

To B or not to B: B cells and the Th2-type immune response to helminths

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Similar T helper (Th)2-type immune responses are generated against different helminth parasites, but the mechanisms that initiate Th2 immunity, and the specific immune components that mediate protection against these parasites, can vary greatly. B cells are increasingly recognized as important during the Th2-type immune response to helminths, and B cell activation might be a target for effective vaccine development. Antibody production is a function of B cells during helminth infection and understanding how polyclonal and antigen-specific antibodies contribute should provide important insights into how protective immunity develops. In addition, B cells might also contribute to the host response against helminths through antibody-independent functions including, antigen presentation, as well as regulatory and effector activity. In this review, we examine the role of B cells during Th2-type immune response to these multicellular parasites.

Helminths and the host response

Chronic infection with helminth parasites has a significant impact on global health; more than 2 billion people worldwide are infected, and these parasites can cause high morbidity including malnourishment and anemia. Although drug treatments do exist, reinfection can occur after treatment; typically in parasite endemic areas, and drug resistance is also becoming an issue. As such, the development of effective vaccines against helminths would be a major advance for control and treatment of helminth disease [1]. Engineering vaccines that work is benefited by an understanding of the pathogen-specific immune response, so that specific components of immune protection can be targeted. Both antigen specificity and the desired cytokine response should be considered to optimize protective immunity. For many helminths, the T helper (Th)2-type response mediates protection, but the effective components of this response can differ between parasite species and different developmental stages of infection with the same helminth species. This is a result of the specific ecological niche that is occupied by the invading helminth at different stages of the life cycle, including the microenvironment where the parasite takes up residence and the

specific host–parasite interactions that subsequently occur. Parasitic helminths are classified as cestodes (tapeworms), nematodes (roundworms) or trematodes (flukes). Helminth parasites invade both mucosal and non-mucosal tissues, and comprise a broad spectrum of different pathogens including: microfilaria, *Strongyloides* (threadworms), *Ancylostoma* and *Necator* (hookworms), *Trichuris* (whipworms), *Schistosoma*, *Taenia*, *Trichinella*, *Ascaris*, and *Anisakis*. The course of infection can vary greatly between helminths. For example, certain filarial nematodes are transmitted by mosquitoes and can occupy and obstruct lymphatic vessels with chronic infection that causes elephantiasis, whereas other parasitic nematodes, such as whipworms, are strictly enteric and reside in the epithelial layer of the large intestine. Nematodes do, however, share a basic life cycle that involves: hatching from eggs into pre-parasitic larval stages (L1 and L2), parasitic larval stages that are often tissue dwelling (L3 and L4), and an adult stage with separate males and females. Often, several different components of the host immune response are required for parasite resistance and these might interact synergistically or independently of each other. In this review, we examine the recent identification of B cells as important players in host immune responses to helminths, both in terms of antibody secretion and their potential role in stimulating and controlling Th2-type immune responses.

Vaccination against helminths

Current strategies to control helminth-related morbidity involve regular and mass drug administration, integrated with disease control through improved sanitation and hygiene [2]. Although safe and effective drugs are currently available for the bulk of human parasitic helminth infections, rapid reinfection and the dramatic rise in drug-resistant helminths of veterinary importance have raised concerns over the feasibility of drug administration as a long-term control strategy [2]. Yet, there is evidence for naturally acquired immunity against helminth parasites [3], which indicates that vaccination could offer a viable alternative. The majority of medically important helminths reproduce outside their human host, and parasitic burden increases through reinfection by new larvae. Natural protective immunity is normally most evident for

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Table 1. Recent developments in vaccination against helminths of clinical interest^a.

Parasitic species (common name)	Host		Target antigen ^b	Development ^c
	Definitive	Intermediate		
<i>Necator americanus</i> (hookworm)	Humans	–	Na-ASP-2 Na-GST-1 Na-APR-1	Clinical and Experimental
<i>Schistosoma haematobium</i>	Humans	Freshwater snails	Sh28GST ^d	Clinical
<i>Schistosoma japonicum</i>	Wide range of mammalian hosts including humans	Freshwater snails	Sj23	Veterinary ^e
<i>Schistosoma mansoni</i>	Humans	Freshwater snails	Sm14 Smp80 SmTSP-2 Sm29	Experimental
<i>Taenia solium</i> (tapeworm)	Humans	Pigs	TSOL-18	Veterinary
<i>Taenia saginata</i> (tapeworm)	Humans	Cattle	TSA-9 TSA-18	Veterinary
<i>Ascaris suum</i> (roundworm)	Pigs	Humans	AS24	Veterinary

^aVaccines undergoing development and published within the past 5 years.

^bData were compiled from [4–6,88].

^cVaccines being developed for human use are categorized as clinical (Phase I or II trials) or experimental (antigen discovery and/or testing in animal models). Vaccines listed as veterinary are being developed primarily for use in livestock but might benefit human health by blocking transmission.

^dRegistered as Bilhvax[®], <http://www.bilhvax.inserm.fr/>.

^eVaccine development is aimed at water buffalo in China.

tissue-invasive larval stages [3] – thus a combined approach using drugs to clear existing adult helminths, and vaccination to target newly encountered infectious larvae, might represent an effective method for helminth control.

In the 1960s, several veterinary vaccines that contained irradiated larvae of *Dictyocaulus viviparus* and *Ancylostoma caninum* were developed commercially for use in cattle and dogs, respectively [3]. Since then, recombinant helminth vaccines have shown promise for several ruminant cestodes [4]. No commercial vaccine for human helminths exists. There have, however, been some promising developments over the past 5 years (Table 1). The most advanced human vaccines are among those being developed for schistosomiasis or hookworm, and a number of these have entered clinical development (reviewed in [5,6]). Some vaccines are being primarily developed for veterinary use, but also have clinical relevance (Table 1).

The majority of antigens used for development of recombinant anti-helminth vaccines are selected based on antibody reactivity [3], and protective immunity often associates with a potent antibody response [5,6]. Most successful vaccines work through antibody-mediated mechanisms, and increasing experimental evidence has shown that antibody plays a crucial role in mediating protective immunity against helminths. However, many issues need to be addressed before effective recombinant vaccines against human helminths reach fruition (Box 1). Murine models of helminth infection are becoming increasingly important for identification of mechanisms of antibody-mediated protection and the specific immune effector cells that also contribute to protective immunity.

Antibody function during helminth infection in murine models

A protective role for antibodies?

During helminth infection, polarized Th2-type responses promote B cell class switching to IgE and IgG1. Interleukin

(IL)-4 receptor signaling and cognate T–B cell interactions mediate production of both isotypes. IgE potently activates mast cells and basophils, on which, antigen crosslinks Fc epsilon Receptor 1 (FcεRI)-bound IgE to trigger degranulation and release of soluble mediators from these cells (Figure 1). IgE does not play an essential role in protective immunity against *Heligmosomoides polygyrus* (more recently named *Heligmosomoides bakeri* [7] and hereafter referred to as *Heligmosomoides polygyrus bakeri*) [8], *Nippostrongylus brasiliensis* [9] or *Schistosoma mansoni* [10] infection in mice. By contrast, mast cells are crucial for protection against *Trichinella spiralis* [11,12] or *Strongyloides venezuelensis* [13], but IgE appears to contribute

Box 1. Helminth vaccination: outstanding questions

As helminths afflict only the poor, most current attempts to produce human helminth vaccines necessarily involve the formation of non-profit product development partnerships. These are typically public-private partnerships that involve funding from government or non-profit institutes and one or more private sector companies, with manufacturing procedures established by biotechnology industries present in the target countries. However, the development of effective vaccination programs against human helminths faces many hurdles in addition to the need to raise sufficient financial support. The most pressing requirements for improved vaccine design are outlined below:

- Better understanding and further development of animal models of human disease
- Greater understanding of the mechanisms of host immunity
- New programs for antigen discovery. This would ideally involve the integration of antigen discovery programs with completed and ongoing genome sequencing projects (see <http://www.sanger.ac.uk/resources/downloads/helminthes/>)
- Optimization of adjuvant formulations
- Addressing requirements of delivery to developing world countries (important parameters include cheap production, vaccine stability and adequate distribution).
- An awareness of the influence of maternal antibodies, or pre-existing immune reactivity, on vaccination efficacy
- An understanding of the effects of ongoing infections with other pathogens on helminth vaccine efficacy.

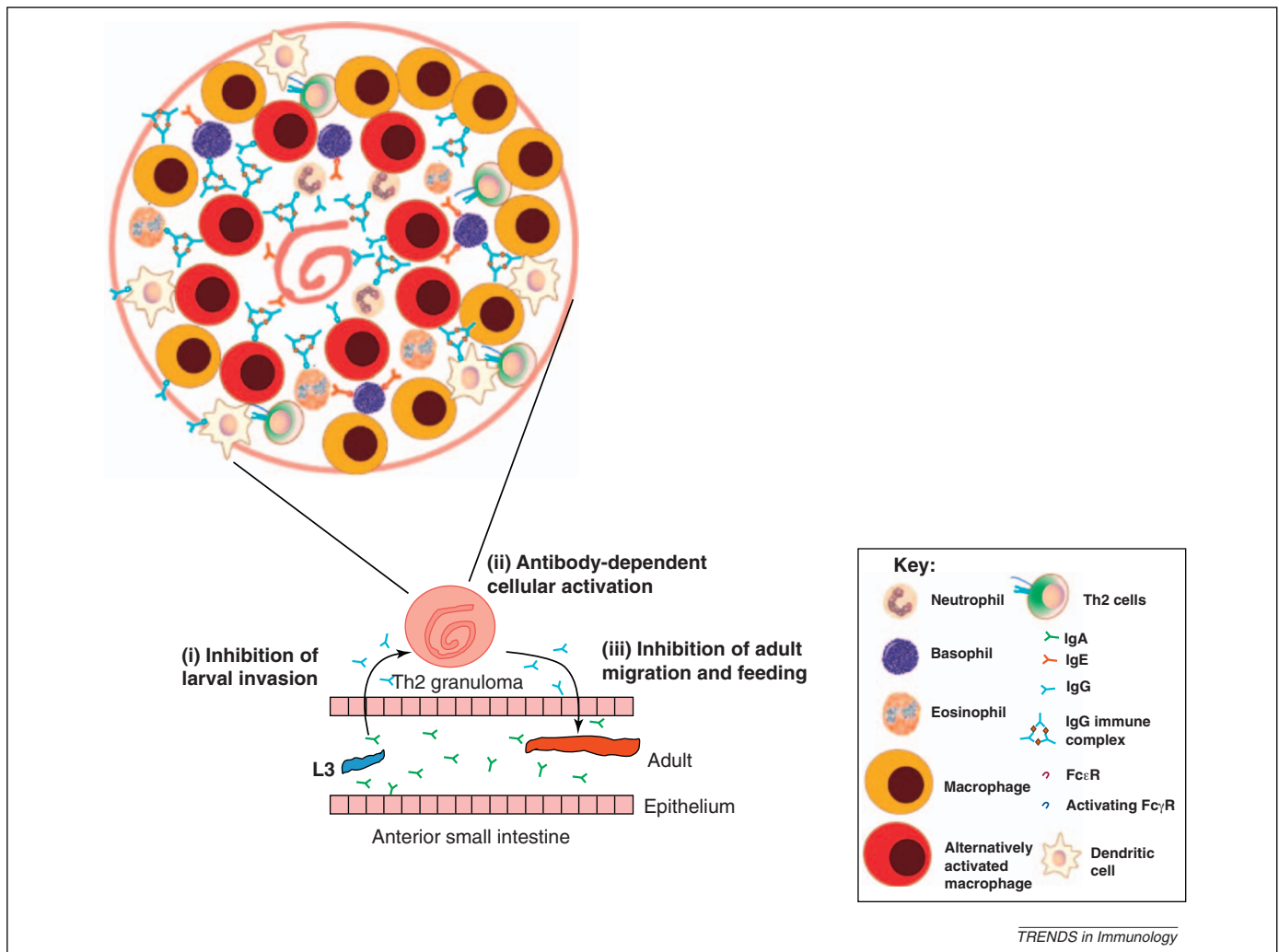


Figure 1. Protective role for antibodies during challenge infections with *H. polygyrus bakeri*. Antibodies could potentially provide protective immunity at three points of the parasite life cycle. (i) Antibodies present in the intestinal lumen or inflamed mucosal tissue might interfere with parasitic enzymes or other essential processes that are required for L3 invasion and migration to the sub-mucosa. (ii) Leukocytes present within the Th2-type granuloma that forms around the invading larva might be activated by the surface-bound antibodies IgG or IgE, or antibody (IgM and IgG)-dependent complement activation could result in additional leukocyte infiltration, cytokine production and cytotoxicity. (iii) Antibodies present within the inflamed mucosal tissue of the small intestine might interfere with essential processes that are involved in feeding and/or migration of adult worms out of the granuloma and into the intestinal lumen.

only partially to *Trichinella*-induced mast cell responses [14] (Table 2).

Despite this seemingly limited role for IgE in mediating protective immunity against helminths, parasite burdens are increased in the absence of B cells following challenge (secondary) infection with *Litomosoides sigmodontis* [15], *S. mansoni* [16], *Trichuris muris* [17] or *H. polygyrus bakeri* [8,18,19] (Table 2). Although B cell-derived cytokines have been reported to play a role in Th2 cell development and sustained antibody production, a direct role for antibodies themselves in mediating protective immunity against *H. polygyrus bakeri* has been demonstrated using AID deficient mice [8,19] (Table 2). IgG has been identified as the antibody isotype that provides the most effective protective immunity against *H. polygyrus bakeri* [8], whereas IgM, which is typically produced in a T cell-independent manner, has been linked to the timely expulsion of filarial parasites [20] (Table 2). These data indicate that antibodies, particularly IgG and IgM, can act as potent mediators of protective immunity following helminth infection.

These findings are supported by observations that protective immunity against helminths is passively transferred to naive experimental animals using immune serum, or purified IgG, (Table 2). Antibody-mediated passive immunity has been demonstrated for *A. caninum* [21], *Schistosoma* species [16], *Taenia* species [22], *Ascaris suum* [23], *Strongyloides ratti* [24], *T. muris* [17], *Trichostongylus colubriformis* [25], *N. brasiliensis* [18] and *H. polygyrus bakeri* [8,18,19,26–28]. Passive immunity has also been shown using: IgG monoclonal antibodies (mAbs) specific for *Fasciola hepatica* [29] and *S. mansoni* [30]; IgM mAbs specific for *Brugia malayi* [31]; and IgG or IgA mAbs specific for *Tr. spiralis* [32–36]. Maternal antibodies provide effective passive immunity against a variety of pathogens and parasite-specific maternal IgG has been reported to protect neonates against infection with the helminths *Tr. spiralis* [37] or *H. polygyrus bakeri* [38].

It is important to note, however, that not all studies that have used passive transfer of immune serum or mAbs have reported a protective effect [18,19,25]. This indicates that the ability of antibodies to mediate protective immunity

Table 2. Experimental models used to determine the impact of antibodies on helminth infection.

Experimental model ^a	Explanation of experimental system	Parasitic species ^b	Refs
Altered mast cell responses	Use of mice exhibiting defects in mast cell development (W/W ^v or IL-3 ^{-/-}), mast depletion using anti-c-kit mAb or IgE-mediated mast cell activation (IgE ^{-/-}).	<i>Trichinella spiralis</i> , <i>Strongyloides venezuelensis</i>	[11–14]
B cell deficiency	Use of mice exhibiting a developmental defect in B cells (μ MT ^{-/-} or JHD ^{-/-}) ^c as a result of gene targeting	<i>Litomosoides sigmodontis</i> , <i>Schistosoma mansoni</i> , <i>Trichuris muris</i> , <i>Heligmosomoides polygyrus bakeri</i>	[8,15–19,42]
Specific lack of isotype switched antibodies	Use of mice rendered deficient in activation-induced deaminase (AID). These mice retain naïve B cells but are unable to undergo class switch recombination or somatic hypermutation.	<i>H. polygyrus bakeri</i>	[8,19]
Fc γ R deficiency	Use of mice rendered deficient in the gamma-chain and exhibiting defective expression of all activating Fc receptors (FcRI, FcRIII and FcRIV).	<i>H. polygyrus bakeri</i> , <i>Strongyloides stercoralis</i> , <i>S. mansoni</i>	[8,43,44,74]
Passive immunization	Transfer of protective antibodies to naïve animals in the form of serum (whole or purified antibody components) or monoclonal antibodies.	<i>Ancylostoma caninum</i> , <i>Schistosoma</i> species, <i>Taenia</i> species, <i>Ascaris suum</i> , <i>Strongyloides ratti</i> , <i>T. muris</i> , <i>Trichostrongylus colubriformis</i> , <i>Nippostrongylus brasiliensis</i> , <i>H. polygyrus bakeri</i> , <i>Fasciola hepatica</i> , <i>Brugia malayi</i> , <i>Tr. spiralis</i> , <i>Str. stercoralis</i>	[8,17,18, 20–36,43,44]
Maternal antibody transfer	Transfer of protective maternal antibodies (IgG and SIgA) via milk to suckling neonates	<i>Tr. spiralis</i> , <i>H. polygyrus bakeri</i>	[37,38]

^aOnly experimental models using rodents are listed

^bParasitic species for which antibodies have been reported to afford protection against primary or secondary infections

^cC57BL/6 μ MT-deficient mice carry a stop codon and a neomycin gene cassette in the first transmembrane exon IgM heavy chain, which leads to developmental arrest at the pro-B cell stage and apoptosis. However, IgM-independent B cell development and immunoglobulin isotype switching can occur *in vitro* if the anti-apoptotic gene Bcl-2 is overexpressed in B cell precursors and the appropriate stimulation (IL-4 and anti-CD40) is provided [89]. Helminth infection has been reported to drive IgE production in C57BL/6 μ MT-deficient mice, despite a continued block in B cell development [90]. Deletion of the J μ gene segment in C57BL/6 JHD mice also results in defective B cell development but these mice do not exhibit the 'leakiness' that is apparent in μ MT-deficient mice.

depends on the species investigated. It might also result from differences in the quality and quantity of serum used. Numerous studies have noted a positive correlation between the number of challenge infections given before collection of immune serum and the ability of serum to provide passive immunity to naïve animals [16,28].

Mechanisms of antibody-mediated protection?

Primary inoculation with *H. polygyrus bakeri* results in chronic infection. If, however, adult parasites are cleared from the intestine with an anti-helminthic drug, secondary challenge results in memory Th2-type-dependent worm expulsion 2 weeks after inoculation. Worms are not expelled in B cell-deficient mice, but can be rescued by exogenous antibody administration, which suggests that antibodies contribute to the protective memory response to *H. polygyrus bakeri* [8,18]. Egg production by adult worms remains inhibited in B cell-deficient mice, which suggests that other protective mechanisms mediated by the CD4⁺ T cell-dependent memory response are intact. The immune response is similarly impaired when macrophages are depleted [39], and antibodies and macrophages might mediate protective effects at a similar stage of the *H. polygyrus bakeri* life cycle.

H. polygyrus bakeri enters the intestine after oral ingestion of L3 and infection can be mimicked experimentally by oral L3 inoculation. After 24 h, larvae penetrate the intestinal wall and migrate to the submucosa, where they reside and develop into adult worms over a period of 8 days. After this tissue-dwelling phase, they migrate back to the intestinal lumen. The tissue-dwelling phase is associated with a Th2-type granuloma response, which is primarily composed of alternatively activated macrophages (M2) and other immune cell populations including

granulocytes, CD4⁺ T cells and dendritic cells (DCs) [40,41].

To examine the importance of antibodies during the early stage of *H. polygyrus bakeri* infection, parasite number and length have been examined in the small intestine in B cell-deficient mice [18]. Both parameters increased as early as 4 days after secondary inoculation, which indicates a role for B cells in larval migration to the submucosa and subsequent worm development (Figure 1). Protective immunity during secondary challenge was restored by serum from wild-type (WT) mice, which indicates a role for antibody. Antibody seems to influence worm migration: in WT mice, larvae distribution in the small intestine is different during the primary and protective memory response, but in B cell-deficient mice, the distribution is similar, and administration of immune serum restores distribution associated with protective immunity [18].

These findings indicate a protective role for antibodies during the tissue-dwelling stage of *H. polygyrus bakeri* infection, although antibodies might also contribute to immunity against other parasitic stages (Figure 1). In an experimental murine model of *Strongyloides* infection, antibodies are essential for the killing of larvae housed in diffusion chambers implanted subcutaneously into mice for a 24-h period that allows transfer of serum and cells, but not of larvae [42–44]. It is possible that antibodies might bind directly to parasites, which impairs their capacity to migrate or develop properly. Studies with *Tr. spiralis* have shown that antibodies can bind to specific parasite structures and soluble (excretory secretory) products, and can impair migration, possibly by interfering with chemosensory reception [32,45]. Alternatively, antibodies might work through more indirect mechanisms, perhaps by recruiting other immune cell populations, which then

mediate direct effects on the parasite. Antibodies are abundant in the Th2-type granuloma that surrounds the developing *H. polygyrus bakeri* larvae [8]. As B cells are infrequent in the granuloma, the antibody presumably binds Fc gamma receptors (FcγRs) on the innate immune cells that surround the parasite. FcγRs are expressed on innate immune cells including basophils, eosinophils, mast cells, monocytes and macrophages, and depending on the effector cell type, FcγR crosslinking can result in cell degranulation, release of cytokines and chemokines, enhanced phagocytosis or antibody-dependent cellular cytotoxicity (Figure 1) [46]. The impact of antibodies on M2 macrophages during *H. polygyrus bakeri* infection is of interest given the recent identification of this cell type as an important mediator of larval killing [39].

The dependence on antibody for protective immunity against *H. polygyrus bakeri* is not strictly applicable to responses to all helminth infections. Although exogenously administered antibody can impair successful *Trichinella* invasion, the natural protective response that leads to adult worm expulsion is effective without B cells, although mast cell degranulation is reduced by as much as 50% [47]. However, parasite-specific IgE contributes to the killing of L1 present in tissues, which suggests some effectiveness of antibody at the early stages of development [14]. The rapidly developing CD4⁺ T cell-dependent protective response that leads to *N. brasiliensis* expulsion after primary inoculation is also intact in the absence of B cells. Furthermore, the more rapid memory Th2-type response is equally effective in *N. brasiliensis*-inoculated WT and B cell-deficient mice [18]. One important difference between the life cycle of *N. brasiliensis* and *H. polygyrus bakeri* is that larval stages of the former migrate through the lung, whereas those of the latter dwell within the intestinal tissue. Both parasites then develop into adult worms that reside within the intestinal lumen. Given that antibody can have important protective effects that result in impaired parasite development in the tissue-invasive stages, it is thus possible that antibodies differ in their ability to attack larvae that are present in the lung or intestine, and that adult parasites restricted to the lumen are not as readily damaged by antibody. Instead, other effector mechanisms might preferentially mediate protection at these later stages during infection. For example, resistin-like molecule β, which is secreted into the lumen by goblet cells and can inhibit parasite feeding, is most effective after adult parasites enter the lumen [48].

Helminth-induced production of polyclonal antibodies: help or hindrance?

Helminth infection has long been associated with the marked production of polyclonal IgE antibodies. Formal proof that helminth infection can lead to the production of irrelevant antibody specificities has been provided by *H. polygyrus bakeri* infection of TgH(VI10)xYEN mice [8]. Almost all B cells in these mice express a neutralizing immunoglobulin against the vesicular stomatitis virus glycoprotein (VSV-GP). In these mice, the immunoglobulin heavy chain locus can undergo class switch recombination to all isotypes, and *H. polygyrus bakeri* infection of TgH(VI10)xYEN mice results in the robust production of VSV-GP-specific IgE and IgG1 antibodies. The exact func-

tion of polyclonal antibody production is not known, but polyclonal IgG in response to *H. polygyrus bakeri* infection reduces the fecundity of female worms [8]. Thus, polyclonal antibody production in response to helminth infection might represent an ancient evolutionary mechanism to benefit both the parasite and host by allowing the parasite to avert the production of protective antibody specificities, while at the same time, reducing worm fecundity and limiting transmission through the host population.

IgG1 antibodies exhibit a higher affinity for the inhibitory FcγRIIB than towards the activating FcγRs, and can thus induce a higher activation threshold in innate immune cells that express both types of receptors [46]. Alterations in IgG glycosylation can also alter the activating versus inhibitory potential of IgG antibodies, with sialic acid-rich IgG glycovariants reported to exhibit potent anti-inflammatory activity [46,49]. The latter finding appears to explain the anti-inflammatory activity of high-dose IgG therapy [49]. Thus, helminth-induced polyclonal IgG production might also serve to restrict excessive inflammatory responses during chronic infection.

Polyclonal antibody production during helminth infection might also provide an explanation for the lowered efficacy that is observed for some vaccines in helminth-infected animals and humans (reviewed in [50,51]). Although this possibility has not been directly investigated, there have been reports of helminth infection reducing antigen-specific antibody production following vaccination of humans [52–55], pigs [56] or rodents [57,58]. However, the relationship between helminth infection and antibody production is complex because, although *H. polygyrus bakeri* infection impairs antibody production following DNA vaccination against *Plasmodium falciparum*, it does not have an impact on responses raised against irradiated sporozoites [58]. Moreover, individuals infected with *Onchocerciasis volvulus* have been shown to mount attenuated humoral responses to Bacillus Calmette-Guerin (BCG) vaccine, whereas they exhibit normal antibody production in response to rubella vaccine [54] and tetanus toxoid [59]. Nevertheless, the exact impact of helminth infection on specific antibody production elicited by vaccination deserves further attention.

Do B cells enhance Th2-type responses

B cells have several important activities in addition to antibody production, including antigen presentation, co-stimulatory molecule signaling, and cytokine production. However, the importance of B cells in driving a T cell-dependent response can vary with the particular antigen and the type of immune microenvironment. In draining lymph nodes, antigen-presenting DCs first interact with naïve T cells in the T zone, and activated T cells then migrate to the B zone [60,61]. In the T:B zone [62], and also in the B zone [63], IL-4-expressing T cells can develop and it has been proposed that here, B cells provide the sustained co-stimulatory molecule interactions that are required for Th2 cell differentiation. In one study, OX40L but not IL-4 expression by B cells was required for Th2 cell differentiation [64]. Interactions of T cells with B cells might be preferentially important for Th2 cell development, because skewing of Th2 to Th1 differentiation occurs in the absence of B cells in

different models [17,64,65], including immunization of B cell-deficient mice with *S. mansoni* eggs [66]. Some helminth infections, including immune responses to *N. brasiliensis* and *H. polygyrus bakeri*, are strongly polarized towards Th2 cytokine production and blockade of co-stimulatory molecules does not deviate the response towards a Th1 cytokine pattern [67,68]. To examine whether these Th2-type responses were refractory to B cell deficiency, mice were inoculated in the ear with *N. brasiliensis*. This blocked the Th2-type response in the draining cervical lymph nodes, but an alternative Th1-type response was not observed [69]. This suggests that B cells are required for Th2 cell differentiation through mechanisms other than blockade of Th1-type cytokine production. In this system, B cell IL-4 production is not required because the Th2-type response was rescued after adoptive transfer of WT or IL-4^{-/-} B cells. However, B cell surface B7 was required, which is consistent with other studies that have suggested an important role for B cell co-stimulatory signals in Th2 cell differentiation [64]. These studies thus suggest that expression of co-stimulatory molecules rather than Th2 cytokines by B cells can contribute to the development of Th2 cells, although it is certainly possible that B cell-derived IL-4 might be important in other *in vivo* systems, as previously suggested from *in vitro* findings [70].

Until recently, few studies had examined the role of B cells in the mucosal immune response to helminths in the enteric region. One early study found that, in the absence of B cells, the Th2-type response deviated to a Th1-type response after *T. muris* infection. In this system, B cells apparently blocked IL-12 upregulation, thereby creating an environment that was permissive for Th2 cell differentiation [17]. However, only recently has the role of B cells in the development of the highly polarized Th2-type mucosal responses to *H. polygyrus bakeri* and *N. brasiliensis* been examined [8,18,19]. Although one study in B cell-deficient mice has concluded that Th2 cytokine production might be compromised in response to *H. polygyrus bakeri* [19], further analyses have indicated that the development of Th2 cells and associated Th2 cytokine production are intact in the immune responses to *H. polygyrus bakeri* and *N. brasiliensis* [8,18]. In the immune response to *H. polygyrus bakeri*, CD4⁺ T cell cytokine gene and protein expression are comparable in the primary and secondary immune responses. Furthermore, in the memory response to *H. polygyrus bakeri*, peripheral Th2 cytokine expression in the granuloma that surrounds the developing larvae is also unaffected. Consistent with this finding, the Th2-cytokine dependent immune cell architecture at the host-parasite interface, which includes CD4⁺ Th2 cells and M2 macrophages, is similar in *H. polygyrus bakeri*-inoculated B cell-deficient and WT mice [18]. These studies suggest that B cells are not essential for the development of polarized enteric Th2-type responses to helminth parasites. Apparently, other signaling pathways can compensate for the absence of B cells in this milieu. The *H. polygyrus bakeri* Th2-type enteric polarized Th2-type response is also refractory to a requirement for thymic stromal cell lymphopoietin interactions [71], a cytokine that has been shown to be essential for Th2 cell differentiation in response to antigens in other immune microenvironments. Recently, several novel enteric im-

mune cell populations, including nuocytes [72] and natural helper cells [73], have been discovered that can support Th2 cell differentiation. It will be important to examine whether these populations contribute to the pathways that support robust B cell-independent development of the polarized Th2-type gut immune response that can be induced during certain helminth infections.

Helminth-induced regulatory B cells

Immune regulation by B cells was first recognized for autoimmune conditions (Box 2). Regulatory B cells also play a role during helminth infection. B cell deficiency results in enhanced Th2-dependent immunopathology following experimental *S. mansoni* infection. A similar increase in immune pathology is observed in mice deficient for FcγRs, which indicates a complex relationship between antibody secretion and B cell function in this model [74]. Regulatory B cells also play a role during *Schistosoma* infection, where their activity correlates with enhanced FasL expression and increased apoptosis of activated CD4⁺ T cells [75]. Although data that show a regulatory role for B cells in suppression of immunopathology after helminth infection have been limited to schistosomiasis, B cells also negatively regulate neutrophil infiltration and parasite clearance following infection with the intracellular protozoan *Leishmania donovani* [76]. Thus, induction of regulatory B cells might represent a broad mechanism of immune modulation by parasites.

Box 2. A brief history of regulatory B cells

Immune regulation by B cells was first recognized for autoimmune conditions and has been reported for rodent models of experimental autoimmune encephalomyelitis (EAE), inflammatory bowel disease (IBD), collagen-induced arthritis (CIA), type I diabetes, lupus, contact hypersensitivity (CHS), anti-tumor immunity and oral tolerance [91,92]. In addition, human B cell markers have recently been found to correlate with spontaneous tolerance of kidney grafts, which has raised speculation that regulatory B cells facilitate transplantation tolerance [93]. Although the ability of regulatory B cells to suppress CIA and EAE could be related to the selective suppression of Th1-type cytokines, regulatory B cells play an equally important role in modulating Th2-driven intestinal inflammation in T cell-receptor-α-deficient mice and helminth infection, which indicates that the action of regulatory B cells is likely to be pleiotropic [94].

The majority of these studies associate regulatory B cell function with IL-10 production. IL-10 is known to exert broad anti-inflammatory effects [95] and B cell-derived IL-10 has been reported to be essential for regulation of IBD, EAE, CIA, lupus and CHS [91,92]. The exact mechanisms by which IL-10 acts differs between studies but includes suppression of pro-inflammatory cytokine production by macrophages or DCs [91,94], and has been predicted to involve additionally modulation of regulatory T cells [94]. IL-10-producing B cells often express the markers CD5 and CD1d, which are found on B-1a cells or marginal zone B cells and transitional-2 B cells, respectively [91,94]. However, ascribing regulatory function to any one B cell subset has been difficult and regulatory function might lie within other B cell populations [94]. Whether regulatory B cells represent a distinct lineage or whether they acquire regulatory potential in response to environmental cues is also not clear. Signals reported to play an important role in the development and/or activation of regulatory B cells include triggering of the B cell receptor, CD40 ligation and Toll-like receptor engagement [91,94]. Finally, in addition to producing IL-10, regulatory B cells have been reported to produce transforming growth factor-β [91,92] and to express Fas ligand [91], which indicates that multiple mechanisms of suppression might exist.

Helminths are potent modulators of chronic inflammatory diseases [77], which might, in part, lie in their ability to invoke regulatory B cells. In support of this, *S. mansoni*-induced regulatory B cells can attenuate allergic disease through an IL-10-dependent mechanism [78–80]. *S. mansoni* infection promotes the expansion of peritoneal B1 cells and splenic B cells, and egg-derived oligosaccharides promote B cell proliferation and IL-10 production [81,82]. *H. polygyrus bakeri* infection also induces regulatory B cells that are capable of attenuating ovalbumin-induced allergic airway disease. In this case, however, the regulatory population is contained within a follicular (B2) cell population and does not involve B cell production of IL-10 [83]. Thus, multiple mechanisms of immune modulation by helminth-induced regulatory B cells are likely to exist.

Although these studies have all utilized experimental models, helminth therapy represents a new, but increasingly popular, tool for the treatment of chronic inflammatory diseases [84]. It will be of interest to assess the ability of human helminths to invoke regulatory B cells, and to determine the potential contribution of these cells to helminth-induced modulation of inflammatory diseases. One recent study has reported that B cell production of IL-10 is increased in multiple sclerosis patients with natural helminth infection [85], which correlates with reduced disease severity [86,87].

Concluding remarks

It is clear that B cells are important in protective immunity against helminths. However, their significance and function can differ greatly depending on the specific parasite. During mucosal responses to helminths, where B cells are essential for protective immunity, antibody secretion appears most significant, and exogenous antibody administration can largely substitute for B cell deficiency. Other helminths elicit host protective responses in the absence of B cells, although even in these cases, administration of exogenous antibody can accelerate expulsion. Passive maternal antibody transfer is also protective against helminths in neonatal infants, which further demonstrates the importance of antibody-mediated protection in the gut. In other immune microenvironments, B cells appear to be more important in promoting Th2 cell differentiation. More studies are needed to examine how antibodies actually mediate protective effects against helminths and why mucosal Th2-type responses can develop through B cell-independent mechanisms. B cells have also emerged as playing an important role in the control of harmful inflammatory responses, and helminths might be powerful elicitors of such regulatory B cells. Elucidation of the immune microenvironments, types of helminth parasites, and stages of parasite development when antibodies are most efficacious in protective immunity might provide an important basis for the development of more effective helminth vaccines.

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