Chapter 16
Can the Study of Helminths Be Fruitful for Human Diseases?

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Abstract Parasitic helminths have an inclination to be long-lived invaders with certain human parasites reported as surviving for in excess of a decade. Such longevity tends to be associated with an apparent lack of pathology and one contributor to this perhaps somewhat surprising situation is likely to be the secretion of anti-inflammatory immunomodulators by the worms. Such molecules act to dampen and effect the polarization of immune responses and this invariably potent immunomodulation frequently extends to responses to third party antigens, vaccines and other diseases. Relating to the latter, a particularly serendipitous consequence of worm infection that is being increasingly recognized, is its effect on human conditions that are associated with aberrant inflammation. For this reason, helminths have within the last decade attracted substantial attention in the research community as a potential source of novel therapies against allergic and autoimmune diseases. In this article we describe the effects of helminths on five such diseases—asthma, rheumatoid arthritis, multiple sclerosis, type I diabetes and inflammatory bowel disease. In particular, we consider the immunological mechanisms that underlie helminth-mediated protection against these diseases and in addition, highlight individual helminth molecules that may have therapeutic potential.

16.1 Introduction

Parasitic helminths such as nematodes, tapeworms, and flukes are large, multicellular invaders that are generally very well adapted to their hosts. One of the consequences of such adaptation was the development of a very particular immunological environment that provides a range of benefits to the hosts. This is manifested by an apparent lack of pathology in most humans infected with

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helminths (Hayes et al. 2004) and, more importantly from the point of view of this article, dampened down immune responses to third-party antigens such as allergens and autoantigens (Elliott and Weinstock 2012). Due to this development, helminths have attracted a substantial attention in the research community as a potential source of novel therapies against diseases associated with aberrant immune/inflammatory responses, e.g., allergies and autoimmune diseases (Harnett and Harnett 2010). Further support for this idea is provided by epidemiological data in which researchers surveyed human cohorts exposed to helminths to determine if protection against inflammatory disorders could be found. Indeed, observational studies of natural helminth infections in patients with multiple sclerosis that spanned over almost 5 years revealed a remarkable beneficial effect of worms on the course of the disease (Correale and Farez 2007, 2011). In addition, a protective association of prior hookworm infection with Crohn's disease was found in studies published by Kabeerdoss et al. (2011). In a similar manner, a negative association between worms and inflammatory diseases was reported by Panda et al. (2013) in that they showed that rheumatoid arthritis patients were free of filarial infection in an area where filariasis was endemic. Also recently, hookworm infection was found to be a protective factor against atopy as patients harboring the worm had lowered reactivity to house dust mite in a skin prick test (Hamid et al. 2013). It should be noted however that other reports rejected the idea that worm presence can indeed limit the burden of different types of inflammatory disease (Bager et al. 2012; van der Werff et al. 2013). In the face of sometimes contradictory data derived from the field studies, researchers have thus started focusing on studying the beneficial impact of helminths on inflammatory disorders using well-controlled laboratory models. In consequence, there is an abundance of experimental data to support the phenomenon, as will be shown in the course of this book chapter.

Infection with helminths does not go unnoticed by the host immune system. Indeed, a strong response from the host is launched shortly after the parasite has entered its body and relies largely on the response of antigen presenting cells of the host such as dendritic cells (DCs) to molecules secreted by the worms. In the following steps of the immunological cascade, priming of a specific T-helper cell response takes place with the subsequent occurrence of Th2 cells that produce IL-4, IL-5, IL-9, and IL-13 cytokines. These cytokines then increase numbers of eosinophils, basophils, mast cells, and alternatively activated macrophages, both in affected tissue and systemically (Allen and Maizels 2011). The immune responses triggered during the worm invasion are sufficient to attenuate the infection and potentially lead to worm expulsion (Anthony et al. 2007); however, this is rarely the case and the parasite very often establishes a chronic infection within the host (Hayes et al. 2004). Thus, worms are able to counteract the host immune responses, and this helminth-driven immune regulation can be extrapolated to unrelated antigens, as mentioned earlier. In practical terms, deciphering the way parasites modulate the host immune responses can be used to create novel drugs to combat allergies and autoimmune disorders. This involves model studies on how live infections with parasites limit pathologies, characterization of parasitic products

with anti-inflammatory activities, both in native and recombinant form, and finally design of drugs based on their structure that can be commercialized.

16.2 Worms and Asthma

Numerous helminth species have been reported to attenuate symptoms of experimentally induced allergic airway inflammation in mice (Table 16.1). Eosinophil influx into the lungs, and especially eosinophil numbers in the bronchoalveolar lavage, is a useful cellular marker in determining the intensity of allergic inflammation in the laboratory setting. Decreased number of these cells in helminth-treated diseased mice has been therefore often reported in the scientific literature. Thus, for example, infection with *Schistosoma mansoni* was shown to reduce eosinophil numbers in the lungs of mice in which asthma-like symptoms were provoked by a combination of systemic immunization with ovalbumin (OVA) in aluminum hydroxide adjuvant (Alum) and a series of intranasal OVA challenges (Pacífico et al. 2009). In addition, production of Th2 cytokines, IL-4 and IL-5, as well as IgE antibodies was inhibited in mice infected with the parasite. Interestingly, protection against asthma was also achieved when schistosome eggs were injected into the sick mice (Pacífico et al. 2009). In this case, mice that received the eggs showed elevated numbers of CD4+ CD25+ Foxp3+ T cells and levels of IL-10. Subsequent neutralization studies, using anti-CD25 and anti-IL-10R antibodies, concluded that regulatory T cells but not IL-10 are responsible for the ability of worm eggs to suppress asthma. In an attempt to further dissect the mechanism by which this helminth can ameliorate asthma, three of the *S. mansoni* antigens were tested in the OVA-induced airway inflammation model (Cardoso et al. 2010). All three proteins could ameliorate the total cell counts and eosinophil numbers in the bronchoalveolar lavage and decrease the levels of IgE antibody. In addition, two proteins, PIII and Sm22.6 lowered levels of IL-4 and IL-5. The frequencies of regulatory T cells, on the other hand, were increased in the groups of mice that received the proteins; however, only Sm22.6 could upregulate IL-10. In conclusion, it could be said in these studies that induction of regulatory T cells might be an important mechanism contributing to the suppression of asthma by helminth products, whereas IL-10 seems to play no or a minor role in this process. It was reported that infection of asthmatic mice with another species of fluke, *S. japonicum*, also resulted in suppressed lung eosinophilia, decreased IL-4 and IL-5 levels, and reduced concentration of allergen-specific IgE antibodies (Liu et al. 2010; Mo et al. 2008). Mechanistically, the authors showed that the transfer of DCs isolated from *S. japonicum*-infected mice greatly contributed to the protective effect of the parasite on asthma in the studied model (Liu et al. 2010). Similar to *S. mansoni* eggs, injection of egg antigens of *S. japonicum* into asthmatic mice also reversed the disease parameters (Yang et al. 2007). The proposed mechanism of action was studied and showed to be CD4+ CD25+ T cell dependent.

Table 16.1 Helminth species and their products displaying beneficial effects on the course of experimental asthma

Helminth species	Infection/Antigen/Cells	Disease model	Reference
Schistosoma mansoni	Antigens: PIII, Sm22.6, Sm29	OVA-induced airway inflammation	Cardoso et al. (2010)
	Infection	OVA-induced airway inflammation	Pacífico et al. (2009)
	Injection of eggs	OVA-induced airway inflammation	Pacífico et al. (2009)
Schistosoma japonicum	Infection	OVA-induced airway inflammation	Liu et al. (2010), Mo et al. (2008)
	Egg antigens	OVA-induced airway inflammation	Yang et al. (2007)
Trichinella spiralis	Infection	OVA-induced airway inflammation	Aranzamendi et al. (2013), Park et al. (2011)
Acanthocheilonema viteae	Product: ES-62	OVA-induced airway inflammation	Rzepecka et al. (2013), Melendez et al. (2007)
	Recombinant product rAv-17	OVA-induced airway inflammation	Schnoeller et al. (2008)
		Grass pollen-specific allergic responses	Daniłowicz-Luebert et al. (2013)
Heligmosomoides polygyrus	Excretory-secretory products	OVA-induced airway inflammation	McSorley et al. (2012)
	B cells from helminth-infected mice	OVA-induced airway inflammation	Wilson et al. (2010)
	Infection	OVA-induced airway inflammation	Hartmann et al. (2009), Rzepecka et al. (2007), Kitagaki et al. (2006)
Anisakis simplex	Recombinant product: macrophage migration inhibitory factor-like protein	OVA-induced airway inflammation	Park et al. (2009)
Ascaris suum	Product: PAS-1	OVA-induced airway inflammation	Araújo et al. (2008)
		APAS-3-induced airway inflammation	Itami et al. (2005)

(continued)

Table 16.1 (continued)

Helminth species	Infection/Antigen/Cells	Disease model	Reference
	Pseudocoelomic fluid	Sensitization with ragweed	McConchie et al. (2006)
	Adult worm extract	OVA-induced airway inflammation	Lima et al. (2002)
Toxascaris leonina	Excretory-secretory products	OVA-induced airway inflammation	Lee et al. (2008)
	Total protein	OVA-induced airway inflammation	Lee et al. (2008)
Litomosoides sigmodontis	Infection	OVA-induced airway inflammation	Dittrich et al. (2008)
Nippostrongylus brasiliensis	Excretory-secretory products	OVA-induced airway inflammation	Trujillo-Vargas et al. (2007)
	Infection	OVA-induced airway inflammation	Wohlleben et al. (2004)
Angiostrongylus costaricensis	Extract	OVA-induced airway inflammation	Pinto et al. (2006)
	Infection	OVA-induced airway inflammation	Pinto et al. (2004)
Strongyloides stercoralis	Infection	OVA-induced airway inflammation	Wang et al. (2001)

Several species of nematode were also demonstrated to protect mice from experimentally induced asthma. *Heligmosomoides polygyrus* is one of the most intensely studied nematodes in the context of asthma and has been shown to have beneficial effects on the course of disease in many publications. Regarding the mechanisms of action, it was shown that protection is IL-10-dependent and that adoptive transfer of cells from helminth-infected/OVA-exposed mice suppressed OVA-induced eosinophilic inflammation, suggesting a role for regulatory cells (Kitagaki et al. 2006). Elevated numbers of Foxp3 regulatory T cells in helminth-infected mice that were subjected to OVA-induced airway inflammation were also reported in the study published by Hartmann et al. (2009). Regulatory T cells are not the only cell population that can play a role in the suppression of allergy by *H. polygyrus*, as shown by Wilson et al. (2010). In this paper, suppression of airway eosinophilia, IL-5 secretion, and pathology following allergen challenge was also achieved upon transfer of CD4– CD19+ B cells isolated from lymph nodes of infected mice. Transferred B cells from IL-10 knockout mice could also mediate the therapeutic effect on asthma suggesting the presence of a yet to be identified

mechanism that allows this regulatory cell population to suppress lung pathology. It is worth mentioning at this point that even though *H. polygyrus*-mediated suppression of asthma might involve a regulatory B cell population that does not require IL-10 to exert its beneficial effect in the course of allergic lung inflammation, regulatory B cells expressing IL-10 have been shown to be induced by infection of mice with *S. mansoni* and contribute to the protection against allergic inflammation afforded by the fluke (Amu et al. 2010; van der Vlugt et al. 2012).

Continuing on the *H. polygyrus*-induced downregulation of asthma in mice, excretory–secretory products of the worm (HES) were tested for their potential to prevent and treat asthma in the OVA/Alum model (McSorley et al. 2012). In both cases, reduction in eosinophil numbers was noted in the nematode products-treated mice; however, only application of HES at the sensitization stage decreased pathogenic T cell responses. HES had previously been shown to induce differentiation of regulatory T cells in vitro and transfer of these cells into asthmatic mice suppressed allergic airway inflammation (Grainger et al. 2010).

Similar to the excretory-secretory products derived from *H. polygyrus*, products released from *Trichinella spiralis* could induce expansion of CD4+ CD25+ Foxp3 regulatory T cells in an in vitro assay (Aranzamendi et al. 2012). Expansion of regulatory T cells in vivo during the chronic stage of *T. spiralis* infection is very significant, and transfer of splenic CD4+ T cells from helminth-infected mice could afford protection from experimental allergic airway inflammation (Aranzamendi et al. 2013). Consistent with this, infection of mice with *T. spiralis* protected them from asthma development, and this beneficial effect coincided with increased recruitment of regulatory T cells into the lungs and elevated levels of IL-10 and TGF-β (Park et al. 2011).

Worm extracts from the porcine parasite, *Ascaris suum*, were shown to suppress accumulation of eosinophils in the airways and decreased levels of IL-4, IL-5, and eotaxin in a model of lung inflammation (Lima et al. 2002). Subsequent studies revealed that *A. suum* adult worms contain an anti-allergenic protein PAS-1 that could inhibit eosinophilic airway inflammation and hyper-responsiveness induced by a pro-allergenic molecule APAS-3 (also found in the *A. suum* extract) (Itami et al. 2005). The suppressive effects of PAS-1 were also demonstrated in the OVA model of asthma and shown to be dependent on IL-10 and IFN-γ (Araújo et al. 2008). McConchie et al. (2006) worked with a distinct fraction of molecules originating from *A. suum*. They showed that the pseudocoelomic fluid of the parasite can effectively decrease immunological parameters of asthma in mice sensitized with ragweed and that this protection is IL-10-independent. Extracts from another nematode *Angiostrongylus*, were reported to protect mice from asthma (Pinto et al. 2006), in agreement with the fact that infection with the same parasite also had potential to mediate beneficial effects on the course of disease (Pinto et al. 2004).

ES-62 is a native molecule purified from excretory–secretory products of a filarial nematode *Acanthocheilonema viteae*. This tetrameric protein with complex, immunologically active posttranslational modifications, in particular phosphorylcholine (PC) attachment to an N-type glycan, was shown to inactivate mast

cells via a TLR-4-dependent mechanism and to suppress eosinophil recruitment in mice with experimentally induced asthma (Melendez et al. 2007). Subsequent work confirmed the ability of ES-62 to subvert eosinophil influx into the lungs, and in addition, it was revealed that the molecule also attenuated infiltration of neutrophils, inflammatory cells that are usually associated with severe, steroid-resistant asthma (Rzepecka et al. 2013). Mechanistically, ES-62 was shown to protect from asthma via IFN-γ-mediated suppression of pathogenic Th2/Th17 responses.

A considerable amount of data has been obtained when applying a recombinant filarial cystatin in two different asthma models. This work showed that the molecule could inhibit eosinophil recruitment, reduce levels of OVA-specific and total IgE, and downregulate IL-4 production in the OVA-induced airway inflammation model (Schnoeller et al. 2008). Depletion of macrophages by clodronate-containing liposomes and blocking of IL-10R signaling restored the number of infiltrating cells and the levels of OVA-specific IgE in the cystatin-treated asthmatic mice. Also administration of the filarial immunomodulator into mice with grass pollen-induced asthma, suppressed allergen-specific Th2-responses and airway inflammation, inhibited local recruitment of eosinophils, reduced levels of allergen-specific IgE, and downregulated IL-5 and IL-13 in the bronchoalveolar lavage (Daniłowicz-Luebert et al. 2013). Interestingly, incubation of human peripheral blood mononuclear cells isolated from timothy grass pollen allergic patients, with cystatin suppressed allergen-specific IL-13 and increased IFN-γ suggesting that IFN-γ, as was reported earlier with ES-62, could promote helminth-induced regulation of asthma.

16.3 Worms and Arthritis

Several species of helminth have been shown to be able to attenuate symptoms of arthritis in a mouse model (Table 16.2). In particular, two species of fluke *S. japonicum* and *S. mansoni* significantly reduced the severity and/or the incidence of experimental autoimmune collagen-induced arthritis (Song et al. 2011; He et al. 2010; Osada et al. 2009). Such protection was mostly associated with reduction in production of the pro-inflammatory cytokines TNF-α, IL-6, IL-1β, IFN-γ, and IL-17, which appeared with concomitant induction of the Th2 cytokine, IL-4, and the anti-inflammatory cytokine, IL-10. Collectively, it could be proposed that the Th2/regulatory cytokine milieu stimulated by infection with *Schistosome* species counteracts pro-arthritic Th1/Th17 cell activation. DCs represent an important cell type in arthritis due to their ability to sense the immunogens, e.g., collagen, presented to them in the inflammatory context. Such priming induces collagen-specific T-helper cell responses and production of collagen-specific autoantibodies, which in turn causes joint swelling and inflammation. There are reports that helminth molecules can subvert these initial processes that lead to pro-arthritic responses in mice. A total extract from *Fasciola hepatica* was shown to induce tolerogenic properties in CpG-ODN-maturated DCs, which when transferred into

Table 16.2 Helminth species and their products displaying beneficial effect on the course of experimental arthritis

Helminth species	Infection/Antigen	Disease model	Reference
Schistosoma mansoni	Infection	Collagen-induced arthritis	Osada et al. (2009)
Schistosoma japonicum	Infection	Collagen-induced arthritis	He et al. (2010), Song et al. (2011)
	Recombinant antigen: rSj16	CFA-induced arthritis	Sun et al. (2010)
Hymenolepis diminuta	Infection	CFA-induced arthritis	Shi et al. (2011)
Fasciola hepatica	Extract	Collagen-induced arthritis	Carranza et al. (2012)
Heligmosomoides polygyrus	Infection	MRL/lpr model	Salinas-Carmona et al. (2009)
Nippostrongylus brasiliensis	Infection	MRL/lpr model	Salinas-Carmona et al. (2009)
Ascaris suum	Extract	Zymosan-induced artrhritis; collagen-induced arthritis	Rocha et al. (2008)
Acanthocheilonema viteae	Product: ES-62; structural moiety within ES-62 (PC)	Collagen-induced arthritis	Pineda et al. (2012), McInnes et al. (2003), Harnett et al. (2008)

DBA/J1 mice with collagen-induced arthritis (CIA) diminished the severity and incidence of CIA symptoms (Carranza et al. 2012). The therapeutic effect correlated with significantly lower levels of IL-17 and IFN-γ but enhanced production of TGF-β and IL-10 from draining lymph node cells. The authors showed that the improvement of the disease upon the transfer of helminth extract-stimulated DCs could be due to the action of regulatory T cells and TGF-β.

Apart from flukes, a tapeworm species *Hymenolepis diminuta* exerted anti-arthritic effects in CFA-injected mice. This required a viable infection and was found to be dependent on adaptive immunity, as infection with *H. diminuta* did not protect mice lacking T cells and B cells or the IL-4 receptor α chain (Shi et al. 2011).

In addition to Platyhelminthes that have been shown to reduce arthritis, two nematode species, namely, *H. polygyrus* and *Nippostrongylus brasiliensis*, were reported for their beneficial effects in protecting MRL/lpr mice from spontaneously developing an autoimmune disease affecting joints (Salinas-Carmona et al. 2009).

The *A. viteae*-derived molecule, ES-62, was found to inhibit priming and polarization of IL-17 responses in CIA by targeting a complex IL-17-producing network, involving signaling between dendritic cells and γ/δ or CD4+ T cells (Pineda et al. 2012). This recent paper confirms and expands initial observation of the protective effects of ES-62 in the CIA model that, at that time, was mostly shown to correlate with inhibition of collagen-specific pro-inflammatory/Th1 cytokine (TNF-α, IL-6, and IFN-γ) release (McInnes et al. 2003). In the more recent study, bone marrow-derived DCs from healthy DBA/1 mice and mice with CIA

pretreated with ES-62 before being matured with LPS showed significant downregulation of the pro-inflammatory cytokine TNF-α and two other cytokines that are involved in the polarization and maintenance of Th17 cells, IL-6 and IL-23 accordingly. Consistent with these findings, ES-62-treated DCs showed a reduced ability to skew naive OVA-specific T cells toward a Th17 phenotype in vitro (Pineda et al. 2012). Interestingly and in addition to the effects of ES-62 on DCs, this molecule could also directly target in vitro-differentiated Th17 cells to produce lower levels of IL-17. The anti-inflammatory actions of ES-62 in CIA appear to be dependent on the PC moiety as indicated by the reduction in severity of disease and also suppression of collagen-specific T-helper 1 cytokine production observed when testing PC conjugated to the carrier protein ovalbumin (Harnett et al. 2008).

In another set of studies, an extract from *A. suum*, given orally, protected from arthritis severity in CIA and also zymosan-induced arthritis (ZYA) (Rocha et al. 2008).

16.4 Worms and Multiple Sclerosis

Similar to the studies discussed in the previous paragraphs, different *Schistosome* species and their products have been used intensely to study the impact of helminths on multiple sclerosis (Table 16.3). In the early studies performed by La Flamme et al. (2003), it was shown that *S. mansoni* significantly reduced the incidence and delayed the onset of experimental autoimmune encephalomyelitis (EAE) in C57BL/6J mice immunized with myelin oligodendrocyte glycoprotein (MOG) (35–55) peptide. Analysis of cytokine production revealed lowered levels of IFN-γ and TNF-α as well as nitric oxide in the helminth-treated groups of mice. In the subsequent study, it was shown that immunization with *S. mansoni* eggs decreased the severity of EAE as measured by decreased clinical scores and CNS cellular infiltrates (Sewell et al. 2003). Disease suppression in this case was associated with decreased IFN-γ and increased IL-4, TGF-β, and IL-10 in the periphery and enhanced percentage of IL-4-producing autoantigen-specific T cells in the brain. Importantly, the authors also showed that the helminth-induced protection from EAE could only be achieved in Stat6-sufficient mice, which points toward the involvement of the Th2 environment in this process.

More recently, SEA from *S. japonicum* was shown to prevent EAE while downregulating IFN-γ and/or increasing IL-4 levels (Zheng et al. 2008). Subsequent studies by Correale and Farez (2009) shed light on how SEA modulates phenotype and effector functions of DCs and B cells isolated from patients with MS. Namely, SEA suppressed the LPS-induced DCs' production of pro-inflammatory cytokines and enhanced TGF-β and IL-10 production. In addition, it also diminished LPS-induced expression of co-stimulatory molecules. The effect of SEA was mediated via regulation of TLR2 and ERK1/2 MAP kinase signaling.

Table 16.3 Helminth species and their products displaying beneficial effect on the course of experimental multiple sclerosis

Helminth species	Infection/Antigen/ Cells	Disease model	Reference
Schistosoma mansoni	Infection	Experimental autoimmune encephalomyelitis	La Flamme et al. (2003)
	Injection of eggs	Experimental autoimmune encephalomyelitis	Sewell et al. (2003)
Schistosoma japonicum	Egg antigens	Experimental autoimmune encephalomyelitis	Zheng et al. (2008)
Fasciola hepatica	Infection	Experimental autoimmune encephalomyelitis	Walsh et al. (2009)
Taenia crassiceps	Infection	Experimental autoimmune encephalomyelitis	Reyes et al. (2011)
Trichinella spiralis	Infection	Experimental autoimmune encephalomyelitis	Gruden-Movsesijan et al. (2008)
	Soluble products	Experimental autoimmune encephalomyelitis	Kuijk et al. (2012)
	Excretory-secretory products	Experimental autoimmune encephalomyelitis	Sofronic-Milosavljevic et al. (2013)
Trichinella pseudospiralis	Infection	Experimental autoimmune encephalomyelitis	Wu et al. (2010)
Strongyloides venezualensis	Infection	Experimental autoimmune encephalomyelitis	Chiuso-Minicucci et al. (2011)
Heligmosomoides polygyrus	Infection	Experimental autoimmune encephalomyelitis	Donskow-Łysoniewska et al. (2012a)
	B cells from helminth-infected mice	Experimental autoimmune encephalomyelitis	Wilson et al. (2010)

The immunomodulatory effect of helminth products on DC function and its importance in ameliorating EAE was also studied by Sofronic-Milosavljevic et al. (2013). They showed that DCs stimulated with excretory–secretory products released from encysted muscle larvae of *T. spiralis* (ES L1) and transferred into rats with EAE ameliorated the disease symptoms. Increased production of IL-4, IL-10, and TGF-β and decreased production of IFN-γ and IL-17 were observed. This study is a follow-up on the initial observation performed by the group that infection with *T. spiralis* L1 stage muscle larvae (TSL1) reduced the severity of the autoimmune disease as judged by lower maximal clinical score, cumulative index, duration of illness, and degree of mononuclear cell infiltration in *T. spiralis*-infected animals compared to the control, EAE-induced group (Gruden-Movsesijan et al. 2008). A close relative of *T. spiralis*, *T. pseudospiralis* was also shown to be able to suppress EAE by reducing the inflammatory infiltration in CNS, and this is likely associated with the inhibition of Th17 and Th1 responses by the infection (Wu et al. 2010). Interestingly, also in this case, the beneficial effects of the parasite correlated with enhanced Th2 responses in the EAE-suffering mice.

In agreement with the above-described studies is the publication by Kuijk et al. (2012). Similar to the previous observations, treatment of mice with EAE with soluble products from *T. spiralis* resulted in significant suppression of the disease symptoms. The same effects could be achieved when the mice were injected with *Trichuris suis* soluble extract.

H. polygyrus is another nematode species that has been shown to reduce the symptoms of EAE (Donskow-Łysoniewska et al. 2012a). A potential mechanism for the therapeutic effects of *H. polygyrus* in EAE as well as in asthma might involve induction of a functionally distinct, to naïve mice, population of B cells that when transferred into the EAE-suffering mice reduced the disease symptoms in an IL-10-independent manner (Wilson et al. 2010).

Apart from different nematode species shown to improve the course of EAE, some *Platyhelminthes* have been shown to share this ability. For example, infection with *T. crassiceps* reduced the severity of EAE with concomitant downregulation of IL-17 and TNF-α and upregulation of IL-4 and IL-10 (Reyes et al. 2011). Also, *F. hepatica* infection attenuated the clinical signs of EAE, and this effect correlated with the suppression of Th1 and Th17 responses (Walsh et al. 2009). The beneficial effect of *F. hepatica* infection was also present when IL-10-deficient mice were infected with the parasite. The effect however was reversed when EAE-suffering mice infected with the helminth were treated with neutralizing anti-TGF-β antibodies, providing strong evidence for the involvement of TGF-β rather than IL-10 in the modulation of the autoimmune disease.

16.5 Worms and Type 1 Diabetes

As early as 1999, Anne Cooke showed that infection with *S. mansoni* or application of parasite eggs alone significantly decreased the spontaneous incidence of insulin-dependent diabetes mellitus in NOD mice (Table 16.4). Later on, it was shown that soluble extracts of *S. mansoni* worms or eggs completely prevented the onset of type 1 diabetes in these mice (Zaccone et al. 2003). SEA in this model acted to induce functional changes in antigen presenting cells and expand Th2 cells and T regulatory cells (Zaccone et al. 2009, 2010). Subsequently, one of the major glycoproteins present in SEA known as ω-1 was shown to condition DCs to drive Th2 responses and induces Foxp3 T cells from NOD mouse naïve T cells (Zaccone et al. 2011). This raises a possibility that a single helminth molecule acting by multiple mechanisms can inhibit onset of diabetes in NOD mice and as such might be a strong candidate for therapeutic modulation of autoimmunity.

Recently another SEA-derived molecule, lacto-N-fucopentaose III (LNFPIII), a Lewis(X)-containing immunomodulatory glycan, was reported to improve glucose tolerance and insulin sensitivity in diet-induced obese mice (Bhargava et al. 2012).

In a model of multiple low-dose streptozotocin-induced diabetes, *T. crassiceps*-infected mice had lower blood glucose levels throughout the study, no insulitis, and normal insulin content in the pancreas. In terms of immunological parameters,

Table 16.4 Helminth species and their products displaying beneficial effect on the course of experimental type 1 diabetes

Helminth species	Infection/ Antigen/ Cells	Disease model	Reference
Schistosoma mansoni	Infection	Non-obese diabetic mice	Cooke et al. (1999)
	Soluble products	Non-obese diabetic mice	Zaccone et al. (2003)
	Egg antigens	Non-obese diabetic mice	Zaccone et al. (2009), Zaccone et al. (2010)
	Antigen: ω-1	Non-obese diabetic mice	Zaccone et al. (2011)
	Antigen: LNFPIII	Diet-induced obese mice	Bhargava et al. (2012)
Taenia crassiceps	Infection	Multiple low dose streptozotocin-induced diabetes	Espinoza-Jiménez et al. (2010)
Heligmosomoides polygyrus	Infection	Non-obese diabetic mice	Saunders et al. (2007), Liu et al. (2009), Mishra et al. (2013)
Trichinella spiralis	Infection	Non-obese diabetic mice	Saunders et al. (2007)
Litomosoides sigmodontis	Infection	Non-obese diabetic mice	Hübner et al. (2009), Hübner et al. (2012)
Strongyloides venezualensis	Infection	streptozotocin-induced diabetes	Peres et al. (2013)

helminth infection induced greater numbers of alternatively activated macrophages and IL-4 levels than found in uninfected mice, with no increase of regulatory T cells (Espinoza-Jiménez et al. 2010). In the same model of type 1 diabetes, immunization with soluble *Strongyloides venezuelensis* antigen in complete Freund's adjuvant followed by infection with the parasite protected mice from developing the disease (Peres et al. 2013).

Infection with live *L. sigmodontis* protected NOD mice against diabetes development, and the protection correlated with upregulated IL-4, IL-5, and insulin-specific IgG1 antibodies as well as increased numbers of splenic CD4+ CD25+ Foxp3 T cells suggesting a shift toward Th2/T regulatory-type immune responses (Hübner et al. 2009). These findings were further explored using IL-4-deficient NOD mice (Hübner et al. 2012). These mice failed to generate the shift toward Th2 immunity after infection but were also protected from the disease arguing that the presence of type 2 cytokines and antibodies might be an epiphenomenon and does not play an active role in dampening diabetes in this model. Both strains of NOD mice, IL-4-deficient and non-manipulated, when infected with the helminth upregulated frequencies and numbers of regulatory T cells and continuous depletion of TGF-β but not IL-10, prevented the beneficial effect of *L. sigmodontis* in the diabetes model. In a similar fashion, neither the in vivo depletion of CD4+ CD25+ T cells nor blocking of IL-10 signaling affected *H. polygyrus*-induced protection

against type I diabetes (Liu et al. 2009). Using the same model system, Mishra et al. (2013) showed that *H. polygyrus* inoculation of NOD and NOD-IL4$^{-/-}$ mice markedly downregulated the development of type I diabetes, pancreatic β-cell destruction, and components of the Th1-type inflammatory immune response. This once again shows that there is no absolute requirement for IL-4 in the helminth-mediated amelioration of diabetes in NOD mice. However, contrary to the previous publication, which dismissed the role of IL-10 in the process, IL-10 blockade in NOD-IL-4-deficient mice inhibited *H. polygyrus*-induced prevention of type I diabetes, but not in NOD-IL-4-sufficient strain (Mishra et al. 2013). This suggests that in the absence of a Th2-type response, IL-10 can still be induced and have potent inhibitory effects on pancreatic β-cell destruction and type I diabetes development.

16.6 Worms and Inflammatory Bowel Disease

Reardon et al. in 2001 published one of the first reports describing beneficial effects of helminths on colitis (Table 16.5). In this study, mice were infected with *H. diminuta* and colitis was provoked by administration of DSS in the drinking water. Infected mice had reduced colitis-induced abnormalities in epithelial ion transport, which suggested that helminths indeed might confer protection in the colitic mice. The same parasite was later used prophylactically and therapeutically in mice with DNBS-induced colitis and was shown to protect from the disease in both models as measured by reduced clinical disease, histological damage score, and myeloperoxidase levels (Hunter et al. 2005). Mechanistically, it was shown that the protective effect of *H. diminuta* in this model depended on IL-10 and did not predispose to enhanced enteric sensitivity to a third-party antigen which suggested perhaps that there is minimal risk of side effects associated with potential application of helminths to colitic patients. More recently, *H. diminuta* was shown to be superior to dexamethasone in preventing DNBS-induced colitis and did not result in additional side effects (i.e., collagen deposition) (Melon et al. 2010). Either a high molecular mass fraction of adult *H. diminuta* or excretory/secretory products, reduced macrophage production of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α after LPS challenge and injection of the fraction into the colitic mice caused less inflammatory disease (Johnston et al. 2010).

Schistosome species have been intensely tested in colitis models. For example, *S. mansoni* egg exposure attenuated TNBS-induced colitis and protected mice from lethal inflammation (Elliott et al. 2003). Protected mice showed reduced production of colonic IFN-γ but increased levels of IL-4 and IL-10, and the therapeutic effect was dependent on Stat-6 signaling. In a similar manner, injection of TNBS-treated mice with *S. japonicum* eggs reduced the inflammation in the colon and suppressed IFN-γ levels, while IL-4, IL-5, and IL-10 cytokines were increased (Mo et al. 2007). In this report, the percentage of regulatory T cells was shown to increase in the colitis-protected mice; however, their involvement in the parasite-induced

Table 16.5 Helminth species and their products displaying beneficial effect on the course of experimental inflammatory bowel disease

Helminth species	Infection/Antigen/Cells	Disease model	Reference
Schistosoma mansoni	Injection of eggs	TNBS colitis	Elliott et al. (2003)
	Infection	DSS-induced colitis	Smith et al. (2007), Bodammer et al. (2011)
	Soluble products	TNBS colitis	Ruyssers et al. (2009), Ruyssers et al. (2010)
Schistosoma japonicum	Injection of eggs	TNBS colitis	Mo et al. (2007), Mo et al. (2007), Zhao et al. (2009), Xia et al. (2011)
Hymenolepis diminuta	Infection	DSS-induced colitis	Reardon et al. (2001)
	Infection	DNBS colitis	Hunter et al. (2005), Melon et al. (2010)
	Soluble products	DNBS colitis	Johnston et al. (2010)
Trichinella spiralis	Infection	DSS-induced colitis	Khan et al. (2002)
	Soluble products	DNBS colitis	Motomura et al. (2009)
	Recombinant product: rTsP3	TNBS colitis	Du et al. (2011)
Heligmosomoides polygyrus	Infection	Piroxicam-induced colitis	Elliott et al. (2004)
	CD8+ T cell from infected mice	Piroxicam-induced colitis	Metwali et al. (2006)
	Infection	TNBS colitis	Setiawan et al. (2007), Sutton et al. (2008)
		Colitic IL-10-deficient mice	Elliott et al. (2008)
	Infection	Rag IL-10-/- transfer model of colitis	Hang et al. (2010), Blum et al. (2012)
	Infection	pan-enterocilitis triggered by feeding with ovalbumin	Leung et al. (2012)
	Infection	DSS-induced colitis	Donskow-Łysoniewska et al. (2012b)
Acanthocheilonema viteae	Recombinant product: rAv-17	DSS-induced colitis	Schnoeller et al. (2008)
Ancylostoma caninum	Soluble products	TNBS colitis	Ruyssers et al. (2009)
	Excretory-secretory products	DSS-induced colitis	Ferreira et al. (2013)
Ancylostoma ceylanicum	Soluble products	DSS-induced colitis	Cançado et al. (2011)
	Excretory-secretory products	DSS-induced colitis	Cançado et al. (2011)

(continued)

Table 16.5 (continued)

Helminth species	Infection/Antigen/Cells	Disease model	Reference
Anisakis simplex	Recombinant product: macrophage migration inhibitory factor-like protein	DSS-induced colitis	Cho et al. (2011)

protection from colitis was not assessed. Zhao et al. (2009) reported similar findings indicating that *S. japonicum* eggs could prevent TNBS colitis. In addition to decreased IFN-γ and increased IL-4 and IL-10, helminth egg-treated mice showed decreased expression of TLR4 and reduced intestinal bacterial translocation. These data are in tune with a more recent publication that showed that *S. japonicum* eggs maintained epithelial barrier function through increasing tight junction proteins, thus causing less exposure of NOD2 (intracellular pattern recognition receptors which recognize a peptidoglycan constituent of bacteria) to the luminal antigens which may activate a series of inflammatory factors and induce colitis (Xia et al. 2011). Active infection with *S. mansoni* has also been shown to make DSS-exposed mice refractory to colitis via a novel mechanism dependent on macrophages rather than by simple modulation of Th2 responses, or via induction of regulatory CD4+ or CD25+ cells, IL-10, or TGF-β (Smith et al. 2007). Soluble proteins from *S. mansoni* can also reverse intestinal inflammation as shown in mice with TNBS-induced colitis. This positive effect driven by helminth proteins correlated with decreased pro-inflammatory cytokine production (IFN-γ and IL-17) and increased anti-inflammatory cytokines (IL-10 and TGF-β) (Ruyssers et al. 2009). In addition, there was evidence that *S. mansoni* proteins also ameliorated motility disturbances during murine colitis (Ruyssers et al. 2010).

Prior infection with *T. spiralis* also reduced the severity of colitis together with a decreased mortality in mice and was correlated with a downregulation of MPO activity, Th1-type cytokine expression in colonic tissue, and emergence of a Th2-type immune response (Khan et al. 2002). In a subsequent study, *T. spiralis* antigens were assessed for their ability to modify intestinal inflammation in mice and were shown to reduce the severity of the disease (Motomura et al. 2009). One of the *T. spiralis* proteins in a recombinant form known as rTsP53 was shown to ameliorate TNBS-induced colitis in mice (Du et al. 2011). A similar effect was attributed to a secreted protease inhibitor of filarial nematodes that modulated macrophage-mediated inflammation in a murine model of DSS-induced colitis (Schnoeller et al. 2008). Also, a recombinant protein type II MIF (As-MIF) from *Anisakis simplex* 3rd stage larvae was found to ameliorate DSS-induced colitis (Cho et al. 2011).

Therapeutic potential of adult hookworm, *Ancylostoma ceylanicum*, and also crude and excretory-secretory products was shown in DSS-colitis (Cançado et al. 2011). Similar to previous observations, treatment with the helminth decreased production of Th1 and Th17 cytokines in the inflamed colon. This protective effect of hookworms was confirmed in a subsequent publication that

showed a beneficial role of excretory-secretory products of *A. caninum* (Ferreira et al. 2013). Interestingly, diminishing protein activity within this antigenic mixture resulted in loss of anti-colitic effect and reversed helminth product-induced upregulation of a CD4+ IL-4+ IL-10+ cell population.

H. polygyrus has been widely used in the studies investigating the potential of helminths to influence experimental colitis. A paper by Elliott et al. (2004) showed that *H. polygyrus* inhibited ongoing piroxicam-induced colitis in IL-10-deficient mice in part through blocking mucosal Th1 cytokine production and that resolution of inflammation was associated with increased IL-13 production and could be adoptively transferred by MLN T cells. In addition to dampening down the Th1 arm of immunity, colonization of colitic IL-10-deficient mice with *H. polygyrus* also suppressed lamina propria mononuclear cell-derived IL-17 production, another pathogenic cytokine in this model (Elliott et al. 2008).

In a similar model of colitis, it was shown that the worm could reverse piroxicam-induced gut inflammation in Rag mice (T and B cell deficient) reconstituted with IL-10-deficient T cells (Metwali et al. 2006). It appears that in this model, *H. polygyrus* induces regulatory CD8+ lamina propria T cells that are potent suppressors of T cell proliferation. Interestingly, these regulatory cells were shown to act independently of IL-10 and TGF-β signaling; however, their mechanism of action has not been addressed so far. In the Rag IL-$10^{-/-}$ T cell transfer model of colitis, *H. polygyrus* prevented and reversed intestinal inflammation with concomitant downregulation of IFN-γ and IL-17 responses (Hang et al. 2010; Leung et al. 2012). In this model, the worm infection changed the phenotype of lamina propria DCs from Rag mice such that the cells displayed lower expression levels of CD80 and CD86, heightened levels of plasmacytoid DC marker Ag-1 and CD40, and impaired ability to present antigen to antigen-specific T cells. This impact of *H. polygyrus* on DC functions was further investigated in a paper published by Blum et al. (2012). These authors showed that intestinal DCs isolated from *H. polygyrus*-infected Rag mice blocked antigen-specific production of IFN-γ/IL-17 from lamina propria mononuclear cells in vitro. More importantly, transfer of the worm-primed DCs into Rag mice reconstituted with IL-10-deficient T cells protected animals from colitis.

H. polygyrus also blocked colitis in the TNBS-treated mice by decreasing Th1 and increasing Th2 cytokines (Setiawan et al. 2007). Blocking of IL-10 signaling in vitro restored Th1 cytokine secretion from lamina propria mononuclear cells (LPMC), whereas in vivo intervention worsened colitis in *H. polygyrus*-infected mice.

16.7 Conclusions

As can be seen in this book chapter, there is an abundance of experimental data confirming the potential of helminths to treat asthma, rheumatoid arthritis, inflammatory bowel disease, type 1 diabetes, and multiple sclerosis. Interestingly, some

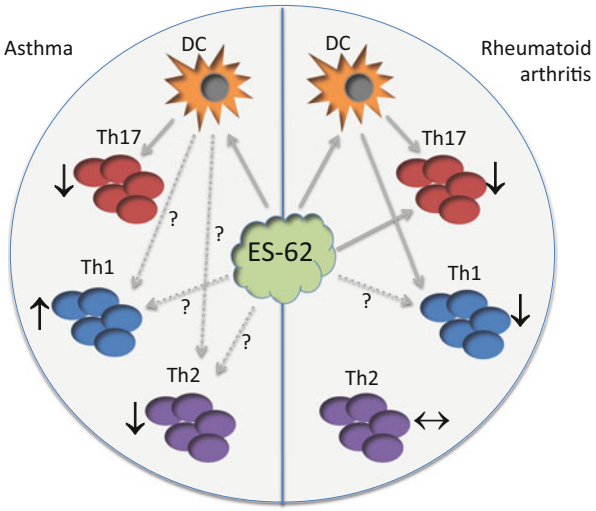

Fig. 16.1 ES-62-mediated immune regulation that confers protection of mice from experimentally induced asthma and rheumatoid arthritis. ES-62 alters the potential of dendritic cells (DCs) to prime naïve CD4+ T cells to differentiate into Th17 cells that are pathogenic in both disease models (IL-17 released by Th17 cells is associated with increased numbers of neutrophils in afflicted tissue; application of ES-62 therefore leads to decreased numbers of these cells in the asthmatic lungs and arthritic joints). ES-62 can also act directly on Th17 cells via TLR4 to attenuate IL-17 release in vitro. Interestingly, ES-62 has opposing effects on Th1 cells depending on the immunological context – Th1 cells are pathogenic in RA and in this model ES-62 down-regulates IFNγ-producing cells; in asthma, on the other hand, Th1 cells might counterbalance pathogenic Th2 responses and ES-62 in this model leads to enhanced numbers of IFNγ-positive cells. Neutralization of IFNγ in the asthma model reversed the protection afforded by ES-62 and increased frequencies of Th2 and Th17 cells. Th2 cells are not increased in the RA model upon injection with ES-62, whereas, as suggested earlier, ES-62 reduces numbers of Th2 cells in the model of asthma

species of helminths or their products can be effective against more than one disease. ES-62, for example, is effective against asthma and rheumatoid arthritis, two types of inflammatory disorders whose pathologies are shaped by different arms of immunity (Fig. 16.1). This implies that there might be one mechanism, not necessarily dependent on regulatory T cells as these are not upregulated by ES-62, that can bring the right balance to the deregulated immune responses in different types of inflammatory disorders. Alternatively, worms or their molecules can exert multiple mechanisms simultaneously or depending on the disease context that ultimately protect from a range of inflammatory disorders. Further work is needed in this area, but based on the literature review as presented in this book chapter, the following patterns of helminth-induced immunoregulation emerge with respect to the different disease conditions:

Asthma: helminths improve asthma-like disease in mice by decreasing the Th2 type of immune response via employment of regulatory T and B cell populations; in addition some helminth molecules such as PAS-1 from *A. suum*, ES-62 from

A. viteae, and filarial cystatin can counteract allergic immune responses by increasing the IFN-γ axis and thus resetting the Th1/Th2 balance.

Arthritis: improvement of arthritis by helminths is mostly associated with a decreased ability of DCs to prime for pathogenic Th1/Th17 responses.

Multiple sclerosis: therapeutic potential of helminths in the murine model of multiple sclerosis correlates with decreased Th1/Th17 and upregulated Th2/Tregs axes.

Type I diabetes: symptoms of type 1 diabetes and appearance of pathogenic Th1 cells are reduced by helminths via induction of regulatory T lymphocytes.

IBD: helminths protect mice from experimentally induced IBD by decreasing the potential of DCs to prime Th1/Th17 responses and inducing Tregs. In addition helminths seem to alter the composition of gut microbiota and shield the immune system from being exposed to bacterial products by improving mucosal barrier functions.

Considerable progress has been made in elucidating the beneficial effects of different helminth species and helminth product(s) on the course of inflammatory disorders. In the near future, we should find out if any of the helminth-based therapies have found their way into the clinic. The need for new solutions to treat inflammatory diseases is so great that some UK patients with Crohn's disease are sourcing helminths in an attempt to relieve the disease symptoms that could not be treated by commercially available drugs (Flowers and Hopkins 2013). Certainly, initial trials conducted by Summers et al. (2005a, b) showed that ingestion of live eggs from *T. suis* reduced symptoms of Crohn's disease and ulcerative colitis in the studied group of patients, and more recently, it was shown that such treatment is well tolerated and did not result in short- or long-term treatment-related side effects (Sandborn et al. 2013). In addition, more trials using *T. suis* eggs to treat different inflammatory disorders are planned or undergoing across the world (Weinstock 2012).

References

Allen JE, Maizels RM (2011) Diversity and dialogue in immunity to helminths. Nat Rev Immunol 11:375–88

Amu S, Saunders SP, Kronenberg M, Mangan NE, Atzberger A, Fallon PG (2010) Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. J Allergy Clin Immunol 125:1114–1124

Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC (2007) Protective immune mechanisms in helminth infection. Nat Rev Immunol 7:975–87

Aranzamendi C, Fransen F, Langelaar M, Franssen F, van der Ley P, van Putten JP et al (2012) Trichinella spiralis-secreted products modulate DC functionality and expand regulatory T cells in vitro. Parasite Immunol 34:210–23

Aranzamendi C, de Bruin A, Kuiper R, Boog CJ, van Eden W, Rutten V et al (2013) Protection against allergic airway inflammation during the chronic and acute phases of Trichinella spiralis infection. Clin Exp Allergy 43:103–15

Araújo CA, Perini A, Martins MA, Macedo MS, Macedo-Soares MF (2008) PAS-1, a protein from Ascaris suum, modulates allergic inflammation via IL-10 and IFN-gamma, but not IL-12. Cytokine 44:335–41

Bager P, Vinkel Hansen A, Wohlfahrt J, Melbye M (2012) Helminth infection does not reduce risk for chronic inflammatory disease in a population-based cohort study. Gastroenterology 142: 55–62

Bhargava P, Li C, Stanya KJ, Jacobi D, Dai L, Liu S et al (2012) Immunomodulatory glycan LNFPIII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. Nat Med 18:1665–72

Blum AM, Hang L, Setiawan T, Urban JP Jr, Stoyanoff KM, Leung J et al (2012) Heligmosomoides polygyrus bakeri induces tolerogenic dendritic cells that block colitis and prevent antigen-specific gut T cell responses. J Immunol 189:2512–20

Bodammer P, Waitz G, Loebermann M, Holtfreter MC, Maletzki C, Krueger MR et al (2011) Schistosoma mansoni infection but not egg antigen promotes recovery from colitis in outbred NMRI mice. Dig Dis Sci 56:70–8

Cançado GG, Fiuza JA, de Paiva NC, Lemos Lde C, Ricci ND, Gazzinelli-Guimarães PH et al (2011) Hookworm products ameliorate dextran sodium sulfate-induced colitis in BALB/c mice. Inflamm Bowel Dis 17:2275–86

Cardoso LS, Oliveira SC, Góes AM, Oliveira RR, Pacífico LG, Marinho FV et al (2010) Schistosoma mansoni antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation. Clin Exp Immunol 160:266–74

Carranza F, Falcón CR, Nuñez N, Knubel C, Correa SG, Bianco I et al (2012) Helminth antigens enable CpG-activated dendritic cells to inhibit the symptoms of collagen-induced arthritis through Foxp3+ regulatory T cells. PLoS One 7:e40356

Chiuso-Minicucci F, VAN DB, Zorzella-Pezavento SF, Peres RS, Ishikawa LL, Rosa LC et al (2011) Experimental autoimmune encephalomyelitis evolution was not modified by multiple infections with Strongyloides venezuelensis. Parasite Immunol 33(5):303–8

Cho MK, Lee CH, Yu HS (2011) Amelioration of intestinal colitis by macrophage migration inhibitory factor isolated from intestinal parasites through toll-like receptor 2. Parasite Immunol 33:265–75

Cooke A, Tonks P, Jones FM, O'Shea H, Hutchings P, Fulford AJ et al (1999) Infection with Schistosoma mansoni prevents insulin dependent diabetes mellitus in non-obese diabetic mice. Parasite Immunol 21:169–76

Correale J, Farez M (2007) Association between parasite infection and immune responses in multiple sclerosis. Ann Neurol 61:97–108

Correale J, Farez M (2009) Helminth antigens modulate immune responses in cells from multiple sclerosis patients through TLR2-dependent mechanisms. J Immunol 183:5999–6012

Correale J, Farez MF (2011) The impact of parasite infections on the course of multiple sclerosis. J Neuroimmunol 233:6–11

Daniłowicz-Luebert E, Steinfelder S, Kühl AA, Drozdenko G, Lucius R, Worm M et al (2013) A nematode immunomodulator suppresses grass pollen-specific allergic responses by controlling excessive Th2 inflammation. Int J Parasitol 43:201–10

Dittrich AM, Erbacher A, Specht S, Diesner F, Krokowski M, Avagyan A et al (2008) Helminth infection with Litomosoides sigmodontis induces regulatory T cells and inhibits allergic sensitization, airway inflammation, and hyperreactivity in a murine asthma model. J Immunol 180:1792–9

Donskow-Łysoniewska K, Krawczak K, Doligalska M (2012a) Heligmosomoides polygyrus: EAE remission is correlated with different systemic cytokine profiles provoked by L4 and adult nematodes. Exp Parasitol 132:243–8

Donskow-Łysoniewska K, Majewski P, Brodaczewska K, Jóźwicka K, Doligalska M (2012b) Heligmosmoides polygyrus fourth stages induce protection against DSS-induced colitis and change opioid expression in the intestine. Parasite Immunol 34:536–46

Du L, Tang H, Ma Z, Xu J, Gao W, Chen J et al (2011) The protective effect of the recombinant 53-kDa protein of Trichinella spiralis on experimental colitis in mice. Dig Dis Sci 56:2810–7

Elliott DE, Weinstock JV (2012) Helminth-host immunological interactions: prevention and control of immune-mediated diseases. Ann N Y Acad Sci 1247:83–96

Elliott DE, Li J, Blum A, Metwali A, Qadir K, Urban JF Jr et al (2003) Exposure to schistosome eggs protects mice from TNBS-induced colitis. Am J Physiol Gastrointest Liver Physiol 284: G385–91

Elliott DE, Setiawan T, Metwali A, Blum A, Urban JF Jr, Weinstock JV (2004) Heligmosomoides polygyrus inhibits established colitis in IL-10-deficient mice. Eur J Immunol 34:2690–8

Elliott DE, Metwali A, Leung J, Setiawan T, Blum AM, Ince MN et al (2008) Colonization with Heligmosomoides polygyrus suppresses mucosal IL-17 production. J Immunol 181:2414–9

Espinoza-Jiménez A, Rivera-Montoya I, Cárdenas-Arreola R, Morán L, Terrazas LI (2010) Taenia crassiceps infection attenuates multiple low-dose streptozotocin-induced diabetes. J Biomed Biotechnol 2010:850541

Ferreira I, Smyth D, Gaze S, Aziz A, Giacomin P, Ruyssers N et al (2013) Hookworm excretory/secretory products induce interleukin-4 (IL-4)+ IL-10+ CD4+ T cell responses and suppress pathology in a mouse model of colitis. Infect Immun 81:2104–11

Flowers S, Hopkins M (2013) Autoimmune disease: patients self-treat with parasitic worms. Nature 493:163

Grainger JR, Smith KA, Hewitson JP, McSorley HJ, Harcus Y, Filbey KJ et al (2010) Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway. J Exp Med 207:2331–41

Gruden-Movsesijan A, Ilic N, Mostarica-Stojkovic M, Stosic-Grujicic S, Milic M, Lj S-M (2008) *Trichinella spiralis*: modulation of experimental autoimmune encephalomyelitis in DA rats. Exp Parasitol 118:641–647

Gruden-Movsesijan A, Ilic N, Mostarica-Stojkovic M, Stosic-Grujicic S, Milic M, Sofronic-Milosavljevic L (2010) Mechanisms of modulation of experimental autoimmune encephalomyelitis by chronic Trichinella spiralis infection in Dark Agouti rats. Parasite Immunol 32: 450–9

Hamid F, Wiria AE, Wammes LJ, Kaisar MM, Djuardi Y, Versteeg SA, Wahyuni S, van Ree R, Sartono E, Supali T, Yazdanbakhsh M (2013) Risk Factors Associated with the Development of Atopic Sensitization in Indonesia. PLoS One 8:e67064

Hang L, Setiawan T, Blum AM, Urban J, Stoyanoff K, Arihiro S et al (2010) Heligmosomoides polygyrus infection can inhibit colitis through direct interaction with innate immunity. J Immunol 185:3184–9

Harnett W, Harnett MM (2010) Helminth-derived immunomodulators: can understanding the worm produce the pill? Nat Rev Immunol 10:278–84

Harnett MM, Kean DE, Boitelle A, McGuiness S, Thalhamer T, Steiger CN et al (2008) The phosphorycholine moiety of the filarial nematode immunomodulator ES-62 is responsible for its anti-inflammatory action in arthritis. Ann Rheum Dis 67:518–23

Hartmann S, Schnoeller C, Dahten A, Avagyan A, Rausch S, Lendner M et al (2009) Gastrointestinal nematode infection interferes with experimental allergic airway inflammation but not atopic dermatitis. Clin Exp Allergy 39:1585–96

Hayes KS, Bancroft AJ, Grencis RK (2004) Immune-mediated regulation of chronic intestinal nematode infection. Immunol Rev 201:75–88

He Y, Li J, Zhuang W, Yin L, Chen C, Li J et al (2010) The inhibitory effect against collagen-induced arthritis by Schistosoma japonicum infection is infection stage-dependent. BMC Immunol 11:28

Hübner MP, Stocker JT, Mitre E (2009) Inhibition of type 1 diabetes in filaria-infected non-obese diabetic mice is associated with a T helper type 2 shift and induction of FoxP3+ regulatory T cells. Immunology 127:512–22

Hübner MP, Shi Y, Torrero MN, Mueller E, Larson D, Soloviova K et al (2012) Helminth protection against autoimmune diabetes in nonobese diabetic mice is independent of a type 2 immune shift and requires TGF-β. J Immunol 188:559–68

Hunter MM, Wang A, Hirota CL, McKay DM (2005) Neutralizing anti-IL-10 antibody blocks the protective effect of tapeworm infection in a murine model of chemically induced colitis. J Immunol 174:7368–75

Itami DM, Oshiro TM, Araujo CA, Perini A, Martins MA, Macedo MS et al (2005) Modulation of murine experimental asthma by Ascaris suum components. Clin Exp Allergy 35:873–9

Johnston MJ, Wang A, Catarino ME, Ball L, Phan VC, MacDonald JA et al (2010) Extracts of the rat tapeworm, Hymenolepis diminuta, suppress macrophage activation in vitro and alleviate chemically induced colitis in mice. Infect Immun 78:1364–75

Kabeerdoss J, Pugazhendhi S, Subramanian V, Binder HJ, Ramakrishna BS (2011) Exposure to hookworms in patients with Crohn's disease: a case-control study. Aliment Pharmacol Ther 34:923–30

Khan WI, Blennerhasset PA, Varghese AK, Chowdhury SK, Omsted P, Deng Y et al (2002) Intestinal nematode infection ameliorates experimental colitis in mice. Infect Immun 70: 5931–7

Kitagaki K, Businga TR, Racila D, Elliott DE, Weinstock JV, Kline JN (2006) Intestinal helminths protect in a murine model of asthma. J Immunol 177:1628–35

Kuijk LM, Klaver EJ, Kooij G, van der Pol SM, Heijnen P, Bruijns SC et al (2012) Soluble helminth products suppress clinical signs in murine experimental autoimmune encephalomyelitis and differentially modulate human dendritic cell activation. Mol Immunol 51:210–8

La Flamme AC, Ruddenklau K, Bäckström BT (2003) Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. Infect Immun 71:4996–5004

Lee KH, Park HK, Jeong HJ, Park SK, Lee SJ, Choi SH et al (2008) Immunization of proteins from Toxascaris leonina adult worm inhibits allergic specific Th2 response. Vet Parasitol 156: 216–25

Leung J, Hang L, Blum A, Setiawan T, Stoyanoff K, Weinstock J (2012) Heligmosomoides polygyrus abrogates antigen-specific gut injury in a murine model of inflammatory bowel disease. Inflamm Bowel Dis 18:1447–55

Lima C, Perini A, Garcia ML, Martins MA, Teixeira MM, Macedo MS (2002) Eosinophilic inflammation and airway hyper-responsiveness are profoundly inhibited by a helminth (Ascaris suum) extract in a murine model of asthma. Clin Exp Allergy 32:1659–66

Liu Q, Sundar K, Mishra PK, Mousavi G, Liu Z, Gaydo A et al (2009) Helminth infection can reduce insulitis and type 1 diabetes through CD25- and IL-10-independent mechanisms. Infect Immun 77:5347–58

Liu P, Li J, Yang X, Shen Y, Zhu Y, Wang S et al (2010) Helminth infection inhibits airway allergic reaction and dendritic cells are involved in the modulation process. Parasite Immunol 32:57–66

McConchie BW, Norris HH, Bundoc VG, Trivedi S, Boesen A, Urban JF Jr et al (2006) Ascaris suum-derived products suppress mucosal allergic inflammation in an interleukin-10-independent manner via interference with dendritic cell function. Infect Immun 74:6632–41

McInnes IB, Leung BP, Harnett M, Gracie JA, Liew FY, Harnett W (2003) A novel therapeutic approach targeting articular inflammation using the filarial nematode-derived phosphorylcholine-containing glycoprotein ES-62. J Immunol 171:2127–33

McSorley HJ, O'Gorman MT, Blair N, Sutherland TE, Filbey KJ, Maizels RM (2012) Suppression of type 2 immunity and allergic airway inflammation by secreted products of the helminth Heligmosomoides polygyrus. Eur J Immunol 42:2667–82

Melendez AJ, Harnett MM, Pushparaj PN, Wong WS, Tay HK, McSharry CP et al (2007) Inhibition of Fc epsilon RI-mediated mast cell responses by ES-62, a product of parasitic filarial nematodes. Nat Med 13:1375–81

Melon A, Wang A, Phan V, McKay DM (2010) Infection with Hymenolepis diminuta is more effective than daily corticosteroids in blocking chemically induced colitis in mice. J Biomed Biotechnol 2010:384523

Metwali A, Setiawan T, Blum AM, Urban J, Elliott DE, Hang L et al (2006) Induction of CD8+ regulatory T cells in the intestine by Heligmosomoides polygyrus infection. Am J Physiol Gastrointest Liver Physiol 291:G253–9

Mishra PK, Patel N, Wu W, Bleich D, Gause WC (2013) Prevention of type 1 diabetes through infection with an intestinal nematode parasite requires IL-10 in the absence of a Th2-type response. Mucosal Immunol 6:297–308

Mo HM, Liu WQ, Lei JH, Cheng YL, Wang CZ, Li YL (2007) Schistosoma japonicum eggs modulate the activity of CD4+ CD25+ Tregs and prevent development of colitis in mice. Exp Parasitol 116:385–9

Mo HM, Lei JH, Jiang ZW, Wang CZ, Cheng YL, Li YL, Liu WQ (2008) Schistosoma japonicum infection modulates the development of allergen-induced airway inflammation in mice. Parasitol Res 103:1183–9

Motomura Y, Wang H, Deng Y, El-Sharkawy RT, Verdu EF, Khan WI (2009) Helminth antigen-based strategy to ameliorate inflammation in an experimental model of colitis. Clin Exp Immunol 155:88–95

Osada Y, Shimizu S, Kumagai T, Yamada S, Kanazawa T (2009) Schistosoma mansoni infection reduces severity of collagen-induced arthritis via down-regulation of pro-inflammatory mediators. Int J Parasitol 39:457–64

Pacífico LG, Marinho FA, Fonseca CT, Barsante MM, Pinho V, Sales-Junior PA et al (2009) Schistosoma mansoni antigens modulate experimental allergic asthma in a murine model: a major role for CD4+ CD25+ Foxp3+ T cells independent of interleukin-10. Infect Immun 77:98–107

Panda AK, Ravindran B, Das BK (2013) Rheumatoid arthritis patients are free of filarial infection in an area where filariasis is endemic: comment on the article by Pineda et al. Arthritis Rheum 65:1402–3

Park SK, Cho MK, Park HK et al (2009) Macrophage migration inhibitory factor homologs of anisakis simplex suppress Th2 response in allergic airway inflammation model via CD4 + CD25 + Foxp3+ T cell recruitment. J Immunol 182:6907–14

Park HK, Cho MK, Choi SH, Kim YS, Yu HS (2011) Trichinella spiralis: infection reduces airway allergic inflammation in mice. Exp Parasitol 127:539–44

Peres RS, Chiuso-Minicucci F, da Rosa LC, Domingues A, Zorzella-Pezavento SF, França TG et al (2013) Previous contact with Strongyloides venezuelensis contributed to prevent insulitis in MLD-STZ diabetes. Exp Parasitol 134:183–9

Pineda MA, McGrath MA, Smith PC, Al-Riyami L, Rzepecka J, Gracie JA et al (2012) The parasitic helminth product ES-62 suppresses pathogenesis in collagen-induced arthritis by targeting the interleukin-17-producing cellular network at multiple sites. Arthritis Rheum 64: 3168–78

Pinto LA, Pitrez PM, Fontoura GR, Machado DC, Jones MH, Graeff-Teixeira C et al (2004) Infection of BALB/c mice with Angiostrongylus costaricensis decreases pulmonary inflammatory response to ovalbumin. Parasite Immunol 26:151–5

Pinto LA, Dias AC, Rymer BL, Fernandes FF, Barbosa GL, Machado DC et al (2006) Effect of Angiostrongylus costaricensis extract on eosinophilic pulmonary response in BALB/c mice. Parasitol Res 98:295–8

Reardon C, Sanchez A, Hogaboam CM, McKay DM (2001) Tapeworm infection reduces epithelial ion transport abnormalities in murine dextran sulfate sodium-induced colitis. Infect Immun 69:4417–23

Reyes JL, Espinoza-Jiménez AF, González MI, Verdin L, Terrazas LI (2011) Taenia crassiceps infection abrogates experimental autoimmune encephalomyelitis. Cell Immunol 267:77–87

Rocha FA, Leite AK, Pompeu MM, Cunha TM, Verri WA Jr, Soares FM et al (2008) Protective effect of an extract from Ascaris suum in experimental arthritis models. Infect Immun 76: 2736–45

Ruyssers NE, De Winter BY, De Man JG, Loukas A, Pearson MS, Weinstock JV et al (2009) Therapeutic potential of helminth soluble proteins in TNBS-induced colitis in mice. Inflamm Bowel Dis 15:491–500

Ruyssers NE, De Winter BY, De Man JG, Ruyssers ND, Van Gils AJ, Loukas A et al (2010) Schistosoma mansoni proteins attenuate gastrointestinal motility disturbances during experimental colitis in mice. World J Gastroenterol 16:703–12

Rzepecka J, Donskow-Schmelter K, Doligalska M (2007) Heligmosomoides polygyrus infection down-regulates eotaxin concentration and CCR3 expression on lung eosinophils in murine allergic pulmonary inflammation. Parasite Immunol 29:405–13

Rzepecka J, Siebeke I, Coltherd JC, Kean DE, Steiger CN, Al-Riyami L et al (2013) The helminth product, ES-62, protects against airway inflammation by resetting the Th cell phenotype. Int J Parasitol 43:211–23

Salinas-Carmona MC, de la Cruz-Galicia G, Pérez-Rivera I, Solís-Soto JM, Segoviano-Ramirez JC, Vázquez AV, Garza MA (2009) Spontaneous arthritis in MRL/lpr mice is aggravated by Staphylococcus aureus and ameliorated by Nippostrongylus brasiliensis infections. Autoimmunity 42:25–32

Sandborn WJ, Elliott DE, Weinstock J, Summers RW, Landry-Wheeler A, Silver N et al (2013) Randomised clinical trial: the safety and tolerability of Trichuris suis ova in patients with Crohn's disease. Aliment Pharmacol Ther 38:255–63

Saunders KA, Raine T, Cooke A, Lawrence CE (2007) Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection. Infect Immun 75:397–407

Schnoeller C, Rausch S, Pillai S, Avagyan A, Wittig BM, Loddenkemper C et al (2008) A helminth immunomodulator reduces allergic and inflammatory responses by induction of IL-10-producing macrophages. J Immunol 180:4265–72

Setiawan T, Metwali A, Blum AM, Ince MN, Urban JF Jr, Elliott DE et al (2007) Heligmosomoides polygyrus promotes regulatory T-cell cytokine production in the murine normal distal intestine. Infect Immun 75:4655–63

Sewell D, Qing Z, Reinke E, Elliot D, Weinstock J, Sandor M et al (2003) Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. Int Immunol 15: 59–69

Shi M, Wang A, Prescott D, Waterhouse CC, Zhang S, McDougall JJ et al (2011) Infection with an intestinal helminth parasite reduces Freund's complete adjuvant-induced monoarthritis in mice. Arthritis Rheum 63:434–44

Smith P, Mangan NE, Walsh CM, Fallon RE, McKenzie AN, van Rooijen N et al (2007) Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism. J Immunol 178:4557–66

Sofronic-Milosavljevic LJ, Radovic I, Ilic N, Majstorovic I, Cvetkovic J, Gruden-Movsesijan A (2013) Application of dendritic cells stimulated with Trichinella spiralis excretory-secretory antigens alleviates experimental autoimmune encephalomyelitis. Med Microbiol Immunol 202:239–49

Song X, Shen J, Wen H, Zhong Z, Luo Q, Chu D et al (2011) Impact of Schistosoma japonicum infection on collagen-induced arthritis in DBA/1 mice: a murine model of human rheumatoid arthritis. PLoS One 6:e23453

Summers RW, Elliott DE, Urban JF Jr, Thompson RA, Weinstock JV (2005a) Trichuris suis therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology 128: 825–32

Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV (2005b) Trichuris suis therapy in Crohn's disease. Gut 54:87–90

Sun X, Liu YH, Lv ZY, Yang LL, Hu SM, Zheng HQ et al (2010) rSj16, a recombinant protein of Schistosoma japonicum-derived molecule, reduces severity of the complete Freund's adjuvant-induced adjuvant arthritis in rats' model. Parasite Immunol 32:739–48

Sutton TL, Zhao A, Madden KB, Elfrey JE, Tuft BA, Sullivan CA et al (2008) Anti-Inflammatory mechanisms of enteric Heligmosomoides polygyrus infection against trinitrobenzene sulfonic acid-induced colitis in a murine model. Infect Immun 76:4772–82

Trujillo-Vargas CM, Werner-Klein M, Wohlleben G, Polte T, Hansen G, Ehlers S et al (2007) Helminth-derived products inhibit the development of allergic responses in mice. Am J Respir Crit Care Med 175:336–44

van der Vlugt LE, Labuda LA, Ozir-Fazalalikhan A, Lievers E, Gloudemans AK, Liu KY, Barr TA, Sparwasser T, Boon L, Ngoa UA, Feugap EN, Adegnika AA, Kremsner PG, Gray D, Yazdanbakhsh M, Smits HH (2012) Schistosomes induce regulatory features in human and mouse CD1d(hi) B cells: inhibition of allergic inflammation by IL-10 and regulatory T cells. PLoS One 7(2):e30883

van der Werff SD, Twisk JW, Wördemann M, Ponce MC, Díaz RJ, Núñez FA et al (2013) Deworming is not a risk factor for the development of atopic diseases: a longitudinal study in Cuban school children. Clin Exp Allergy 43:665–71

Walsh KP, Brady MT, Finlay CM, Boon L, Mills KH (2009) Infection with a helminth parasite attenuates autoimmunity through TGF-beta-mediated suppression of Th17 and Th1 responses. J Immunol 183:1577–86

Wang CC, Nolan TJ, Schad GA, Abraham D (2001) Infection of mice with the helminth Strongyloides stercoralis suppresses pulmonary allergic responses to ovalbumin. Clin Exp Allergy 31:495–503

Weinstock JV (2012) Autoimmunity: The worm returns. Nature 491:183–5

Wilson MS, Taylor MD, O'Gorman MT, Balic A, Barr TA, Filbey K et al (2010) Helminth-induced CD19 + CD23hi B cells modulate experimental allergic and autoimmune inflammation. Eur J Immunol 40:1682–96

Wohlleben G, Trujillo C, Muller J, Ritze Y, Grunewald S, Tatsch U et al (2004) Helminth infection modulates the development of allergen-induced airway inflammation. Int Immunol 16:585–596

Wu Z, Nagano I, Asano K, Takahashi Y (2010) Infection of non-encapsulated species of Trichinella ameliorates experimental autoimmune encephalomyelitis involving suppression of Th17 and Th1 response. Parasitol Res 107:1173–88

Xia CM, Zhao Y, Jiang L, Jiang J, Zhang SC (2011) Schistosoma japonicum ova maintains epithelial barrier function during experimental colitis. World J Gastroenterol 17:4810–6

Yang J, Zhao J, Yang Y, Zhang L, Yang X, Zhu X et al (2007) Schistosoma japonicum egg antigens stimulate CD4 CD25 T cells and modulate airway inflammation in a murine model of asthma. Immunology 120:8–18

Zaccone P, Fehérvári Z, Jones FM, Sidobre S, Kronenberg M, Dunne DW et al (2003) Schistosoma mansoni antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. Eur J Immunol 33:1439–49

Zaccone P, Burton O, Miller N, Jones FM, Dunne DW, Cooke A (2009) Schistosoma mansoni egg antigens induce Treg that participate in diabetes prevention in NOD mice. Eur J Immunol 39:1098–107

Zaccone P, Burton OT, Gibbs S, Miller N, Jones FM, Dunne DW et al (2010) Immune modulation by Schistosoma mansoni antigens in NOD mice: effects on both innate and adaptive immune systems. J Biomed Biotechnol 2010:795210

Zaccone P, Burton OT, Gibbs SE, Miller N, Jones FM, Schramm G et al (2011) The S. mansoni glycoprotein ω-1 induces Foxp3 expression in NOD mouse CD4+ T cells. Eur J Immunol 41:2709–18

Zhao Y, Zhang S, Jiang L, Jiang J, Liu H (2009) Preventive effects of Schistosoma japonicum ova on trinitrobenzenesulfonic acid-induced colitis and bacterial translocation in mice. J Gastroenterol Hepatol 24:1775–80

Zheng X, Hu X, Zhou G, Lu Z, Qiu W, Bao J et al (2008) Soluble egg antigen from Schistosoma japonicum modulates the progression of chronic progressive experimental autoimmune encephalomyelitis via Th2-shift response. J Neuroimmunol 194:107–14