Current perspectives

The role of eosinophils in host defense against helminth parasites

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The precise function of eosinophils in parasitic infection in vivo remains poorly understood despite eosinophils having been shown to be potent effectors in killing parasites in vitro. Although it has long been held that the primary function of the eosinophil is protection against helminth parasites, there are little data to prove this unequivocally. Moreover, eosinophils are responsible for a considerable amount of inflammatory pathology accompanying helminth infections. This article will provide an overview of our current knowledge about eosinophils and their role, both protective and pathogenetic, in parasitic helminth infections. (J Allergy Clin Immunol 2004;113:30-7)

Key words: *Immunity, resistance, cytotoxicity, nematodes*

Parasitic infections are caused by unicellular protozoa or multicellular helminths (worms), the extraordinary worldwide prevalence of which exacts a major medical and economic burden. Each parasite has an exceedingly diverse and unique biology. Protozoa are usually a few micrometers in size, whereas helminths are typically centimeters to meters in length. Tissue-dwelling protozoa are often intracellular parasites at some stage of infection, whereas helminths, being larger than most tissue cells, are almost always extracellular pathogens, with the significant exception being *Trichinella spiralis*. Although protozoa usually replicate during infection of a single host, helminths do not reproduce without the passage through either intermediate hosts or through soil or water.

Helminths have many developmental stages present during infection.[1] In the course of a single infection, the host might be repeatedly exposed to larval-, adult-, or egg-stage antigens. For example, free-swimming cercariae of the trematode *Schistosoma mansoni* penetrate the skin of human subjects immersed in infested water and evolve into tissue-stage schistosomula that migrate to the liver and mesenteric veins for further differentiation into sexually dimorphic adult worms. Eggs are laid that migrate through tissues into the lumen of the bowel or bladder for environmental release. Similarly, filarial infection involves repeated exposure to arthropod-borne infective larvae and parasitization by long-lived adult worms capable of continuously releasing microfilariae that circulate in the bloodstream or migrate through subcutaneous tissues.

Because each stage of parasite development can be antigenically distinct, the host response to helminth infection is often characterized by a series of discrete immune responses that evolve at different times during the course of infection. Protective immunity directed against a single stage might be circumvented by parasite differentiation, assisting in the survival of the pathogen and posing a significant challenge to immune-mediated resistance. Each stage of parasite development might also entail a change in tissue trophism, introducing a compartmental feature to immune or inflammatory responses. For example, a variety of distinct cutaneous, pulmonary, and intestinal inflammatory or hypereosinophilic syndromes are associated with different stages of Ascaris and Strongyloides species as they migrate through the skin and lung before reaching adulthood in the gastrointestinal tract.[2]

Because complex parasitic life cycles can only be maintained by sequential passage through intermediate and definitive hosts, parasites have adapted methods of optimizing transmission by prolonging infection. Perhaps because of this evolutionary pressure, chronicity, latency, or both are hallmarks of helminth infections. For example, adult schistosomes and filariae can survive in host tissues for as long as 20 to 30 years, continuously producing eggs and larvae. Strongyloides species, because of their ability to "autoinfect," maintain their life cycle for decades.[3] Chronicity might also reflect pressure toward "true parasitism" in that inducing mortality in the host would disrupt parasite transmission if the host were to die before egg laying or larval release could occur. Adaptations for chronicity can be so successful among parasites that naturally acquired protective immunity is only observed rarely in areas endemic for a given disease. Specifically, schistosomiasis results in an incomplete form of protection in which reinfection is limited but adult worms are tolerated for years (concomitant immunity).[4]

Whereas pathology and protective immunity to most protozoa are believed to reflect T cell–, B cell–, and macrophage-dependent (or perhaps cytokine-mediated) mechanisms, infection with helminth parasites induces immune effector responses that are characterized by IgE

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Received for publication October 20, 2003; revised October 20, 2003; accepted for publication October 21, 2003.

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doi:10.1016/j.jaci.2003.10.050

antibody production, tissue and peripheral blood eosinophilia, and the participation of inflammatory mediator–rich tissue mast cells, qualitatively not unlike that seen in atopic diseases. For the atopic state, these responses have clearly been implicated in the pathogenesis of allergic diseases. In parasitic infection, although these types of responses can certainly induce pathologic reactions, they have also been implicated in mediating protective immunity to the helminth parasites.[5-7]

GENERAL FEATURES OF THE IMMUNE RESPONSE TO HELMINTHS

Whereas the cytokine response to helminth infection is clearly more complex than initially appreciated,[8] the eosinophilia and increased serum IgE levels characteristic of helminth infection are associated with the production of IL-4 and IL-5. The critical role of IL-5 in this eosinophilic response has been clearly demonstrated in animal models, including transgenic and knockout mice infected with a wide variety of intestinal and tissue helminths.[9,10] Whereas IL-5 alone is sufficient to cause helminth infection–mediated eosinophilia and eosinophil activation in most instances, a number of other cytokines (eg, IL-4 and IL-13)[11,12] and chemokines (eg, RANTES and eotaxin)[13,14] appear to be necessary for the recruitment of eosinophils to the tissues.

A number of transcription factors involved in the regulation of *IL5* gene expression, including nuclear factor (NF) IL-6 (C/EBP/β), NF-AT, and GATA-3, have been recently identified.[15] At least one of these, GATA-3, appears to play a direct role in helminth-induced eosinophilia, as demonstrated by enhanced IL-5 production and eosinophilia in GATA-3–transgenic mice infected with *Heligosomoides polygyrus*, but not in uninfected GATA-3–transgenic control mice.[16] The role of other transcription factors in the eosinophilia of helminth infection remains to be explored.

Eosinophils in the blood and tissues of helminth-infected patients and experimental animals exhibit morphologic and functional changes associated with eosinophil activation in vitro.[17] These include decreased density,[18,19] upregulation of surface activation molecules (eg, CD69, CD25, CD44, and HLA-DR),[20] enhanced cellular cytotoxicity, and release of granule proteins, cytokines, leukotrienes, and other mediators of inflammation.

The kinetics of eosinophilia after infection with any parasitic infection have been difficult to define because the time of initial infection can be defined only rarely. However, information from both single-source outbreaks[21,22] and experimental human infection has shed light on the initiation of eosinophilia and its natural regulation. In a study of 5 volunteers infected with the hookworm *Necatur americanus*,[23] it was shown that blood eosinophil counts increased from baseline between 2 and 3 weeks after infection and peaked between weeks 7 and 9 (Fig 1). This increase was accompanied by a parallel increase in total leukocyte counts. In those exposed to lymphatic filariae,[24] peak eosinophil levels occur 11 to

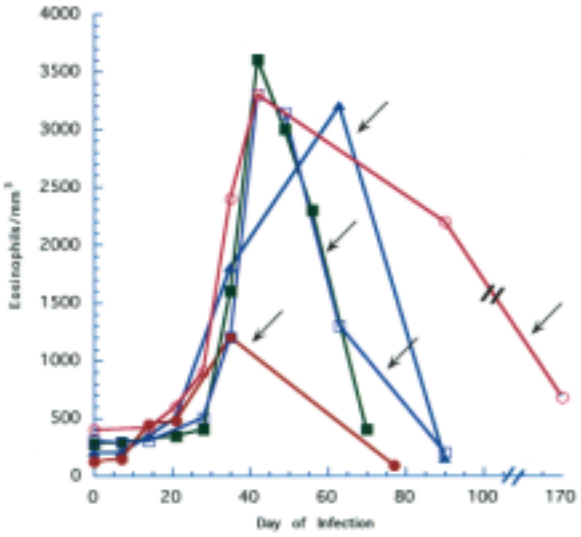

FIG 1. Blood eosinophil response in 5 normal volunteers after exposure to *Necator americanus* third-stage larvae. Each *line* represents an individual patient's eosinophil levels after experimental infection with hookworm. The *arrows* indicate the time of anthelmintic treatment. Adapted from data of Maxwell et al.[23]

30 weeks after infection, which is consistent with the differences in life cycles between these parasites (Table I). More interesting was a spontaneous decrease in eosinophil numbers in the absence of treatment, suggesting active downregulation of the eosinophilia, a process seen in other experimental volunteer infections.[25,26] This active (and spontaneous) modulation of eosinophilia generally occurred when migration of the larval forms ceased and the infections became patent (sexually mature adults began egg laying [eg, hookworm[23] or schistosomes]) or larval release (eg, filariae[24]).

EOSINOPHILS AND PROTECTIVE IMMUNITY

Epidemiologic evidence for the role of eosinophils in the immune response to helminth infections

As early as 1939, eosinophils were postulated to play a role in the immune response to helminth infection.[27] Such hypotheses were based primarily on histopathologic evidence of eosinophils surrounding dying parasites in tissue biopsy specimens. Later, in vitro killing of parasites by eosinophils (in the presence of antibodies, complement, or both)[28-30] and eosinophil granule products[31,32] was demonstrated. Despite these in vitro findings and epidemiologic evidence correlating high eosinophil counts with resistance to posttreatment reinfection with *Schistosoma haematobium* and *S mansoni* in human subjects,[33,34] the in vivo role of eosinophils in immunity to helminth infection has been much more difficult to define. There has been conflicting evidence[35,36] on the protective efficacy of eosinophils in animals depleted of eosinophils and subsequently challenged with a variety of parasites

TABLE I. Blood eosinophil response in human volunteers infected with lymphatic filariae

Parasite	Peak WBC	Peak eosinophil count (/μL)	Time to peak eosinophil count (wk)	Reference
Brugia malayi	20,400	12,840	14	84
B malayi	8800	2640	13	85
B malayi	7600	1234	14	85
Brugia pahangi	8200	1330	12	85
B pahangi	9600	1692	12	85
B malayi	32,000	22,400	26	86
B malayi	10,000	<500	20	87
B malayi	11,880	3020	30	87
B malayi	10,800	1728	11	88

WBC, White blood cell count.

TABLE II. The role of the eosinophil in mediating protection in selected helminth infections

Parasite	Protection		References
	Animal model	Human model	
Cestode			
Mesocestoides corti	–		89-91
Hymenolepsis diminuta			92
Trematode			
Fasciola species			35, 92
Schistosoma species	–	++	33, 34, 41, 43, 77, 93, 94
Nematodes			
Angiostrongylus species	++		48, 49, 95
H polygyrus	–		9, 96
Brugia species	++		97
Litomosoides species	++		98
Nippostrongylus species	±		10, 99
Onchocerca species	++	±	50-53, 60, 71, 73, 75, 100, 101
Strongyloides species	++		44-46
Toxocara species	–		102-105
Trichinella species	±		39, 42, 106-108
Trichuris species	–		96, 109-111

–, Shown to play no role; ++, documented role; ±, conflicting information.

(Table II). Overall, the discrepancy seen in the various studies examining the role of eosinophils in protective immunity in different animal models of parasite infections has been attributed to the redundancy of effector immune responses or to the basic differences in the animal models used.[37]

Animal models

Animal models of helminth infection have allowed for the assessment of the eosinophil's role in vivo. Treatment with anti–IL-5 antibody prevents the tissue and blood eosinophilia that occurs in animal models of gastrointestinal helminth infection.[10,38] However, such treatment has not been shown to increase susceptibility to infection in murine models of *T spiralis*,[39] *Trichuris muris*,[40] *Nippostrongylus brasiliensis*,[10] *H polygyrus*,[9] or schistosomiasis.[41] Similarly, IL-5 transgenic mice that have persistent increases of eosinophil levels do not demonstrate enhanced immunity to *T spiralis* or *S mansoni* infection.[42,43]

Eosinophils do appear to play a role in the killing of some helminths, particularly those with tissue-migratory stages. Larval stages of many of the intestinal helminths, including Strongyloides, Nippostrongylus, and Ascaris species, migrate through the lungs or skin on their way to the gastrointestinal tract. Although treatment with anti–IL-5 antibody had no effect on primary infection with *Strongyloides venezuelensis* in mice, recovery of worms from the lungs after challenge infection was increased in anti-IL-5–treated mice compared with that in control animals.[44] The most conclusive in vivo evidence for the role of eosinophils in helminth killing comes from experiments with diffusion chambers containing infective *Strongyloides stercoralis* larvae implanted in the subcutaneous tissue of mice. Not only were eosinophils the only cells that accumulated in the chamber concomitant with larval killing,[45,46] but such killing could be prevented by means of inhibition of eosinophil migration into the chamber or treatment of the mice with anti–IL-5 antibody.[45-47]

In *Angiostrongylus cantonensis* infection in mice, immature worms develop in the brain before migrating to the lungs. Prolonged survival of intracranial worms resulting in a larger parasite burden in the lungs has been demonstrated in normal mice treated with anti–IL-5 antibody,[48] as well as in IL-5 receptor α–deficient mice,[49] which is again consistent with a role for eosinophils in tissue-based larval killing.

TABLE III. The role of the eosinophil in mediated pathology in selected parasitic helminth infections

Parasite	Host	Organ	Eosinophil role in pathology	Reference
Angiostrongylus species	Mouse	Brain	+	112, 113
	Guinea pig		+	
	Rat		–	
Anisakis species	Human	Intestine	+	69
Baylisascaris species	Human	Brain	+	70, 114
Fasciola species	Mouse	Liver	+	92
Mesocestoides species	Mouse	Lung	–	91
Nippostrongylus species	Mouse	Lung	–	10
Onchocerca species	Mouse	Eye	+	60, 71, 73, 100, 115
		Skin	+	
Schistosoma species	Mouse	Liver	–	11, 77, 116, 117
	Human	Bladder	+	93
Toxocara species	Mouse	Lung	+	102, 103

Animal models of protective immunity to the exclusively tissue-dwelling nematode Onchocerca species further support this hypothesis. In diffusion chamber studies of infective larval survival in immune mice, eosinophils were the only cells that accumulated in the chambers at the time of larval killing.[50] Furthermore, similar to the results with Strongyloides species larvae, eosinophil larval contact was necessary for larval destruction to occur.[50] In a different murine model system, mice infected with *Onchocerca lienalis* depleted of eosinophils (but not macrophages or neutrophils) with mAb showed delayed clearance of primary infection and abrogation of resistance to secondary infection.[51]

Overall, despite the prevailing dogma, the animal models do not strongly support an indispensable role for eosinophils in immunity to most helminth parasites in vivo.

Human studies

In human subjects with onchocerciasis, the picture is somewhat more complex. Some studies of in vitro cytokine production in response to parasite antigens have demonstrated increased levels of IL-5 production by PBMCs from putatively immune individuals living in areas endemic for onchocerciasis in comparison with infected individuals,[52] whereas others have detected no difference in parasite antigen-induced IL-5 production between these groups.[53] The most compelling association between eosinophilia and protective immunity in human subjects comes from the elegant posttreatment reinfection studies in schistosomiasis that demonstrated a direct relationship between the lack of reinfection and the level of peripheral blood eosinophils in patients from areas endemic for both *S mansoni* and *S haematobium* infections.[33,34]

Mechanism of eosinophil-mediated killing of helminth parasites

The mechanism by which eosinophil-mediated protection against helminth infection occurs is incompletely understood, but in most cases it appears to involve antibody-induced release, complement-induced release, or both of toxic granule proteins and reactive oxygen intermediates by activated eosinophils. Interestingly, evidence suggests that there might be significant differences in the susceptibility to and mechanisms of eosinophil-mediated killing between different life-cycle stages of the same parasite.[54,55] Unlike protozoa, helminths are too big to be engulfed by phagocytes and can only be killed by these cells when the latter have been activated by products of the adaptive immune response.

Eosinophils, which frequently accumulate in tissues soon after worm invasion, might also play a role in innate defense against this type of parasitic pathogen. For example, L3 larvae of *S stercoralis* are killed within 72 hours when implanted in chambers in susceptible mice,[45,47] with eosinophils being prominent among the cells infiltrating the chambers. Larval attrition is greatly reduced when worms are transferred instead into IL-5–deficient mice. This rapid and nonspecific eosinophilic response might be a barrier-limiting mechanism active against the invasion of many tissue-dwelling helminths.

EOSINOPHILS AND PATHOGENESIS

Eosinophils have also been implicated in the pathogenesis of helminth infection (Table III). Similarities between the sequelae of hypereosinophilic syndrome, a syndrome characterized by extremely high levels of eosinophilia and eosinophil-mediated end-organ damage,[56] and the pathologic consequences of infection with helminths, including loiasis and lymphatic filariasis[57,58] and toxocariasis,[59] suggest a primary role for eosinophils in the pathogenesis of these infections. The association between the severity of clinical manifestations and the degree of eosinophil activation, as exemplified by increased eosinophil responsiveness to platelet-activating factor in patients with sowda, a hyperreactive form of onchocerciasis associated with severe dermal pathology,[60] provides additional indirect support for this hypothesis.

Although eosinophils can cause tissue damage through a number of different mechanisms, including direct cellular cytotoxicity, physical damage caused by tissue infiltration (space occupying), and thromboembolic phenomena caused by eosinophil-induced hypercoagulability,[56] it

is the release of eosinophil granule proteins and other cytotoxic substances that has been best correlated with helminth-induced tissue damage. Eosinophil cationic proteins (including major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin) are toxic to various normal tissues and cells both in vitro and in vivo.[7,61,62] Other potentially toxic inflammatory mediators that are released by activated eosinophils include leukotrienes,[63] platelet-activating factor,[64] reactive oxygen species,[65] and lysosomal hydrolases.[62] These mediators not only damage the parasite and surrounding tissues (reactive oxygen intermediates[66,67] and hydrolases) but play a role in the perpetuation of the local immune response (platelet-activating factor and leukotrienes).

Serum and urine levels of eosinophil granule proteins provide an indirect measure of degranulation in the tissues and are markedly increased in many helminth-infected patients.[68] Furthermore, deposition of eosinophil granule proteins in the affected tissues of patients with helminth infection has also been described.[69,70] One of the characteristics of the posttreatment reaction in onchocerciasis is an increase in blood and tissue levels of eosinophils and eosinophil granule proteins that is temporally related to the onset of symptomatology[71,72] and preceded by an increase in serum levels of IL-5.[73] Although localization of eosinophils and their products in the skin is most pronounced at sites of microfilarial degeneration,[71] which is consistent with the hypothesis that eosinophil-mediated parasite killing plays a major role in pathogenesis, the possibility that recruitment of eosinophils occurs secondarily in response to parasite death cannot be excluded.

More direct evidence that eosinophils contribute to the pathogenesis of helminth infection comes from animal models. Eosinophilic infiltration of the cornea and conjunctival tissue, a characteristic component of onchocercal sclerosing keratitis in human subjects, occurs within 72 hours of intrastromal injection of sensitized mice with onchocercal antigens.[74] Attenuation of both the eosinophil response and ocular inflammation is observed in IL-4 knockout mice on a C57BL/6 background,[74] whereas eosinophil infiltration and corneal opacification is increased in mice treated with IL-12 at the time of corneal sensitization to onchocercal antigens despite decreased local expression of T_H2 cytokines, including IL-5.[75] The presence of eosinophil infiltration in a T_H1 environment could be accounted for by a marked increase in corneal chemokines, including RANTES and eotaxin.[75] Taken together, these data suggest that eosinophils, rather than the cytokine milieu, are the crucial factor in the development of onchocercal keratitis.

Although it seems clear that eosinophils play a role in some models of helminth-induced pathology, they do not appear to be essential to the development of pathologic sequelae in all cases. Liver granulomas in schistosomiasis typically contain a high percentage of activated eosinophils, the major source of T_H2-associated cytokines in the granuloma milieu.[76] Despite this, anti-IL-5–treated mice infected with schistosomiasis produce normal-sized granulomas, despite the absence of eosinophils.[77] Similarly, lung infiltrates in *N brasiliensis*–infected mice treated with anti–IL-5 differ from those of *N brasiliensis*–infected control mice in the absence of eosinophils but not in size or number of lesions.[10] Finally, in contrast to the attenuation of ocular disease observed in IL-$4^{-/-}$ C57BL/6J mice, corneal opacification is exacerbated in sensitized IL-$4^{-/-}$ BALB/cJ, STAT$6^{-/-}$ BALB/cJ, and IL-$5^{-/-}$ C57BL/6J[78,79] mice after intrastromal injection of onchocercal antigen and is accompanied by increased tissue infiltration by neutrophils (but not eosinophils).[79] Whether these findings reflect a true lack of involvement of eosinophils in the pathologic sequelae of natural infection with these parasites or the induction of alternative mechanisms of pathogenesis in the setting of dysregulation of the cytokine milieu remains to be elucidated.

CONCLUSIONS

Much has been learned about the biology of the eosinophil[80] and its role in the pathogenesis of allergic,[81] gastrointestinal,[82] and hypereosinophilic disorders[83] since its discovery in 1879. The importance of its role in parasitic infections remains a large question, despite the plethora of studies in animals and human populations. Although we wait for unequivocal evidence for the salutary role of the eosinophil in helminth infections, we are left still with strong epidemiologic support for the eosinophil's benefit. Although unsatisfying as a conclusion, we must endeavor to elucidate its role more definitively if we consider the billions of persons worldwide infected with these multicellular and elusive parasitic organisms.

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