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Eosinophils in helminth infection: defenders and dupes

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Abstract

Eosinophilia is a central feature of the host response to helminth infection. Larval stages of parasitic worms are killed *in vitro* by eosinophils in the presence of specific antibodies or complement. These findings established host defense as the paradigm for eosinophil function. Recently, studies in eosinophil-ablated mouse strains have revealed an expanded repertoire of immunoregulatory functions for this cell. Other reports document crucial roles for eosinophils in tissue homeostasis and metabolism, processes that are central to the establishment and maintenance of parasitic worms in their hosts. In this review, we summarize current understanding of the significance of eosinophils at the host-parasite interface, highlighting their distinct functions during primary and secondary exposure.

Keywords

eosinophil; helminth; type 2 immunity

Eosinophils in the host-parasite interface

Helminths are highly complex, macroscopic animals that incorporate nervous, excretory, gastrointestinal, muscular, and reproductive systems. Despite their complexity, parasitic helminths depend upon a host to compensate for lost functions that enable a free-living lifestyle. The host provides a supportive physical and metabolic environment for growth, reproduction, and maturation, often for months or years, even though the size and motility of worms is such that tissue trauma is a common feature of infection.

In parasitic infections of animals, a potent Th2-driven immune response occurs that is coincident with the metabolic and physical interaction between host and worm. Effector

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Conflict of interest

The authors have no conflicts of interest.

mechanisms that clear worms from the body vary depending upon the habitat and niche of the parasite. Best understood in the intestine, in some instances goblet cells are critical [1], in others mast cells [2] or antibodies [3–5] play important roles in host defense. Immune mediators may cause displacement [3, 4], deprive the worm of energy or nutrients [1], or disrupt the habitat [6]. By a similarly varied array of mechanisms, worms evade or co-opt the immune response in order to complete their life cycles and be transmitted to the next host [7].

Representing less than 5% of blood leukocytes in healthy animals, tissue and blood eosinophils increase dramatically during worm infection [8]. The type 2 cytokine IL-5 plays a central role in inducing activation and recruitment of eosinophils to sites of infection, while eotaxin also promotes eosinophilia [9, 10]. It has been widely accepted that eosinophils are defensive effector cells downstream of Th2 activation. This paradigm is supported by early studies that showed the capacity of eosinophils to kill helminth larvae *in vitro* [11, 12]. The availability of eosinophil-ablated mouse models has enabled experimentation to test functions of eosinophils *in vivo*. Data from several mouse models show that eosinophils are often dispensable, in some instances protect the host, and, surprisingly, in other instances protect the worm. The properties and functions of eosinophils have been reviewed in recent years [9, 13, 14]. In this review we will summarize recent progress in our understanding of the role of eosinophils in worm infection, emphasizing differences in function during primary and secondary exposure.

Mouse models that disrupt eosinophil function

Early studies of eosinophil function in mouse models of worm infection relied upon antibody-mediated depletion of eosinophils or IL-5 [15, 16]. Subsequently, mouse strains were engineered to be deficient in IL-5 [10], in eotaxins [17–19] or their receptor CCR3 [17–20], or to overexpress IL-5 (IL-5Tg) [21]. Although expansion and recruitment of eosinophils are profoundly altered in these strains, the lineage is not ablated and this complicates the interpretation of negative results. Two eosinophil-ablation models, PHIL and dblGATA [22, 23], enabled definitive experiments that confirmed some prior findings but also revealed previously unknown properties of eosinophils (Table 1). The two strains were engineered using different approaches. In PHIL mice, diphtheria toxin (DT) A chain was inserted downstream of the eosinophil peroxidase promoter, resulting in eosinophil apoptosis [22]. In contrast, dblGATA mice bear a deletion of the high-affinity double GATA site in the GATA1 promoter that blocks the development of eosinophil lineage [23]. In addition, mice bearing dual deficiencies in the eosinophil secretory granule proteins MBP-1 [24] and EPX [25] were shown to manifest a specific and near complete loss of peripheral and tissue eosinophils [26]. This model offers an additional resource for testing eosinophil contributions to disease.

Adoptive transfer of eosinophils to eosinophil-ablated mice is an experimental design that considerably expands the utility of the model. Applied widely and creatively in recent years, transfer of eosinophils isolated from IL-5Tg mice into eosinophil-ablated or other genetically deficient recipients allows for testing of the significance and temporal requirements for eosinophil-derived factors *in vivo* [27]. The method can be elaborated

upon, for example, by crossing IL-5Tg mice with major histocompatibility complex II deficient (MHCII^{-/-}) mice to test the significance of antigen presentation by eosinophils in immunity [28]. Alternatively, bone marrow-derived eosinophils can be propagated *in vitro* for adoptive transfer experiments [29]. Although the number of cells produced by this method is limited, it provides a more rapid way to generate eosinophils with targeted genetic deficiencies.

A disadvantage of ablation models is that they do not enable contextual or temporal evaluation of eosinophil function in an otherwise eosinophil-replete animal. This challenge was addressed using knock-in strategies that inserted the gene for human diphtheria toxin receptor into the EPX locus [30, 31]. Depletion of eosinophils is induced by injection of the toxin. In the mouse model of asthma, inducible PHIL (iPHIL) mice were used to show that eosinophils were dispensable for sensitization to ovalbumin (OVA), although they were critical to the manifestation of disease following antigen challenge [30]. Although the approach has value for time-limited depletion of eosinophils, complete or sustained depletion is not readily achieved [30].

Eosinophils display a variety of receptors and produce an array of chemokines and cytokines that are known to be key regulators in different physiological and pathological processes [9, 32]. Their broad distribution across immune and other cell types makes it difficult to determine whether the eosinophil is the critical source of a particular mediator in the mechanism under investigation. A mouse model that addresses this need is one in which a gene knock-out is targeted specifically to eosinophils using Cre recombination [33].

As with any experimental approach, caution must be applied in interpreting results from available mouse models, as different gene targeting strategies, depletion methods and transfer designs may create artifacts. For example, *dblGATA* mice display impaired generation and function of basophils [34], which should be taken into account when interpreting results. The growing collection of mouse strains available for eosinophil manipulation provides an array of tools and approaches that reduce this risk and significantly advance study of eosinophil function *in vivo*. A comprehensive summary of mouse strains for studying functions of eosinophil can be found in a recent review by Jacobsen et al [35]. Fortunately, the value of new mouse strains is combined with a generosity of spirit shown by their inventors in a way that has allowed the field to advance rapidly.

Secondary infection: confirming the dogma *in vivo*

Early in the study of parasitic disease, histological examination revealed that large numbers of eosinophils infiltrated sites of worm infection [36]. In the 1970s, eosinophils were shown to be capable of killing helminth larvae *in vitro* when combined with specific antibodies or complement [12, 37, 38], a form of antibody-dependent cellular cytotoxicity (ADCC), a process where immune effector cells kill target cells coated by specific antibodies. These findings engendered a dogma that the role of eosinophils is to protect the host against parasitic worms [11, 12, 39]. Indeed, helminth infection in mice induces a robust and diverse

antibody response [40] and passive immunization with specific antibodies protects against some worm infections [3, 4, 41, 42].

Eosinophil-ablated mice have allowed testing of their effector function in secondary infection. In secondary subcutaneous larval infection with *Nippostrongylus brasiliensis*, increased numbers of larvae migrate to the lungs in IL-5^{-/-} and dblGATA mice [43]. Similarly, migration of *Trichinella spiralis* newborn larvae is increased in ablated versus WT mice and muscle larvae burdens are increased in dblGATA mice upon secondary infection [45]. Earlier reports showed that eosinophils attach to newborn *T. spiralis* via specific antibodies, degranulating and killing the larva. Protection by eosinophils and antibodies has been documented *in vivo* by transferring sera or immunoglobulin from infected mice together with eosinophils to naïve dblGATA mice prior to infection [45]. Although both EPX and MBP are toxic for newborn *Trichinella in vitro*, mice deficient in EPX or MBP show similar resistance to reinfection that is comparable to wild type mice (WT) (Figure 1). Although it is possible that MBP and EPX are functionally redundant, testing double knockouts is not feasible because such mice do not generate eosinophils [26].

An alternative to ADCC is a mechanism in which antibodies impede the movement of larvae in tissue, enabling binding and further impedance by eosinophils. Recent reports show that specific antibodies are capable of cooperating with basophils or alternatively activated macrophages to impede the mobility of *N. brasiliensis* or *Heligmosomoides* larvae, respectively [46, 47]. Larvae are also immobilized by eosinophils that are activated to release extracellular traps comprised of DNA fibers and eosinophil granule proteins [48, 49]. These mechanisms merit further investigation.

Specific antibodies are clearly important to protection against secondary helminth infection [40, 50–52]. Plasma cells in the bone marrow are the main source of circulating antibodies and prolonged survival of these cells is crucial to sustaining a state of immunity. A variety of cell types that provide contact-dependent and secreted survival signals to plasma cells populate niches within the bone marrow. These include mesenchymal stromal cells [53], megakaryocytes [54], dendritic cells (DCs) [55, 56], macrophages [57], basophils [58] and eosinophils [59]. Bone marrow eosinophils have recently been implicated as a critical cellular source of the plasma cell survival factors, APRIL and IL-6 [59]. Using adoptive transfer protocols in eosinophil-ablated recipient mice, it was shown that plasma cell survival is further enhanced when eosinophils are first activated with adjuvant [60], consistent with a role for eosinophils in bone marrow plasma cell survival during infection. Eosinophils are also capable of regulating peripheral B cell homeostasis and proliferation in mice [61]. Although eosinophils are dispensable for the parasite-specific IgM response during primary infection by *Strongyloides stercoralis* [62], they are required for production of specific IgG2c in *Trichinella* infection [45]. Taken together, the findings suggest that eosinophils may guarantee their protective effector function by sustaining the cellular source of antibodies that are capable of binding Fc receptors on innate effector cells.

Primary infection: Location matters

Recent findings reveal a marked disparity in eosinophil influence among worms that colonize intestinal versus extra-intestinal sites (summarized in Figure 2). In PHIL and dblGATA mice undergoing primary infection with *Schistosoma mansoni*, there are no obvious defects in immune responses, worm burdens, or egg deposition [63]. Furthermore, egg-induced liver lesions, including granuloma formation and fibrosis, are not affected by eosinophil ablation [63]. Larvae survival and development of primary and secondary immune responses are normal in PHIL mice infected with *S. stercoralis* [62]. Similarly, eosinophils are dispensable for the development of intestinal Th2 responses to *Trichuris muris* and worm expulsion is normal in dblGATA mice [64]. Immune responses induced by primary infection with *N. brasiliensis*, are only marginally affected in dblGATA mice [43]. Infection of either PHIL or dblGATA mice with *T. spiralis* showed that eosinophils are dispensable for intestinal immunity that clears adult worms during primary infection [65]. Mechanisms of immunity across several enteric infections vary considerably, relying upon different effector cells and cytokines, yet a common feature is that eosinophils are generally unnecessary for immunity that clears primary infection. A notable exception was reported in a recent study of *Heligmosomoides polygyrus* infection that revealed a protective role of eosinophils in clearing intestinal adult worms [44].

Primary infection: eosinophils, immune regulation and IL-10

Eosinophils enter tissues during inflammation induced by helminth infection [66] or hypersensitivity [67], providing them opportunity to interact with other responding cells. As the diversity of receptors and soluble mediators produced by eosinophils has been revealed, their potential to regulate immunity and influence disease has been tested at several levels.

In both allergic and helminth disease, eosinophils demonstrate the characteristics of antigen presenting cells. Exposure to allergens and worm antigens *in vitro* induces upregulation of co-stimulatory molecules CD80, CD86 and CD40 [68, 69]. In mice infected intraperitoneally with the human filarial parasite *Brugia malayi*, eosinophils upregulate surface MHC II enabling cognate interaction with T cells [69]. During *T. muris* infection, eosinophils display an activated phenotype with increased expression of Siglec-F, MHC II, and CD80 [64]. Studies of *S. stercoralis* infection clearly demonstrate that mouse eosinophils are capable of presenting antigens and initiating antigen specific T cell responses *in vitro* and *in vivo* [28]. Furthermore, eosinophils accumulate in mesenteric lymph nodes (MLN), preferentially localizing in the medulla and T cell zone during *T. muris* infection. Although receptor expression and tissue distribution are consistent with antigen presentation, there is no compelling, direct evidence that antigen presentation by eosinophils impacts the course of worm infection.

Recruitment of T cells is influenced by eosinophils. For example, T cell migration to the lung during allergic asthma is dependent upon eosinophil-derived chemokines [71, 72]. In addition, granules released by eosinophils have profound effects on recruitment and activation of dendritic cells, which in turn promote the development of Th2 responses [71, 72]. More recently, eosinophils have been shown to contribute to suppression of goblet cell

mucus production in the lung and the IgE response in infection by the filarial nematode, *B. malayi*, providing indirect evidence for eosinophil-derived mediators that support immune regulation [70]. Importantly, eosinophils are required for clearance of *B. malayi* microfilariae in primary infection [70].

A dramatic impact of eosinophil-mediated immune regulation is evident during muscle infection by *T. spiralis*. Among parasitic worms, *Trichinella* is unusual in two ways: it completes its life cycle within one animal host and growth and development of all life-stages occurs intracellularly. First-stage larvae are ingested and develop into adult worms in the intestinal epithelium, where they reproduce. Newborn larvae (NBL) migrate from the intestine to skeletal muscles, invade myotubes, grow rapidly, and establish chronic, intracellular infection. Eosinophilia is prominent during both intestinal and extra-intestinal phases of infection; however, eosinophils do not contribute to intestinal immunity that expels worms from the gut or that which limits their fecundity [65, 73], nor do they influence liver injury caused by NBL as they migrate from the intestine [74, 75]. These findings are consistent with those from other mouse models of intestinal helminth infection, described above.

In stark contrast, eosinophils have a profound effect on the growth and survival of intracellular larvae in skeletal muscle. In both *dblGATA* and *PHIL* mice, larval burdens in skeletal muscle are significantly reduced [65, 76]. Unlike the results obtained in secondary infection, NBL arrive in skeletal muscles of eosinophil-ablated mice in numbers comparable to wild-type mice. Poor larval survival in primary infection correlates with reduced infiltration of infected muscle by Th2 cells, associated with increased local production of nitric oxide (NO) [76]. NO is toxic for growing larvae [76].

Among leukocytes that infiltrate infected muscle, IL-10 from CD4⁺CD25⁻ T cells suppresses interferon- γ (IFN- γ) production and induction of inducible nitric oxide synthase (iNOS) in macrophages and neutrophils [77]. Importantly, larval burdens are reduced in IL-10 deficient mice [65, 77, 78] confirming a central role for IL-10 in promoting survival of intracellular larvae. In the absence of eosinophils, IL-10⁺CD4⁺ T cells are reduced, implicating eosinophils as drivers of the IL-10 response [76, 79]. Notably, MHCII expression on eosinophils is not required for the expansion of IL-10⁺CD4⁺ T cells, suggesting that antigen presentation is not important to the function of eosinophils during infection [79]. Transfer of IL-10 competent eosinophils to ablated mice, at the initiation of muscle infection, expands IL-10-producing myeloid DCs and CD4⁺ T cells which in turn inhibit the production of local NO and protect muscle larvae (Figure 1). Indeed, transfer of IL-10 competent DCs or CD4⁺ T cells compensates for eosinophil deficiency [79]. These findings are consistent with an earlier report that eosinophils were required for DC recruitment and accumulation in lung draining lymph nodes in the allergy model [27]. Unsolved questions include the mechanism of DC recruitment by eosinophils.

Primary infection: Eosinophils, worm growth, and IL-4

In addition to evading host defense, successful colonization of the host requires that the parasite secure nutrients that support its growth. This demand for energy often occurs

concurrently with tissue injury. In recent years, important roles for eosinophils have been revealed in both metabolism and tissue regeneration, for example, eosinophils are critical to maintaining homeostasis in white adipose tissue (WAT) and orchestrating biogenesis of beige fat [80–82]. IL-4, a critical cytokine for Th2 immunity, is a central mediator in each case [80–84]. Worms are strong inducers of IL-4. Following the intraperitoneal injection of schistosome eggs, mouse eosinophils are recruited and produce IL-4 [85], demonstrating that eosinophils have potential to initiate or polarize Th2 immunity to worms. Results from 4get mice (IL-4-EGFP reporter mice) infected with *N. brasiliensis* further confirmed the capacity of eosinophils for rapid production of IL-4 [86].

Eosinophils have been linked to IL-4 driven regenerative responses to tissue injury [83, 84]. Following cardiotoxin injection into skeletal muscle, eosinophils are dominant among IL-4-secreting cells that infiltrate the site, and the regenerative response is severely compromised in *dblGATA* mice. Fibro/adipogenic progenitor cells (FAPs) at the site of injection are induced to express IL-4R α . IL-4 drives proliferation of FAPs, preventing their differentiation into adipocytes and supporting myogenic differentiation. Additional findings support the conclusion that FAPs promote muscle regeneration through their ability to phagocytose necrotic fibers [83]. In the liver, infiltrating eosinophils are responsible for promoting IL-4-dependent regenerative responses to injury caused by CCl₄ [84]. Liver regeneration in this model is dependent, in part, on IL-4R α expression on hepatocytes. These observations provide evidence that eosinophils and IL-4 contribute to tissue repair in two highly regenerative organs. In each instance, IL-4 binds to resident or recruited, non-immune cells.

In contrast with the findings in models of liver injury described above, experiments with parasitic worms provide evidence that responses to injury vary, depending on both the insult and the tissue. For example, liver injury is a prominent feature of the granulomatous response to *S. mansoni*, yet eosinophils do not influence the development of granulomas in the livers of infected mice [63]. Similarly, eosinophils do not play a role in protecting the liver against damage caused by migrating *Trichinella* larvae [74, 75]. In contrast, eosinophils are highly active in skeletal muscle invaded by *Trichinella* [65, 76, 87].

Following entry into skeletal muscle cells, NBL undergo a period of growth over the course of 20 days. Larvae grow poorly in muscles of eosinophil-ablated mice. This effect is distinct from NO mediated killing and occurs independently of an adaptive immune response [76, 79]. Host gene expression in skeletal muscle following NBL invasion reflects a regenerative response. Specifically, the presence of eosinophils suppresses local inflammation (e.g. a STAT1 signature) while also influencing nutrient homeostasis. Eosinophils are known to regulate glucose homeostasis [82]. In wild-type mice, nurse cells redistribute glucose transporter 4 (GLUT4) and phosphorylate Akt, correlating with glycogen storage by larvae that is known to occur. The data are consistent with a mechanism in which eosinophils promote larval growth by an IL-4 dependent mechanism that limits local interferon-driven responses that otherwise alter nutrient availability in infected muscle. (Figure 1).

Although IL-5 is a critical cytokine in regulating activity of eosinophils, *Trichinella* larval growth is not affected by IL-5 deficiency. This is an important observation in the context of

glucose uptake, as recent reports showing that lineage negative, innate lymphoid type 2 cell (ILC2) produces large quantities of type 2 cytokines IL-5 and IL-13. Accumulation of eosinophils and alternatively activated macrophages (arginase-1 positive; AAMs) in mouse visceral adipose tissue (VAT) depends upon IL-5 and IL-13, respectively [82, 88]. ILC2s in VAT are the major source of these cytokines [88]. IL-4 dependent AAMs in VAT are associated with metabolic syndrome, type 2 diabetes, and insulin resistance. Eosinophils are the predominant source of local IL-4 in VAT [82]. The recruitment of IL-4-producing eosinophils to VAT by IL-5-producing ILC2s further promotes alternative activation of adipose resident macrophages, which are required for increasing systemic insulin sensitivity. This model may be relevant to infection with *Litosomoides sigmodontis*. *L. sigmodontis* larvae show impaired growth in the absence of either eosinophils or IL-5 [89]. In contrast, ILC2-dependence is not supported for *Trichinella* infected muscle. Larvae grow normally in either ILC2 or IL-5 deficient mice, suggesting that a distinct mechanism is at work in skeletal muscle [87].

Concluding remarks

Mouse models of eosinophil ablation and related research tools have enriched our understanding of eosinophil function in health and disease. Across several models of worm infection, the influence of eosinophils ranges from insignificant to pivotal. Dramatic differences in function are evident in primary versus secondary infection and also in intestinal versus non-intestinal infections. The dogma that eosinophils are defenders clearly applies in secondary infection, while in primary infection of extra-intestinal sites, eosinophils protect the parasite. By promoting delivery of essential host resources that support parasite growth and preventing a toxic immune response that causes larval destruction, eosinophils appear to be duped by worms. Further exploration of this developing story is an exciting prospect. Determining whether eosinophils and worms are similarly complicit in other animal species will be of particular interest, with important implications for managing and preventing infections in people and animals.

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Outstanding questions

How does the antibody-mediated effector function of eosinophils compromise larvae: adherence, impedance by extracellular traps, killing by toxic granules?

What accounts for the tissue-dependent variation in eosinophil function and influence on parasitic worm infection?

How are eosinophils called into skeletal muscle within hours of colonization by *Trichinella* larvae?

How does IL-4 support larval growth: by a direct effect on infected cells, by influencing cells adjacent to the nurse cell, by inhibiting STAT1 dependent responses that limit glucose availability to infected cells?

What is the relevance of findings in mouse models to diseases in humans and other animals?

Trends

Parasitic worms exploit cells and cytokines of the innate immune system that are critical to metabolic homeostasis and responses to injury.

The influences of innate and adaptive immune mediators on parasitic infection vary among tissue/organ systems, e.g. IL-4 promotes expulsion of worms from the intestine, yet supports worm survival in extra-intestinal sites.

Eosinophils sometimes have a profound influence on helminth infection that is independent of eosinophilia and Th2 driven immunity.

Figure 1. Model of eosinophil mediated influences on primary and secondary infection by *Trichinella spiralis*

In primary infection, *Trichinella* induces a mixed Th1 and Th2 immune response associated with eosinophilia in skeletal muscle. By secreting IL-10, eosinophils promote the expansion of IL-10-producing myeloid dendritic cells (DCs), and enhance the production of IL-10 by CD4⁺CD25⁻ T cells. In macrophages and neutrophils, the production of nitric oxide (NO) by interferon- γ (IFN- γ)-activated inducible nitric oxide synthase (iNOS) is suppressed by IL-10, thereby promoting survival of muscle larvae. Independent of the adaptive immune response, eosinophils and IL-4 promote larval growth by controlling activation of STAT1, a

signaling molecule that is known to attenuate insulin sensitivity and impair glucose uptake. The identity of cell(s) responsible for STAT1-dependent gene expression is not known. In secondary infection, eosinophils cooperate with antibodies to interfere with migratory newborn larvae (NBL), preventing their colonization of skeletal muscle and completion of the life-cycle.

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Figure 2. Life cycles of selected helminths

Different phases of infection in the mammalian host are highlighted in red. Symbols indicate life stages impacted by eosinophils in primary (I) or secondary (#) infection.

Table 1

Documented influence of eosinophils on helminth infections in eosinophil-ablated PHIL or dblGATA mice.

Helminth	Primary	Secondary	References
<i>Nippostrongylus brasiliensis</i>	- increased parasite egg production in intestinal phase	- more larvae in the lung. - impaired worm expulsion when CD4 ⁺ T cells were depleted	[43,90]
<i>Heligmosomoides polygyrus</i>	-impaired worm expulsion	- more worms trapped in the intestinal wall of mice immunized with excretory/secretory products	[44]
<i>Trichinella spiralis</i>	- impaired growth of muscle larvae - decreased burden of muscle larvae	- increased number of migratory newborn larvae - increased muscle larvae burden	[45, 65, 76]
<i>Brugia malayi</i>	- increased number of microfilarae	no impact	[70]
<i>Litomosoides sigmodontis</i>	- impaired development of filariae	unknown	[89]
<i>Trichuris muris</i>	no impact	unknown	[64]
<i>Strongyloides stercoralis</i>	no impact	no impact	[62]
<i>Schistosoma mansoni</i>	no impact	unknown	[63]