefore be due to "adverse effects" on other cell types. Recently two eosinophil-deficient mouse strains have been described, the ΔdblGATA-1 [29] and the TgPHIL [30] strains of mouse. ΔdblGATA-1 mice are deficient in eosinophils due to the deletion of a high-affinity binding site for GATA-1 in the GATA-1 promoter [29], while TgPHIL mice lack eosinophils due to expression of the cytocidal diphtheria toxin (DT) A-chain under control of the EPO promoter [30]. These mice appear to have no apparent phenotype apart from eosinophil-deficiency, and continued work in these models are likely to be important in confirming previous work and unequivocally determine the role of eosinophils.

Anti-parasitic effector cells

From very early on (dates back at least to 1939 [31]), eosinophils have been associated in particular with immune responses towards infection with helminthic parasites. This association derives at least partly from the well-accounted accumulation of eosinophils in tissues infected with parasites, observations of degranulating eosinophils in the close vicinity of parasites *in vivo* [32], and eosinophil-specific mediators being directly anti-parasitic *in vitro* [33]. The eosinophil mode of parasite killing has been

suggested to occur predominantly via release of their toxic granule content in response to activation via antibody-antigen complexes binding to Fc receptors or complement [32]. Despite the association between parasites and tissue eosinophilia, unequivocal evidence for a direct role of eosinophils in anti-parasite defense remains relatively scarce, in particular for the population of eosinophils in the gastrointestinal tract. Below is a summary of investigations of the potential role of eosinophils in the most commonly utilized models of gastrointestinal nematode infections in experimental mice, the nematodes *T. spiralis* (infects the duodenal or jejunal epithelium; acute infection), *T. muris* (infects the caecal/proximal colonic epithelium; acute infection in most mouse strains)*, Heligmosomoides bakeri* (previously *H. polygyrus* [34]; as adults free-living in the small intestinal lumen; chronic infection), *N. brasiliensis* (as adults free-living in the small intestinal lumen; acute infection) and *Strongyloides ratti* (adults infect the small intestine) and *S. venezuelensis* (adults infect the small intestinal epithelium), that spend all or parts of their life cycles is the intestine. While all these nematodes spend their adult stages in the intestine, *N. brasiliensis*, *S. ratti* and *S. venezuelensis* larvae go through a complex migratory pathway via the lung in order to reach the intestine, and *T. spiralis* infection gives rise to larvae that migrate from the intestine to the muscle tissue where they encyst.

The overall results following primary infection with these parasites in a variety of eosinophil-reducing/-ablating models have demonstrated that eosinophils play no major role during expulsion of a primary infection. Thus expulsion remained unaltered after infection of anti-IL-5-treated mice (*T. spiralis*: [35]; *T. muris*: [36]; *H. bakeri*: [37], *N. brasiliensis*: [38], *S. ratti*: [39], *S. venezuelensis*: [40]), IL-5-deficient (*T. spiralis*: [41], *S.*

ratti: [42]), and IL-5/ CCL11 double-deficient mice (*T. muris*: [18]) that have reduced number of eosinophils, and in CCL11-deficient (*T. muris*: [18]; *H. bakeri*: [43]) and CCR3-deficient (*T. spiralis*: [19]) mice that have defective eosinophil migration to the infected intestinal mucosa. Consistent with results in these models, and providing conclusive evidence to demonstrate that eosinophils are dispensable for worm expulsion during primary infection with these parasites, TgPHIL and ΔdblGATA-1 mice that are genetically deficient in eosinophils display a normal expulsion pattern following infection with *T. spiralis* [44], *T. muris* [9] and *N. brasiliensis* [45, 46], respectively. Despite displaying kinetically normal worm expulsion, *S. ratti* and *S. venezuelensis*-infected IL-5-deficient [42] or anti-IL-5-treated [39, 40] mice, and *N. brasiliensis*-infected ΔdblGATA-1 mice had increased intestinal worm burden and egg production [39, 40, 42, 45], suggesting a potential role of eosinophils in limiting these processes. In mice that have increased numbers of eosinophils due to overexpression of IL-5, expulsion occurred normally in a primary *T. spiralis* infection [47], and egg production remained unchanged in *H. bakeri*-infected IL-5-Tg mice [43]. In contrast, following infection with *N. brasiliensis*, *S. ratti* and *S. venezuelensis* that have larval migratory stages prior to the intestinal stage, IL-5-Tg mice had enhanced larval killing during primary infection; however larval killing occurred prior to the intestinal stage of the infection and therefore independently of intestinal eosinophils [48, 49].

During challenge *T. spiralis* infection, IL-5-deficient mice that successfully cleared the primary infection had delayed worm expulsion during the secondary infection [50], indicating a role of IL-5, and potentially eosinophils, during the response to secondary infection. There is also evidence for a role of eosinophils in secondary *N.*

brasiliensis [45, 46] and *S. ratti* [39] infection, however similar to as in IL-5-Tg mice this likely occurs prior to the intestinal stage.

In conclusion, surprisingly given the dogmatic function of eosinophils as one of the major effectors during parasite infection, the overall results from infection of experimental mice with a panel of gastrointestinal nematode parasites suggest that intestinal eosinophils play a limited role during the expulsion of these parasites. However importantly, the large majority of these studies examine worm expulsion as a measurement of a potential role of eosinophils in the anti-parasite immune response; therefore potential effects of eosinophils on other aspect of the immune response to these infections may have been over-looked.

Anti-bacterial effector cells

Recently a novel anti-bacterial characteristic of intestinal eosinophils was described, suggesting that eosinophils could rapidly release, or "catapult", mitochondrial DNA [51]. The DNA was released in response to inflammatory trigger, e.g. LPS or complement, and served to trap bacteria in a DNA network formed in the extracellular matrix of the intestinal lamina propria. To further promote bacterial killing, the released DNA contained the eosinophil-derived effector molecule MBP. A similar feature has previously been described for neutrophils, the so-called NET (neutrophil extracellular traps), although the source of DNA and mechanism of release appear different. The relevance and physiological importance of this mechanism remains to be established.

Non-beneficial role of eosinophils during intestinal inflammatory disorders

Eosinophils accumulate in the gastrointestinal tract during several human intestinal inflammatory disorders, e.g. eosinophil-associated gastrointestinal disorders (EGID; e.g. food allergy) and inflammatory bowel disease (IBD) [52]. The tissue-toxic nature of some of the contents of the eosinophil cytoplasmic vesicles, and a correlation between the number of infiltrating eosinophils and/or eosinophil degranulation with the extent of intestinal tissue damage has led to suggestions of a detrimental contribution from eosinophils in mediating tissue damage during these disorders. Although difficult to study in humans, the role of eosinophil during gastrointestinal disorders has been studied in murine experimental models that mimic human intestinal inflammation.

DSS colitis is a chemical-induced model of colonic intestinal inflammation that mimics many of the clinical manifestations typical for human ulcerative colitis. Within days of DSS administration in the drinking water, damage is inflicted to the epithelial layer and mice display signs of colonic inflammation, e.g. shortening of the colon tissue, diarrhea, weight loss, and leukocyte infiltration. As one of the dominating infiltrating cell types is the eosinophil, the DSS colitis model has been utilized to study the role of eosinophils and eosinophil-derived effector molecules in mediating tissue damage [14, 22, 53]. DSS colitis caused colonic eosinophilia and eosinophil degranulation, with a direct correlation between eosinophil numbers in the intestine and disease score [14]. Disease was attenuated in eosinophil-deficient mice [14], and in models where eosinophil migration to the intestinal mucosa was abrogated [22, 53], with reduced clinical scores and pathology. The eosinophil effector molecule EPO appeared important in inflicting tissue damage, as disease was also attenuated in EPO-deficient mice but not in MBP-deficient mice [53].

The role of eosinophils during small intestinal inflammation was examined in the oral allergy model previously described. Here, apart from infiltration of eosinophils into the lamina propria of the small intestinal mucosa, stomach and esophagus, mice developed several signs of intestinal inflammation, including weight loss, reduced villus/crypt ratio, gastric dysmotility and gastromegaly in response to oral delivery of OVA particles to OVA/alum-sensitized mice [54]. It appeared that eosinophils were involved in mediating these signs of inflammation as CCL11-deficient mice that had disrupted accumulation of eosinophils in the intestinal mucosa displayed attenuated disease [54]. Together these studies demonstrate a detrimental role of eosinophils in experimental models of colonic and small intestinal inflammation. Noteworthy these are only experimental models that mimic certain features of human disease, however the results are consistent with a suggested role for eosinophils as contributors to tissue-destruction and gastrointestinal dysfunction in patients with gastrointestinal disorders characterized by intestinal eosinophilia. The detrimental role of eosinophils in mediating tissue-destruction in various gastrointestinal inflammatory disorders has gained interest also from a therapeutic point of view. Recently, a phase I/II trail with a humanized anti-IL-5 antibody yielded promising results in hyper-eosinophilic syndrome (HES), including eosinophilic esophagitis [55].

Potential alternative functions of intestinal eosinophils

The documented functions of eosinophils described above all refer to the role of eosinophil during infection and inflammatory conditions, be it beneficial or non-beneficial. Furthermore, the described role of eosinophils does not appear consistent with

the widely held belief that eosinophils are effector cells in the anti-parasite defense. Given the constitutive presence of eosinophils in the intestinal mucosa at steady state, and suggestions of a possible function of eosinophils in adaptive immune responses, several additional functions of intestinal eosinophils can be envisaged at steady state and during inflammation/infection. This is further supported by the versatile range of soluble mediators that eosinophil can produce and release in response to various stimuli, e.g. cytokines (including IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-18, TGFβ, GM-CSF, TNFα, IFNγ), chemokines (CCL11, CCL5, CCL3) and proinflammatory (leukotrienes, PAF) [56] and inflammation-resolving (protectin D1; PD1) [57] substances. Below a number of potential additional functions of intestinal eosinophils are discussed.

Support lamina propria tissue integrity and lamina propria cell homeostasis?

Given their constitutive presence in the gastrointestinal tract, it is conceivable that eosinophils take part in regulating homeostatic processes, such as supporting maintenance and/or function of epithelial cells or other immune cells present in the intestinal mucosa at steady state. In a recent review article (with a very informative introduction discussing why eosinophils probably aren't merely anti-parasitic), the authors put forward the *LIAR* (Local Immunity And/or Remodeling/Repair) hypothesis, suggesting that eosinophils accumulate at sites characterized by simultaneous cell apoptosis (that stimulate eosinophil infiltration) and proliferation events (which would provide eosinophil survival factors) [58]. Within the tissues, eosinophils are suggested to take part in immunomodulation in a variety of events, e.g. intestinal tissue remodeling,

with the role being pro- or anti-inflammatory depending on the situation [58]. Consistent with a potential role on regulating the local environment, intestinal eosinophils have been suggested to regulate colonic epithelial barrier function [59]. Furthermore, the fact that eosinophils constitutively reside in the intestinal lamina propria in substantial numbers suggest that they may interact also with other cells in the intestinal lamina propria, e.g. macrophages and dendritic cells, and potentially influence the function, survival and/or recruitment of these cells (see further below). Finally, eosinophils produce cytokines, e.g. TGFβ and IL-13 that have the capacity to influence various cells in the intestinal mucosa. TGFβ is involved in a variety of processes in the intestinal mucosa, including isotype switching (see further below), immunoregulation, and induction of fibrosis as a consequence of chronic inflammatory processes in the mucosa. Increased production of IL-13 is thought play a role in the anti-helminth defense by inducing goblet cell hyperplasia and increased mucous production. However, the relevance of eosinophil-derived IL-13 and TGFβ, or other cytokines, versus cytokines produced by other cell types during these and other processes needs to be further investigated.

Resolution of inflammatory responses?

In addition to their known capacity to produce proinflammatory substances, eosinophil may also contribute to resolution of inflammation. In a mouse model of acute peritonitis induced by treatment of mice with zymosan, eosinophil accumulation at the site of inflammation correlated in time with the resolution of inflammation [57]. The peritoneal eosinophils were demonstrated to be involved during the resolution of inflammation, via production of the pro-resolving mediators, including PD1 [57].

Furthermore, adoptive transfer of wild-type eosinophils, but not 12/15 lipoxygenase-deficient eosinophils that cannot produce PD1 and other similar pre-resolving mediators, supported resolution of inflammation in mice that had been depleted of eosinophils by anti-IL-5 treatment [57]. It is plausible that eosinophils that are recruited to the site of intestinal inflammation may also contribute to the resolution of inflammation by production of pro-resolving mediators, however this remains to be determined.

Involvement in the generation of adaptive T cell responses?

Several recent publications have demonstrated expression of MHC-II and costimulatory molecules (e.g. CD80, CD86) on the surface of eosinophils isolated from various organs, e.g. the airways [60] and MLN [9]. This has led to suggestions of a possible function of eosinophils in direct antigen-presentation to T cells [60-62], although it is unclear whether eosinophils can stimulate naïve T cells [62], or if this capacity is limited to previously activated T cells [61]. In the latter case, one possibility is that eosinophils in the intestinal lamina propria can present antigen to effector T cells that have localized to the intestinal mucosa after activation in MLN, to promote effector T cell functions, e.g. cytokine production. The ability of intestinal eosinophils to present antigens and mediate T cell activation has not been directly tested, however intestinal eosinophils express relatively low levels of MHC-II [7, 9], which would suggest that they have a limited capacity to directly present antigens to T cells compared to eosinophils at other sites.

As an alternative to a role of eosinophils in direct interaction with T cells, intestinal eosinophils may play a more indirect role in adaptive immune responses.

Eosinophils are a constitutive component of the intestinal lamina propria cell repertoire; as such they reside in close vicinity of dendritic cells that have a well documented function in antigen uptake, transport to local MLN and presentation to mediate activation of antigen-specific T cells. Interestingly EDN, one of the hallmark eosinophil-derived effector molecules, can act as an endogenous ligand to TLR2 on the surface of dendritic cells. The interaction between EDN and TLR2 promotes the ability of DC to promote T cell activation and skew the response to a Th2 response [63]. Finally eosinophils may also contribute to shaping the nature of the adaptive immune response e.g. by providing cytokines that are known to be involved in regulating induction of Th1 vs. Th2 responses, as has previously been suggested [60].

Support antibody-secreting cell development and function?

Several recent publications have associated eosinophils with regulation of antibody responses [64, 65]. Eosinophils were demonstrated critical for the maintenance of plasma cells in the bone marrow, via production of the plasma cell growth, maturation and survival factors APRIL and IL-6 [64]. Furthermore, splenic eosinophils have been demonstrated important for the early stage IgM antibody response following alum administration [65]. The abundant production and importance of IgA antibodies at intestinal mucosa sites and the constitutive presence of eosinophils at this site suggests a potential role of eosinophils in this process. A role of intestinal eosinophils in antibody-responses can be envisaged at several stages, e.g. in mediating class switch to the IgA isotype and/or in maintenance of IgA-secreting cells in the lamina propria, analogous to their function in the bone marrow. Apart from its role in the maintenance of bone marrow

plasma cells, APRIL has been demonstrated important for the *in situ* class switching that has been proposed to occur directly in the intestinal lamina propria tissue in response to T cell independent antigens [66]. Furthermore eosinophils produce TGFβ, a cytokine known to be involved in mediating isotype switching to the IgA isotype in germinal centers in response to T cell-dependent antigens [67]. Thus a potential role of intestinal eosinophils in regulating IgA antibody responses is plausible for both T cell dependent and T cell independent antigens, however remains to be demonstrated.

Concluding remarks

From a historical perspective eosinophils in general, and in the gastrointestinal tract in particular, have been considered to be end-stage effector cells, predominantly accumulating in the intestinal mucosa in response to parasite infection and during inflammatory disorders of the gastrointestinal tract. Recent reports however suggests differently and the text-book dogma stating that eosinophils are anti-parasitic effector cells that only localize to tissues in response to parasite infection, as well as during certain inflammatory disorders of the intestinal tract where they contribute to tissue damage, appears to be about to change. Firstly, eosinophils are present in substantial numbers in the entire gastrointestinal tract even at steady state. Secondly, although there is indeed a marked infiltration of eosinophils into the intestinal mucosa in response to infection with gastrointestinal nematode parasites, there is very little evidence at present to demonstrate that eosinophils, or eosinophil-derived effector molecules, play a major role in expulsion of the intestinal stage during a primary infection with these parasites. Importantly, this does not exclude a role of eosinophils in other aspects of the anti-

parasite response. Finally, although it appears increasingly clear that eosinophils do contribute significantly to the tissue-damage that characterizes several intestinal inflammatory disorders this is obviously not what these cells developed to do, but rather an adverse effect to the circumstances. Given the attention this non-beneficial role of eosinophils has been getting, perhaps in part because of the relatively recent increase in incidence of these disorders, examination and discussion of the physiological role(s) of eosinophils has been overlooked. The recent identification and development of novel tools to study eosinophils will facilitate the study of these cells to delineate the function of eosinophils and determine their role(s) in the gastrointestinal immune system.

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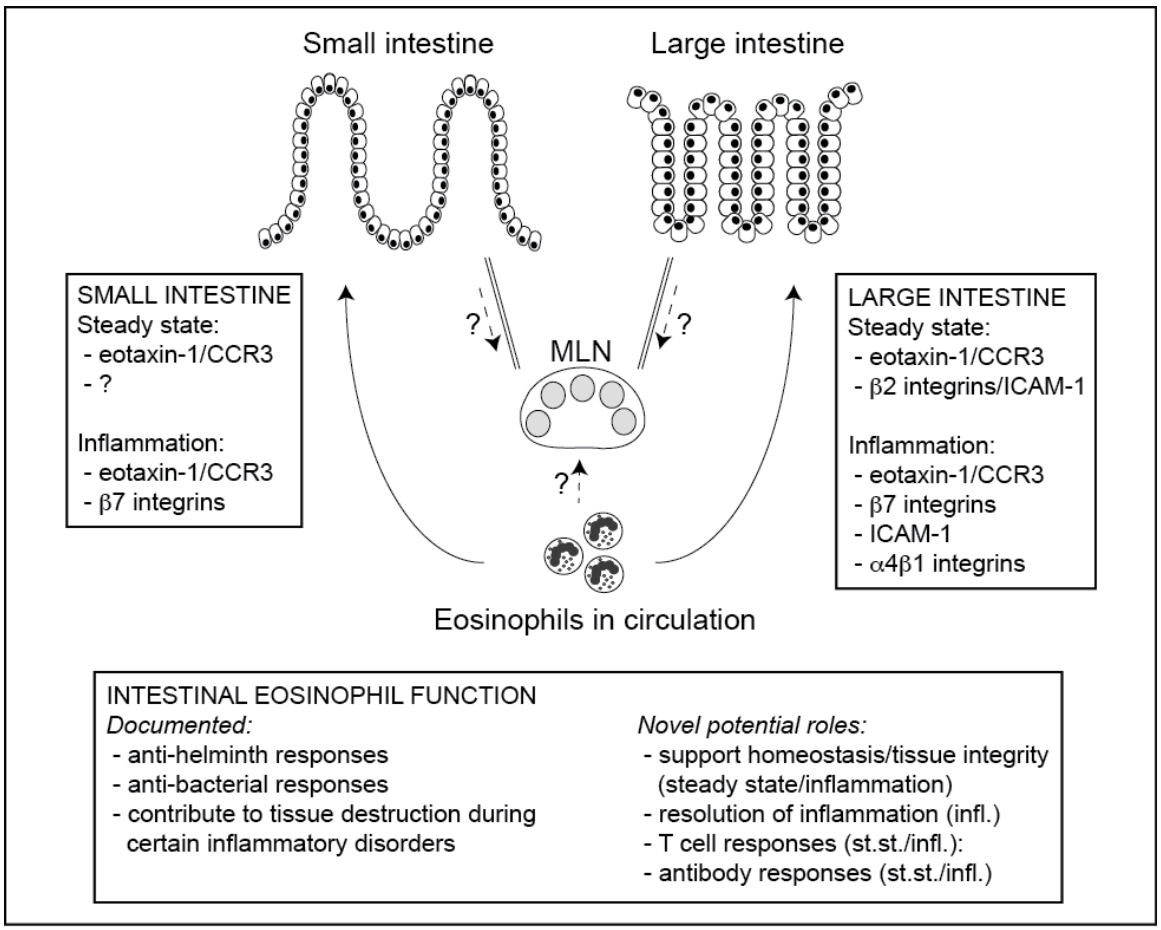
Figure legends

Figure 1. *Eosinophil accumulation and function in the intestinal mucosa.* Documented and potential novel functions of intestinal eosinophils, and a schematic overview of the migrational cues that guide eosinophil localization from the blood circulation to the intestinal mucosa and/or eosinophil retention in the intestinal tissue. Peyer's patches have been omitted for simplicity.

FIGURE 1.


Small intestine
Large intestine
SMALL INTESTINE
Steady state:
- eotaxin-1/CCR3
- ?
Inflammation:
- eotaxin-1/CCR3
- β7 integrins
?
?
MLN
?
LARGE INTESTINE
Steady state:
- eotaxin-1/CCR3
- β2 integrins/ICAM-1
Inflammation:
- eotaxin-1/CCR3
- β7 integrins
- ICAM-1
- α4β1 integrins
Eosinophils in circulation
INTESTINAL EOSINOPHIL FUNCTION
Documented:
- anti-helminth responses
- anti-bacterial responses
- contribute to tissue destruction during certain inflammatory disorders
Novel potential roles:
- support homeostasis/tissue integrity (steady state/inflammation)
- resolution of inflammation (infl.)
- T cell responses (st.st./infl.):
- antibody responses (st.st./infl.)