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Acquired Immunity to Helminths

DAVID ARTIS, AND RICK M. MAIZELS

INTRODUCTION

Helminth parasites are multicellular worms belonging to the Nematode, Trematode, and Cestode taxa which have co-existed with the vertebrate immune system for a long evolutionary time. Consequently, an impressive array of host innate and adaptive immune mechanisms have developed to control helminth infections and the pathologies they engender. Despite these defenses, a number of important parasitic helminths have become adept at evading immunity and continue to pose major health problems in humans and animals.

In humans, the pattern of helminth infections amply illustrates the key characteristics of these parasites and the courses of disease they cause. Most species do not multiply in the mammalian host, and helminth infections accumulate through repeated transmission, reflecting both chronic susceptibility and remarkable parasite longevity (often >10 years). Infections can reach very high prevalence, particularly among children, and even today over 25% of the human population carries one or more helminth parasite species (Hotez et al., 2008). Infections are often overdispersed, with a small number of highly infected carriers, and polymorphisms controlling susceptibility or resistance to helminth infections are now being identified (Quinnell, 2003).

In this chapter, we focus on adaptive immunity to helminth parasites, first in the context of naturally acquired immunity in human populations, and subsequently in terms of experimental studies. Laboratory models for the study of protective immunity, in both intestinal tract or tissue systems, present common and specific features; in general, the Th2 arm of cellular immunity is seen to orchestrate the anti-helminth immune response, acting through pivotal inducer and activating cytokines including IL (interleukin)-4, IL-5, IL-9, and IL-13. These mediators promote effector mechanisms ranging from antibodies, macrophages, and granulocytes, to epithelial cell responses that have less prominence in microbial and protozoal infections (Anthony et al., 2007). In the final part of the chapter, we will consider how understanding the different roles of key immune components will help us achieve the optimal balance of immune responsiveness to maximize immunity while avoiding deleterious immunopathological consequences.

IMMUNITY TO HELMINTH PARASITES IN HUMANS

The strongest indication that acquired immunity to helminth infections may develop in humans comes from the changing patterns of infection with age. Peak intensities of schistosome infection, for example, are observed in juveniles, whereas older individuals are more likely to have low-level or undetectable infections (Bundy & Medley, 1992). Moreover, the higher the level of transmission in a community, the younger the age of peak infection intensity, implying that immunity is acquired following a certain quantum of exposure to the parasite (Woolhouse, 1992). While intensity declines with age in all common helminth infections, prevalence is often asymptotic, indicating a degree of acquired immunity that limits the survival of new waves of parasites and/or reduces the fecundity of those that are able to establish themselves. With each helminth species following different developmental and migratory pathways within the host and using different transmission life history strategies, unanswered questions remain about the targets and locales for protective immunity in humans and how partial immunity may act to limit, but not eliminate, the presence of parasitic organisms.

The most common group of human helminth parasites are the soil-transmitted geohelminths, principally the gastrointestinal nematodes *Ascaris*, hookworm (*Ancylostoma* and *Necator* species), and *Trichuris* (Hotez et al., 2008). These infections show the classic age-intensity profile, which declines after the childhood years (Bundy & Medley, 1992). To explore whether intensity is related to immune responsiveness in humans, Turner and colleagues (2003) measured cytokine responses of peripheral blood T cells challenged with *Ascaris* antigen among individuals above the age of peak intensity. Within this subgroup, intensity (measured by fecal egg counts) was inversely related to the strength of the Th2 cytokine (IL-4, IL-9, IL-10, and IL-13) response. The same investigators also conducted treatment/reinfection studies, in which therapeutic drug clearance of gastrointestinal helminths was performed and worm burdens were assessed 8 to 9 months later, after natural reacquisition

David Artis, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104-4539. **Rick M. Maizels,** Centre for Immunity, Infection and Evolution, and Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh, UK.

of infection from the contaminated environment (Jackson et al., 2004). In this setting, the degree of resistance to re-infection with either *Ascaris* or *Trichuris* again correlated with Th2 cytokine responses (in this case, IL-5 and IL-13), as well as with the IL-4-dependent IgE isotype (Bradley & Jackson, 2004).

Schistosomes are the most prevalent tissue-dwelling hel-minths of humans, with adult worms residing in the mes-enteric vasculature (e.g., *S. japonicum, S. mansoni*) or the bladder wall (e.g., *S. haematobium*). Again, Th2 immunity appears to be critical in minimizing worm burdens, as first indicated by IgE and eosinophil studies, and subsequently by cytokine assays (summarized by Walter et al., 2006). In classic treatment/reinfection studies, those most resistant to reinfection following chemotherapy had significantly higher IgE levels in both *S. haematobium* (Hagan et al., 1991) and *S. mansoni* (Dessein et al., 2004) areas. Eosinophils, a char-acteristic feature of the Th2 response to helminths, have long been associated with enhanced killing of larval schisto-somes (Capron et al., 1982), with recent studies demonstrat-ing greater immunity in eosinophilic humans (Ganley-Leal et al., 2006). At the cytokine level, Th2 polarization is evi-dent in resistant individuals (assessed through T-cell clones specific for larval schistosome antigens), while susceptible patients' cells are more IFN-γ/Th1 biased (Dessein et al., 2004). The importance of IL-13, in particular, also emerges from genetic studies, in which polymorphisms raising the level of IL-13 responsiveness are associated with greater re-sistance to schistosomal infection (Dessein et al., 2004);

In lymphatic filariasis and onchocerciasis, adult para-sites reside in the lymphatics and skin respectively, while releasing microfilariae for onward transmission through blood-sucking vectors. Immunity to filarial nematodes is not readily apparent in human populations, for two inter-related reasons: responsiveness is greatly dampened by these parasites, and most of the exposed population carry infection for a long period of their life. In general, infec-tion intensities increase through adolescence, but reach a stable plateau level of infection once the peak is attained (Day et al., 1991). This illustrates an intriguing example of "concomitant immunity" in which the existing adult worm load is tolerated but new waves of invading larval parasites are thought to be eliminated. In individuals with detectable circulating microfilariae, suppression of T-cell reactivity can be profound (Maizels & Yazdanbakhsh, 2003), so that both Th1 (IFN [interferon]-γ) and Th2 (IL-5) signature cytokine responses to parasite antigens are lost (Sartono et al., 1997). Reactive individuals, however, may progress to dermal or lymphatic pathology, which has very recently been linked to heightened antiparasite Th1 and Th17 responses (Babu et al., 2009), suggesting by default that Th2 immunity may be the most beneficial to protect humans from filarial nema-tode infection.

Overall, these studies show a convincing quantitative protective effect of Th2 immunity in suppressing hyper-infection in endemic populations, accompanied by some uncertainty of how, if ever, sterile immunity to helminths may be achieved. But the question is raised of why Th2 immunity fails to be sufficiently mobilized in so many indi-viduals to attain an immune status. Part of the answer lies in the ability of parasites to profoundly modulate the host immune system, so that immunity is only expressed after a long-haul process of overcoming parasite immunoregula-tion. The restoration of peripheral T-cell responsiveness to parasite antigens after curative drug therapy indicates that live parasites actively down modulate host immune capac-ity. Interestingly, IL-10 responses are not reduced and in-deed antibodies to IL-10 or TGF-β are able to rescue the in vitro proliferative response of T cells from infected patients (King et al., 1993). Moreover, one of the few immune re-sponses to be greatly enhanced in filariasis and schistoso-miasis is production of IgG4 isotype antibodies, which are known to be promoted by IL-10 (Satoguina et al., 2005). In the treatment/reinfection studies mentioned above, in-dividuals with high IgG4:IgE ratios did not display immu-nity to reinfection, arguing that helminth-induced IgG4 will act as a blocking antibody to protect parasites from immune attack.

Both IL-10 and TGF-β are also closely linked to regu-latory T cell (Treg) activity, which is now becoming more evident in a number of human helminth infections (e.g., in filariasis [Babu et al., 2006]). It is possible that Tregs act di-rectly to impede immune clearance of parasites (as indicated in animal models discussed below), and that an unfavorable excess of Tregs in human infection may take a long time to correct. Alternatively, the action of Tregs may primarily be to limit the pathological consequences of infection, as sug-gested by the finding that lymphatic filariasis patients who progress to inflammatory sequelae such as lymphedema have reduced Treg expression (Babu et al., 2009). Resolution of these questions will be critically important in designing fu-ture intervention studies to promote protective immunity in humans.

ANIMAL MODELS OF HELMINTH INFECTION

Our understanding of acquired immunity to helminths is based on a rich substrate of animal models, most impor-tantly, the mouse (Table 1). Some human pathogens, in-cluding *S. mansoni,* are fully infective in murine models, which reproduce many of the clinical features of infection such as hepatic fibrosis. While many other human parasites (such as the common intestinal worms) are not infective to rodents, closely related species are available that parasitize mice and therefore provide excellent model systems. As will be detailed below, studies across a range of helminth infec-tions have uncovered specific mechanisms that are impor-tant in different settings; hence, while some general features of protective immunity to helminths can be identified, the exact combination of factors that mediate elimination differ between the various species in question. Arguably, the most important factor in dictating which immune mechanisms are in play is the host tissue niche occupied by the parasite.

At this stage, our understanding of the mechanisms of immunity is still relatively fragmented. Studies with gene-deficient mice, with cytokines and antibodies, have helped delineate which components are required for immunity and which are dispensable. Advancing towards a more holistic picture of the most effective combinations requires more painstaking work and more consideration of the physiologi-cal settings in which protective immunity will be required to operate in vivo.

ACQUIRED IMMUNITY IN THE GASTROINTESTINAL TRACT

CD4+ Th2 Cell-Dependent Immunity to Intestinal Nematode Parasites

As discussed in Chapters XX, immune-mediated control of **AQ1** viral, bacterial, fungal, and protozoan pathogens is associ-ated with the development of pathogen-specific CD4+ or CD8+ T-cell responses that are characterized by either the production of proinflammatory cytokines (including IFNγ, IL-17A, and TNF [tumor necrosis factor]α) and/or direct cy-totoxic activity. In contrast, studies in murine model systems

TABLE 1 Major human helminth parasites and corresponding model systems

Species	Common name; disease	Taxon	Human infections	Mouse model species	Life cycle	Notes
Ancylostoma caninum/ Necator americanus	Hookworms; anemia	Nematoda	580 million	*Heligmosomoides polygyrus*; *Nippostrongylus brasiliensis*	Ac/Na/Nb: skin-penetrating larvae, migrate through lungs to gut; Hp: oral ingestion of larvae, remains intestinal	Closely related to ruminant parasites (e.g., *Haemonchus contortus*)
Ascaris lumbricoides	Common roundworm	Nematoda	800 million	–	Faecal-oral transmission of eggs; larvae hatch in stomach but migrate through lungs and return to gut	Closely related to pig roundworm *Ascaris suum*
Brugia malayi/ Wuchereria bancrofti	Filariasis; elephantiasis	Nematoda	120 million	*Litomosoides sigmodontis*	Mosquito transmission of larvae through skin; migration to lymphatics; release of newborn microfilariae into blood	Less prevalent human species include *Loa loa* and *B. timori*
Echinococcus granulosus/E. multilocularis	Hydatid cyst	Cestoda	>3 million	Both directly infective	Ingestion of eggs by humans (or mice) as intermediate host, harbor protoscoleces in cysts which infect definitive canid hosts	Among most life-threatening of helminth infections
Onchocerca volvulus	River blindness	Nematoda	20 million	–	Blackfly transmission of larvae through skin; parasites remain subcutaneous; and release newborn microfilariae into skin	Blindness results from microfilariae in eye; generalized dermatitis
Schistosoma mansoni/ S. japonicum/ S. haematobium	Schistosomiasis; Bilharzia	Trematoda	200 million	*S. mansoni* directly infective	Cercariae invade from aquatic snail, migrate through lung to vascular sites (Sh: bladder wall; Sm, Sj: hepatic portal vein)	Pathology largely due to eggs either in egress (e.g., through bladder wall) or lodged in liver
Trichinella spiralis	Pork worm, Trichinosis	Nematoda	1000s p.a.	Directly infective	Larvae in undercooked meat; adults in intestine release newborn larvae which encyst in muscle	Infects very wide range of host species
Trichuris trichiura	Whipworm	Nematoda	600 million	*T. muris*	Faecal-oral transmission of eggs	

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have identified a critical role for CD4$^+$ Th2 cells that produce IL-4, IL-5, IL-9, and IL-13 in resistance to intestinal nematode infection. The results of these studies support the reported correlations between expression of Th2 cytokines, type 2 cytokine-mediated effector mechanisms and immunity to helminths in humans (discussed above), and identify functional roles for Th2 cell-derived cytokines in expulsion of intestinal nematode parasites.

The mechanisms of recognition of helminth parasites by the innate immune system remain poorly defined. However, current models suggest that intestinal epithelial cells (IECs) and professional antigen presenting cells such as dendritic cells (DCs) and macrophages are critical in initiating and regulating antiparasite immune responses (Perrigoue et al., 2008). These cell types have been proposed to directly recognize nematode parasites and parasite-derived ES products via a number of mechanisms including recognition of nematode glycans, proteases, and chitin (see chapter X, this volume). In addition to direct innate recognition, nonspecific tissue damage created by intestinal nematode parasites may provoke danger signals that influence innate responses to infection.

Th2 cells express IL-4, IL-5, IL-9, and IL-13 and their differentiation is influenced by a number of factors including IL-4 itself, the Notch pathway, and IEC-derived cytokines which, as shown in Figure 1, include IL-17E (IL-25), IL-33, and thymic stromal lymphopoietin (TSLP) (Saenz et al., 2008). Recent studies suggest all these cytokines play an important role in initiating a program of gene expression required for the development of Th2 cytokine responses. For example, IL-17E can be produced by Th2 cells, IECs, monocytes, and granulocytes and appears to be particularly important in the development of antiparasite Th2 responses following exposure to *Trichuris muris* or *Nippostrongylus*
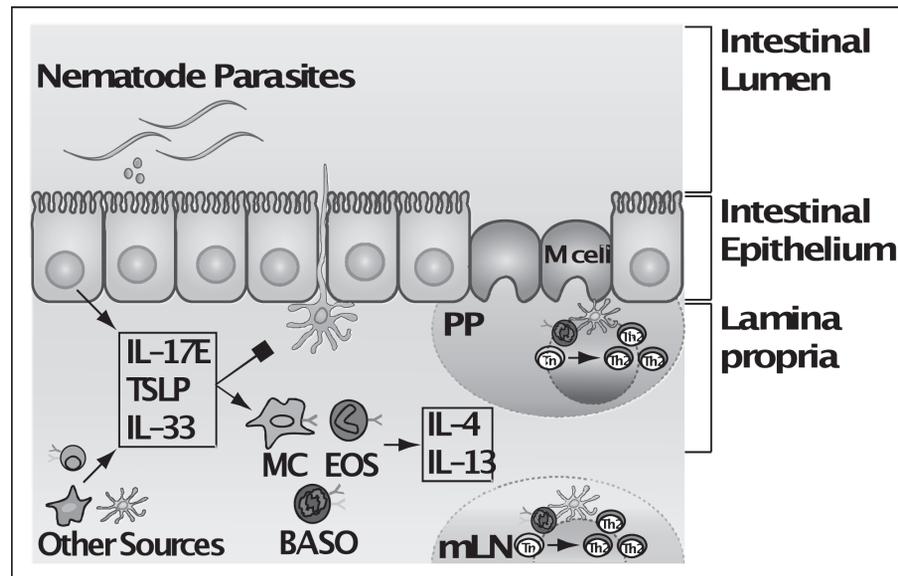
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FIGURE 1 Initiation and regulation of parasite-specific Th2 cell responses following exposure to intestinal nematode infection. Following infection with intestinal nematode parasites, intestinal epithelial cells express a wide range of chemokines and immunoregulatory cytokines, including IL-17E, thymic stromal lymphopoietin (TSLP), and IL-33. These cytokines can promote expression of IL-4 and/or IL-13 in granulocyte populations including mast cells (MC), eosinophils (EOS), and basophils (BASO). IL-17E and TSLP can also create a permissive environment for Th2 cell differentiation by limiting expression of proinflammatory cytokines in dendritic cells (DCs). Naïve CD4$^+$ T cells are activated in the draining mesenteric lymph nodes and, in the presence of the appropriate signals, differentiate into host protective Th2 cells that express IL-4, IL-5, IL-9, IL-13, and IL-25.

brasiliensis infections. Eliciting the expansion of non-B or non-T cells that produce abundant amounts of IL-4 and IL-13 is one mechanism through which IL-17E has been proposed to promote immunity to infection. IEC-derived IL-33 and TSLP are also important in not only influencing the development of Th2 cell responses following *T. muris* infection, but also appear to be dispensable in the context of other intestinal nematode infections. TSLP is expressed by epithelial cells, keratinocytes, and granulocytes and promotes Th2 cell differentiation by simultaneously limiting expression of proinflammatory cytokine production by DCs and inducing IL-4 expression in mast cells and basophils. In addition to expressing high levels of IL-4, emerging studies suggest basophils also coexpress MHC class II and costimulatory molecules and can act as antigen presenting cells that promote Th2 cell differentiation (Sullivan & Locksley, 2009). In contrast, IL-33 expression appears to be restricted to epithelial cells, macrophages, and DCs, however, it acts on multiple granulocyte populations to elicit IL-4 production. Associated with production of cytokines and chemokines by IECs, activation and recruitment of basophils, eosinophils, mast cells, NK (natural killer) cells, and NK T cells are hallmarks of the early immune response to intestinal nematode infections. All of these cell lineages have been proposed as early sources of IL-4, IL-13, and/or TSLP that promote and sustain optimal Th2 cell responses following exposure to intestinal nematodes (Stetson et al., AQ3 2004) (see Fig. X).

The importance of Th2 cells and IL-4 in expulsion of intestinal nematodes was first demonstrated by Urban and Finkelman following challenge with *Heligmosomoides polygyrus* AQ4 infection of mice. Subsequent studies employing IL-4 deficient mice or anti-IL-4 monoclonal antibody treatment of normally resistant wild-type mice confirmed an

important role for IL-4 in immunity to *T. muris* (Finkelman et al., 1997; Grencis, 1997). Although initial expression of AQ5 Th2 cytokines can occur in the absence of IL-4, it appears that maintenance of optimal Th2 cell responses require IL-4-IL-4Rα signaling that results in the phosphorylation of STAT-6. The transcription factor GATA-3 is also critical in the differentiation of Th2 cells. However, expulsion *of N. brasiliensis* and *Trichinella spiralis* was unaffected by depletion or genetic deletion of IL-4, suggesting a redundancy in its protective role against infection. Consistent with this, subsequent studies identified that the requirement for IL-4 in immunity to *T. muris* was dependent on mouse genetic background. For example, C57BL/6 IL-4$^{-/-}$ mice were susceptible to infection while BALB/c IL-4$^{-/-}$ exhibited normal expulsion of parasites (Cliffe & Grencis, 2004). Although independent of IL-4 itself, a number of studies highlighted the importance of IL-4Rα and STAT-6 expression in immunity to multiple intestinal nematode parasites. The IL-4-related cytokine, IL-13, shares the IL-4Rα chain and, similar to IL-4, promotes STAT-6 phosphorylation. Indeed, the importance of IL-13 in immunity to intestinal nematode infection, independent of IL-4, was subsequently demonstrated in a number of nematode infections. Mice deficient in IL-13 exhibit impaired expulsion of *N. brasiliensis*, *T. spiralis*, and *T. muris* infections. In most cases, mice lacking both IL-4 and IL-13 exhibit even slower expulsion of parasites than IL-13 deficiency alone, highlighting the cooperative nature of the IL-4 and IL-13 pathways (Grencis & Bancroft, 2004). These findings are supported by the demonstration that mice deficient in STAT-6 signaling exhibit delayed expulsion following exposure to *N. brasiliensis*, *T. spiralis,* and *H. polygyrus* infection. Notably, although STAT-6 expression was important for immunity to most intestinal nematodes, deletion of STAT-6 had variable effects

on the magnitude of the antiparasite Th2 cell response, suggesting STAT-6 influences both Th2 cells and the effector response elicited against specific parasites.

In addition to IL-4 and IL-13, other Th2-associated cytokines such as IL-3, IL-5, and IL-9 can influence immunity to some, but not all, intestinal nematode infections. For example, although the mechanisms of action remain unclear, administration of exogenous IL-3 could promote immunity to *T. spiralis* and infections with *Strongyloides* species. IL-5 appears to play a minor contributory role in the response following primary *H. polygyrus* infection, although expulsion of *N. brasiliensis*, *T. spiralis*, *T. muris*, *Strongyloides* spp., and secondary *H. polygyrus* appears to be independent of IL-5 (Grencis, 1997). A more substantial role for IL-9 in immunity to intestinal nematode infection was demonstrated in the context of *T. muris* and *T. spiralis* infections. Transgenic overexpression of IL-9 resulted in enhanced expulsion of both parasites, whereas blockade of IL-9 responses resulted in protracted *T. muris* infection. In the case of *T. spiralis* infection, protective effects of IL-9 appear to be partially dependent on mast cells (Pennock & Grencis, 2006).

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Collectively, the emerging paradigm in the development of protective immunity following intestinal nematode infection is that IEC-derived cytokines such as IL-25, IL-33, and TSLP play a critical role in the early innate events that promote the development of Th2 cells (Artis, 2008; Artis & Grencis, 2008). IECs can influence DC activation and provoke production of IL-4 and IL-13 from nonlymphocyte sources including basophils, eosinophils, and mast cells (Fig. 1). Depending on the infection, NK and NK T cells may also produce IL-4 and/or IL-13. These cell populations subsequently influence the differentiation of Th2 cells that express IL-3, IL-4, IL-5, IL-9, and IL-13 (Stetson et al., 2004). All these Th2 cell-derived cytokines can influence immunity to infection, with a central role of IL-4/IL-13 signaling through IL-4Rα and STAT-6 (Finkelman et al., 2004; Urban et al., 1992).

Th2 Cytokine-Dependent Immune Effector Mechanisms in Expulsion of Intestinal Nematode Parasites

While the development of adaptive immunity to intestinal nematodes is dependent on parasite-specific CD4$^+$ Th2 cells, the immune effector mechanisms that mediate expulsion of nematodes appear to be nonparasite-specific in nature. Rather, Th2 cytokine-dependent intestinal inflammation and associated alterations in tissue physiology are general features associated with expulsion of most intestinal nematodes. Following their development in draining lymph nodes, some parasite-specific Th2 cells recirculate to intestinal tissue where they elicit local production of cytokines, chemokines, and other inflammatory mediators that promote recruitment of myeloid cell lineages including mast cells, basophils, eosinophils, and alternatively activated macrophages (Else, 2002). Associated with this inflammatory cell infiltrate, Th2 cytokines derived from either Th2 cells or infiltrating myeloid cells provoke significant changes in intestinal physiology that are a common response associated with expulsion of most nematodes. For example, Th2 cytokines elicit alterations in smooth muscle contractility and dysregulated IEC function including proliferation, differentiation, permeability, and ion exchange (see Fig. 2). These inflammatory responses and associated changes in tissue physiology, rather than having a direct cytotoxic on the parasites or parasite-infected cells, are thought to create a local habitat unsuitable for optimum survival of intestinal nematodes. Consistent with this hypothesis, most intestinal nematodes are expelled from their immune host live and can survive in nonimmune hosts upon surgical transfer.

Although stereotypic Th2 cytokine-dependent inflammatory and physiologic changes in the intestine are associated with immunity to most intestinal nematodes, the relative importance of each effector mechanism appears to depend on the biology of specific nematode parasites. In the following sections, the influence of Th2 cytokine-dependent regulation of mast cells, alternatively activated macrophages (AAMac), and IEC function on expulsion of specific nematode parasites will be discussed.

MAST CELLS

The influence of mast cells on immunity to intestinal nematode infection is species specific. For example, Th2-cell-dependent optimal expulsion of primary *T. muris, N. brasiliensis,* and *H. polygyrus* infections appears to be independent of mast cells. In contrast, mast cells are critical for rapid expulsion of *T. spiralis* that inhabits an intracellular niche in the small intestine (see Table 1). Similar to other nematode parasites, rapid expulsion on challenge infection is associated with a robust CD4$^+$ Th2 cell response, and stereotypic Th2 cytokine-associated inflammation, including elevated serum IgE responses, peripheral blood eosinophilia, goblet cell hyperplasia, altered IEC proliferation, changes in intestinal muscle contractility, and mucosal mast cell hyperplasia. Grencis et al. systematically dissected these responses and revealed that intestinal mast cells are essential for efficient expulsion of *T. spiralis*. In infected mice and rats, there is a strong temporal association between intestinal mast cell hyperplasia and worm expulsion (Miller, 1996). Mast-cell-deficient mice (W/W^v or W$^{Sh/Sh}$) show a delayed worm expulsion (Ohnmacht & Voehringer, 2010) and depletion of mast cells in infected mice using anti-ckit or antistem cell factor (SCF) antibody significantly delays worm expulsion. Secretion of mast cell proteases, in particular mouse mast cell protease 1, MMCP1, appears to be critical for the host protective effects of mast cells as mice deficient in MMCP1 exhibit delayed worm expulsion (Pennock & Grencis, 2006).

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Intestinal mastocytosis in *T. spiralis*-infected mice is regulated by hematopoietic growth factors such as stem cell factor (SCF) and by CD4$^+$ T$_H$2 cell-derived cytokines including IL-3, IL-4, and IL-9. SCF appears to regulate proliferation, differentiation, and migration of mast cell precursors in bone marrow. Upon exit from bone marrow, IEC-derived chemokines, including CCL2, promote recruitment of mast cells into the intraepithelial compartment of the intestine where local production of TGF [transforming growth factor]β regulates expression of MMCP1. Secretion of mast cell-derived proteases, including MMCP1, is thought to promote worm expulsion through regulation of IEC tight junction proteins that results in elevated epithelial cell permeability and increased secretion of fluid into the lumen (McDermott et al., 2003). Coupled with Th2 cytokine-dependent changes in smooth muscle contractility, mast cell responses are thought to promote a "weep and sweep" response, rendering the IEC interface between the host and parasite unsuitable for colonization (see Fig. 1).

ALTERNATIVELY ACTIVATED MACROPHAGES

Macrophage activation and effector functions, such as expression of iNOS, have been traditionally associated with expression of proinflammatory cytokines and immunity to bacterial and protozoan pathogens. However, recent findings highlight the heterogeneity and potential plasticity of macrophage responses. Activation and recruitment of AAMac are a hallmark of Th2 cytokine responses associated with intestinal nematode infection and allergy (Maizels
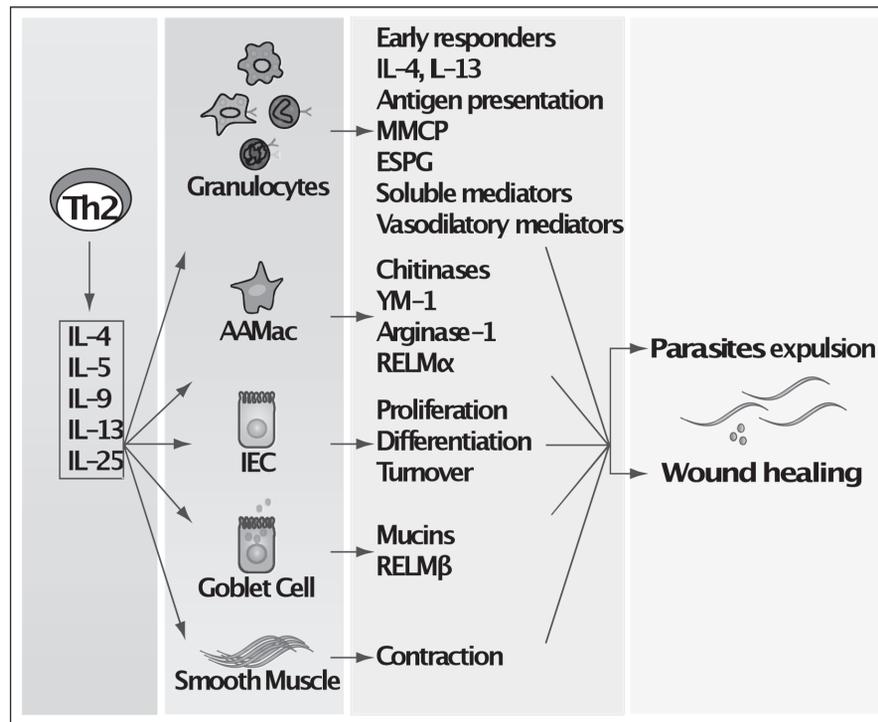
FIGURE 2 Th2 cell-mediated immune effector mechanisms in expulsion of intestinal nematode infection. Th2 cell-derived cytokines can promote granulocyte activation including expression of IL-4, MHC class II, costimulatory molecules, and mouse mast cell protease (MMCP). In addition, Th2 cytokines can promote alternative activation of macrophages (AAMac) that express chitinases, chitinase-like molecules (YM-1), arginase-1, and RELMα that can promote parasite expulsion, wound healing, and can limit the magnitude of Th2 cytokine responses. Th2 cells can also influence nonhematopoietic cell lineages, including promoting smooth muscle contractility and alterations in intestinal epithelial cell (IEC) proliferation, differentiation, and migration. Therefore, parasite-specific CD4^{+} T cells elicit nonparasite-specific inflammation and changes in intestinal physiology that create an environment that is unfavorable for parasite persistence. The relative importance of each pathway depends on the particular species of nematode parasite that infects the host.

et al., 2004). Alternatively activated macrophages can be induced by IL-4, IL-13, IL-10, and IL-21 and are characterized by expression of multiple genes, including arginase-1, RELMα, chitinases, and chitinase-like molecules such as Ym-1 (Nair et al., 2006). The expression of chitin in the cuticle of nematodes suggests that chitinases or chitinase-like molecules may be important immune effector molecules in the expulsion of intestinal nematodes, while other AAMac-associated genes may have potent immunoregulatory and tissue-protective functions.

A host-protective role for AAMac in immunity to intestinal nematodes was first illustrated by Gause and **AQ7** colleagues following infection with the tissue-dwelling nematode *H. polygyrus*. This is a natural intestinal nematode infection of mice that is commonly used as a model for human hookworm infection. Primary infections with *H. polygyrus* occur following ingestion of the larval parasites that transiently invade the mucosa before establishing a luminal niche (see Table 1). Primary infections are typically chronic, however most experimental studies employ drug clearance of primary infection and analysis of subsequent secondary responses. The importance of AAMacs in immunity to secondary or challenge *H. polygyrus* infection was revealed by chemical depletion of macrophages. Inhibition of arginase-1 had a similar effect on impairing resistance to infection, suggesting a critical

role for AAMac-derived arginase activity in worm expulsion (Anthony et al., 2007; Patel et al., 2009). Whether other AAMac-associated genes, including chitinases or RELMα, contribute to immunity to the tissue-dwelling phase of *H. polygyrus* and related parasites has not been examined to date.

In addition to a direct role in immunity to intestinal nematode infection, AAMacs exhibit potent immunoregulatory and wound healing functions in the context of Th2 cytokine-mediated inflammation. For example, AAMacs are critical in limiting potentially lethal intestinal inflammation associated with experimental *S. mansoni* infection and RELMα derived from eosinophils and AAMacs can limit the magnitude of Th2 cytokine responses following infection with multiple intestinal nematode infections. RELMα also induces collagen deposition from tissue-resident myofibroblasts, suggesting that this molecule could play a role in wound healing associated with intestinal nematode infection (Artis, 2006). Given that nematode parasites are large and either undergo developmental stages that require a transient intracellular stage or a tissue-invasive stage, it is not surprising that these infections are associated with a marked inflammatory response and significant tissue damage. Therefore, the immunoregulatory and wound healing properties of AAMacs can also be considered host-protective (see Fig. 2).

TH2 CYTOKINE-MEDIATED REGULATION, IEC PROLIFERATION, AND DIFFERENTIATION

In addition to recruitment of inflammatory cells, expulsion of most intestinal nematodes is commonly associated with dramatic alterations in IEC proliferation, turnover, and/or differentiation. Until recently, these changes in IEC function were thought to be a pathologic "off-target" effect associated with worm expulsion, however, it is now clear that Th2 cytokine-dependent regulation of IECs plays a critical contributory role in expulsion of some nematode parasites. Each crypt within the intestine is an individual proliferative unit, with pluripotent stem cells located near the base of the crypt dividing by asymmetric division to undergo self-renewal and give rise to a number of cell lineages, including Paneth cells, enteroendocrine cells, and goblet cells. Migrating IECs are shed at the luminal surface of the crypts and the rates of IEC proliferation, migration, and shedding are a tightly regulated process that is critical for maintenance of tissue homeostasis.

Trichuris spp. inhabit a partially intracellular niche within IECs of the cecum and proximal colon and appear to continually invade new cells in order to counter the constant proliferation, migration, and shedding of IECs. Recent studies indicate that Th2 cytokine-dependent changes in IEC proliferation, migration, and differentiation contribute to expulsion of *T. muris* and perhaps other intestinal nematode parasites. Most inbred strains of mice expel larval stages of *T. muris* between days 17 to 21 post infection. As discussed above, immunity to infection is dependent on non-T-cell-derived cytokines, including TSLP, IL-25, and IL-33 that promote CD4$^+$ Th2 cell production of IL-4, IL-9, and IL-13, all of which are important in optimal expulsion of the parasite. Immunity to infection is associated with elevated serum IgE levels and the presence of AAMacs, mast cells, and eosinophils in the lamina propria of the intestine. However, it is clear that worm expulsion can occur independently of mast cells, eosinophils, and AAMacs as expulsion is normal in animals depleted of these cell lineages. Further, adoptive transfer of purified CD4$^+$ T cells into lymphocyte-deficient mice is sufficient to induce worm expulsion indicating that B cells and antibody production are not necessary for expulsion of *T. muris* (Else & Finkelman, 1998).

Characteristic changes in the intestinal epithelium are associated with expulsion of *T. muris*, including changes in IEC proliferation, turnover, and differentiation into goblet cells, and recent studies implicate these physiologic changes in IEC biology in the expulsion of the parasite. For example, in mice expelling their parasites, almost a doubling of the rate of IEC turnover occurs whereas in animals that do not expel the worms, only a slight elevation in turnover is apparent. These changes in IEC turnover appear to be regulated, at least in part, by IL-13 (Cliffe et al., 2005). In contrast, in genetically susceptible strains that harbor persistent infection, IFNγ counter regulates the potential protective T$_H$2 cytokine response and slows down the IEC turnover rate through induction of the chemokine CXCL10 (Artis & Grencis, 2008). Accordingly, a slow moving epithelium is established, presumably promoting maintenance of parasites. Critically, blockade of CXCL10 in susceptible mice raised the turnover rate of IECs that was associated with worm expulsion. Based on these findings, a model of "epithelial escalator" was proposed in which Th2 cytokines promote expulsion of the parasite via rapid IEC turnover that propels the parasite from its favored intraepithelial niche (Artis & Grencis, 2008) (Fig. 2).

In addition to alterations in IEC turnover, increased differentiation of goblet cells is a hallmark of expulsion of many intestinal nematodes. Goblet cells secrete mucus, trefoil peptides, and other bioactive molecules including intelectins and resistin-like molecule-beta (RELMβ/FIZZ2) (Artis, 2006). In the steady state, goblet cell-derived mucus is known to create a physical barrier at the mucosal surfaces of the intestine and a number of studies have demonstrated that intestinal-dwelling nematodes do not establish, are impeded from doing so, or can be physically trapped in secreted mucus. There are a number of reports that expression of mucin genes, including Muc-2 and Muc-3, is up regulated following intestinal nematode infection. Changes in glycosylation status of mucins also occur following intestinal nematode infection, although at present, there is limited evidence to support a direct role for mucins in expulsion of intestinal nematode parasites. Similarly, goblet cells express intestinal trefoil factor 3 (TFF3), chloride channel, calcium activated 3 (mCLCA3, Gob5), and intelectins. Although expression of these molecules is up regulated following infection with a number of intestinal nematode parasites and Th2 cytokines appear to regulate their expression, a direct role for these molecules in worm expulsion remains undefined.

In contrast, goblet cell-derived RELMβ appears to play both an immunoregulatory and host protective role following intestinal nematode infection. RELMβ expression is restricted to IECs in the intestine and production can be regulated by a number of factors including Th2 cytokines. Maximal secretion of RELMβ into the intestinal lumen is associated with worm expulsion following exposure to multiple intestinal nematode infections including *N. brasiliensis*, *T. spiralis*, and *T. muris*. Rapid expression of RELMβ is also a hallmark of Th2 memory responses following secondary challenge with *T. muris*. Secreted RELMβ was found to associate with the chemosensory apparatus of some nematodes and to interfere with host-finding behavior. Therefore, it has been hypothesized that RELMβ may contribute to worm expulsion through disorientation of the parasites (Artis, 2006). Recent studies demonstrated that RELMβ$^{-/-}$ mice exhibit impaired immunity to *N. brasiliensis*, confirming an important role in immunity to infection (Herbert et al., 2009). Critically, although the magnitude of the antiparasite immune response appears to be regulated by RELMβ, expulsion of *T. muris* and *T. spiralis* appear to be independent of RELMβ, highlighting the potential redundancy that operates in the context of effector mechanisms involved in expulsion of nematode parasites.

Taken together, studies in diverse parasitic nematode infections have demonstrated multiple Th2 cytokine-dependent effector mechanisms including mastocytosis, recruitment of AAMacs, and regulation of IEC permeability, proliferation, turnover, and differentiation. Therefore, as discussed above, parasite-specific CD4$^+$ Th2 cell responses elicit nonspecific "modular" responses characterized by type 2 inflammation and changes in intestinal physiology, all of which can create an unfavorable environment for this diverse group of parasites (Fig. 1).

ACQUIRED IMMUNITY TO TISSUE-DWELLING HELMINTHS

Immunity to parasites within the internal tissues of the body operates very differently than in the gastrointestinal locale. However, the generation of a dominant Th2 response forms a strong common theme between intestinal helminths and those parasitizing the tissues and, as described above, cells of the innate immune system are instrumental in shaping the mode of T-cell reactivity. The distinction between intestinal helminths and those living in the various somatic organs

of the host is further blurred by the fact that the larval stages of many gut-dwelling helminths traverse the tissues (e.g., in hookworms, larvae penetrate the skin before migrating to the lung and reaching the stomach via the esophagus). Moreover, intestinal parasites can encompass significant tissue-dwelling phases such as the larval forms of *H. polygyrus*, which temporarily encyst in the submucosal muscle wall (see Table 1). Even entirely tissue-dwelling helminths may occupy different niches as they progress through the developmental cycle, such as seen with the lung-stage schistosomulae and vascular plexus adult schistosomes. Hence, a key issue is when and where tissue stages of helminth parasites may be intercepted and killed by the host immune system.

Where parasites are directly skin-penetrating, there is evidence of localized trapping. For example, serum antibodies from animals vaccinated with irradiated *A. caninum* L3 were able to inhibit larval migration through skin in vitro (Fujiwara et al., 2006), while mice that overexpress IL-5 mounted a subcutaneous eosinophil-rich response that trapped the majority of *N. brasiliensis* larvae at the site of entry (Daly et al., 1999). Such reactions are unlikely in the naïve host however and, in primary schistosome infections, the skin phase is thought primarily to serve as a stimulus to systemic Th2 immunity, which acts to eliminate schistosomals at the subsequent lung stage (Mountford & Trottein, 2004).

In many tissues—particularly the lungs, liver, and gut—the immune system forms granulomas around parasitic organisms. In the case of secondary infection with *H. polygyrus*, as discussed above, these focus AAMacs to eliminate parasites while still in the submucosal wall (Anthony et al., 2006). In the liver, however, where granulomas form around trapped Schistosome eggs, they protect the host from the toxic effects of egg secretions but do not mediate immunity to the adult parasites remaining in the portal vasculature (Anthony et al., 2007). Quite possibly, effective killing of helminths in tissue sites is achieved by direct actions of hematopoietic cells and granuloma formation occurs when killing cannot be accomplished. Arguably, the closest representation of immune-mediated lethality can be visualized in chamber experiments, in which helminths are implanted in cell-permeable constructs and killing can be correlated with ingress of eosinophils and other host cells (Rotman et al., 1996).

Certain helminth parasites inhabit the vascular and lymphatic systems. Although it is difficult to observe immune killing in these dispersed organs, substantial data has been built up on immune-dependent clearance of *Brugia* microfilariae (Mf) from the bloodstream. While innate immune attack can be mediated by eosinophils, the adaptive immune response is dependent both on antibody production and on FcR expression, indicating that ADCC-like mechanisms are in play in removing these parasites from the bloodstream (Gray & Lawrence, 2002). Because *xid* mice deficient in T-independent IgM responsiveness cannot control blood microfilaremia and other mutant mice with IgM priming defects are similarly susceptible, evidence argues for an exclusive role for IgM in immunity to bloodstream Mf.

These three examples of skin, muscle wall, and vasculature serve to illustrate the diversity of biological environments and immunological mechanisms that are likely to be mobilized against helminth parasites. However, as in the intestinal locale, a unifying component remains the $CD4^+$ T cell; hence mice deficient in any gene linked to $CD4^+$ T-cell development (including RAG, MHC class II, or CD4 itself) are highly susceptible to infection and are generally unable to express any form of protective immunity. In contrast, the $CD8^+$ population (as well as other, less conventional T-cell types) are rarely implicated in acquired immunity. Within

the $CD4^+$ population, it is often assumed that Th2 cells are the mediators of immunity to helminths; however, this is not always the case. For example, vaccine-induced immunity to the cercarial larvae of *S. mansoni* in mice requires a Th1 response in the dermis (James & Glaven, 1989), while immunity to larvae of the cestode *Taenia crassiceps* is Th1-mediated and IL-12p35-deficient animals are highly susceptible to infection (Rodriguez-Sosa et al., 2003). Interestingly, in both schistosomiasis and cestode infections, an early Th1 response, which can play a protective role, appears to be a supplanted by a later dominant Th2 response, which is not so effective at eliminating parasites (Pearce & MacDonald, 2002; Zhang et al., 2008). This scenario stands in contrast to the more general observation in nematode infections that disruption of Th2-associated genes such as IL-4, IL-13, or STAT-6 will drastically impair antihelminth immunity, as described above.

As mentioned above, a further common feature with intestinal immunity is the partnership between adaptive and innate components which first dictates the mode of T-cell subset differentiation, and comes again into play when nonparasite-specific cells act to eliminate helminths in the tissues (Cadman & Lawrence, 2010). This relationship has been most clearly expounded in murine schistosomiasis, in which dendritic cells selectively induce a Th2 response to schistosome egg antigen (Pearce & MacDonald, 2002). In addition, in murine filariasis models, AAMacs are also able to induce Th2 responses, a contribution likely to play increasing significance in the upkeep of Th2 reactivity during the chronic phase of infections in which the upkeep of Th2 reactivity (Maizels et al., 2004). Most recently, a role for basophil-derived IL-4 in the initiation of Th2 responsiveness to tissue helminths has become clearer, with the discovery that both *S. mansoni* and the tissue cyst-forming *Echinococcus multilocularis* release ligands that stimulate basophils to release this cytokine through nonantigen-specific interactions with surface-bound IgE (Schramm et al., 2007). **AQ8**

The generation of Th2 immunity in this way can, in a manner that differs exquisitely according to the precise parasite/tissue combination in question, mediate immune killing of helminths. For example, in mice carrying a genetic deletion of eosinophils, *N. brasiliensis* had a much greater propensity to reach the lungs and progress to the gut (Knott et al., 2007), consistent with earlier work from the same group showing that constitutively eosinophil-rich IL-5 overexpressing mice permitted fewer larvae to reach the intestinal site. However, the same eosinophilic mice could not attack *Toxocara canis* larvae, and earlier studies had established that eosinophil-deficient mice mounted equivalent vaccine-induced immunity to *S. mansoni* (Sher et al., 1990). Interestingly, in an elegant illustration of the importance of parasite niche, $eotaxin^{-/-}$ mice in which eosinophils cannot enter the peritoneal cavity show increased killing of blood microfilariae of *Brugia malayi*, alongside failure to kill peritoneal parasites (Simons et al., 2005). Hence, eosinophils can mediate immunity to some extent, and against some parasites, but not in every case. Indeed, as evidence is now emerging that eosinophils may secrete proteins that dampen Th2 responses (Pesce et al., 2009), and survival of *Trichinella spiralis* is actually reduced in the absence of eosinophils (Fabre et al., 2009), it can be appreciated that eosinophils may strongly influence the outcome of infection in alternative ways, depending crucially on the precise setting in question. **AQ9**

Neutrophils are often assumed to be exclusively involved in antibacterial immunity, but in some instances have proven essential in antihelminth immunity. One example is in the mouse filarial parasite, *Litomosoides sigmodontis*.

Both IFN-γ-deficient and IL-5-deficient mice suffer increased worm and microfilarial loads, with doubly deficient mice being highly susceptible (Saeftel et al., 2003). In this instance, TNF-α appears to be an essential mediator that activates neutrophils for antiparasite immunity and there is a requirement for Th1/Th2 synergy rather than antagonism for the successful resolution of this infection. Several other examples have been reported, in which neutrophils are important in acting through either an antibody-dependent or antibody-independent mechanism (reviewed by Cadman & Lawrence, 2010) suggesting that, as with eosinophils, there are defined settings in which the action of neutrophils will prove to be crucial for immunity.

AQ10   Macrophages have long been known to attack schistosome larvae through classical activation and the production of nitric oxide (James & Glaven, 1989), but the newcomer to our understanding of innate cell response to helminth infection is certainly the AAMph, as discussed above. These cells mediate immunity to the tissue-invasive stage of *H. polygyrus* (Anthony et al., 2006), but in other contexts, such as the thoracic cavity niche parasitized by adult *L. sigmodon-*
AQ10   *tis*, the role of AAMphs is more in dampening host T-cell
AQ10   reactivity (Taylor et al., 2006). Indeed the AAMph phenotype has been strongly associated with immune suppression as with the dampening of liver pathology in schistosome-
AQ9   infected mice (Pesce et al., 2009) and with the potent antiproliferative suppressive effects in filarial infections (MacDonald et al., 1998). Hence, as with other innate cell types, the contribution of macrophages depends entirely on the context of host signaling and helminth biology.

Where innate cells can effectively deal with tissue helminth infections, they will have been stimulated and armed by the adaptive immune system; cytokines from both T cells and B cells play crucial roles in achieving the necessary level of response (Wojciechowski et al., 2009), even if antibodies are not crucial in many cases. To date, most studies have used μMT B-cell-deficient mice, which while being generally more susceptible to helminth infection, do not help identify the alternate roles of B cells as antibody or cytokine producers (or indeed, as APCs [antigen-presenting cells]). More exacting studies have employed secIgM⁻/⁻ mice deficient only in IgM secretion; such mice are more susceptible to infective larvae of *B. malayi*, and data indicate a role for IgM in mediating macrophage adherence to the surface of the infective larval stage (Rajan et al., 2005). Perhaps surprisingly, in view of the evidence from human studies discussed above, there is conspicuously little evidence that IgE fulfills a protective role in the mouse, possibly because of the more restricted expression of IgE Fc receptors on murine leukocytes, including eosinophils.

AQ11   ## CONCLUSIONS

Successful resolution of helminth infections requires the appropriate mode and degree of immune responsiveness if full immunity is to be achieved without severe pathological consequences. This intricate balance between protection against infection and forestalling immunopathology can too often go awry, as in the 5% to 20% of the helminth-infected population progressing to severe disease such as hepatosplenic fibrosis in schistosomiasis, blindness in onchocerciasis, and elephantiasis in lymphatic filariasis. The vital importance of striking this balance may be reflected in the number of regulatory controls and checkpoints in the immune system that are invoked (including regulatory T and B cells, alternatively activated macrophages at the cellular level, and IL-10 and TGF-β at the cytokine level). It is entirely plausible that the halting nature of human

immunity to helminths can be ascribed to the strength of regulatory restraints that have evolved to minimize pathological outcomes. This promises to be a fascinating area for future research, and one that may allow us to understand human responsiveness to helminths and to intervene in an appropriate and beneficial manner.

REFERENCES

Anthony, R. M., J. F. Urban, Jr., F. Alem, H. A. Hamed, C. T. Rozo, J. L. Boucher, N. Van Rooijen, and W. C. Gause. 2006. Memory T$_H$2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. *Nat. Med.* **12:**955–960.

Anthony, R. M., L. I. Rutitzky, J. F. Urban, Jr., M. J. Stadecker, and W. C. Gause. 2007. Protective immune mechanisms in helminth infection. *Nat. Rev. Immunol.* **7:**975–987.

Artis, D. 2006. New weapons in the war on worms: identification of putative mechanisms of immune-mediated expulsion of gastrointestinal nematodes. *Int. J. Parasitol.* **36:**723–733.

Artis, D. 2008. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat. Rev. Immunol.* **8:**411–420.

Artis, D., and R. K. Grencis. 2008. The intestinal epithelium: sensors to effectors in nematode infection. *Mucosal Immunol.* **1:**252–264.

Babu, S., C. P. Blauvelt, V. Kumaraswami, and T. B. Nutman. 2006. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. *J. Immunol.* **176:**3248–3256.

Babu, S., S. Q. Bhat, N. Pavan Kumar, A. B. Lipira, S. Kumar, C. Karthik, V. Kumaraswami, and T. B. Nutman. 2009. Filarial lymphedema is characterized by antigen-specific Th1 and th17 proinflammatory responses and a lack of regulatory T cells. *PLoS Negl. Trop. Dis.* **3:**e420.

Bradley, J. E., and J. A. Jackson. 2004. Immunity, immunoregulation and the ecology of trichuriasis and ascariasis. *Parasite Immunol.* **26:**429–441.

Bundy, D. A. P., and G. F. Medley. 1992. Immunoepidemiology of human geohelminthiasis: ecological and immunological determinants of worm burden. *Parasitology* **104:**105–119.

Cadman, E. T., and R. A. Lawrence. 2010. Granulocytes: effector cells or immunomodulators in the immune response to helminth infection? *Parasite Immunol.* **32:**1–19.

Capron, A., J.-P. Dessaint, M. Capron, M. Joseph, and G. Torpier. 1982. Effector mechanisms of immunity to schistosomes and their regulation. *Immunol. Rev.* **61:**41–66.

Cliffe, L. J., and R. K. Grencis. 2004. The *Trichuris muris* system: a paradigm of resistance and susceptibility to intestinal nematode infection. *Adv. Parasitol.* **57:**255–307.

Cliffe, L. J., N. E. Humphreys, T. E. Lane, C. S. Potten, C. Booth, and R. K. Grencis. 2005. Accelerated intestinal epithelial cell turnover: a new mechanism of parasite expulsion. *Science* **308:**1463–1465.

Daly, C. M., G. Mayrhofer, and L. A. Dent. 1999. Trapping and immobilization of *Nippostrongylus brasiliensis* larvae at the site of inoculation in primary infections of interleukin-5 transgenic mice. *Infect. Immun.* **67:**5315–5323.

Day, K. P., B. Grenfell, R. Spark, J. W. Kazura, and M. P. Alpers. 1991. Age specific patterns of change in the dynamics of *Wuchereria bancrofti* infection in Papua New Guinea. *Am. J. Trop. Med. Hyg.* **44:**518–527.

Dessein, A., B. Kouriba, C. Eboumbou, H. Dessein, L. Argiro, S. Marquet, N. E. Elwali, V. Rodrigues, Y. Li, O. Doumbo, and C. Chevillard. 2004. Interleukin-13 in the skin and interferon-gamma in the liver are key players in immune protection in human schistosomiasis. *Immunol. Rev.* **201:**180–190.

Else, K. J., and F. D. Finkelman. 1998. Intestinal nematode parasites, cytokines and effector mechanisms. *Int. J. Parasitol.* **28:**1145–1158.

Else, K. J. 2002. Chemokines and leucocyte migration in parasitic disease. *Parasite Immunol.* **24:**281–283.

Fabre, V., D. P. Beiting, S. K. Bliss, N. G. Gebreselassie, L. F. Gagliardo, N. A. Lee, J. J. Lee, and J. A. Appleton. 2009. Eosinophil deficiency compromises parasite survival in chronic nematode infection. *J. Immunol.* **182:**1577–1583.

Finkelman, F. D., T. Shea-Donohue, J. Goldhill, C. A. Sullivan, S. C. Morris, K. B. Madden, W. C. Gause, and J. F. Urban, Jr. 1997. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu. Rev. Immunol* **15:**505–533.

Finkelman, F. D., T. Shea-Donohue, S. C. Morris, L. Gildea, R. Strait, K. B. Madden, L. Schopf, and J. F. Urban, Jr. 2004. Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites. *Immunol. Rev.* **201:**139–155.

Fujiwara, R. T., A. Loukas, S. Mendez, A. L. Williamson, L. L. Bueno, Y. Wang, A. Samuel, B. Zhan, M. E. Bottazzi, P. J. Hotez, and J. M. Bethony. 2006. Vaccination with irradiated *Ancylostoma caninum* third stage larvae induces a Th2 protective response in dogs. *Vaccine* **24:**501–509.

Ganley-Leal, L. M., P. N. Mwinzi, C. B. Cetre-Sossah, J. Andove, A. W. Hightower, D. M. Karanja, D. G. Colley, and W. E. Secor. 2006. Correlation between eosinophils and protection against reinfection with *Schistosoma mansoni* and the effect of human immunodeficiency virus type 1 coinfection in humans. *Infect. Immun.* **74:**2169–2176.

Gray, C. A., and R. A. Lawrence. 2002. A role for antibody and Fc receptor in the clearance of *Brugia malayi* microfilariae. *Eur. J. Immunol.* **32:**1114–1120.

Grencis, R. K. 1997a. Enteric helminth infection: immunopathology and resistance during intestinal nematode infection. *Chem. Immunol.* **66:**41–61.

Grencis, R. K. 1997b. Th2-mediated host protective immunity to intestinal nematode infections. *Philosophical Transactions of the Royal Society, Series B* **352:**1377–1384.

Grencis, R. K., and A. J. Bancroft. 2004. Interleukin-13: a key mediator in resistance to gastrointestinal-dwelling nematode parasites. *Clin. Rev. Allergy Immunol.* **26:**51–60.

Hagan, P., U. J. Blumenthal, D. Dunn, A. J. G. Simpson, and H. A. Wilkins. 1991. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* **349:**243–245.

Herbert, D. R., J.-Q. Yang, S. P. Hogan, K. Groschwitz, M. V. Khodoun, A. Munitz, T. Orekov, C. Perkins, Q. Wang, F. Brombacher, J. F. Urban, M. E. Rothenberg, and F. D. Finkelman. 2009. Intestinal epithelial cell secretion of RELM-β protects against gastrointestinal worm infection. *J. Exp. Med.* **206:**2947–2957.

Hotez, P. J., P. J. Brindley, J. M. Bethony, C. H. King, E. J. Pearce, and J. Jacobson. 2008. Helminth infections: the great neglected tropical diseases. *J. Clin. Invest.* **118:**1311–1321.

Jackson, J. A., J. D. Turner, L. Rentoul, H. Faulkner, J. M. Behnke, M. Hoyle, R. K. Grencis, K. J. Else, J. Kamgno, M. Boussinesq, and J. E. Bradley. 2004. T helper cell type 2 responsiveness predicts future susceptibility to gastrointestinal nematodes in humans. *J. Infect. Dis.* **190:**1804–1811.

James, S. L., and J. Glaven. 1989. Macrophage cytotoxicity against schistosomula of *Schistosoma mansoni* involves arginine-dependent production of reactive nitrogen intermediates. *J. Immunol.* **143:**4208–4212.

King, C. L., S. Mahanty, V. Kumaraswami, J. S. Abrams, J. Regunathan, K. Jayaraman, E. A. Ottesen, and T. B. Nutman. 1993. Cytokine control of parasite-specific anergy

in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. *J. Clin. Invest.* **92:**1667–1673.

Knott, M. L., K. I. Matthaei, P. R. Giacomin, H. Wang, P. S. Foster, and L. A. Dent. 2007. Impaired resistance in early secondary *Nippostrongylus brasiliensis* infections in mice with defective eosinophilopoeisis. *Int. J. Parasitol.* **37:**1367–1378.

MacDonald, A. S., R. M. Maizels, R. A. Lawrence, I. Dransfield, and J. E. Allen. 1998. Requirement for in vivo production of IL-4, but not IL-10, in the induction of proliferative suppression by filarial parasites. *J. Immunol.* **160:**4124–4132.

Maizels, R. M., and M. Yazdanbakhsh. 2003. Regulation of the immune response by helminth parasites: cellular and molecular mechanisms. *Nat. Rev. Immunol.* **3:**733–743.

Maizels, R. M., A. Balic, N. Gomez-Escobar, M. Nair, M. Taylor, and J. E. Allen. 2004. Helminth parasites: masters of regulation. *Immunol. Rev.* **201:**89–116.

McDermott, J. R., R. E. Bartram, P. A. Knight, H. R. Miller, D. R. Garrod, and R. K. Grencis. 2003. Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc. Natl. Acad. Sci. USA* **100:**7761–7766.

Miller, H. R. 1996. Mucosal mast cells and the allergic response against nematode parasites. *Vet. Immunol. Immunopathol.* **54:**331–336.

Mountford, A. P., and F. Trottein. 2004. Schistosomes in the skin: a balance between immune priming and regulation. *Trends Parasitol.* **20:**221–226.

Nair, M. G., K. J. Guild, and D. Artis. 2006. Novel effector molecules in type 2 inflammation: lessons drawn from helminth infection and allergy. *J. Immunol.* **177:**1393–1399.

Ohnmacht, C., and D. Voehringer. 2010. Basophils protect against reinfection with hookworms independently of mast cells and memory Th2 cells. *J. Immunol.* **184:**344–350.

Patel, N., T. Kreider, J. F. Urban, Jr., and W. C. Gause. 2009. Characterisation of effector mechanisms at the host:parasite interface during the immune response to tissue-dwelling intestinal nematode parasites. *Int. J. Parasitol.* **39:**13–21.

Pearce, E. J., and A. S. MacDonald. 2002. The immunobiology of schistosomiasis. *Nat. Rev. Immunol.* **2:**499–511.

Pennock, J. L., and R. K. Grencis. 2006. The mast cell and gut nematodes: damage and defence. *Chem. Immunol. Allergy* **90:**128–140.

Perrigoue, J. G., F. A. Marshall, and D. Artis. 2008. On the hunt for helminths: innate immune cells in the recognition and response to helminth parasites. *Cell Microbiol.* **10:**1757–1764.

Pesce, J. T., T. R. Ramalingam, M. M. Mentink-Kane, M. S. Wilson, K. C. El Kasmi, A. M. Smith, R. W. Thompson, A. W. Cheever, P. J. Murray, and T. A. Wynn. 2009a. Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLoS Pathog.* **5:**e1000371.

Pesce, J. T., T. R. Ramalingam, M. S. Wilson, M. M. Mentink-Kane, R. W. Thompson, A. W. Cheever, J. F. Urban, Jr., and T. A. Wynn. 2009b. Retnla (relma/fizz1) suppresses helminth-induced Th2-type immunity. *PLoS Pathog.* **5:**e1000393.

Quinnell, R. J. 2003. Genetics of susceptibility to human helminth infection. *Int. J. Parasitol.* **33:**1219–1231.

Rajan, B., T. Ramalingam, and T. V. Rajan. 2005. Critical role for IgM in host protection in experimental filarial infection. *J. Immunol.* **175:**1827–1833.

Rodriguez-Sosa, M., A. R. Satoskar, J. R. David, and L. I. Terrazas. 2003. Altered T helper responses in CD40 and interleukin-12 deficient mice reveal a critical role for Th1 responses in eliminating the helminth parasite *Taenia crassiceps*. *Int. J. Parasitol.* **33:**703–711.

Rotman, H. L., W. Yutanawiboonchai, R. A. Brigandi, O. Leon, G. J. Gleich, T. J. Nolan, G. A. Schad, and D. Abraham. 1996. Strongyloides stercoralis: eosinophil-dependent immune-mediated killing of third stage larvae in BALB/cByJ mice. *Exp. Parasitol.* **82:**267–278.

Saeftel, M., M. Arndt, S. Specht, L. Volkmann, and A. Hoerauf. 2003. Synergism of gamma interferon and interleukin-5 in the control of murine filariasis. *Infect. Immun.* **71:**6978–6985.

Saenz, S. A., B. C. Taylor, and D. Artis. 2008. Welcome to the neighborhood: epithelial cell-derived cytokines license innate and adaptive immune responses at mucosal sites. *Immunol. Rev.* **226:**172–190.

Sartono, E., Y. C. M. Kruize, A. Kurniawan-Atmadja, R. M. Maizels, and M. Yazdanbakhsh. 1997. Depression of antigen-specific interleukin-5 and interferon-γ responses in human lymphatic filariasis as a function of clinical status and age. *J. Infect. Dis.* **175:**1276–1280.

Satoguina, J. S., E. Weyand, J. Larbi, and A. Hoerauf. 2005. T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J. Immunol.* **174:**4718–4726.

Schramm, G., K. Mohrs, M. Wodrich, M. J. Doenhoff, E. J. Pearce, H. Haas, and M. Mohrs. 2007. IPSE/alpha-1, a glycoprotein from *Schistosoma mansoni* eggs, induces IgE-dependent, antigen-independent IL-4 production by murine basophils in vivo. *J. Immunol.* **178:**6023–6027.

Sher, A., R. L. Coffman, S. Hieny, and A. W. Cheever. 1990. Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fails to affect immunity against *Schistosoma mansoni* in the mouse. *J. Immunol.* **145:**3911–3916.

Simons, J. E., M. E. Rothenberg, and R. A. Lawrence. 2005. Eotaxin-1-regulated eosinophils have a critical role in innate immunity against experimental *Brugia malayi* infection. *Eur. J. Immunol.* **35:**189–197.

Stetson, D. B., D. Voehringer, J. L. Grogan, M. Xu, R. L. Reinhardt, S. Scheu, B. L. Kelly, and R. M. Locksley. 2004. Th2 cells: orchestrating barrier immunity. *Adv. Immunol.* **83:**163–189.

Sullivan, B. M., and R. M. Locksley. 2009. Basophils: a nonredundant contributor to host immunity. *Immunity* **30:**12–20.

Taylor, M. D., A. Harris, M. G. Nair, R. M. Maizels, and J. E. Allen. 2006. F4/80^{+} alternatively activated macrophages control CD4^{+} T cell hyporesponsiveness at sites peripheral to filarial infection. *J. Immunol.* **176:**6918–6927.

Turner, J., H. Faulkner, J. Kamgno, F. Cormont, J. Van Snick, K. Else, R. Grencis, J. Behnke, M. Boussinesq, and J. E. Bradley. 2003. Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. *J. Infect. Dis.* **188:**1768–1775.

Urban, J. F., K. B. Madden, A. Svetica, A. Cheever, P. P. Trotta, W. C. Gause, I. M. Katona, and F. D. Finkelman. 1992. The importance of Th2 cytokines in protective immunity to nematodes. *Immunol. Rev.* **127:**205–220.

Walter, K., A. J. Fulford, R. McBeath, S. Joseph, F. M. Jones, H. C. Kariuki, J. K. Mwatha, G. Kimani, N. B. Kabatereine, B. J. Vennervald, J. H. Ouma, and D. W. Dunne. 2006. Increased human IgE induced by killing *Schistosoma mansoni* in vivo is associated with pretreatment Th2 cytokine responsiveness to worm antigens. *J. Immunol.* **177:**5490–5498.

Wojciechowski, W., D. P. Harris, F. Sprague, B. Mousseau, M. Makris, K. Kusser, T. Honjo, K. Mohrs, M. Mohrs, T. Randall, and F. E. Lund. 2009. Cytokine-producing effector B cells regulate type 2 immunity to *H. polygyrus*. *Immunity* **30:**1–13.

Woolhouse, M. E. 1992. A theoretical framework for the immunoepidemiology of helminth infection. *Parasite Immunol.* **14:**563–578.

Zhang, W., A. G. Ross, and D. P. McManus. 2008. Mechanisms of immunity in hydatid disease: implications for vaccine development. *J. Immunol.* **181:**6679–6685.

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