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## Induction of regulatory cells by helminth parasites: exploitation for the treatment of inflammatory diseases

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**Summary:** Helminth parasites are highly successful pathogens, chronically infecting a quarter of the world's population, causing significant morbidity but rarely causing death. Protective immunity and expulsion of helminths is mediated by T-helper 2 (Th2) cells, type 2 (M2) macrophages, type 2 innate lymphoid cells, and eosinophils. Failure to mount these type 2 immune responses can result in immunopathology mediated by Th1 or Th17 cells. Helminths have evolved a wide variety of approaches for immune suppression, especially the generation of regulatory T cells and anti-inflammatory cytokines interleukin-10 and transforming growth factor- $\beta$ . This is a very effective strategy for subverting protective immune responses to prolong their survival in the host but has the bystander effect of modulating immune responses to unrelated antigens. Epidemiological studies in humans have shown that infection with helminth parasites is associated with a low incidence of allergy/asthma and autoimmunity in developing countries. Experimental studies in mice have demonstrated that regulatory immune responses induced by helminth can suppress Th2 and Th1/Th17 responses that mediate allergy and autoimmunity, respectively. This has provided a rational explanation of the 'hygiene hypothesis' and has also led to the exploitation of helminths or their immunomodulatory products in the development of new immunosuppressive therapies for inflammatory diseases in humans.

**Keywords:** helminths, immune regulation, regulatory T cells, Th1/Th2/Th17 cells, autoimmunity, allergy, hygiene hypothesis

### Introduction

The mammalian immune system has evolved to control infection and prevent the development of cancer. It does this by a combination of innate and adaptive effector cells and molecules which must be tightly regulated to prevent unwanted infection-induced immunopathology (1). Regulatory T (Treg) cells play a key role in controlling immunopathology and associated collateral damage to host tissues. The immune system can also mount antibody and T-cell responses to self-antigen and allergens, leading to the development of autoimmune and allergic disorders. These responses are normally regulated by mechanisms involving

immunological tolerance and active immune suppression mediated by Treg cells. However, evidence is emerging that cells of the innate immune system, including certain subpopulations of macrophages, dendritic cells (DCs) and innate lymphoid cells (ILCs), can also have immunosuppressive functions, producing anti-inflammatory cytokines and other mediators that act either directly on T cells or antigen presenting cells (APCs).

Infection with helminth parasites represents one of the greatest challenges to our immune system, both in the context of the physical size of the organisms and their capacity to produce immunomodulatory molecules that are directly immunosuppressive or confer innate and adaptive immune cells with regulatory function. Helminths have evolved potent and varied immune subversive strategies that have allowed them to evade host immune responses and establish persistent infections. Indeed helminths are highly successful pathogens, usually inducing chronic infections that do not kill the host, thus ensuring a niche for their survival and transmission to their next host. Helminths have classically been considered to be T-helper 2 (Th2)-inducing pathogens, and in the Th1/Th2 model first described by Mossman and Kauffman (2), helminths were considered to be the archetypal pathogen that promoted interleukin-4 (IL-4)-producing CD4<sup>+</sup> T cells (Th2 cells). It is now known that the anti-helminth 'type-2' responses also include innate immune cells that respond to or secrete the type 2 cytokines IL-4, IL-5, IL-10, and IL-13. These innate cells include eosinophils, basophils, mast cells, alternatively activated or type 2 (M2) macrophages, and type 2 ILC2 (3). M2 macrophages have low expression of IL-12, high expression of IL-10, arginase-1, RELM- $\alpha$ , and YM-1 and have anti-inflammatory and immunosuppressive function and are involved in tissue repair, whereas M1 macrophages express high Nos-2 and low IL-10 and have pro-inflammatory function (4). ILCs are a recently described population of lymphocytes that lack lineage markers and have been divided into three subtypes: ILC1 that secrete IFN- $\gamma$ , ILC2 that secrete IL-5 and IL-13 and ILC3 that secrete IL-17 and IL-22 (5). M1 and ILC1 together with Th1 cells mediate protective immunity to viruses, bacteria, and tumors, whereas ILC2 and M2 macrophages together with Th2 cells play a role in the expulsion of helminth parasites.

Despite the induction of ILC2, M2 macrophages, and Th2 cells, helminth parasites can persist in the host in the face of these type-2 immune responses. This reflects the potent immune subversive properties of many helminths including the production of immunomodulatory molecules that inhibit

the function of APCs or T cells, or promote the induction of Treg cells. The activation of regulatory networks by helminths also suppresses immune responses to other pathogen antigens, self-antigens, and allergens. Consequently, infection with helminths in humans and domestic animals can be associated with suppression of protective immunity to viruses and bacteria, where Th1 and IL-17-secreting CD4<sup>+</sup> T cells (Th17 cells) are required for protection. Furthermore, there is growing evidence that a high incidence of infection with helminths is associated with lower incidence of allergy/asthma and autoimmune diseases (6). It appears that Treg cells induced by helminths can suppress Th2 responses that mediate allergy and the Th1 and Th17 responses that mediate many autoimmune diseases. However, parasite-induced type 2 immune responses may also be involved in suppressing disease mediated by Th1/Th17 cells. This review discusses the regulatory cells and molecules that control protective anti-parasite immunity but also exert bystander immunosuppression against pathogenic T cells that mediate allergy and autoimmunity and how helminth-derived molecules can be exploited as therapeutics for immune-mediated diseases in humans.

### Helminths

Worms are invertebrate animals that occupy a variety of ecological niches. Free living worms include those that live in the soil such as the common earthworm *Lumbricus terrestris* and the model organism *Caenorhabditis elegans*. Other worm species have evolved to live within host animals on which they rely for nutrients. These parasitic worms are known as helminths and are members of two phyla, *Nematoda* and *Platyhelminthes*.

The nematodes (roundworms) are elongated and cylindrical in shape with a bilateral symmetrical arrangement. The outer surface consists of a non-nucleated cuticle secreted by the underlying hypodermis. Unlike platyhelminthes, nematodes have tubular digestive systems with openings at both ends, and those that are parasitic in humans are unisexual (7). Platyhelminthes (flatworms) are bilaterally symmetrical and are compressed dorsoventrally to give a flat leaf-like (trematodes) or tape-like (cestodes) shape. Unlike nematodes, they lack a body cavity and they have no specialized circulatory or respiratory organs, which restricts them to flattened shapes to enable oxygen and nutrients to pass through their bodies by diffusion. Instead, they are covered in a tegument, a biologically active outer layer of multinucleated cells covered with a carbohydrate glycocalyx. The tegument

performs all the vital activities such as protection, absorption, excretion, and secretion. For parasitic flatworms, the tegument is also the key host-parasite interface, protecting the worm from digestive enzymes and the host's immune response.

Many helminth species are noted for their complex life cycles, with many requiring more than one host species. A definite host is one in which the adult worm reproduces while an intermediate host houses one or more larval life cycle stages. There are, however, helminth species that do not require an intermediate host, namely the soil-transmitted helminths, including hookworms, which exist as independent soil-living worms in their larval stage. In this review, we discuss the interactions between the mammalian immune system and a variety of helminth species. It is therefore important that we give adequate consideration to the morphological and genetic differences and to the unique ecological niches that specific helminth species inhabit as well as their different methods of transmission.

### Helminthiasis

It is estimated that one quarter of the world's population are infected with helminth parasites, with the vast majority of infected living in the developing world (8, 9). Between 750 million–1300 million people are infected with hookworms, and this is almost entirely restricted to sub-Saharan Africa, Latin America, and South-East Asia (9, 10). While there is a crude geographical correlation between the natural range of many helminth intermediate hosts and areas of the world with the majority of human infectees, the major reason for the disparity in the geographic spread of helminthiasis is poverty, with many people relying on untreated water supplies that act as a source of transmission for soil-transmitted helminths or as a habitat for the intermediate mollusk host of schistosomes. Moreover, these regions generally lack adequate education regarding disease prevention, access to anti-helminthic drugs, and basic medical care (11, 12).

Helminths are highly successful parasites that establish long lasting infections in their definitive host. In humans, infections with helminths can last for years, during which time the parasite can inflict considerable harm. Helminths, which compete with the host for nutrients, cause mechanical and enzymatic damage during their migration through tissues, and in many cases can also drive immune-mediated pathology. The most common symptoms of helminth infection include chronic anemia, malnutrition, and fatigue.

However, certain species of helminths cause significant morbidity, disability, and a reduction in life expectancy. In particular, schistosomes and the filarial nematodes are associated with severe pathology. *Brugia malayi* and *Onchocerca volvulus* are the causative agents of the disabling and disfiguring diseases, lymphatic filariasis (elephantiasis) and river blindness, respectively. In some cases, infection with these parasites results in a chronic inflammatory disease, tissue damage, and fibrosis, which may persist even after the worm is expelled (13). Furthermore, children are more vulnerable to infection, and children and adolescents harbor a greater number of worms than adults (13, 14). Helminth infection of children has more insidious effects including growth retardation, diminished physical fitness, and even impairment in cognitive development (15).

There is a high natural variation in helminth infections within endemic regions, with some individuals being particularly susceptible to infection (13), while others appear to be immune to infection despite continued exposure, others may control the infection but may suffer from severe immune pathology (16). Interestingly, a major feature of many helminth infections is that they are often totally asymptomatic in otherwise healthy adults (17). In most cases, infection with species of the genera *Schistosomes*, *Necator*, and *Trichuris*, which collectively infect over a billion people, cause mild or benign disease. The reasons why different individuals are either immune, susceptible, or tolerant to helminth infections is a product of the complex interaction of the parasite with host immune factors and perhaps reflects the co-evolution of helminths with their human hosts.

### Host protective immunity against helminths: type 2 immune responses

Historically, it was thought that helminth infections did not elicit immune responses. Indeed, adult helminths are rarely killed by the immune system. It was not until the 1920s that Norman Stoll demonstrated that sheep previously infected with gastrointestinal nematodes were resistant to re-infection. In the 1970s, it was shown that CD4<sup>+</sup> T cells were the canonical cell type involved in driving this memory immune response. Following the discovery of Th1 and Th2 cells, it was reported that protective immunity to helminths is mediated by Th2 cells and the cytokines they produce, namely IL-4, IL-5, IL-9, IL-10, and IL-13 (18). While Th2 responses rarely kill the parasites, they limit infection, reducing the viability and reproductive capacity of helminths

and can physically expel them from the mucosal membranes in which they reside. The importance of the Th2 response in helminth immunity is supported by population genetics which have demonstrated an association between loci that control Th2 responses or their effector cytokines with susceptibility to infection (19). Indeed, persistently susceptible individuals have been shown to mount weak Th2 responses to parasite antigens (20).

Much of our understanding of Th2-mediated protective immunity to helminths comes from the study of mouse models of infection. *Nippostrongylus brasiliensis* is a rat gastrointestinal nematode infection that establishes an acute infection in wildtype mice with clearance of adult worms generally achieved by day 14. The depletion of CD4<sup>+</sup> cells in this model results in chronic infection, highlighting the importance of T-helper cells (21). Consistent with epidemiological studies that have reported higher worm burdens in infected children than infected adults, young mice infected with *N. brasiliensis* exhibit impaired clearance of worms and attenuated Th2 responses when compared with adult mice (22, 23). Another much used mouse model of helminth infection is *Heligmosomoides polygyrus*, a murine gastrointestinal nematode which establishes a chronic infection in mice and is seen as a more representative model of human helminth infection. Interestingly, severe combined immunodeficient mice infected with *H. polygyrus* have significantly increased worm burdens when compared with normal mice (24), demonstrating that the adaptive immune response can control the infection, even when parasite clearance is not achievable.

Th2 cells orchestrate anti-helminth immunity through the production of their signature cytokines that direct and activate other cell types (Fig. 1). IL-4 binds to both the IL-4R and IL-13R, which results in STAT6 activation. Indeed, both receptors share the IL-4R $\alpha$  subunit, which is a critical component of anti-helminth immune responses. IL-4 drives isotype class switching in B cells, resulting in the production of parasite-specific immunoglobulin G1 (IgG1) and IgE. Basophils, mast cells, and eosinophils all express Fc $\epsilon$ R and are activated upon IgE cross linking (25).

IL-4 also promotes alternatively activated or M2 macrophages (26, 27), which are a major constituent, together with eosinophils, of the type 2 granulomas that surround helminths, helminth larvae, and helminth eggs. M2 macrophages, that expressed arginase-1, were found to be essential for protective immunity during secondary infection with *H. polygyrus* (28). M2 macrophages secrete a multitude of anti-helminthic compounds that reduce the overall viability of the parasites (28). Chitinase has been shown to degrade

the glycan coats of helminths such as *B. malayi* (26), while RELM- $\beta$ , produced in response to IL-13, interferes with the chemosensory apparatus and inhibits the chemotactic function of nematodes (29). M2 macrophages are often defined by the high expression of arginase-1, which depletes the tissue microenvironment of L-arginine, thus starving the helminth of this essential amino acid (28).

Helminths cause considerable damage while migrating through the host and M2 macrophages also play an important role in tissue repair (17, 30). Secreted RELM- $\alpha$  (FIZZ1) has proliferative and angiogenic properties while it also regulates type-2 inflammation to prevent immunopathology (31, 32). Following infection with *N. brasiliensis* (33), M2 macrophages are thought to induce fibrosis through the expression of profibrotic factors, including fibronectin, matrix metalloproteinases, IL-1 $\beta$ , and TGF- $\beta$  (17). Fibrosis during helminth infection is supported by IL-3 and IL-9, which induce the expression of pro-fibrotic mediators by mast cells that in turn activates fibroblasts (26).

While IL-4 plays a role in orchestrating Th2 immune responses, it is not required for expulsion of the helminth *N. brasiliensis*. However, IL-13, IL-4R $\alpha$ , and STAT6 are all critical for worm clearance; suggesting that separate facets of type-2 immune responses work in tandem to mediate protective immunity against helminths (34). Indeed, IL-13 and IL-5 appear to be more important for the expulsion of helminths. Liang et al. (35) have shown that IL-13-producing cells in mice infected with *N. brasiliensis* home to the infected tissue, while IL-4-producing cells remain in the lymph node to provide 'help' to B cells.

IL-13 is involved in mediating changes to the physiology of target organs and is particularly associated with the induction of goblet cell hyperplasia, mucus production, epithelial shedding, and smooth muscle contraction that act to physically expel helminths from these membranes, in what is referred to as the 'weep and sweep' response (26). During *T. muris* infection, IL-13 increases epithelial cell turnover in the gut, which removes parasitized cells (36). Similar flushing effects are controlled by the action of IL-13 and IL-25 in response to *H. polygyrus*, and *N. brasiliensis* infection (26). IL-13 also induces the production of a mucin, MUC5AC in the gut, which has direct detrimental effects on gut dwelling nematodes (37). Absence of the mucin prevented expulsion of worms even in the presence of an otherwise robust Th2 response (37).

IL-5 recruits and activates eosinophils (38), and a classic but simplified understanding is that eosinophils directly kill helminths. Helminths are too large to be phagocytosed and

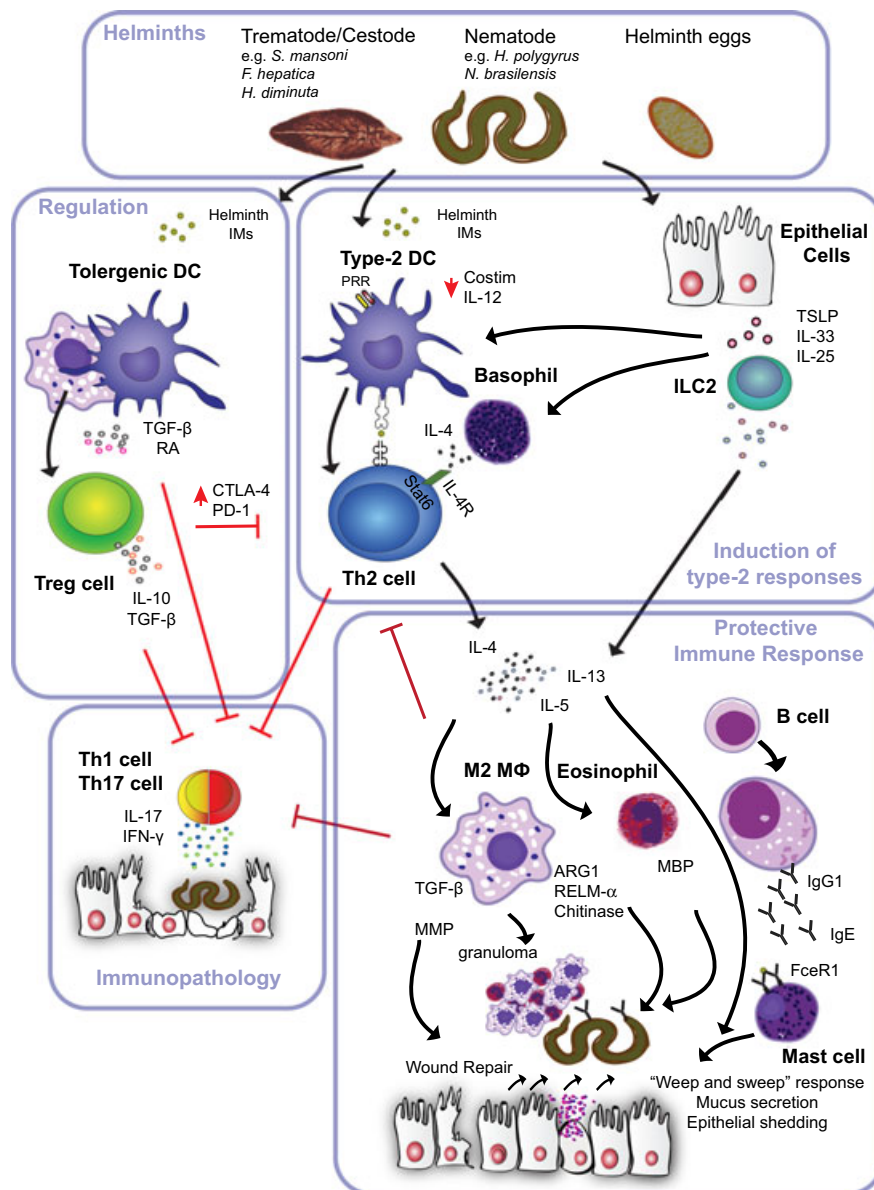


**Fig. 1. Helminths induce type-2 and regulatory immune responses.** Helminths: Helminths are classified as either nematodes (roundworms), trematodes (flukes), or cestodes (tapeworms). They stimulate immune responses via tissue damage, egg production, and the secretion of immunomodulatory molecules (IMs). Detection and Th2 cell induction: The alarmin cytokines IL-25, IL-33, and TSLP, produced by epithelial cells in response to helminth infection, activate innate lymphoid type-2 (ILC2) cells that provide an early source of the protective type-2 cytokines IL-5 and IL-13. Helminth-derived IMs can bind to pattern recognition receptors on DCs and inhibit costimulatory molecule expression and production of IL-12; this favors the differentiation of Th2 cells. TSLP induces IL-4 production by basophils; IL-4 is critical in the differentiation of Th2 cells. Protective immune response: Anti-parasite Th2 cells produce IL-4, IL-5, and IL-13. IL-4 and IL-5 act on B cells to induce IgG1 and IgE class switching. Anti-parasite IgE binds and activates mast cells, which release inflammatory molecules. IL-5 promotes the expansion and activation of eosinophils, which produce granule proteins, such as MBP that can cause direct damage to helminths. IL-4 and IL-13 promote the differentiation of M2 macrophages. M2 macrophages together with eosinophils encase antibody bound helminths in type-2 granulomas. M2 macrophages also produce molecules that directly harm helminths, including RELM- $\alpha$  and chitinase. Arginase expressed by M2 macrophages starves helminths by depleting L-arginine. M2 macrophages play a role in promoting wound repair during infection by release of TGF- $\beta$ , RELM- $\alpha$ , and matrix metalloproteinases. IL-13 has direct effect on mucosal tissues; it promotes epithelial shedding and mucus secretion by goblet cells that aid the removal of helminths. Immunopathology: An inappropriate Th1 or Th17 response to a helminth can lead to inflammatory destruction of host tissues. A prolonged or pronounced Th2 response may also induce immunopathology. Regulation: Helminths induce regulatory responses not only to suppress protective Th2 responses but also to prevent immunopathology that is deleterious for both host and parasite. Helminths induce T-cell hyporesponsiveness by enhancing the expression of co-inhibitory molecules on T cells, including PD-1 and CTLA-4. M2 macrophages can also regulate T-cell responses via arginase. Helminth IMs promote the differentiation of tolerogenic DCs that preferentially induce the differentiation of Treg cells. Treg cells produce the anti-inflammatory cytokines IL-10 and TGF- $\beta$ . These cytokines can regulate both anti-parasite Th2 cells and inflammatory Th1/Th17 cells that mediate immunopathology.

are thought to be killed by eosinophils through the release of preformed or metabolic mediators, such as major basic protein (MBP), or through antibody-dependent cell-mediated cytotoxicity. When used as a purified protein for *in vitro* studies, MBP has been shown to have toxicity against a number of parasites, including schistosomes spp. (25). However, these studies have been difficult to replicate *in vivo*, and it is still unclear whether eosinophils can kill worms. During infection, worms are commonly found coated with eosinophils, but the chronic nature of helminth infections argues against direct killing mechanisms (18). However, the action of eosinophils in coating the parasite and releasing toxic substances may have evolved to reduce the viability of parasites, restricting their growth and fecundity.

### The induction of type 2 immune responses

While the importance of Th2 cells in anti-helminth immunity is well established, the mechanisms of induction of immune responses following helminth infection and how the initial innate type 2 immune response translates to Th2 cell induction is only beginning to be understood. Immune responses against most bacteria and viruses are initiated following PRR activation by well-defined pathogen-derived PAMPs (39). Although less is known about helminth-derived PAMPs and their respective PRRs, studies are beginning to identify specific helminth-derived products that promote Th2 responses.

Omega-1, a glycoprotein derived from the *S. mansoni* egg antigens (SEA), can prime DCs to induce Th2 responses *in vivo*, even in the absence of IL-4 (40). It has been recently shown that omega-1 binds to the mannose receptor via its glycan residues (41). *N. brasiliensis* excretory-secretory (ES) products also drive Th2-biased responses and can act as a Th2-inducing adjuvant for unrelated antigens, even in the presence of complete Freund's adjuvant, a powerful Th1-inducing adjuvant (17, 42). Moreover, ES-62, a molecule derived from the secreted products of the filarial helminth *Acanthocheilonema viteae* can prime DCs to induce Th2 cells (17). IL-4 is essential for the differentiation of Th2 cells; however, DCs are poor producers of this cytokine. Thus, there have been considerable efforts to identify the source of helminth-induced innate IL-4 that promotes type 2 immune responses. IPSE/ $\alpha$ -1, another glycoprotein from *S. mansoni* eggs, induces IgE-dependent IL-4 production by basophils (43). Furthermore, 2-Cys peroxiredoxin, an antioxidant from *F. hepatica*, expands Th2 cells via promoting alternative activation of macrophages that provide a source of IL-4 (44).

ILC2 that secrete type 2 cytokines IL-5 and IL-13 are induced by helminths and may play a role in host anti-helminth protective immunity, especially at the early stages of infection. The 'alarmin' cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), produced primarily by epithelial cells, have been shown to drive ILC2 responses. ILC2 provide a major early innate source of IL-5 and IL-13 that are required for clearance of *N. brasiliensis* infection in mice (45, 46). Further studies have identified interactions between ILC2s and other innate immune cells in driving parasite clearance. Yatsuda *et al.* (47) recently demonstrated that the lung/intestinal nematode *Strongyloides venezuelensis* was expelled in the absence of T cells and B cells by eosinophils that were recruited by IL-5 produced by ILC2 cells, suggesting that, in this model at least, innate effector cells are sufficient to control infection. TSLP, in contrast, has been shown to elicit a distinct population of basophils that are required for optimal Th2 responses against *T. muris* and *T. spiralis* infections in mice (48).

### Immunopathology

Failure to mount a type-2 response to helminths may result in an adverse outcome. For example, susceptibility to *T. muris* is linked to the development of a Th1 response and IFN- $\gamma$  production, which can suppress protective type-2 immune responses, including the weep and sweep response (49, 50). Paradoxically, the induction of 'protective' type-2 responses can in some cases be advantageous to both the host and the helminth. The induction of Th1/Th17 responses is significantly more inflammatory and destructive than Th2 responses, damaging the mucosal membranes that they inhabit.

Infection with *S. mansoni* provides a striking example of an inappropriate, pathological Th1/Th17 response during infection that leads to liver damage, splenomegaly, portal hypertension and even death. The immunopathology associated with *S. mansoni* infection is mediated by inflammatory responses to *Schistosoma* eggs deposited in the liver. However, the severity of immunopathology differs greatly between individuals and between different strains of mice. Initially, SEA induces a Th1 response in the host, which in most cases converts to a non-damaging Th2 response. Pathologically, this Th2 response presents as small fibrotic type-2 granulomas in the liver which surround the eggs. Failure to develop an effective Th2 response in some individuals and certain strains of mice, results in exacerbated type-1 granulomatous inflammation driven by Th1 and Th17 cells (51). In one

study, Th1-predisposed CBA mice, lacking IL-23p19 and thus Th17 responses, were protected from this immunopathology. In these mice, the expression of IL-5 and IL-10 was increased, while IL-17 was suppressed. In addition, the granulomas formed were smaller in size and no longer type-1 in phenotype (composed of neutrophils and M1 macrophages), but instead displayed a type-2 phenotype, similar to those induced during a non-damaging Th2 response (51). These observations are consistent with those seen in humans, where severe bladder pathology in patients infected with *S. haematobium* correlated with Th17 responses (52).

It has also been reported that M2 macrophages are essential for the development of an appropriate Th2 response to SEA; removal of M2 macrophages, using macrophage specific IL-4Ra<sup>-/-</sup> mice, resulted in death caused by severe Th1 responses (53). Likewise, infection with *N. brasiliensis* initially induces a Th17 response that damages the lung, which is suppressed by IL-4R $\alpha$  signaling that subsequently prevents further immunopathology and helps maintain tissue homeostasis (33).

#### Immune evasion and regulation: modified type 2/regulatory response

Despite the induction of the type 2 immune responses described above, the majority of helminth infections remain chronic and do so without compounding immunopathology. This reflects a combination of active host immunoregulatory mechanisms and parasite immune evasion strategies (26, 54). Helminths employ a variety of mechanisms to successfully suppress the immune response to prolong their survival, ideally without killing the host.

#### Immunosuppressive cytokines and co-inhibitory molecules

Helminths cause considerable damage to the host in the acute stage of infection and provide a high antigenic stimulus for the immune system. However, T cells from humans and animals infected with helminths typically display weak antigen-specific responses. Furthermore, population studies have shown that the intensity of helminth infection correlates with the production of IL-10 and TGF- $\beta$  by peripheral blood cells; infected patients who produce higher amounts of these cytokines have less potent effector T-cell responses (55). Individuals with heavy *Onchocerca* spp infections typically have weak Th1/Th2 responses with concurrent robust production of IL-10 and TGF- $\beta$  (56). However, individuals with natural

immunity to *Onchocerca* spp display a pronounced Th2 response, with less IL-10/TGF- $\beta$  (56). In animal models, chronic infection with *F. hepatica* suppresses the production of IL-4 but enhances IL-10 and TGF- $\beta$  by peripheral blood mononuclear cells (PBMCs) in response to parasite antigen (57–59). Neutralization of IL-10 and TGF- $\beta$  results in increased production of both IL-4 and IFN- $\gamma$ , suggesting that the parasite may induce these anti-inflammatory cytokines to evade the immune response (59). However, these anti-inflammatory cytokines may also benefit the host by preventing immunopathology. IL-10 appears to be critical in preventing immunopathology following infection with schistosomes (60).

Helminths also target co-inhibitory molecules expressed on T cells and APCs to maintain hyporesponsiveness. *N. brasiliensis* exploits the expression of cytotoxic T-lymphocyte antigen-4 (CTLA-4) to inhibit proliferative responses and Th2 cytokine production and thus maintain weak anti-helminth responses (61). Likewise, *L. sigmodontis* induces Th2 cell hyporesponsiveness via PD-1, another inhibitory molecule expressed by T cells (62). Neutralization of PD-1 restored IL-4 production and enhanced worm clearance (62).

#### Regulatory T cells

Several helminth species exploit the function of Treg cells to evade the host immune response. Long-term persistence of *H. polygyrus* is associated with enhanced frequencies of Treg cells in the host during infection (17, 26). Likewise, infection of mice with the filarial helminth *Litomosoides sigmodontis* induces hyporesponsiveness that can be reversed by depletion of Treg cells *in vivo* (63). The induction of Treg cells is required for the establishment of chronic infection and depletion of Treg cells leads to the induction of a protective immune response and parasite expulsion (64). Furthermore, neutralization of Treg-derived IL-10 and TGF- $\beta$  *in vivo* also restored anti-parasite responses, suggesting that immunosuppressive cytokines are the crucial mediators of Treg-induced immunoregulation in helminth infection (17). These observations also translate to human infections, where it has been demonstrated that patients infected with filarial nematodes or schistosomes had higher frequencies of Treg cells in the peripheral blood (18, 65).

The induction of Treg cells during helminth infection may also benefit the host by preventing both Th1/Th17 and Th2-mediated immunopathology. A striking example is seen in hyper-reactive onchocerciasis or sowda, a severe skin complication in patients infected with *Onchocerca volvulus*.

Sowda occurs when the Th2 response effectively kills the filarial worm, keeping the infection to low numbers, but leading to a severe inflammatory reaction to the dead parasites (66). Indeed, a variant of IL-13 has been linked with hyper-reactive form of onchocerciasis (67). Patients that do not display such complications typically have higher worm burdens, yet they avoid pathology by the inhibitory effect of TGF- $\beta$  on Th2 responses (68). Chronic pathology in humans with schistosomiasis was also associated with a reduction in IL-10 and a corresponding increase in IL-13 (66). In mouse models, Treg cells induced during the chronic phase of *S. mansoni* infection in mice protected the host from uncontrolled Th2-mediated immunopathology (69). Similarly, depletion of Treg cells from mice during infection with *T. muris* enhanced the expulsion of worms but at the cost of significant intestinal pathology (70).

Treg cells are also critical in suppressing immunopathology mediated by type-1 responses. Elephantiasis seen in some patients infected with *Wuchereria bancrofti* was associated with high Th1 and Th17 responses (71). Pathology did not correlate with Th2 responses, but was associated with reduced Treg cells (71).

The role of TGF- $\beta$  in promoting the expansion of inducible Treg cells is well established, and TGF- $\beta$  has been implicated in the helminth-mediated expansion of Treg cells (72–74). *H. polygyrus* enhances the expression of host TGF- $\beta$  during infection (73, 74), while *Leishmania* spp activate latent-TGF- $\beta$  using parasite-derived cathepsins, a process which is essential for the survival of the parasite (75). Remarkably, *H. polygyrus* secretes a TGF- $\beta$  homologue that acts to enhance the expression of FOXP3 in naive T cells (76). In certain cases, *Onchocerca* spp and *B. malayi* also express distinct molecules homologous to mammalian TGF- $\beta$  (72). In vitro studies have shown that SEA induces the expression of FOXP3 in naive T cells via TGF- $\beta$ , which confers Treg cell-expanding, or tolerogenic properties on DC (77–79).

#### Dendritic cells that drive Th2/Treg cells

Fitting with their central role in directing adaptive immunity (39), DCs are major targets for helminth-induced immune suppression. Helminth products such as SEA and ES62 modulate DCs to selectively induce Th2 cells (80, 81). However, some of these products, including ES62, achieve this by inhibiting the DC response to classic Th1-inducing PAMPs (82). Likewise, omega-1 also inhibits TLR-induced DC activation and suppresses the development of Th1 cells, thus

amplifying type-2 responses. (40). Likewise, the ES products of *Taenia crassiceps* inhibited lipopolysaccharide (LPS)-induced DC maturation and secretion of IL-12 in a cRAF-dependent manner and promoted Th2 cell differentiation through TLR2 and the mannose receptor (83).

The tegument and the ES products of *F. hepatica* was found to suppress DC activation in response to microbial PAMPs (57, 84, 85). Cathepsin L from *F. hepatica* was subsequently shown to suppress LPS-induced cytokine production in vivo (86). The cysteine protease degraded Toll-like receptor 3 (TLR3), leading to an impairment in the TRIF-dependent signaling pathway (86). Another protein from *F. hepatica*, helminth-defense molecule-1, was recently shown to inhibit antigen processing and presentation by preventing acidification of endolysosomes, a critical step in the preparation of antigens for presentation on MHC class II (87).

Helminth-derived molecules have been shown to promote tolerogenic DCs that promote T-cell anergy and the induction of Treg cells. DCs matured in the presence of *F. hepatica* ES promoted the differentiation of T cells with a Th2/Treg cell phenotype; these cell functionally suppressed Th1 cells (85). Lysophosphatidylserine from *S. mansoni* interacts with DCs via TLR2 and promotes the differentiation of IL-10-producing Treg cells (88). Indeed, schistosomal infected TLR2-deficient mice are less capable at inducing Treg cells and have more pronounced immunopathology than infected wildtype mice (89).

#### M2 macrophages

Despite playing a protective role in certain anti-parasite type 2 immune responses (28), the anti-inflammatory properties of M2 macrophages can also be exploited by helminths (53). Arginase-1 produced by M2 macrophages depletes L-arginine from the tissue microenvironment, which inhibits T-cell function, particularly effector T cells, which have a high metabolic turnover. Rodriguez et al. (90, 91) demonstrated that T cells stimulated in the absence of L-arginine lose their expression of the CD3 $\zeta$ -chain, which is required for T-cell receptor (TCR) signaling and results in the suppression of T-cell proliferation and cytokine production. Similarly, SEA can suppress pathological inflammation by promoting the expression of arg1 in M2 macrophages (92). M2 macrophages from mice infected with *L. sigmodontis* were shown to suppress T-cell responses via IL-4 and IL-13 (17). In addition to their characteristic expression of arginase-1, M2 macrophages express IL-4R, TGF- $\beta$ , and chitinases, including RELM- $\alpha$ /FIZZ1 (93, 94). Macrophage-derived

IL-10 and TGF- $\beta$  can inhibit LPS induced pro-inflammatory cytokine release, induce Treg cells and inhibit Th1 responses (95). RELM- $\alpha$ , is a secreted protein that helps to control inflammation induced by SEA, and mice lacking RELM- $\alpha$  develop SEA-induced inflammation of the liver. This was associated with enhanced Th2 responses and could be reversed upon treatment with recombinant RELM- $\alpha$  (96).

### Type 2 immune responses

In almost all cases of helminth infection, type-2 immune responses are associated with regulatory mechanisms, be they host or helminth in origin. This may even reflect co-evolution of the parasite with the host (26, 54, 97). Thus, a revision of terminology may be necessary as these modified type-2/regulatory responses may not be as 'protective' as they are 'appropriate', ideally balancing susceptibility with immunity and immunopathology. Interestingly, the evolutionary origin of the type-2 response is believed to be an expansion of ancient wound repair mechanisms and there is a view that rather than trying to kill the pathogen, Th2 responses are more concerned with the damage they cause (98). Thus, preventing the induction of inflammatory Th1/Th17 responses and favoring tolerance of infection may have originated from an evolutionary cost/benefit analysis; this is perhaps best understood if one considers the primary role of the immune system in maintaining homeostasis rather than that of pathogen killing.

### Helminths regulation of immunity to unrelated pathogens or tumors

The potent regulatory and type 2 immune response induced by helminths actively suppress protective immune responses raised against unrelated pathogens, especially those that elicit Th1 type immune responses. Consequently, helminth infection is often accompanied by increased susceptibility to infection with bacteria, viruses, or even other parasites.

Epidemiological studies have demonstrated a link between intestinal hookworm infections and an increased incidence of malaria (99). This observation was confirmed in an experimental model of malaria where co-infection of mice with *H. polygyrus* resulted in impaired Th1 responses and severe mortality. The protective anti-malaria responses were restored by treatment with anti-helminthic drugs (73). Furthermore, *N. brasiliensis* infection of mice was shown to compromise resistance to mycobacterium, by enhancement of M2 macrophages (100). However, other studies have demonstrated that infection of cotton rats with *L. sigmodontis* did

not affect *M. tuberculosis*-specific IFN- $\gamma$  production or the formation of protective granulomas (101). Conversely, studies in a natural host showed that infection of cattle with the trematode, *F. hepatica* suppressed immune responses against *M. bovis* infection; PBMCs from co-infected calves had reduced Th1 responses to *M. bovis* antigen (102). In another respiratory infection model, we reported that *F. hepatica* infection of mice suppressed Th1 responses directed against *Bordetella pertussis* and delayed bacterial clearance from the lung (103). In contrast, Miller et al. (104) demonstrated that co-infection of mice with *F. hepatica* and the intracellular parasite *Toxoplasma gondii*, which typically induces strong Th1 response, did not affect the production of NO or IFN- $\gamma$  in vivo. However, infection with *T. gondii* suppressed the production of IL-4, IL-5, and the alternative activation of macrophages associated with *F. hepatica* infection. These findings suggest that the immunomodulatory effects of helminths on bacterial infections are species-specific and may work both on and via helminth infection.

Helminth infections can also impair immune responses to viral pathogens. Schistosomiasis has detrimental effects on patients infected with hepatitis C virus (105) or human immunodeficiency virus (106), including exacerbation of clinical symptoms of the viral infection and accelerated disease progression. The mechanism is likely to involve suppression of protective IFN- $\gamma$ -producing T cells as a result of regulatory or Th2-biased immune responses associated with helminth infection.

It has also been reported that helminth infections can suppress immunity generated by vaccination and may contribute to vaccine failures in developing countries. In Gabon, helminth-infected children developed poorer influenza-specific T-cell responses following vaccination compared with uninfected children (30, 103). We have reported that infection of mice with *F. hepatica* suppresses *B. pertussis*-specific Th1 responses induced by a pertussis whole cell vaccine, which resulted in delayed bacterial clearance from the lung following respiratory challenge with the live bacteria (103).

There are limited data to suggest that helminth infections can also inhibit immune responses to tumors and promote the development to cancer. Epidemiological studies have linked trematode infections with carcinogenesis in human populations (107). Experimentally, the total antigen of *Schistosoma haematobium* transformed Chinese hamster ovary cells in vitro into metastatic cells capable of forming tumors in recipient mice (108). We have demonstrated that both infection with *F. hepatica* or administration of its ES products

can exacerbate growth of B16 melanoma in a mouse model (K. Galvin and K. H. G. Mills, unpublished observations). The effect was associated with TGF- $\beta$ -mediated expansion of Treg cells and the suppression of anti-tumor Th1 and Th17 responses by the helminths.

### The hygiene hypothesis

The incidence of autoimmune and allergic diseases in the developed world has increased dramatically since the beginning of the 20th century (109, 110). This has coincided with a dramatic change in lifestyle and healthcare, including the eradication of many infectious diseases, a trend of migration from rural to urban areas and a decrease in the number of children per family (110). In contrast, autoimmune and allergic diseases are rare in rural areas of the developing world where infectious diseases are endemic (16).

Hay fever, the most common allergic disease is a relatively modern disease, the first description of which dates to the early 19th century; however, by the end of that century the disease was commonplace (111). In 1989 David Strachan reported a correlation between the incidence of hay fever in children and low family size or an older position in the family. He used the term 'hygiene', postulating that 'unhygienic' contact with older siblings lead to unspecified infections in early life that conferred protection against atopy (112). Similarly, epidemiological studies dating back 40 years have linked low rates of infections or sanitation with autoimmune diseases (113, 114).

The 'hygiene hypothesis' has attributed the rise in autoimmune and atopic diseases to a simultaneous decrease in infectious disease and in particular infection with helminth parasites. The main tenet of this hypothesis is that helminth infection induces a variety of immunoregulatory networks that aid in parasite survival by suppression of anti-helminth immune responses, but have systemic side-effect of suppressing inflammation associated with allergic and autoimmune disorders.

A seminal study by Correale and Farez (115) demonstrated that helminth infections could influence the severity of multiple sclerosis (MS) in patients in South America. Over a 5-year period, MS patients naturally infected with parasites had significantly lower rate of relapse, reduced disability scores and a reduction in the number of brain lesions compared with uninfected MS patients. T cells from infected MS patients produced less IL-12 and IFN- $\gamma$ , but more IL-10 and TGF- $\beta$  in response to myelin basic protein, compared with those from uninfected MS patients. The frequency of

Treg cells also increased in patients infected with helminths (115). A follow up study demonstrated that B cells from infected but not uninfected MS patients produced high concentrations of IL-10 (116).

A recent study reported that patients infected with schistosomes had lower levels of autoantibody compared with patients free of parasites. Moreover, the frequency of autoantibodies increased in infected patients in response to treatment with anti-helminthic drugs (117). Interestingly, polymorphisms in cytokine genes that have been previously correlated with autoimmune disease also correlated positively with selection pressure imposed by microorganisms, but not with helminths (97). This suggests that helminths can over time shift the immune genome toward greater regulation or at least away from inflammatory responses mediated by Th1 and Th17 cells.

A number of studies have shown that helminth-infected cohorts have a lower propensity to develop allergic disorders. In a Brazilian study, a lower frequency of children infected with schistosomes tested positive for skin-prick tests for house dust mite allergy compared with uninfected children (118). A recent meta-analysis of published data provided a statistically significant correlation between *Trichuris* and *Ascaris* infections and a reduction in atopy (119). However, caution must be taken when interpreting such results, as protection from allergy may be related to susceptibility of infection due to polymorphisms in immunoregulatory genes. For example, polymorphisms in the IL-10 locus that are associated with increased resistance to helminth infection may predispose individuals to allergic sensitization (120). However, in support of a direct protective effect of infection, a study in Gabon found that eradication of worms resulted in increased skin reactions to allergens among children in a follow up study (121).

### Helminth infections and allergy

There is strong epidemiological evidence to support the premise that the dramatic increase in atopic disease in the developed world is a direct consequence of the eradication of helminth infections. Experimental studies performed in murine allergy models have strengthened this assertion (Table 1).

#### Asthma and airway hyper-responsiveness

The mouse model of asthma is established via sensitization with an antigen/allergen and Th2-promoting adjuvant, alum. Allergic inflammation in the lung is then induced following the aerosolization of the immunized antigen.

**Table 1. Examples of helminth-induced suppression of autoimmunity or allergy in mouse models**

| Disease                   | Mice                               | Helminth                                    | Treatment                                 | Association                                                              | Mechanism                                                                 | Ref.                                                             |
|---------------------------|------------------------------------|---------------------------------------------|-------------------------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------|
| T1D                       | NOD                                | <i>S. mansoni</i>                           | Infection or eggs                         | ↑IgM ↓IgG                                                                |                                                                           | (146)                                                            |
|                           | NOD                                | <i>S. mansoni</i>                           | Eggs                                      | ↑Th2 cytokines, IL-10, DC, NKT cells; ↓DC IL-12                          |                                                                           | (147)                                                            |
|                           | NOD                                | <i>S. mansoni</i>                           | SEA                                       | ↑FOXP3, IL-4, IL-10, tolerogenic DC, T cell TGF-β; ↓T cell proliferation | CD25 <sup>+</sup> Treg cells                                              | (77)                                                             |
|                           | NOD                                | <i>Dirofilaria immitis</i>                  | Recom. Antigen                            | IgE associated with protection                                           |                                                                           | (158)                                                            |
|                           | NOD                                | <i>L. sigmodontis</i> (female worms)        | Infection/crude homogenate                | ↑Th2 cytokines, IgE, FOXP3, TGF-β, IL-10                                 | TGF-β-dependant; IL-4, IL-10, Treg-independent                            | (145, 163)                                                       |
|                           | NOD                                | <i>T. spiralis</i> /<br><i>H. polygyrus</i> | Infection                                 | ↑IgE, IL-4                                                               |                                                                           | (144)                                                            |
| Colitis                   | NOD                                | <i>H. polygyrus</i>                         | Infection                                 | ↑FOXP3, M2 M0, T cell IL-4, CD62L <sup>+</sup> T cells                   | Treg cell and IL-10 independent                                           | (166)                                                            |
|                           | NOD                                | <i>H. polygyrus</i>                         | Infection                                 | ↑Phos-Stat6, Th2 cells, T cell IL-10 (not Treg)                          | T cell, IL-10 and IL-4 dual-dependent.                                    | (167)                                                            |
|                           | IL-10 <sup>-/-</sup>               | <i>H. polygyrus</i>                         | Infection                                 | ↑T cell IL-13, FOXP3                                                     | T cells                                                                   | (204)                                                            |
|                           | TGF-βRII RN                        | <i>H. polygyrus</i>                         | Infection                                 | ↑IL-4, IL-10, TGF-β                                                      | No protection in TGF-βRII                                                 | (169)                                                            |
|                           | RAG + IL-10 <sup>-/-</sup> T cells | <i>H. polygyrus</i>                         | Infection cleared prior to reconstitution | ↑DC CD40; ↓DC CD80/86, T cell IFN-γ, IL-17 (in vitro).                   | DCs                                                                       | (173, 174)                                                       |
|                           | BALB/c                             | <i>S. mansoni</i>                           | Egg treatment                             | ↑IL-4; ↓IFN-γ                                                            | STAT6                                                                     | (150)                                                            |
|                           | BALB/c                             | <i>S. mansoni</i>                           | Infection, eggs had no effect             | ↑TGF-β, IL-10                                                            | M0-dependent; IL-10, TGF-β, Treg, T cell, IL-4/IL-13 independent          | (180)                                                            |
|                           | BALB/c                             | <i>Hymenolepis diminuta</i>                 | Infection and glycoproteins               | ↓M0 response to LPS                                                      |                                                                           | (205, 206)                                                       |
|                           | C57BL/6                            | <i>Trichinella spiralis</i>                 | Infection                                 | ↓IL-12, IFN-γ, MPO                                                       |                                                                           | (207)                                                            |
|                           | C57BL/6                            | <i>Acanthocheilone vitae</i>                | AvCystatin                                |                                                                          |                                                                           | (129)                                                            |
| EAE                       | C57BL/6J                           | <i>S. mansoni</i>                           | Infection,                                | ↓IL-12, IFN-γ, M0 and T cells in CNS                                     |                                                                           | (141)                                                            |
|                           | SJL/J                              | <i>S. mansoni</i>                           | Eggs                                      | ↑IL-4, IL-5, IL-10; ↓IFN-γ                                               | STAT-6                                                                    | (159)                                                            |
|                           | C57BL/6J                           | <i>F. hepatica</i>                          | Infection                                 | ↑Treg cells TGF-β, IL-10                                                 | TGF-β-dependant; IL-10 independent                                        | (140)                                                            |
|                           | C57BL/6J                           | <i>F. hepatica</i>                          | ES products                               | ↑Th2 response, eosinophilia, DC and T cell modulation                    | IL-5-dependant; IL-4, TGF-β, IL-10, Treg-independent                      | C. M. Finlay, A. M. Stefanska and K. H. G. Mills, In preparation |
|                           | C57BL/6J                           | <i>S. mansoni</i>                           | SEA                                       | ↑IL-4; ↓IFN-γ                                                            |                                                                           | (143)                                                            |
|                           | C57BL/6J                           | <i>T. suis</i> + <i>T. spiralis</i>         | ES products                               | DC induce Th2 responses                                                  |                                                                           | (208)                                                            |
| CIA                       | Dark Agouti rats                   | <i>T. spiralis</i>                          | Infection                                 | ↑IL-4, IL-10, FOXP3; ↓IL-17, IFN-γ                                       | T cells                                                                   | (138)                                                            |
|                           | DBA/I                              | <i>A. viteae</i>                            | Secreted protein ES-62                    | ↓TNF-α; ↑IL-10                                                           |                                                                           | (137)                                                            |
|                           | DBA/I                              | <i>S. japonicum</i>                         | Infection                                 | ↑IgG1, Th2/Treg genes; ↓IgG2a, collagen-specific responses               |                                                                           | (134)                                                            |
|                           | DBA/I                              | <i>S. mansoni</i>                           | Infection                                 | ↓IFN-γ, IL-17, IL-6, IL-1β; ↑IL-4                                        |                                                                           | (133)                                                            |
|                           | DBA/I                              | <i>F. hepatica</i>                          | Total extract + Ag- pulsed DCs            | ↓IFN-γ, IL-17, DCs promote Treg cells                                    | CD25 <sup>+</sup> Treg cells                                              | (178)                                                            |
|                           | DBA/I                              | <i>F. hepatica</i>                          | Infection                                 | ↑IL-4, IL-10; ↓TNF-α                                                     |                                                                           | (135)                                                            |
| CFA-induced arthritis AHR | BALB/c                             | <i>Hymenolepis diminuta</i>                 | Infection                                 | ↑IL-4, IL-10; ↓TNF-α                                                     | Partially IL-10 mediated, IL-4Ra-dependent                                | (135)                                                            |
|                           | BALB/c                             | <i>A. viteae</i>                            | AvCystatin                                | ↑IL-10, Tregs                                                            | M0-dependent, IL-10-dependent                                             | (129)                                                            |
|                           | BALB/c and C57BL/6                 | <i>H. polygyrus</i>                         | Infection                                 | ↓IL-5, IL-13, lung cell infiltrate                                       | CD4 <sup>+</sup> CD25 <sup>+</sup> T cells and B cells; IL-10 independent | (124, 139)                                                       |
|                           | BALB/c                             | <i>H. polygyrus</i>                         | ES products                               | ↓IL-5, IL-13, lung cell infiltrate                                       |                                                                           | (126)                                                            |
| Anaphylaxis               | BALB/C and C57BL/6                 | <i>S. mansoni</i>                           | Infection                                 | ↓Mast cell degranulation                                                 | IL-10-producing IL-4 deficient B cells                                    | (130)                                                            |

CIA, collagen-induced arthritis; T1D, spontaneous diabetes in NOD mice; EAE, experimental autoimmune encephalomyelitis; AHR, airway hypersensitivity (OVA-induced).

Infection of mice with numerous helminths, including *N. brasiliensis*, *S. mansoni*, *L. sigmodontis*, and *H. polygyrus*, have been shown to suppress the development of airway hyper-responsiveness (AHR), with helminth-infected animals displaying reduced eosinophilia in the lung, lower titers of allergen-specific IgE and attenuated airway inflammation (122–125). Furthermore, the ES products of the helminths *H. polygyrus*, *N. brasiliensis*, and *F. hepatica* ES (126, 127, C. Finlay and K. H. G. Mills, unpublished observation) confer protection from the development of asthma when co-administered with the antigen and adjuvant at sensitization. Indeed McSorley et al. (126) have recently shown that HES is also effective at preventing allergic disease when co-administered with the antigen during aerosolization. Definitive molecules within ES products that are responsible for the suppression of airway inflammation are emerging and include cystatins from the filarial nematode *A. viteae* (128, 129).

#### Anaphylaxis

The ability of helminth infection to attenuate other forms of atopic disease has been less well studied. Mangan et al. (130) demonstrated that infection of mice with *S. mansoni* could attenuate anaphylaxis, an extreme form of allergic inflammation. Mice infected with male-only worms were completely protected from the drop in core temperature and mortality associated with disease (130). The effect of helminth infection on atopic dermatitis has also been examined. *H. polygyrus* infection attenuated allergic inflammation in an AHR model, but infection with the nematode did not confer protection against the development of the atopic lesions associated with atopic dermatitis (131).

#### Helminth and protection against autoimmunity and inflammatory diseases in mice

There is a significant body of experimental evidence from mouse models to support the hypothesis that helminth infections can suppress the inflammatory responses that lead to autoimmunity (Table 1).

#### Collagen-induced arthritis (CIA)

Pearson and Taylor reported in 1975 (132) that infection with the nematode *Symphacia obvelata* attenuated the severity of CIA in rats, a model of human rheumatoid arthritis. Subsequently, it was shown that infection with *S. mansoni* (133), *S. japonicum* (134), *Hymenolepis diminuta* (135), or *H. polygyrus* (136) reduced joint erosion and inflammation in the mouse

CIA model. In some studies, protection was associated with a reduction in pathogenic cytokines TNF- $\alpha$ , IFN- $\gamma$ , IL-6, RANK-L, IL-1 $\beta$ , and IL-17 (133, 134). ES-62, a glycoprotein from *A. viteae* with immunomodulatory phospholipid modifications, also protected mice from CIA and was notably efficacious even when given at onset of symptoms (137).

#### Experimental autoimmune encephalitis (EAE)

EAE is a model of multiple sclerosis that is mediated by myelin-specific Th1 and Th17 cells that infiltrate the central nervous system (CNS) and damage the myelin sheath resulting in progressive paralysis. Experimental infection with several nematode species, including *T. spiralis*, *H. polygyrus*, and *S. mansoni*, have been shown to attenuate the severity of disease and delay paralysis (138, 139). We and others have shown that infection with the trematodes *F. hepatica* and *S. mansoni* also protected mice against EAE (140, 141). Protection was associated with a reduction in myelin-specific IFN- $\gamma$  and IL-17, and the infiltration of these cells into the brain (138, 140, 141). The protective effect of helminths against EAE (and CIA) provide evidence that helminth infections, which are often localized in specific organs (Fig. 2), can regulate immune responses in distal parts of the body and are not limited to the tissue microenvironment (e.g. gut). However, not all studies have reported a protective effect of helminths against CNS autoimmunity; for example, infection with *Strongyloides venezuelensis* did not alter the progression of EAE (142).

The ES products of helminths also have protective roles in EAE. We have demonstrated the ES products of *F. hepatica* protects against EAE (C. M. Finlay, A. M. Stefanska, and K. H. G. Mills, unpublished observations). In this model, protection was observed when treatment was initiated after onset of symptoms. In contrast, the protective effect of SEA from *S. mansoni* in this model was lost when SEA was given after disease onset (143).

#### Type 1 diabetes (T1D)

Several helminths have been shown to protect mice from diabetes. Infection with *T. spiralis* (144), *H. polygyrus* (144), *Litomosoides sigmodontis* (145), and *S. mansoni* all protect against hyperglycemia mediated by the Th1-induced destruction of  $\beta$ -islet cells in the pancreas of non-obese diabetic (NOD) mice. Interestingly, mice infected with *S. mansoni* had granulomas surrounding eggs in the pancreas, suggesting that *S. mansoni* eggs reached the pancreas from the portal vascula-

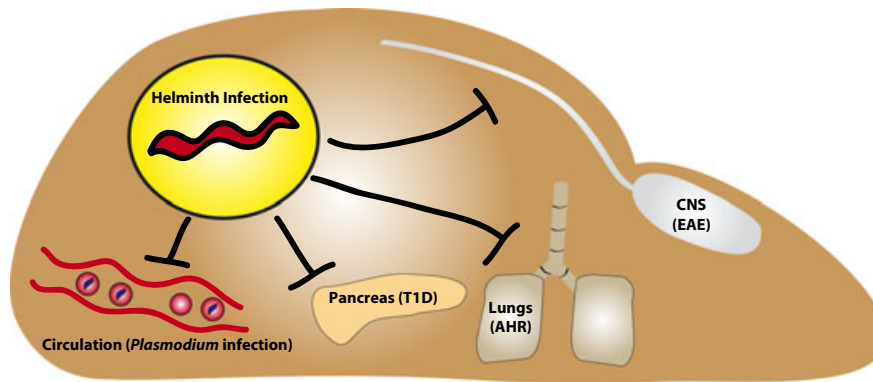


**Fig. 2. Helminth infections modulate immune responses against bystander antigens and suppress inflammatory disease in different organs.** Helminth infections modulate a variety of phenotypically different immune responses against bystander antigens in spatially alternate organs/tissues within the host. Studies in mice have shown that helminth parasites suppress the immune responses that drive immunity to *Plasmodium chabaudi* in red blood cells in the blood, the demyelination that results in the clinical symptoms of experimental autoimmune encephalomyelitis (EAE), the destruction of  $\beta$ -islet cells in the pancreas that leads to type 1 diabetes (T1D) and the allergen-specific inflammation in the lungs in models of airway hyper-responsiveness (AHR).

ture where the adult flukes reproduce. These granulomas had a high proportion of eosinophils, suggesting that they were of a type-2 phenotype maintained by IL-4 and IL-5. The presence of a type-2 granuloma in the vicinity of an anti-islet response may explain the potent protective effect of infection with *S. mansoni* (146). Indeed, almost all helminths that have been shown to protect against diabetes have been shown to be associated with type 2 responses (144, 145, 147) (Table 1).

#### Colitis

Inflammatory bowel disease (IBD), which in humans includes Crohn's disease (CD) and ulcerative colitis (UC), is believed to be caused by inappropriate inflammatory reactions to the gut microbiota. Although not autoimmune diseases in the strictest sense, they do share many clinical and immunological features of autoimmunity, including chronic inflammation, and irreversible tissue damage. The local interplay between the gut immune system, the microbiota and the helminth raises the possibility that helminth may attenuate IBD through the sustained and localized release of immunoregulatory factors. There are several mouse models of colitis, including those induced by dextran sodium sulfate (DSS) that degrade the gut mucosa or the haptens oxazalone, dinitrobenzenesulphonic acid (DNBS), or trinitrobenzenesulfonic acid (TNBS) that induce reactivity to gut microbiota. Alternatively, colitis can be induced by inoculation with the bacteria *Citrobacter rodentium* (148). Infection with *S. mansoni* (149) or exposure to *Schistosoma* eggs (150) has been shown to protect against TNBS-induced colitis. Protection delayed the duration of disease and prevented

mortality and inflammation in the gut, decreasing colonic IFN- $\gamma$  and enhancing IL-10 and IL-4 production (149, 150). Likewise, infection of mice with *H. polygyrus* was shown to reduce IL-17 production, and suppress the development of spontaneous colitis in IL-10<sup>-/-</sup> mice (151). Crude antigens isolated from *T. spiralis* reduced the severity of DNBS-induced colitis and was associated with a reduction in MPO activity, IL-1 $\beta$  production, and iNOS expression, and an upregulation of IL-13 and TGF- $\beta$  production in the colon (152).

Not all helminths have a protective effect in colitis. Despite its protective effect against spontaneous colitis in IL-10<sup>-/-</sup> mice, *H. polygyrus* exacerbated *C. rodentium*-induced colitis (153). Furthermore, the tapeworm *Hymenolepis diminuta* was shown to exacerbate oxazalone-induced colitis (154). However, oxazalone-induced colitis is partly mediated by IL-5-dependent eosinophilia, which may be enhanced by helminth-induced Th2 responses (155).

#### Other diseases with an inflammatory basis

Helminth infections have been shown to have protective roles in murine models of metabolic diseases, including atherosclerosis (156) and type-2 diabetes (157). This may be due to the direct effects of helminths on metabolism and adipose tissue. Wu et al. (157) recently demonstrated that helminth infection promoted IL-4 production by eosinophils, which maintained the M2 phenotype of macrophages in adipose tissue and thus lowered insulin levels and protected mice from diet-induced diabetes. SEA from *S. mansoni* was also shown to be protective in a model of atherosclerosis in mice (156). Atherosclerosis is potentiated

by an inflammatory macrophage-mediated response to lipid deposits in the vasculature. Protection was associated with an M2 response and an increase in IL-10 production by macrophages, with a corresponding reduction in TNF- $\alpha$ , MCP-1, and an associated reduction in neutrophils, and inflammatory monocytes. The protective effect was independent of a change in cholesterol levels and thought to be solely dependent on immune modulation (156). These studies suggest that helminths may provide homeostatic control of metabolism, and their absence may partially explain the increase in lifestyle-associated diseases.

### Mechanisms of suppression

Each species of helminth secretes distinct molecules with immunomodulatory activity, which can target different aspects of innate and adaptive immune responses. While much of the research focus has been on the ability of helminths to modulate bystander immune responses through the induction of Treg cells, fewer studies have investigated the cross-regulation of Th1 responses by helminth-specific Th2 cells. We believe that helminth-induced suppression of autoimmunity can be classified into two categories, those that inhibit host-antigen-specific Th1 and Th17 responses, via Treg cell induction and those that inhibit these responses through activating parasite-specific type 2 responses (Fig. 3). There is now a significant body of experimental evidence that supports the view that these mechanisms can work separately or in concert to exert immunosuppressive effects.

### Type-2 responses

Most reports on helminth-induced protection against autoimmunity have demonstrated an association between the attenuation of autoimmune inflammation and the induction of Th2 cells. For instance, the gastrointestinal nematodes *T. spiralis* and *H. polygyrus* prevented the onset of Th1-mediated type-1 diabetes in NOD mice following induction of profound parasite-specific Th2 responses (144). Furthermore, protection from diabetes in NOD mice induced with a recombinant protein from the filarial nematode *Diriofilaria immitis* was found to be associated with the induction of Th2 responses (158). Similarly, La Flamme et al. (141) demonstrated that protection of mice from EAE following infection with *S. mansoni* was associated with a reduction in MOG-specific IFN- $\gamma$  with a concurrent increase in systemic IL-4 and IL-5 production. While these early studies concluded that the induction of potent helminth-induced Th2

responses suppressed the development of autoimmune responses (144), they did not address the mechanisms of protection or specifically demonstrate that Th2 cells were required for protection.

A small number of studies have demonstrated an unequivocal requirement for Th2 cells. Protection against TNBS-induced colitis provided by administration of schistosome eggs was reversed in mice deficient in STAT-6, a key transcription factor which mediates IL-4 and IL-13 signaling through the IL-4R $\alpha$  (150). Likewise, infection with the tapeworm *Hymenolepis diminuta*, which was shown to suppress the development of CIA in WT mice, had no effect on disease in mice deficient in the IL-4R $\alpha$  (135). Furthermore, the protective effect of *S. mansoni* eggs on EAE was dependent on either IL-4R $\alpha$  and STAT6 (159). Notably, these studies all involved worms from the phylum Platyhelminthes.

While IL-4 and the IL-4R $\alpha$ , which mediates IL-4 and IL-13 signaling, have been extensively studied as mediators of helminth-induced suppression of autoimmunity, the role of IL-5 has been largely overlooked. Administration of recombinant IL-5 has been shown to ameliorate experimental autoimmune neuritis in rats, even when given at onset of symptoms (160).

It is possible that rather than simply exerting suppressive effects on Th1/Th17 responses, helminths switch autoantigen-specific responses from Th1/Th17 toward a Th2 phenotype. Autoantigen-specific Th2 cells are less capable at promoting autoimmunity than Th1 or Th17 cells; for example, myelin-specific Th1 and Th17 cells differentiated *in vitro*, induced EAE upon passive transfer, while MOG-specific Th2 cells did not transfer disease (161). There are limited data however to suggest that helminths infection promotes Th2 response to bystander antigens. It is also unclear whether parasite infections can alter the phenotype of differentiated T cells or if they act only on naive T cells. In an experimental model, *N. brasiliensis* did not promote bystander differentiation of T cells specific for non-parasite antigens despite the induction of potent parasite-specific Th2 responses (162). In contrast, Zheng et al. (143) showed that administration of SEA from *S. mansoni* to mice with EAE switched MOG-specific responses from IFN- $\gamma$  toward production of IL-4. Furthermore, in co-infection model, we demonstrated that infection of mice with *F. hepatica* exacerbated infection with *B. pertussis* by inducing a switch in the *B. pertussis*-specific response from Th1 to Th2 (103).

Th2 induction may not be sufficient in many cases to inhibit autoimmunity. *Strongyloides venezuelensis* did not alter the progression of disease despite an ongoing Th2 response

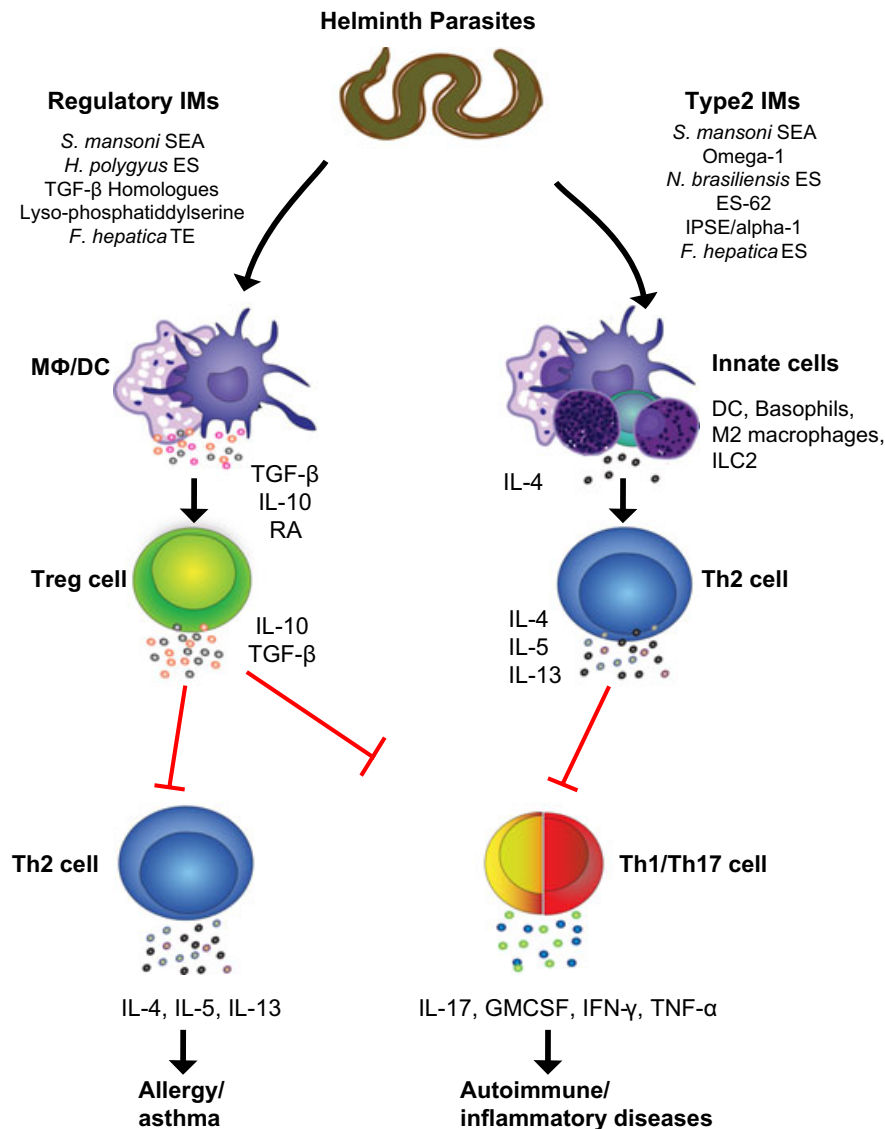


**Fig. 3. Helminths suppress autoimmunity and allergy via type 2 or regulatory immune response.** Different immunomodulatory molecules (IMs) from a variety of helminth parasites activate innate immune cells that promote either Th2 or Treg responses. IMs that induce TGF- $\beta$ , IL-10, or retinoic acid (RA) production by dendritic cells (DCs) or macrophages (M $\Phi$ ) induced IL-10 or TGF- $\beta$ -producing Treg cells that suppress Th2 responses that mediate allergy/asthma and Th1 or Th17 responses that mediate autoimmune or other inflammatory diseases (e.g. colitis). A separate set of helminth-derived IMs promote activate type 2 innate cells, including basophils, M2 macrophages, and type 2 lymphoid cells (ILC2) and induce innate IL-4 production, which drives differentiation of Th2 cells. These Th2 cells and type 2 innate immune cells can also suppress Th1 and Th17 responses that mediate autoimmune or other inflammatory diseases.

in vivo (142). Other studies that originally attributed helminth-induced protection against autoimmunity to induction of Th2 responses have been revised in follow up studies. For example, *L. sigmodontis* was assumed to protect mice against T1D by induction of Th2 responses (144). While there was a shift toward insulin-specific IgG1, there was also an increase in Treg cells (145). A follow up study from Hübner et al. (163) revealed that IL-4-deficient mice, which failed to mount a Th2 response to *L. Sigmodontis*, were still protected from diabetes.

#### Treg cells

Since the paradoxical discovery that Th2-inducing helminths, or their products, suppress the induction of the Th2 responses that mediate allergy, several mechanisms have been proposed to explain this phenomenon. In a revised interpretation of the hygiene hypothesis, it was suggested that regulatory immune responses induced by helminth infection inhibited Th2-driven allergic inflammation (164). Thus, while helminths ameliorate autoimmune processes

using a combination of regulatory and Th2 responses which antagonize Th1 and Th17 responses, their effects on allergy are more dependent on regulatory mechanisms, particularly induction of Treg cells.

Helminth infections have been shown to expand Treg cells, most likely as a mechanism designed to enhance survival of the parasite through regulation of anti-parasite immune responses. We have shown that *F. hepatica* infection recruits FOXP3<sup>+</sup> T cells and that parasite-specific T cells from infected animals expressed CTLA-4 and FOXP3, produced large quantities of IL-5 and IL-10 and could functionally suppress antigen-specific Th1 and Th17 cells *in vitro* (140). These data suggest that helminth infection can suppress not only parasite-specific but also T-cell responses to unrelated antigens, through bystander suppression. Indeed, Treg cells have been shown to play a crucial role in mediating *H. polygyrus*-induced suppression of autoimmunity and atopy (30).

The induction of Treg cells as well as Th2 cells is key feature of many helminth infections and may explain their potent suppressive function against autoimmune responses. For example, *S. japonicum* infection protected DBA/1 mice from CIA and was associated with the inhibition of Th1 responses and the induction of a mixed Th2/Treg responses (134).

## IL-10

Although helminths induce IL-10-secreting Treg cells and several helminth-derived products have been shown to promote IL-10 production by regulatory B (Breg) cells and DCs, there is limited evidence that IL-10 mediates bystander suppression of immune responses that mediate autoimmunity. We have shown that infection of mice with *F. hepatica* promotes IL-10 production by DCs and induced *F. hepatica*-specific IL-10<sup>+</sup> Treg cells, but suppression of EAE induced by *F. hepatica* infection was maintained in IL-10<sup>-/-</sup> mice. (140). Consistent with this finding, it has been reported that IL-10 is dispensable for suppression of colitis and AHR associated with *H. polygyrus* infection (124, 151). Moreover, infection with *H. polygyrus* was shown to reduce IL-17 production and suppress the development of spontaneous colitis in IL-10<sup>-/-</sup> mice (151), and transfer of Breg or Treg cells from *H. polygyrus*-infected wildtype and IL-10<sup>-/-</sup> mice ameliorated EAE and AHR (123, 139). Furthermore, infection of NOD mice with *L. sigmodontis* conferred protection against diabetes independent of the action of IL-10 (163). In contrast, in a study on TNBS-induced colitis, loss of IL-10 partially reversed the protective effect of *H. polygyrus* infection (165).

Despite the lack of a clear role of IL-10 in different models, this immunosuppressive cytokine may still play a supportive role in immune regulation by helminths. Protection against T1D by *H. polygyrus* is associated with induction of Th2 and Treg response (144, 166), but Treg cells and IL-10 were not essential, suggesting that Th2 responses are important (166). However, a recent study demonstrated that protection in IL-4<sup>-/-</sup> mice did require IL-10 (167). Furthermore, combined inhibition of IL-10R and IL-4 reversed the suppressive effect of *H. polygyrus* infection on mucosal IL-17 responses (151). In addition, the combination of IL-10 and IL-4 was shown to be involved in protection against experimental arthritis induced by infection with *Hymenolepis diminuta* (135).

## TGF-β

Although TGF-β has been implicated in driving differentiation of Th17 cells, this cytokine has potent anti-inflammatory properties. Administration of recombinant TGF-β *in vivo* was shown to attenuate the clinical symptoms of EAE, even when administered during active disease (168). Thus, there has been a major focus on the ability of helminth-induced TGF-β to protect against autoimmunity. Indeed, we have reported (140) that infection with *F. hepatica* attenuated EAE in a TGF-β-dependent manner. This is consistent with a study by Hübner *et al.* (163) that demonstrated that the protection induced by *L. sigmodontis* infection in a model of diabetes was dependent on TGF-β. Mice lacking functional TGF-β receptors on T cells, and thus lacking Treg cells, develop spontaneous colitis, which could not be reversed with *H. polygyrus* infection, suggesting that this helminth may mediate their protective effect through TGF-β production (169).

TGF-β is thought to primarily mediate suppressive effects through its ability to expand Treg cells. SEA from *S. mansoni* enhanced the expression of FOXP3 in naive NOD-specific T cells in a TGF-β dependent manner (77). Grainger *et al.* (76) demonstrated that the secreted products of *H. polygyrus* and *Teladorsagia circumcincta* contained molecules that bound to the mammalian TGF-βRII and induced the expression of FOXP3 in naive T cells. However, infection with *L. sigmodontis*, which protected mice from diabetes in a TGF-β-dependent manner and was associated with the recruitment of Treg cells, was not mediated by Treg cells, as depletion of CD25<sup>+</sup> cells did not impair protection (145, 163). There is also evidence that TGF-β signaling may be required to promote the production of IL-10 during helminth infection

(169). These studies indicate that the immunoregulatory effects of the broadly acting cytokine TGF- $\beta$  are not limited to Treg cells (170).

Infection with *L. sigmodontis* protects mice from AHR through a mechanism involving the expansion of Treg cells (122). This was associated with enhanced production of TGF- $\beta$  by T cells, and protection from AHR was partially reversed by neutralization of TGF- $\beta$ . However, in the broader context, TGF- $\beta$  may not be protective in AHR, as administration of recombinant TGF- $\beta$  at sensitization has no effect on disease outcome (16). In addition, TGF- $\beta$  has been contra-indicated in the treatment asthma, as it contributes to pathology, specifically airway remodeling and fibrosis (171).

### B cells

B cells have been implicated in helminth-induced suppression of allergy. Mangan et al. (130) showed that IL-10-producing B cells induced by *S. mansoni* infection protected mice against experimental induced fatal systemic anaphylaxis; transfer of B cells from IL-4-deficient mice that had been modulated by worms *in vitro* conferred resistance to anaphylaxis (130). Furthermore, a study by Wilson et al. (139) demonstrated that transfer of CD23<sup>high</sup> B cells from allergen-naïve mice infected with *H. polygyrus* transferred protection against AHR in recipients in a similar manner to Treg cells. This suggests that Breg cells can mediate bystander suppression of T-cell responses, independently of antigen or the production of antibodies.

### Dendritic cells

Helminth products are well known to modulate DC activation and inhibit their ability to induce Th1 and Th17 responses, while enhancing induction of Th2 responses (57, 79, 82–86, 172). Certain helminth products are also known to promote the differentiation of tolerogenic DCs that fail to prime effector T cells but promote differentiation of Treg cells (88, 89, 147, 172). Thus, DCs play a major role in mediating helminth-induced suppression of autoimmunity and allergy.

*H. polygyrus* infection induces tolerogenic DC in the gut of infected animals. In a model of colitis induced by transfer of IL-10<sup>-/-</sup> T cells into RAG<sup>-/-</sup> recipient mice, infection with *H. polygyrus* mice prior to transfer of T cells protected against disease, even when mice are cleared of infection before T-cell transfer (173). This finding demonstrates that innate mechanisms are sufficient to protect from disease.

Further phenotyping revealed that these DCs from the infected gut of RAG<sup>-/-</sup> mice are less capable of promoting IFN- $\gamma$  and IL-17 production by T cells than DCs from uninfected animals (173). These DCs were subsequently shown to transfer protection against colitis in recipient mice (174).

Production of retinoic acid (RA) by DCs promotes TGF- $\beta$ -dependent Treg cell differentiation (175) and has a protective role in mouse models of colitis (176). A recent study from Correale and Farez (177) demonstrated that RA may be involved in helminth suppression of autoimmunity; helminth-infected patients that had lower disease scores had high concentrations of RA (177). In mouse models, SEA from *S. mansoni* induced the production of RA by DC and enhance differentiation of Treg cells (177). Omega-1 may be involved, as Zaccone et al. (79) demonstrated that this glycoprotein from SEA conditions DCs to promote FOXP3 expression in an TGF- $\beta$  and RA-dependent manner, while also promoting Th2 responses. These studies raise the possibility that helminth-induced RA may promote resistance to autoimmunity.

Helminth-modulated DCs, with or without antigen loading, have potential as cell-based therapeutic for inducing tolerance to autoantigens or allergens. A recent report demonstrated that transfer of DCs pulsed *in vitro* with CpG and an extract of *F. hepatica* and loaded with collagen delayed the onset and reduced the severity of CIA (178). The protective effect and was associated with a reduction in IL-17 and IFN- $\gamma$  and an increase in IL-10 and TGF- $\beta$  production and was reversed by depleting CD25<sup>+</sup> cells or the neutralization of TGF- $\beta$  during priming *in vitro* (178). DC transfer has also been shown to have a therapeutic effect in OVA-induced AHR. Adoptive transfer of DC isolated from mice infected with *S. japonicum* enhanced Treg cells and IL-10 responses in mice following allergen challenge while suppressing allergen-specific IL-4 and symptoms of disease (179).

### Macrophages

Macrophages may also mediate the protective effects of helminths. *S. mansoni* infection protected mice from DSS-induced-colitis, which was independent of IL-10, TGF- $\beta$ , and T cells, but was mediated by macrophages. Macrophages could transfer protection to uninfected recipients and was maintained in IL-4/IL-13 deficient mice, suggesting that alternative activated M2 macrophages were not involved (180). AvCystatin derived from *A. viteae* protects mice from AHR and DSS-induced colitis in a macrophage dependent manner (181). AvCystatin also induces the production of

IL-10 by macrophages *in vitro* and protection from colitis was partially dependent on IL-10 (181).

### Helminth therapy in humans

The epidemiological and experimental evidence that helminths can modulate immune responses that mediate autoimmunity and allergy has advanced to a point where helminths are now being investigated as potential therapeutics for treating inflammatory diseases in humans. A number of clinical trials involving treatment with live helminth parasites or their eggs have already been initiated or completed (16). The most advanced trials thus far have been conducted in patients with IBD using eggs from the porcine whipworm *Trichuris suis* (182). An initial trial demonstrated safety in patients with IBD and showed temporary clinical benefit in a small treatment group (182). In a Crohn's disease trial, 79% of patients responded to the therapy, and 72% entered remission after 24 weeks of therapy with no major adverse events (183). This was followed by a trial in ulcerative colitis patients which had more modest outcomes: 45% of patients responding and 10% entering remission (184). *T. suis* eggs were chosen, as they do not reproduce and thus cause self-limiting colonization *in vivo*, although the eggs must be repeatedly administered every 3 weeks. Treatment with *T. suis* eggs is currently being investigated in larger phase 2 placebo-controlled trials, with results expected in 2014 (NCT01279577 and NCT01576471).

Infection with the hookworm *Nector americanus*, an intestinal helminth, has also been assessed as a therapy in IBD. *N. americanus* is administered through the skin and can live for up to 5 years in the human host, it therefore does not require repeated dosing (185). In a small cohort of IBD patients, infection with *N. americanus* resulted in all 5 patients entering remission by week 47. While patients developed transient intestinal pain and systemic eosinophilia, no other adverse effects were observed. In an experimental infection of an ulcerative colitis patient, disease remission was associated with the production of IL-22, a cytokine that maintains epithelial integrity, with a corresponding reduction IL-17 in the gut (186).

*N. americanus* has also been investigated in celiac disease, an inflammatory intestinal disease caused by an immune reaction to wheat gluten that has similar clinical features to colitis. While infection did reduce IFN- $\gamma$  and IL-17 responses and enhanced production of IL-5 following gluten challenge (187), it did not confer clinical benefit to the patients (188).

In support of Correale and Farez's (115) demonstration that natural infection with helminths reduced the progression of MS, clinical trials have shown that infection with *T. trichiura*, *Hymenolepis nana*, and *Ascaris lumbricoides* were effective in MS patients, with reduced CNS lesions and disease exacerbations (189, 190). Some of these treatments were associated with an expansion of Treg cells and enhanced production of TGF- $\beta$  and IL-10 (189). However, successful treatment of MS patients using *T. suis* eggs was not associated with induction of TGF- $\beta$ , IL-10, or Treg cells (190) and the mechanism of immune suppression remains to be defined (190).

While helminth therapy has so far shown promise in human IBD and MS, data from animal models have suggested that this approach may be broadly effective in inflammatory diseases. Infection with *H. polygyrus* and *T. spiralis* were particularly protective in models of T1D (144), while *N. brasiliensis*, *S. mansoni*, and *S. japonicum* have shown efficacy in murine models of arthritis (133, 134, 136). The application of helminth therapy for allergic disease has been largely unsuccessful thus far. A trial using *T. suis* eggs in patients with hay fever demonstrated no protective effect, while the adverse events were deemed to be unacceptable (191).

While large scale clinical trials are warranted especially for life-threatening autoimmune diseases, there are significant ethical and standardization issues that need to be addressed before this approach can be accepted for general use in humans. Although the approach of using probiotics in the treatment or prophylaxis of inflammatory diseases may have set a precedent for the use of live organisms in controlling human diseases, their properties differ considerably from that of live metazoans. Helminths, even those that cause asymptomatic infections, will cause at least some damage to the host as they translocate across mucosal barriers. Unfortunately, due to the divergent immune responses within a population, there will always be the potential for immune pathology.

Aside from the ethical concerns, there are many practical considerations that may reduce the efficacy of helminth-based therapy. The original understanding of the hygiene hypothesis was that infections early in life provided protection against allergy and possibly other immune-mediated diseases (112). Thus, it is possible that exposure of the human immune system to helminths early in life is required to confer immunosuppressive activity. One study suggested that perinatal helminth infection influenced the immune response of neonates to produce parasite-specific antibodies, thus raising the possibility that children of helminth-infected

mothers may also have developed helminth-induced immunoregulatory networks in early life that differ from those not exposed in utero (192). In addition, experiments in animal models have suggested that helminths have the greatest effect on immune activation when present at the time of antigen sensitization. Thus, the beneficial effect of helminth therapy may be limited to the induction phase of allergy or autoimmune disease. Furthermore, in an attempt to limit the side effects of therapy, many trials have used what may be the suboptimal doses of helminths, resulting in lack of clinical efficacy. This explanation was put forward to explain the failure of a trial of *N. americanus* in IBD patients (193). It is also quite difficult, or impossible, to conduct these trials using proper placebo controls. This is due to the obvious symptoms of early infection that will reveal to patients whether they are in placebo or treatment groups (16, 193). In comparison with recombinant biologics and small molecules, there will be considerable practical issues around the adherence to good manufacturing practices in the production of live helminth for use in humans.

#### Helminth products as anti-inflammatory drugs

The ethical, regulatory and other hurdles associated with the use of live helminth have motivated attempts to identify immunomodulatory products from helminths with potential to treat inflammatory diseases in humans. Since helminth-derived molecules have potent abilities to inhibit proinflammatory responses to microbial PAMPs, and have potential as therapeutics for sepsis and immune-mediated diseases (194). Indeed the immunosuppressive products secreted by helminths have already been used successfully in animal models, and have generated much interest as potential therapeutics for allergy and autoimmune diseases in humans (195). The best characterized preparation, *S. mansoni* SEA, has been shown to reduce the severity of EAE as well as to prevent the development of TNBS-induced colitis and diabetes in mouse models (77, 146, 150, 159). We have demonstrated that parenteral administration of *F. hepatica* ES is highly effective in suppressing Th1/Th17 and Th2 responses that mediate EAE, AHR, and colitis (C. M. Finlay, A. M. Stefanska and K. H. G. Mills, unpublished data).

However, since SEA and *F. hepatica* ES are a mixture of a large number of distinct proteins and small molecules they may run into insurmountable practical and regulatory hurdles. Therefore, purified or recombinant molecules will have significant advantages. ES-62 is probably the best characterized helminth-derived molecule and has been shown to

attenuate the symptoms of CIA, AHR, and DSS-induced colitis (137, 196). Other candidates include the *S. mansoni*-derived molecule LNFPIII, which was shown to prevent the development of psoriatic-like lesions in flaky skin mice, as well as to promote transplant tolerance in mice (197, 198). AvCystatin derived from *A. vitæ* has also been shown to be protective in AHR and colitis models (129).

#### Conclusions and future perspectives

Helminth parasites differ from many other classes of pathogens in a number of respects. Unlike many viral and bacterial diseases of humans that are well controlled by vaccination, we have yet to see a single licensed vaccine against a helminth infection of humans (199). This is partly a reflection of the fact that helminth parasites are mostly endemic in developing countries where the market forces do not readily promote vaccine development by the major vaccine manufacturers and also a reflection of the complexity of the organisms and their success in evading and subverting immune responses that eliminate the parasites from their host. Indeed, the powerful immunoregulatory strategies developed by many helminths extends to suppression of immune responses to unrelated pathogens, and exacerbation of diseases caused by viruses, bacteria, and even other parasites. This can translate to a failure to mount protective immune responses to viral and bacterial vaccines. Therefore, the future control of helminth parasite using anti-helminthic drugs or vaccines has the potential of reducing not only morbidity and mortality from helminth parasites, but also from infectious diseases in general.

One of the upsides of helminth-induced immunosuppression is that it has the bystander effect of attenuating dysregulated and pathogenic immune responses to allergens and autoantigens. Consequently, individuals infected with helminth parasites have reduced susceptibility to developing allergy/asthma and autoimmune diseases. This formed the basis of the 'hygiene hypothesis', which was originally attributed to Th1-Th2 cross-regulation but now appears to involve potent helminth-induced immunoregulatory networks, including Treg cells, Breg cells, and also cells of the innate immune system, such as ILC2, regulatory/tolerogenic DCs, and M2 macrophages. Although work in small animal models needs to be interpreted with caution, studies with cell transfer and knockout mice have provided convincing evidence that regulatory cells induced by helminth infection (or their products) can suppress inflammatory responses, including the Th2/Th17 responses that mediate

allergy/asthma and Th1/Th17 responses that mediate many autoimmune diseases. This has already translated to clinical studies, where there are reports that infection with helminth parasites that are not pathogenic in humans, can attenuate autoimmune diseases in patients.

While the exploitation of live helminths as therapies for human immune-mediated diseases looks very promising and has been embraced by patients with debilitating and life-threatening diseases, it will face many ethical, regulatory, and manufacturing hurdles before it can be licensed for use in humans. Alternative and perhaps more acceptable approaches already under pre-clinical investigation involve the identification and purification or expression of immunomodulatory molecules from helminth parasites as injectable therapeutics for allergy/asthma and autoimmune diseases. The focus here has been on proteins or small molecules that promote the induction of regulatory cells, especially Treg cells or immunosuppressive cytokines, IL-10, or TGF- $\beta$ . There is already convincing evidence that treatment of mice with helminth-derived immunomodulatory molecules can promote regulatory cells and cytokines that suppress pathogenic Th1, Th2, or Th17 responses that mediate autoimmunity and AHR.

Traditional therapies for autoimmune diseases include steroid and non-steroidal anti-inflammatory drugs, which have considerable side effects and in some cases rather limited efficacy. These are now being replaced with biological drugs, especially antibodies that blocking inflammatory cytokines, including TNF- $\alpha$ , IL-17, IL-12p40, and IL-23

(200). However, the Th1 and Th17 cells that mediate pathogen in autoimmune disease also protect the host against pathogens. Furthermore, IFN- $\gamma$  has an essential role in protective immunity to tumors. Therefore, drugs that chronically suppress Th1 and/or Th17 responses have run the risk of increasing susceptibility to infection. Indeed, there is already evidence of enhanced infection in patients treated with anti-inflammatory biologics that target TNF- $\alpha$ , the Th1 and Th17 pathways (anti-IL-12/23p40), or immune cell migration (201–203). Therefore, broadly immunosuppressive drugs need to be replaced with more targeted therapies that selectively promote host anti-inflammatory responses that do compromise host protective immunity to infection and tumors. Live helminth and helminth-derived immunomodulatory products have the advantage of promoting the host immunoregulatory networks and if properly controlled, it should be possible to re-balance rather than suppress the host immune response, attenuating pathogenic Th17 responses without completely blocking protective Th1 and Th17 responses to infection. It may also be possible to exploit helminth-derived immunomodulants as adjuvants for Treg-based vaccines or tolerance-inducing DC-based therapies. The generation of Treg cells specific for autoantigens or tissue-specific antigens may allow selective inhibition of T-cell responses that mediate organ-specific autoimmune diseases without the side effects of systemic immune suppression associated with many current therapies for immune-mediated diseases in humans.

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