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Review Article

Mucosal immune responses following intestinal nematode infection

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SUMMARY

In most natural environments, the large majority of mammals harbour parasitic helminths that often live as adults within the intestine for prolonged periods (1–2 years) (1). Although these organisms have been eradicated to a large extent within westernized human populations, those living within rural areas of developing countries continue to suffer from high infection rates. Indeed, recent estimates indicate that approximately 2·5 billion people worldwide, mainly children, currently suffer from infection with intestinal helminths (also known as geohelminths and soil-transmitted helminths) (1, 2). Paradoxically, the eradication of helminths is thought to contribute to the increased incidence of autoimmune diseases and allergy observed in developed countries. In this review, we will summarize our current understanding of host–helminth interactions at the mucosal surface that result in parasite expulsion or permit the establishment of chronic infections with luminal dwelling adult worms. We will also provide insight into the adaptive immune mechanisms that provide immune protection against re-infection with helminth larvae, a process that is likely to be key to the future development of successful vaccination strategies. Lastly, the contribution of helminths to immune modulation and particularly to the treatment of allergy and inflammatory bowel disease will be discussed.

Keywords *geohelminth, immune-expulsion, intestinal helminth, mucosal immunity, nematode, type 2 immunity*

INTRODUCTION

Although they rarely kill, helminths often cause chronic infections and impact on human health through effects on nutrition leading to growth retardation, vitamin deficiencies and poor cognitive function (3, 4). Hookworm infection is a major cause of iron-deficiency anaemia in endemic areas (5). While evidence is still limited to support important effects of intestinal helminths on intestinal function and immune responsiveness in humans, there is strong evidence for such effects in natural and experimental infections in animals including impaired immune responses to vaccines, increased susceptibility to other infectious diseases and a reduction in disease severity in experimental models of allergic and autoimmune disorders (6–12). Children living in poor regions of the rural tropics have a particularly high risk of infections with intestinal helminths and may harbour high parasite burdens with one or more of the major parasites, namely hookworm (*Anyclostoma duodenale* and *Necator americanus*), roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and threadworm (*Strongyloides stercoralis*). The disability adjusted life years (DALYs) lost each year as a result of intestinal helminth infections is approximately 39 million and is greater than that estimated for malaria and approaching that attributed to tuberculosis (1).

In contrast to other pathogens, intestinal helminths, with the exception of threadworms, are not able to replicate within their mammalian hosts. Thus, worm burdens tend to increase slowly over time as a result of constant exposure to infection in a faecally contaminated environment and tend to reach a peak during childhood after which infection intensities and prevalence may decline (1). Exceptions are hookworm and *S. stercoralis* for which peak prevalence tends to occur in adults. The convex age-prevalence and age-intensity profiles for intestinal helminths have been suggested to indicate the acquisition of age-dependent protective immunity (13). Such protective immunity may be targeted primarily at larval stages of intestinal helminths. Larvae that survive to mature into adults may survive for periods of years, and it has been suggested that the long-term survival of adults in the intestinal tract can be explained by the modulation of host

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immune responses. The exact mechanisms by which such modulation occurs remain poorly understood.

IMMUNE MECHANISMS LEADING TO EXPULSION OR CHRONICITY OF ADULT WORMS

The majority of human helminths establish chronic infections in their host by subverting the host immune response, an effect that is also beneficial to the host because it minimizes the long-term harmful effects of inflammatory responses directed against the parasites. A better understanding of this complex relationship has been the subject of great interest, and much of the past research in the area of helminth immunobiology has focused on host–parasite relationships following primary infection. Much of our knowledge regarding host immunity has been necessarily derived from experimental murine models. Of these, three widely used models include *Trichuris muris*, *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus bakeri* (see Box 1 for detailed information on similarities and differences between these species). *Trichuris muris* is a murine pathogen closely related to *T. trichuria* (whipworm), the causative agent of human trichuriasis. *Trichuris muris* has been exploited in laboratory model systems for over 60 years to determine many of the immunological mechanisms associated with resistance and susceptibility. *Nippostrongylus brasiliensis* and *H. p. bakeri* belong to the Strongylida order, which includes the human hookworm parasites *A. duodenale* and *N. americanus* (14). While *N. brasiliensis* provides a suitable model for the life-cycle of human hookworm, infecting its host through the skin and migrating to the lung prior to entering the intestinal lumen, this parasite fails to persist and is instead expelled from immune competent animals within several weeks of infection. By contrast, primary infections with *H. p. bakeri* can persist for many months in susceptible strains of mice and thus represent a useful model for chronic intestinal helminthiases.

Box 1: Model Murine Intestinal Helminths

Numerous rodent parasites are routinely used to investigate the immune parameters of intestinal helminth infection. Although all of these helminths reside within the intestinal lumen in their adult form, many important differences exist in terms of their life cycle and chronicity. Indeed, as a result of their unique life cycles, and the likely abundant genetic differences, it is very likely that many features of the host immune response are species-specific and care needs be taken not to make generalized conclusions based on the findings of experiments using individual species. Details of the lifecycles for the three main murine nematode helminths discussed in this review are given below:

Heligmosomoides polygyrus bakeri: is a trichostrongylid nematode naturally infecting small rodents. It is ingested by the host and penetrates the submucosa of the small intestine as an L3 stage. Here is matures to an L4 stage then exists the mucosa to enter the intestinal lumen as an adult. Adults anchor themselves by coiling around intestinal villi and become sexually mature resulting in the production of eggs that are secreted through the faeces. Excreted eggs hatch within the soil where they develop over several weeks to the infective L3 stage and the life cycle continues. Adult worms form chronic infections in susceptible strains of mice and posses potent immune modulatory potential.

Nippostrongylus brasiliensis: is natural parasite of rats that can be adapted to use in murine experiments. Infective larvae enter their host through the skin, then enter the vasculature to be carried to the heart and lungs. Larvae exit the vasculature through small capillaries within the lung and develop into the L4 stage. They then penetrate the alveoli, are coughed up and swallowed, and migrate to the small intestine where they develop into sexually mature adults. Adults produce eggs, which are passed out in faeces, but are typically expelled by the host within a few weeks in a process that is dependent on type 2 immunity. As for *H. p. bakeri* excreted eggs hatch within the soil where they develop over several weeks to the infective L3 stage allowing the lifecycle to continue.

Trichuris muris: the life cycle of *T. muris* is entirely enteric, with orally ingested embryonated eggs hatching in the distal small intestine, releasing L1 larvae that migrate to the caecum and embed in the intestinal mucosa (55). Following four moults to adulthood, male and female worms copulate leading to the production of thousands of eggs per day, which are excreted in the faeces. Excreted embryonated eggs are not immediately infective, as they require approximately 3 weeks for larvae to develop. Infective eggs present in the environment are then ingested and the life cycle continues. Genetically, resistant strains of mice generate a strongly polarized type 2 immune response and reject larval stages of the parasite while susceptible strains exhibit a type 1-dominated response and develop chronic infections with adult worms.

Induction of type 2 immune responses

The majority of intestinal helminths elicit a strongly polarized type 2 immune response, with the exception of *Trichuris* spps that invoke a mixed type 2/type 1 response in many genetic backgrounds. Type 2 immunity is generally associated with protection against intestinal helminths and is characterized by a polarized cytokine response involving the secretion of interleukin (IL)-4, IL-13 and IL-5, B-cell isotype switching to IgG1 (mice), IgG4 (humans) and IgE, eosinophil and basophil haematopoiesis, and the expansion of alternatively activated macrophages, goblet cells and mast cells [reviewed in (15)]. While type 2 cytokines were originally identified as being produced by T cells, an increasing number of studies have identified innate cell populations that contribute to their secretion. Of these, basophils (16) are a major source of IL-4 (17, 18) and a novel population of type 2 innate lymphoid cells (ILC2), which lacks T- or B-cell markers and is expanded by *N. brasiliensis* infection, represents an important early source of IL-13 and IL-5 (19–22). *Trichuris muris* infection results in the expansion of a population of multipotent progenitor cells that produce IL-4 termed $MPP^{type\ 2}$ cells (23). These cells express several haematopoietic stem cell markers, but unlike ILC2, have the ability to differentiate into several cellular lineages including mast cells and macrophages (23). Expansion of ILC2 or $MPP^{type\ 2}$ cells following helminth infection represents one of the earliest events, and these cells likely shape the nature of the ensuing adaptive immune response. A better understanding of the development and function of these cells will undoubtedly be critical to our understanding of immune responses against intestinal helminths.

The mechanisms by which type 2 immunity is initiated in response to helminth infection remain unclear. Most host–pathogen interactions involve recognition of pathogenic molecular patterns by host pattern recognition receptors (PRR), yet the search for PRRs recognizing helminth products has yielded few results. Intestinal epithelial cells (IECs) have been identified as important in the initiation of type 2 immune responses following intestinal helminth infection (24). Mice with an IEC-specific defect in NF-κB activation ($Ikkb^{\Delta IEC}$ mice) are susceptible to *T. muris* infection and produce decreased levels of IL-4, IL-5 and IL-13 and increased levels of IFN-γ leading to a failure to expel worms (24). IECs produce several cytokines that are required for the development of polarized type 2 immunity in response to *T. muris* including thymic stromal lymphopoietin (TSLP), IL-25 and IL-33 (24–28). IEC-intrinsic, NF-κB-dependent, production of TSLP is critical for licensing dendritic cells (DCs) to allow the development of adaptive $CD4^+$ type 2 (Th2) cell responses (24, 25, 28). Mice deficient in the receptor for TSLP (TSLPR KO) are susceptible to *T. muris* infection (25), and antibody blockade of IL-12p40 or IFN-γ in either $Ikkb^{\Delta IEC}$ mice or TSLPR KO mice following *Trichuris* infection renders these susceptible strains resistant (24, 25, 28). However, the development of type 2 immunity following infection with *N. brasiliensis* or *H. p. bakeri* does not require TSLPR (28). Thus, it is likely that TSLP is not required to directly promote protective immunity, but instead limits the development of nonprotective type 1 responses by suppressing the production of IL-12p40.

IL-25 and IL-33 (a member of the IL-1 family) have been shown to be produced by IEC rapidly following helminth infection. These cytokines play a crucial role in the regulation of type 2 cytokine production (26, 27) and protective immunity against multiple helminth parasites including *T. muris* (27), *N. brasilienis* (29) and *H. p. bakeri* (22). IL-25 can also be produced by Th2 cells (30) and can act on a number of cell types including antigen-presenting cells (31), airway smooth muscle cells (32) and invariant natural killer T (iNKT) cells (33). Injection of recombinant IL-25 into naïve mice stimulates the production of type 2 cytokines by ILC2 (19, 29) or $MPP^{type\ 2}$ cells (23), induces IL-4 production by invariant iNKT cells (33) and facilitates the differentiation of Th2 cells (34). IL-25 has recently been shown to elicit both ILC2 and $MPP^{type\ 2}$ simultaneously, although these cell populations are distinct in their transcriptional profile, developmental programs and pluripotency (35). IL-33 is able to induce the secretion of type 2 cytokines (IL-4, IL-5 and IL-13) by Th2 cells (36–38), basophils and mast cells (39, 40) and ILC2 (29).

While ILC2 and $MPP^{type\ 2}$ cells are generally considered to arise directly as a result of IL-25 and IL-33 production, much controversy surrounds the issue of how Th2 cells are activated. DCs are the primary antigen-presenting cell (APC) of the immune system and are typically regarded as necessary for the activation of naïve $CD4^+$ T cells. Yet, despite a clear increase in the frequency and number of DC in the tissues and mesenteric lymph nodes (mLN) following *T. muris* infection, DCs do not appear to be the primary APC required to promote Th2 differentiation. Mice that express MHC class II solely on DC are susceptible to infection with *T. muris* (41), indicating the existence of another APC population. Basophils, a rare granulocyte population (<0·5% of circulating cells), are often associated with helminth infection and can produce the Th2-polarizing cytokine IL-4 (42). Recent studies highlighted this cell as playing a specific and critical role in antigen presentation during *T. muris* infection (41). Infection resulted in the transient appearance of these cells within the mLN where they functioned as APCs (41). Moreover,

antibody depletion of basophils rendered normally resistant mice susceptible to *T. muris* infection. Interestingly, however, expulsion of adult worms following primary infection with *N. brasiliensis* (42, 43) or secondary infection with *H. p. bakeri* (44) does not require basophils. Moreover, Th2 cell differentiation has been shown to be entirely dependent on $CD11c^+$ DCs following *N. brasiliensis* or *H. p. bakeri* infection (45, 46). Thus, depending on the context of the infection, DC or basophils promote Th2 cell differentiation by acting as APCs, with basophils additionally providing a source of IL-4 to boost type 2 immunity. Like DC and basophils, mast cells form a potent arm of the innate immune response and are capable of responding to the presence of pathogens. Of particular interest, mast cell degranulation following *H. p. bakeri* infection was recently reported to be required for the enhanced expression of IL-25, IL-33 and TSLP within the gut indicating that these cells play a role in the early response to helminth infection (47).

Immune mechanisms of worm expulsion

Resistance to infection with *Trichuris* spps in mice and pigs is associated with the activation of Th2 cells that produce the cytokines IL-4 and IL-13 (48–51). As mentioned, the differentiation of Th2 cells is driven by binding of the type 2-associated cytokines IL-4 and IL-13 to IL-4Rα on the T-cell surface, leading to activation of signalling intermediates such as STAT6 and resulting in the activation of the master transcriptional activator, GATA3 (52). Infection of humans also results in a Th2 cell-biased immune response, with elevated levels of IL-4 and IL-13 (49, 50), and immunoglobulin class-switching to IgG4 and IgA (53). Th2 cells and activation of STAT6 signalling pathways are also essential for the expulsion of *N. brasiliensis* and *H. p. bakeri*. Interestingly, transfer of ILC2 into wild type, but not RAG deficient mice (lacking T and B cells), promotes expulsion of *N. brasiliensis* (20, 29), indicating that adaptive immune cells are necessary for the effector function of ILC2. Th2 cells represent a potent source of IL-25 and addition of exogenous IL-25 into RAG mice lacking Th2 cells could restore worm expulsion (29), indicating that the main role of Th2 cells in worm rejection may be to maintain the expansion of ILC2. However, other cells that are induced by IL-25 treatment may also promote ILC2 expansion. The ability of ILC2 to directly mediate worm expulsion likely results from the large quantity of IL-13 produced by these cells because addition of an exogenous source of the related cytokine, IL-4, in the form of immune complexes can also promote worm expulsion in *H. p. bakeri*- or *N. brasiliensis*-infected mice (54).

But what are the mechanisms by which IL-14/IL-13 mediate worm expulsion? These cytokines are well known to activate IL-4Rα-dependent responses in numerous intestinal cell types including epithelial cells (IEC), goblet cells, smooth muscle cells and macrophages. IECs are in a state of constant proliferation resulting in the regeneration of the intestinal epithelium. *Trichuris muris* larvae live embedded within IEC within the caecum (55), and increased epithelial cell proliferation and turnover can result in parasite expulsion. In susceptible strains of mice, increased epithelial proliferation is observed, but IEC turnover is limited, resulting in crypt elongation and a failure to expel the parasite (56). Surprisingly, the control of epithelial turnover during *T. muris* infection is controlled by the IFN-γ-dependent chemokine CXCL10. Antibody blockade of CXCL10 is sufficient to render susceptible mice, including immunodeficient mice, resistant to *T. muris* infection. Yet, signalling through IL-4Rα expressed on IEC is also critical for immunity to *T. muris* (57). Whether IL-4/IL-13 signalling in IEC promotes proliferation has not been examined. However, these cytokines promote intestinal permeability and increased fluid section, which are likely to contribute to the expulsion of luminal worms. Type 2 responses have also been shown to stimulate Paneth-cell growth and secretion of antibacterial products that may harm helminths (58, 59).

IL-4/13 also stimulate goblet cells, and the goblet cell product resistin-like molecule (RELM) β plays an important role in the expulsion of *N. brasiliensis* and *H. p. bakeri*, presumably by interfering with their feeding upon host tissues (57). RELMβ is also highly induced during *Trichuris* infection and has been shown to bind to secretory structures on adult *Trichuris* worms (60). Surprisingly, however, RELMβ is dispensable for resistance to *T. muris* (61). Another major goblet cell product, mucin, has recently been identified as playing a role in parasite expulsion. The mucins Muc2 and Muc5ac are upregulated during worm expulsion and are required for clearance of *T. muris* and *N. brasiliensis* from the intestine (62, 63). In contrast to the well-characterized intestinal mucin Muc2, Muc5ac is primarily expressed in the airways and is specifically induced in the intestine during helminth infection. In keeping with its persistence within the intestinal lumen, *H. p. bakeri* infection does not lead to increased Muc5ac expression (63, 64). Additional goblet cell proteins including intelectin (60, 65), chloride channel calcium-activated 3 (59, 66), pancreatic lipase-related protein 2 and pancreatic colipase (67) are upregulated during *T. muris* infection, but the roles of these proteins in parasite expulsion remain unknown.

IL-4 and IL-13 also induce increased contractility of intestinal longitudinal smooth muscle cells. However, deletion

of the gene encoding the shared receptor subunit, IL-4Rα, specifically on smooth muscle cells only contributes partially to resistance against *N. brasiliensis* (68), indicating that this response plays a secondary or minor role in worm expulsion. Macrophages were recently identified as contributing to expulsion of *N. brasiliensis* (69), although their depletion with clodronate-loaded liposomes has no effect on the expulsion of *T. muris* (41). These cells contribute to smooth muscle hypercontractility following *N. brasiliensis* infection (69); however, their impact on host immunity and physiology may be much broader and should be investigated in more detail.

In addition to the canonical Th2 cell-associated cytokines IL-4 and IL-13, IL-9 has also been shown to be induced by *T. muris* infection and to play an important role in protective immunity (70–73). Transgenic over expression of IL-9 in mice resulted in rapid worm expulsion (73). In contrast, immunization with IL-9-ovalbumin complexes that led to a robust anti-IL-9 response (74), or treatment with anti-IL-9 antibodies (75), in normally resistant C57BL/6 mice rendered the animals susceptible to *T. muris* infection. Functionally, IL-9 can stimulate smooth muscle contractility (75), a potential mechanism of worm expulsion (76) and can potentiate IL-4-dependent immunoglobulin class-switching to IgE (73, 77–79). IL-9 also promotes mucosal mastocytosis (73), a typical feature associated with type 2 immune responses and intestinal helminth infection [reviewed in (80)]. Although mast cells are not required for expulsion of *T. muris* (81), they contribute to the development of type 2 immunity following *H. p. bakeri* infection (47) and are essential mediators of immune expulsion of *Trichinella spiralis*, a natural parasite of mice (82–84). Mucosal mast cells express a variety of effector molecules, including proteases that degrade tight junctions allowing the influx of fluids into the intestinal lumen, a process that likely contributes to their ability to promote worm expulsion (85).

Overall, the literature to date indicates that a variety of mechanisms contribute to the immune expulsion of adult helminths from the intestinal lumen, with the exact means of expulsion being highly dependent on the species present. A generalized summary of possible expulsion mechanisms is shown in Figure 1.

There are limited data on the mechanisms by which lumen-dwelling intestinal helminths are expelled in humans largely because of ethical and practical limitations to the investigation of the human intestinal tract. Histological studies of intestinal biopsy samples from individuals infected with intestinal helminth parasites generally show only mild alterations (86–88), indicating the close adaptation of these parasites to humans. In the case of trichuriasis, heavily infected children may develop colitis (inflammation of the large intestine) (89) or rarely a dysentery-like syndrome (*Trichuris* dysentery syndrome or TDS) (90). Children with TDS have greater numbers of mucosal IgE+ mast cells that show prominent degranulation by electron microscopy and high rates of spontaneous histamine release ex vivo (90), indicating that immediate hypersensitivity reactions per se may be ineffective in expelling *T. trichiura* adult worms. New data, derived from experimental infections of human volunteers with *Necator americanus*, have shed light on the mechanisms by which human hookworm may be expelled from the intestinal tract. Interestingly, most experimentally infected larvae reach the intestinal tract with little evidence of attrition during systemic migration (91, 92). Experimentally infected individuals develop enteritis (inflammation of the small intestine) during primary infections (92), a clinical picture that is typical of human infections with the dog hookworm, *Ancylostoma caninum* (93). In the case of human infections with *N. americanus*, the mucosal inflammatory response appears to be restricted towards immature worms, characterized histologically by an intense eosinophilic inflammation associated with a shortened attachment time of the larvae and their progressive distal expulsion along the gut (92). The intensity of eosinophilic inflammation in biopsy samples was positively associated with the rate of larval expulsion (94). Immature worms are probably the primary target for expulsion mechanisms because, during repeat infections, mucosal histology is normal at sites adjacent to mature worms (92).

Mechanisms of chronicity

Treatment of mice with IL-4 complexes or exogenous recombinant IL-25 can promote worm expulsion in chronically *H. p. bakeri*-infected animals indicating that inadequate type 2 immunity is responsible for the initial failure of mice to expel these worms. This hypothesis is supported by the recent findings that *H. p. bakeri*-infected mice display little expansion of ILC2 (95) and that transfer of IL-13-producing macrophages into mice harbouring a chronic *H. p. bakeri* infection can promote worm expulsion (96). Why host immunity is inadequate in expelling the worm is not clear, but it may be related the potent ability of this parasite to elicit regulatory T-cell expansion as depletion of these cells results in enhanced Th2 immunity (97). Interestingly, a recent study identified the existence of a TGF-β homologue within *H. p. bakeri* that functioned to promote conversion of naïve T cells to Foxp3+ regulatory T cells *in vitro* (98).

Genetics also plays a strong role in resistance or susceptibility to infection with intestinal helminths. For

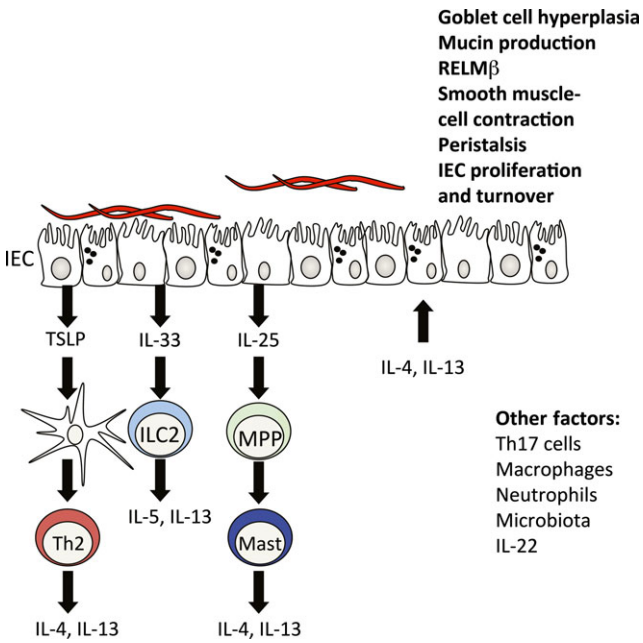

Figure 1 Mechanisms of expulsion of intestinal worms. Although the initial interaction between helminths and the host are poorly defined, infection results in the production of epithelial cell-derived cytokines such as thymic stromal lymphopoietin (TSLP), IL-33 and IL-25. Resistance to some helminth infections is independent of TSLP. Induction of TSLP regulates dendritic cell (DC) production of IL-12 and promotes basophilia (Baso), both leading to priming of type $CD4^+$ T-cell responses (Th2). IL-33 is normally a nuclear protein that is released upon cellular damage. IL-33 is a potent activator of type 2 innate lymphoid cells (ILCs) that occurs early after helminth infection. IL-25 is induced in response to the microbiota and is increased following helminth infection. IL-25 induces a multipotent progenitor cell (MPP) that can give rise to other innate cell lineages. The result of these pathways is to promote a T_H2 cell response and high levels of IL-4 and IL-13. These cytokines promote worm expulsion by inducing physiological changes in the intestinal epithelium. Some expulsion mechanisms include goblet cell hyperplasia and mucus secretion, increased proliferation and turnover and smooth muscle contractility and peristalsis. In addition, although they are not critical for resistance, other factors such as cells (neutrophils, macrophages and Th17 cells), cytokines (IL-22) and the microbiota are dynamically regulated during infection and most likely play a regulatory role in the development of protective immunity to helminth infection.

H. p. bakeri, rapid rejection of adult worms or the establishment of chronicity differs for various mouse strains with SJW and SJL mice expelling primary infection within 4–6 weeks, C57BL/6 and 129/J mice exhibiting an intermediate phenotype and CBA, C3H, SL and AJ mice exhibiting very little resistance to primary or challenge infections [reviewed in (99)]. Infection of most strains of mice including BALB/c, C57BL/6, 129/J and C3H with *T. muris* results in worm expulsion between days 18 and 21. AKR/J mice are currently the only immunocompetent strain that fails to clear *T. muris* infection. A recent study has used F2 intercrosses between resistant BALB/c and susceptible AKR/J mice to identify quantitative trait loci (QTL) associated with chronic infection and inflammation (100). Seven QTL on seven chromosomes were identified (*Tm1*, *Tm3*, *Tm4*, *Tm10*, *Tm11*, *Tm12* and *Tm17*). Consistent with a role in helminth immunity, the Tm1 and Tm17 loci have previously been identified in studies examining susceptibility to *H. p. bakeri* (101). Strikingly, one QTL, Tm3, completely overlaps with a region termed *Cdcs1.1*, previously identified in three unrelated spontaneous colitis models (102–104). Thus, genetic studies have shown that there appear to be genomic sites associated with both immunity to infection and regulation of intestinal inflammation.

The epidemiologic patterns of nonrandom clustering of infections and clustering at family and household levels have been interpreted as evidence for genetic susceptibility to ascariasis (105). Genome wide analyses for genes associated with susceptibility to ascariasis identified as a candidate gene, TNFSF13B, a regulator of B-cell activation and immunoglobulin secretion. Subsequent analyses have suggested additional possible genes associated with immune function (105), but these studies have shed little light so far on the mechanisms of susceptibility and establishment of chronic infections. Insights into the immunological mechanisms associated with the establishment of infections with mature adult hookworms have been provided by experimental infections of individuals with coeliac disease in remission (106). Comparisons of

duodenal mucosal biopsies from such individuals compared with uninfected controls showed that the survival of mature adults in the small intestine during primary infections was associated with an increased protein and/or mRNA expression for Th2 (IL-4, IL-5, IL-9 and IL-13), regulatory (IL-10 and TGF-β) and mucosal healing (IL-22) cytokines (106). Comparison of pre- and post-infection biopsies, also indicated evidence for suppression of IL-23, a key cytokine involved in driving mucosal inflammation (106).

PROTECTIVE IMMUNITY AGAINST RE-INFECTION

For the majority of experimental helminths, repeated infection rounds of mice, of the appropriate genetic background, can elicit protective immunity if chemotherapy is administered between infection rounds to eliminate existing adult worms. Where observed, this protective response is normally targeted against the larval stages of the parasite. There is little evidence, however, that repeated rounds of chemotherapy, given to children living in endemic areas, contribute to the development of protective immunity against intestinal helminths.

Immune memory against intestinal helminths

Most experimental helminths can elicit the development of memory T cells, as evidenced by the more rapid production of IL-4 and IL-13 by $CD4^+$ T cells following re-infection (107). In mice, long-term mucosal immunity against *T. muris* requires $CD4^+$ Th2 cells and these cells persist even in the absence of chronic infection (107, 108). Secondary infections are rapidly rejected (prior to day 12 post-infection), suggesting that infection-induced immunity is directed against larval stages. However, the mechanisms associated with this rapid immunity against re-infection with *T. muris* are unknown.

In humans, the magnitude of the Th2 cell response during *T. trichiura* infection is correlated with the probability of re-infection (49, 50, 109), indicating that protective immunity is mediated by mechanisms requiring type 2 cytokine production. Similarly in a hookworm endemic area of Papua New Guinea, resistance to re-infection following chemotherapy was associated with elevated production of IL-5 by PBMCs stimulated with parasite antigen (110).

For *N. brasiliensis,* clear evidence indicates that protective immunity against challenge infections occurs against larvae present in the lung (111). The development of immunity is dependent on Th2 cells, and many cell types increase in the lung following re-infection, including eosinophils and alternatively activated macrophages. However, again, the identity of the immune effectors that successfully target the helminth larvae has remained elusive. Antibodies are not necessary for immune protection following *N. brasiliensis* re-infection (112); however, passive transfer of serum from immune mice can confer some degree of resistance (113). More information is available for *H. p. bakeri* where protective immunity is targeted at larvae present within the intestinal submucosa. In this case, Th2 cells mediate the development of a granuloma around the larvae that is rich in eosinophils and alternatively activated macrophages, and protection is macrophage dependent (114). Antibodies also form an important arm of protective immunity in this model; however, the mechanisms by which these target larvae are not clear.

By contrast, a role for antibodies in protective immunity against intestinal helminths in humans is not established. Infected individuals including those with ascariasis have high circulating levels of specific antibodies of almost all isotypes and subclasses and antibody levels are generally positively associated with parasite burdens (115). However, levels of specific IgE against *A. lumbricoides* (116, 117) and *T. trichiura* (118) and IgE reactive to larval antigens against hookworm (119) have been associated with resistance to infection with *A. lumbricoides*, *T. trichiura* and hookworm, respectively. Additional evidence for a possible protective role of IgE against intestinal helminth infections came from a placebo-controlled randomized trial of the use of anti-IgE therapy in asthma: in a region of Brazil where intestinal helminths are present, anti-IgE treatment was associated with a trend of increased risk of geohelminth infection, primarily with *A. lumbricoides* (120). It therefore seems reasonable to infer that specific IgE has a role to play in protective immunity either through the initiation of allergic-type responses to the parasites or in the amplification of other Th2-mediated mechanisms.

Vaccination

Vaccination relies on the development of immune memory with successfully vaccinated individuals raising a rapid and strong protective response following encounter with the true pathogen. Yet, despite the increased knowledge of the cellular and molecular requirements for protective immunity to intestinal helminths, there are currently no vaccines available against human species. In mice, subcutaneous vaccination with *T. muris* excretory/secretory (ES) products or adult worm homogenate in the presence of complete or incomplete Freunds adjuvant rendered susceptible mice resistant to infection and was associated with an increased Th2 cell response (121–126). In humans, an interesting vaccine target was identified as a 47 kDa

antigen present in adult *T. trichuria* (43 kDa in *T. muris*) that is strongly recognized by human immune serum (127) and which can form pores in cells (128).

There has been considerable investment into the development of hookworm vaccines, and several promising candidates have been identified (129). A safety and immunogenicity study using *Necator americanus* Ancylostoma-secreted protein 2 (Na-ASP-2) caused generalized urticarial reactions in Brazilian adults previously infected with hookworm (130) and was associated with the presence of pre-existing specific IgE to this antigen. New vaccine candidates have targeted important parasite enzymes such as Na-GST-1 and Na-APR-1 that are required by the parasite for feeding on host blood (129). However, the need to induce Th2 responses for a vaccine to be useful for protection while avoiding such adverse allergic responses poses a major challenge for the development of vaccines against intestinal helminth parasites and it remains to be seen if a vaccine targeted against established adults in the intestine can be safe and effective.

IMPACT ON INFLAMMATORY DISEASES

Evidence of heavy helminth burdens can be found in the mummified remains of early hominids reflecting our long co-evolution with these pathogens (131). Although helminth infections still infect almost one-third of the human population, the introduction of municipal sanitation has largely resulted in their eradication from developed countries. The relatively recent absence of intestinal helminths within developed societies has been hypothesized to be associated with a possible increased incidence of immune-mediated inflammatory diseases, including allergies, autoimmunity and inflammatory bowel disease (IBD) [reviewed in (132)]. In support of this concept, helminths were recently described to represent the main selective force for the selection of human genes associated with autoimmunity and allergy (133).

Immunomodulation by intestinal helminths

There is growing interest in the potential effects of intestinal helminths in modulating inflammatory diseases of the mucosa. Modulatory effects of intestinal helminths have been reported for inflammation in the intestine and in the lungs, but the evidence for a clinically relevant role is still much stronger in experimental murine models than in humans.

Asthma

Temporal trends of increased asthma prevalence over recent decades have been attributed to changes in the living environment that includes declining exposures to infectious diseases and microbial products (134, 135). Helminth parasites have attracted considerable interest as a potential exposure that might modify allergic inflammation and asthma. Murine models have shown clearly that intestinal helminth infections can modulate airway inflammation (7, 136). The intestinal helminth *H. p. bakeri* has been shown to suppress allergen-induced airway eosinophilia (9, 136) and bronchial hyper-reactivity (9) induced by sensitization with ovalbumin (OVA) (9, 136) or *Dermatophagoides pteronyssinus* allergen p 1 (Der p 1) (136). Suppression was transferable to uninfected animals by splenocytes (9) or mesenteric lymph node cells (136) and has been associated with regulatory T cells (136) and regulatory B cells (137). *Nippostrongylus brasiliensis* can induce alternatively activated macrophages during primary infections (138), and infections are associated with a protracted suppression of airway hyper-reactivity and inflammation to *D. pteronyssinus* (139). Findings from human studies have been less clear. In cross-sectional studies, hookworm infection has been associated with a reduced prevalence of wheeze or asthma (140), but *A. lumbricoides* and *T. trichiura,* or markers of infection, have been associated with an increased risk (140–143). So far, no well-controlled trial has shown an effect of periodic antihelminthic treatment on the prevalence of asthma (144, 145). Randomized therapeutic studies using small infective doses with hookworm larvae have not shown clear clinical benefits of infection on symptoms of asthma (146) or allergic rhinitis (146). Similarly, a randomized controlled trial of the efficacy of the pig whipworm, *T. suis,* in the treatment of allergic rhinitis showed no demonstrable benefit (147). The ability to show a clinical benefit in asthma likely requires very marked effects on inflammation in the lungs, and it seems unlikely that helminth treatment, at doses and infection periods that are free of significant adverse effects, will be a useful therapeutic approach. More useful, perhaps, will be the isolation of helminth products that have specific effects in distinct inflammatory pathways.

Inflammatory bowel disease

Inflammatory bowel diseases that include Crohns diseases and ulcerative colitis are associated with impairments in epithelial function and dysregulated immune responses to commensal bacteria in the intestinal tract (148). Increases in the prevalence of IBD over recent years have been attributed to improved hygiene and the disappearance of intestinal helminth infections (149). Experimental murine models have shown that intestinal helminths can reduce inflammation in chemical-induced colitis (8, 130), and the Th2 response induced by *H. p. bakeri* infection can attenu-

ate experimental gastritis caused by *Helicobacter pylori* infection (150). The mechanisms by which helminths may protect against colitis include the induction of a Th2 cytokine environment in the mucosa (151, 152), downregulation of Th1 (96) and Th17 mucosal responses (153), upregulation of IL-10 and TGF-β by colonic regulatory T cells (152, 154), the induction of regulatory DCs (155) or alternatively activated macrophages in the intestinal mucosa (156), and by regulatory effects on mucosal innate immune responses (157–159).

The usefulness of therapeutic infections with intestinal helminths has been evaluated in patients with IBD. Treatment with *T. suis* ova was associated with clinical improvement in a randomized controlled trial of patients with ulcerative colitis (160). Two other studies have reported temporary improvements in symptoms of patients with ulcerative colitis (161) and Crohns disease (160, 161) following *T. suis* therapy, but these studies are difficult to evaluate because no comparison groups were included. The use of *N. americanus* infections for the treatment of IBD has been evaluated for Crohns (162) disease and for coeliac disease (163): in the case of Crohns disease, a small open trial provided some evidence for reductions in disease activity (162), but for coeliac disease, a small randomized controlled trial showed no demonstrable clinical benefit of hookworm infection (163), although there was evidence for reductions in Th1 and Th17 immune responses in the duodenal mucosa of hookworm-treated subjects (132). Therefore, as for asthma, the potential for the clinical use of helminth therapy for the treatment of IBD remains doubtful, and adequately powered, properly controlled, randomized trails will be required to fully evaluate the therapeutic usefulness of such a strategy.

Interactions with the intestinal microbiota

As intestinal helminths and commensal bacteria inhabit the same environmental niche, it is likely that these organisms interact with, and impact on, each other. Intestinal helminths are well known to alter intestinal physiology, permeability, mucous secretion and the production of antimicrobial peptides – all of which may impact on bacterial survival and spatial organization. Yet, despite rapid advances in our understanding of host–intestinal bacteria interactions, the impact of helminths on the relationship has remained largely unexplored. That such interactions do take place was highlighted in a recent report (164), indicating that bacterial interactions are essential for the hatching of embronyated *T. muris* eggs. Such interactions are likely to also be required in the intestine as active *T. muris* infection was not observed in mice infected with embronyated eggs and additionally treated with broad-spectrum antibiotics to diminish the numbers of intestinal bacteria. It also appears that the presence of helminths can alter the nature and complexity of intestinal bacterial communities as *H. p. bakeri* infection of mice was observed to result in an increase in abundance of Lactobacillaceae family members at 14 days post-infection (165). However, whether this bacterial dysbioses resulted from helminth infection per se or from the introduction of bacteria associated with the faecal-hatched larvae was not established. Nevertheless, interactions of helminths with bacterial communities may occur raising the possibility that such interactions contribute to immune modulation and to the general well-being of the host. Increasing evidence suggests that alterations to intestinal bacterial communities (dysbioses) are associated with chronic inflammatory diseases including obesity, IBD, diabetes and allergy (166). Thus, the exact nature of helminth–bacterial interactions – and the contribution of this to host immunity and disease – will be an important topic for future studies in both man and mice.

CONCLUDING COMMENTS AND PERSPECTIVES

The large majority of intestinal helminths elicit polarized type 2 immune responses, and type 2 cytokine production is essential for effective immune expulsion of adult worms or protection against re-infection. The distinction between innate and adaptive immunity has become increasingly blurred, and type 2 immunity following helminth infection is likely to involve important contributions first from innate immune cells and later from T cells that act to both amplify type 2 cytokine secretion and to sustain the activity of innate immune cells. Yet, despite the protective role of type 2 immune responses in animal infections, most human intestinal helminths remain chronically within their host for years and children tend to suffer increasing worms burdens acquired through constant re-infection. These findings indicate that immunity is often inadequate against these macro-parasites. Evidence in mouse models indicates that the immune system can effectively target helminths, but that the response must be both strong and rapid. Helminths efficiently evade host immunity through a number of mechanisms including but not limited to (i) promoting a strong regulatory T-cell response that diminishes type 2 immunity, (ii) immune deviation towards type 1 cytokine production and (iii) production of molecules with immune dampening properties.

Investigations of our relationship with intestinal helminths can thus reveal fascinating information about immune regulation. A better understanding of how intestinal

helminths regulate mucosal inflammatory responses may prove useful for the development of new treatments for chronic inflammatory diseases of the mucosa that are driven by over-exaggerated or inappropriate immune stimulation. By contrast, an improved understanding of protective immune mechanisms against intestinal helminths will be key to the development of vaccines against these important pathogens. Recent studies have also demonstrated the importance of rapid wound healing for host survival following helminth infection (167). Further studies investigating the impact of immune cells on this response are likely to reveal novel insights into this fundamental process. Lastly, we can no longer consider the host–helminth relationship in isolation. Helminths and bacteria live alongside one another within the intestine and both have been shown to have important impacts on the host immune system. Future studies should consider this ménage à trois situation, particularly when investigating the immunomodulatory potential of helminths because bacterial dysbioses is linked to a variety of inflammatory disease.

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