Resistance to Helminth Infection: The Case for Interleukin-5–Dependent Mechanisms

Rick M. Maizels and Adam Balic

Institute of Cell, Animal, and Population Biology, School of Biological Sciences, University of Edinburgh, Edinburgh, United Kingdom

(See the article by Quinnell et al., on pages 430–8.)

It has been >100 years since the link between eosinophils and helminth infections was first noted [1] and nearly 30 years since the eosinophil was proposed to be the principal effector cell for antihelminth protective immunity [2, 3]. Until the late 1980s, the paradigm held sway that eosinophils (perhaps armed with parasite-specific IgE) killed helminths by degranulation, unleashing toxic proteins at the target parasite [4]. This view was supported primarily by associations in vivo (higher eosinophilia in humans resistant to infection [5]) and in vitro (adherence to and killing of parasites by eosinophils [6]). The concept was cemented by data showing loss of resistance in mice experimentally depleted of eosinophils with polyclonal antibodies [3]. However, no observer had witnessed the phenomenon occurring in vivo. Thus, a controversy continued as to whether the observed association was one of cause or effect—for example, eosinophils may be attracted to dead or dying parasites or to those that have strayed into an inappropriate niche.

A turning point was the discovery of interleukin (IL)–5 and its importance in the generation, activation, and survival of eosinophils [7]. Somewhat unexpectedly, the first key experiments using anti–IL-5 antibodies showed that mice retained immunity to *Schistosoma mansoni* in the absence of eosinophils [8]. Treatment with anti–IL-5 antibodies also failed to change the outcome of nematode infection in other mouse models, including *Trichinella spiralis* [9] and *Trichuris muris* [10], and worm burdens were unchanged in IL-5 knockout ($IL\text{-}5^{-/-}$) mice infected with *Toxocara canis* [11]. These and other results (discussed in more detail in recent reviews [12–14]) were influential in shifting sentiment away from a role for eosinophils in parasite immunity. Nevertheless, controversy has continued, particularly concerning the disparity between mice and other species, and interest has been maintained in epidemiological studies of human infections.

It is therefore instructive to revisit this issue in the light of the latest work of Quinnell et al. [15] on hookworm-infected patients from Papua New Guinea, which appears in this issue of *The Journal of Infectious Diseases*. The article by Quinnell et al. represents the most detailed study to date on cytokine-response profiles in human hookworm infection, which currently afflicts hundreds of millions of people worldwide. As in earlier key studies of schistosomiasis [5, 16, 17], patients were administered curative drug therapy and were reexamined many months later to measure susceptibility to reinfection. The key observation is that reinfection following therapy is associated with higher IL-5 responses. The authors also report that hookworm infection downmodulates interferon-γ/Th1 responses to parasite antigen and that coinfection with filarial or malarial parasites interferes or suppresses the antihookworm response: each of these findings is also important in its own right but is beyond the scope of the present discussion.

IL-5 responses to *Necator americanus* antigen do not correlate with intensity of infection before anthelmintic treatment and do not increase after treatment. However, intensity of reinfection is inversely correlated with pretreatment IL-5 responses to *N. americanus* antigen. This result implies that the effect of IL-5 (which, as the authors note, probably is mediated by eosinophils) does not operate against existing worm burdens but against incoming (larval) parasites. This is a significant finding that applies to a number of animal model studies. For example, if IL-5 is overexpressed in mice (through transgenic constructs using the CD2 or similar promoters), eosinophils are sufficiently numerous and/or activated to kill larvae of some parasite species (e.g., *Nippostrongylus brasiliensis* [18–20] and *Litomosoides sigmodontis* [21]) but not others [20, 22]. Thus, quantitative con-

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Reprints or correspondence: Dr. Rick M. Maizels, Institute of Cell, Animal, and Population Biology, Ashworth Laboratories, West Mains Rd., University of Edinburgh, Edinburgh EH9 3JT, UK (rick.maizels@ed.ac.uk).

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siderations may determine whether eosinophils are protective.

Moreover, murine models of infection have demonstrated that the IL-5–deficient phenotype in various infection models is quite specific to certain stages of certain species and that there are examples in which nematode infectivity is enhanced in the absence of IL-5 (e.g., *Strongyloides venezuelensis* [23] and *Angiostrongylus cantonensis* [24]). Furthermore, IL-5–dependent mechanisms may not operate at first exposure but may be mobilized after challenge; thus, *L. sigmodontis*–infected IL-$5^{-/-}$ mice fail to gain immunity after vaccination, although the level of innate protection remains unaltered [25]. Similarly, although an IL-5–deficient phenotype has no effect on the outcome of a primary infection with *T. spiralis,* significantly larger worm burdens are noted in IL-$5^{-/-}$ mice after challenge infection [26]. In humans, most individuals in regions where nematode infection is endemic are undergoing repeated challenge infections, rather than primary infections; therefore, it is precisely this aspect of antinematode immunity that should be examined in rodent models.

In general, the proposition that eosinophils and other IL-5–dependent mechanisms can operate to kill helminth parasites is supported both by this latest study of nematodes [15] and by earlier investigations of human schistosome reinfections [16, 17]. Drawing from the murine model systems, one can conclude that these mechanisms require amplification (whether by innate cell populations or adaptive antibody components) before they are competent to protect the host. Moreover, the human hookworm study now highlights the thesis, drawn from experimental models, that this protection operates primarily to intercept newly invading larval forms and does not kill established adult worms. Before reaching such a conclusion, however, there are some important areas that remain to be defined.

First, IL-5 is not limited to driving eosinophil activity. This cytokine, like most others, is pleiotropic in effect. In the mouse, IL-5 is also a B cell growth factor [7], particularly for the B-1 ("natural") B cell subset associated with autoantibody production [27, 28]. IL-5 also induces B cells to switch to IgA [29], which is of paramount importance in the gastrointestinal tract. Moreover, a recent report attributes the failure of IL-$5^{-/-}$ mice to kill *L. sigmodontis* parasites to a loss of neutrophil function [30].

Second, other cytokines are involved in differentiation, migration, and activation of eosinophils. Principal among these is eotaxin, a chemokine that is released locally at sites of inflammation and that mobilizes eosinophils [31]. IL-5–deficient mice retain small numbers of normal eosinophils, because of the presence of eotaxin, but are unable to expand this population in the event of nematode infection [11]. IL-4 and IL-13 are also implicated in eosinophil extravasation, probably by up-regulating adhesion proteins such as intracellular adhesion molecule–1 [32, 33].

Third, not all eosinophils are the same, and eosinophils can act in more than one way. The "headline" activity of eosinophils—degranulation—may have attracted attention to the detriment of the more steady-state function of these cells. Eosinophils are major producers of cytokines [34]; for example, they are a major source of IL-4 in *N. brasiliensis*–infected mice [35, 36]. Likewise, human eosinophils are a principal source of transforming growth factor–β, which is responsible for airway remodeling in asthma [37], and produce the proinflammatory cytokine macrophage migration inhibitory factor [38]. Not surprisingly, continuous eosinophil activation can have adverse outcomes in a range of tissue contexts [39].

These studies highlight one of the major deficiencies of many studies of eosinophils—that is, the identification of this cell type solely by histochemical methods. Heterogeneity within the eosinophil population (e.g., between quiescent and "activated" eosinophils) is rarely followed by surface staining for markers such as CCR3, the eotaxin receptor. Of interest, it has recently been reported that eosinophils from *Schistosoma japonicum*–infected mice show a prolonged downshift in expression of CCR3 and induction of CXCR3, the receptor for 2 other chemokines (CXCL9 and 10) [40]. The importance of defining an eosinophil's functional state is emphasized by the work of Shinkai et al. [35], who demonstrated that a notable subset of IL-4–positive eosinophils in the lungs of *N. brasiliensis*–infected mice had degranulated, differing significantly in histochemical structure from nondegranulated eosinophils. This raises the questions of how degranulation may be regulated and whether a balance between different chemokines may determine if the full effector potential of the eosinophil is triggered.

One consequence of this heterogeneity in degranulation status is that the gross structure of the eosinophil population will vary markedly; indeed, this has been suggested to be a potential cause of misidentification of eosinophils in earlier studies [35]. Further heterogeneities may well occur by locale (e.g., skin vs. lung) and certainly occur between species, because mouse eosinophils lack the FcεRI, which is postulated to be critical for IgE-dependent recruitment of human eosinophils [41].

From these varied data it is clear that, after a century or more of study, no general role can be assigned to eosinophils in helminth infections. However, the data presented by Quinnell et al. [15] greatly strengthen the case that, if there is a role for eosinophils in resistance to human hookworm infection, it is likely to be against reinfection by larval stages. Definitive data on phenotyped eosinophils acting to kill nematodes or other helminths have yet to be obtained. Further studies of both human and nonhuman systems should address this point.

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