Helminth infections induce immunomodulation : consequences and mechanisms

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General introduction

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Helminths and the immune response

Worldwide, more than a billion people are infected with helminths. These worm infections generally do not lead to mortality, however, they are chronic in nature and can lead to considerable morbidity. Immunologically these infections are interesting; chronic helminth infections are characterized by skewing towards a T helper 2 type response as well as regulatory responses. The regulatory network is associated with chronic helminth infections and is thought to prevent strong immune responses against parasitic worms, allowing their long-term survival and restricting pathology. This regulatory network is thought to also temper responses to non-helminth antigens, like allergens or self antigens, possibly leading to lower prevalence of allergies and autoimmune diseases in subjects that are chronically infected with helminths. This raises the interesting idea that helminths may bear molecules that have potential therapeutic action against allergies and possibly other inflammatory diseases. However, on the other side of the coin, this would predict that helminth infected subjects might not respond strongly to third party antigens like vaccines. This is an important issue, since most vaccines that are being developed against diseases such as HIV, tuberculosis or malaria will be introduced in areas where helminth infections are highly prevalent. Moreover, these vaccines are proving difficult to develop and are often weak, thus any confounder that would affect their efficacy needs to be taken into consideration. Helminth derived molecules have been identified that induce T helper 2 and regulatory responses via modulation of dendritic cells and some appear to do so via TLR signalling. New insights into these pathways could be useful to antagonize suppression and hence boost vaccine efficacy or to optimize suppression induced by helminth derived molecules and control inflammatory diseases.

Scope
Helminth infections are characterized by a strong T helper 2 (Th2) response as well as an overall downregulated immune system. In this general introduction first the immune responses evoked by helminths will be described. Second the effect of helminth infections or helminth derived molecules on responses to unrelated antigens will be discussed and third an overview will be given of the mechanisms involved in helminth induced immune modulation.

Host immune responses to helminths

Helminths are multicellular organisms of which many species are parasitic. Those infecting humans are mainly found in two phyla; the phylum of Platyhelminths includes digenean flukes (trematodes) and tapeworms

(cestodes), and roundworms belong to the phylum of Nematoda (http://www.path.cam.ac.uk/~schisto/General_Parasitology/Hm.helminths.html). Worldwide millions of people are infected, causing mainly indirect effects on health, as they contribute to malnutrition, and hence a decrease in children's growth, and impairment of cognitive functions. Generally, a small population of infected individuals suffer from severe symptoms, but most people remain asymptomatic.

Helminths vary greatly in their biology. Their intermediate hosts range from snails for schistosomes to flies in case of filarial worms. Also the route of infection can differ from oral infection (e.g. *Ascaris lumbricoides*) to direct penetration of the human skin (schistosome species) or infectious bites of a fly (*Onchocerca volvulus*). In addition helminths can exist within the human host in different developmental stages, such as eggs, larvae or adult worms. Finally, different species affect different organs, including the colon, the small intestine, the lymphatics, the lungs and the liver. Despite this broad range of characteristics, most members of the helminth parasites evoke similar adaptive immune responses in their human host. Helminths are known to skew the immune response towards Th2, characterized by Th2 related cytokines, that typically include IL-4, IL-5 and IL-13 that induce B-lymphocytes to switch to IgE antibody production. The active molecules present in helminths that are inducing these responses include not only proteins but also lipids (described in Chapter 2 and Chapter 5). If the role of the Th2 response is to eradicate helminth infections, as shown in animal models [1], the question is why these organisms would carry Th2 inducing molecules? Presumably these molecules are essential for parasite biology.
Helminths are long-lived organisms (up to 10 years for filarial worms) and mostly they are not able to replicate within their human host. Therefore the use of antigenic variation to escape from the hosts' immune attack is not possible. In addition they are too large for sequestration in specialized niches away from the immune system. However, helminths are thought to have developed different strategies for survival in their human host. For example schistosomula can compromise complement function [2] and degrade host immunoglobulins [3], which may weaken a direct immune attack. Helminths may shield themselves by molecular mimicry. For example schistosomula can acquire surface molecules from the host, including blood group determinants and MHC molecules or they can produce cytokine mimics (reviewed by Maizels and coworkers [4]). Their ability to neutralize host derived immune molecules was shown recently when *Schistosoma mansoni* eggs were found to secrete a chemokine binding protein (smCKP) that blocks IL-8 induced neutrophil migration. In a purified recombinant form, smCKP was able to inhibit IL-8 induced pulmonary neutrophilic inflammation in a contact hypersensitivity model [5], indicating that this molecule may protect the parasite from an

inflammatory attack. Finally, helminths can interact with the hosts' adaptive immune response by downregulation of T and B-cell responses via the induction of regulatory T cells or the anti-inflammatory cytokines IL-10 and TGF-β in the chronic phase of infection, as reviewed before [6].

Immunomodulation is hypothesized to be beneficial to both the human host and the parasite, as it could protect helminths from being eradicated, and at the same time protect the host from excessive pro-inflammatory responses that may lead to organ damage. Importantly, immune hyporesponsiveness is evident mostly in case of chronic or high level infections; upon infection the immune system will be activated to try and eradicate the worm, however, as the burden or time after infection increases, the worms seem to modify and downregulate these responses in order to survive. The modulation of the immune response is not only directed to helminths, but also to non-related antigens. Below we will give an overview of how this affects co-infections, vaccinations, as well as allergic and autoimmune diseases.

Spill-over effects of helminth-induced immunomodulation

Helminths and co-infections

Helminths have been shown to induce immune hyporesponsiveness, which might affect the immune reaction to concomitant infections that occur with high frequencies in helminth-endemic areas. The most prevalent and threatening diseases in this respect are malaria, tuberculosis, and HIV, caused by protozoa, bacteria and viruses, respectively.

Helminths are prevalent in tropical areas where malaria parasites thrive and co-infections are common. The effect of helminth infections on the outcome of malaria is the subject of recent studies, however results are variable depending on the type of helminth, the intensity of infection and the age of the study population. An effective immune response against primary malaria infection requires a strong Th1 response. Therefore it would be expected that helminth infections would decrease the development of protective immunity by inducing a Th2 response, and this has been confirmed in several studies of helminth and malaria co-infections [7, 8]. However, the results on schistosome infections indicate that light schistosome infections might be protective in young children as it was observed that children between 4-8 years of age infected with schistosomes showed less malaria, increased time to the first clinical infection and lower parasitemia compared to non-infected controls [9]. Further, the influence of helminth infections on the symptoms of severe malaria could be different from the effects on parasitemia. Although in one study it was found that ascaris infections were associated with an increase

in prevalence of severe malaria [10], others found an association between ascaris infections and protection from cerebral malaria [11] or from renal failure [12]. As cerebral malaria has been associated with increased levels of pro-inflammatory cytokines, a concomitant helminth infection may be able to suppress these cytokines by production of IL-10 and/or TGF-β (upregulated by helminth infections [13]) and therefore decrease the chance of developing severe malarial disease.

Animal models of malaria and helminth co-infection showed that in C57BL/6 mice infected with both *Heligmosomoides polygyrus*, a gastrointestinal nematode, and *Plasmodium chabaudi* AS, a strain that cannot induce cerebral malaria, the proliferation of $CD4^+$ T cells and IFN-γ production was compromised and malaria parasites were not eliminated. Interestingly, treatment of the mice with an antihelminthic drug before malaria infection fully restored protective antimalarial immunity and TGF-β levels were downregulated [14]. However, in another study, infection with *P. chabaudi* in CBA mice resulted in lower parasitemia in the schistosome-infected mice, whereas in the same model schistosome infection did not have an effect on parasitemia caused by another parasite species, *Plasmodium yoelii* [15]. These studies support the notion that helminths could alter the course of malarial infection and disease as discussed in recent reviews [16, 17]. However, there is a need for more extensive studies with uniform methodology to understand the nature of interactions in different stages of malaria co-infections.

Protection from tuberculosis is characterized by strong mycobacterium-specific Th1 responses and it has been hypothesized that co-infections with helminths will prevent the necessary Th1 response by either disturbing the Th1-Th2 balance, or by driving the immune response towards a more anti-inflammatory status. Early observations showed that the incidence of lepromatous leprosy, caused by *Mycobacterium leprae*, was twice as high in areas where onchocerciasis was hyperendemic [18]. Others have shown that PBMC from onchocerciasis patients have decreased IL-4 responsiveness to mycobacterial antigens *ex vivo* [19]. Moreover, in a murine model, an established Th1 response to *Mycobacterium avium* was decreased by a subsequent co-infection with *S. mansoni*, affecting both Th1-cytokine production and IgG2a antibody responses [20].

Although there are not many epidemiological studies on the effects of helminth infections on tuberculosis, more is known on the modification of immune cells by helminths. For example, dendritic cells (DCs) and macrophages exposed to live microfilariae *in vitro* show reduced maturation after subsequent *Mycobacterium tuberculosis* infection, indicating a compromised activation of the immune system by mycobacteria upon interaction with helminths. In addition dendritic-cell-

specific ICAM-3 grabbing non-integrin (DC-SIGN) lectin receptor, used by *M. tuberculosis* to enter DCs, was shown to be downregulated on the surface of these filarial-infected DCs [21], thereby possibly reducing susceptibility of these DCs for infection by *M. tuberculosis*. Thus, *in vitro* studies indicate that helminths can suppress the immune response to mycobacterial infections. However, the *in vivo* relevance of these laboratory-based *in vitro* findings has to be more intensively examined in immuno-epidemiological studies in areas where these two infections are prevalent.

Recently the effects of helminth infections on the course of HIV infection have gained much attention. The question was raised whether it would be beneficial to treat HIV-infected people in helminth endemic areas with anti-helminthics in addition to HIV medication. The results on human studies so far are ambiguous as some studies find no association between treatment of intestinal helminth infections and reduction in viral load [22], whereas others find a decrease in viral load upon de-worming [23] or even find a transient increase [24]. Variation between the studies may be explained by difference in age groups studied, prevalence of helminth species, type and frequency of medication as well as length of time post treatment before determination of viral load. In this respect, infection with hookworm (an intestinal helminth) showed most consistent results. Hookworm infections were shown to be associated with reduced HIV disease progression, represented by higher $CD4^+$ T cell counts and lower mortality [25]. In another study hookworm was negatively associated with sensitivity to HIV infection, whereas in the same study *Wuchereria bancrofti*, a filarial nematode, was positively associated with infection [26]. Several studies have indicated that the characteristic depletion of $CD4^+$ memory T cells in HIV infected subjects does not occur gradually and systemically as originally thought, but at a high speed at the mucosal surfaces of the intestine (reviewed in [27]). Therefore, one could speculate that intestinal parasites like hookworm could play a role in influencing the anatomy of the mucosal surface, and thereby prevent T-cell death. However, both the epidemiological relations and the possible mechanisms involved in the interaction between helminths and HIV need to be further studied in more detail.

Helminth infections and vaccination efficacy

For successful vaccination against most bacterial and viral diseases an efficient Th1 response is required. When it was found that responsiveness to live (attenuated) oral vaccines like cholera, polio and rotavirus was impaired in developing countries it was hypothesized that the presence of helminths in the gastro-intestinal tract might have affected efficient uptake of oral vaccines, as these infections are also associated with reduced absorption of nutrients [28]. It was found that *A. lumbricoides* infections impair immune responses to oral cholera vaccine by decreasing

seroconversion as well as mean antibody titres to the vaccine. In addition, the magnitude of the Th1 cytokine response (IFN-γ and IL-2) to cholera toxin subunit B after oral cholera vaccination was greater in albendazole treated subjects compared to placebo-treated controls [28, 29]. Roughly, two mechanisms can contribute to this. First, helminths could decrease effective uptake of the vaccine by cells present at the mucosal surface of the small intestine by interfering with mucus production and intestinal motility. Second, the Th2 skewing or immunomodulation induced by helminth infections could decrease the strength of the Th1 response mounted against the vaccine. Moreover, these mechanisms might not only add up, but could also be interfering with each other as for example goblet cell hyperplasia and intestinal muscle contractility are considered to be under direct immunological control, and are associated with a Th2 response where CD4 T cells, STAT6, MHCII and CD40L play a role [30].

Considering non-oral vaccines, BCG (Bacille Calmette-Guérin) has received much attention as tuberculosis is still affecting many people worldwide, causing great mortality. In a study in Ethiopia, BCG vaccination improved cellular PPD-specific immune responses in de-wormed young adults, but not in placebo-treated subjects infected with intestinal helminths [31]. In a mouse model it was demonstrated that schistosomal infection reduced the effect of BCG vaccination [32]. In addition it was found that prenatal sensitization to filariasis and schistosomiasis biases T cell immunity away from protective INF-γ responses [33]. This could be of particular interest as currently BCG vaccination is mostly administered directly after birth, indicating that the effect of maternal helminth infections on newborns may be important.

Experimental data on effectiveness of other non-oral vaccines generally support the results seen for BCG. For example, after tetanus vaccination, the responsiveness to tetanus toxoid (TT) is decreased by filarial infections [34]. Infections with filarial worms reduced IFN-γ production and increased TT specific IL-10 production in an Indian population [34]. A study in areas where onchocerciasis was endemic indicated that following vaccination with a single dose of concentrated tetanus toxoid vaccine, 7.1% of onchocerciasis patients seroconverted versus 44.5% of the uninfected control group [35]. In another study of onchocerciasis, infected subjects showed lower antibody, proliferation and IFN-γ responses compared to non-infected subjects, whereas IL-10 levels were elevated in infected subjects [36]. Although light *O. volvulus* infections did not affect the anti-tetanus antibody response, heavier infections impaired the antitetanus IgG response [37]. For schistosomal infections similar results were found in Brazilian adults; TT-specific Th1-like responses, represented by IFN-γ production, were low in schistosome infected subjects in comparison to non-infected controls [38]. We found similar results in a study in Gabon

where rural children with concurrent schistosomiasis and intestinal helminth infections showed reduced IFN-γ responses to TT compared to semi-urban subjects, having less infections, after tetanus vaccination (described in Chapter 4). In addition these children received an influenza vaccine and similarly it was found that the IFN-γ response to influenza was higher in non- or low infected semi-urban children, whereas IL-5 and IL-13 production was increased in infected rural children (described in Chapter 3). Thus, in Chapters 3 and 4 we found differences between semi-urban and rural cohorts, and helminths are likely to play a role, but we find that other factors might be involved as well. Moreover, it needs to be investigated whether the differences can be attributed to helminth infections, and whether the effect is dependent on their actual presence at time of vaccination, (chronic) helminth infections in the past and / or the worm or egg load. For this, larger studies are needed, that for example also include groups of children that are treated with anti-helminthics before vaccination.

The influence of helminth infections on vaccination efficacies should be taken in consideration when designing new vaccines. It has for example proven difficult to design an effective vaccine against HIV. Notably, most trials on HIV vaccines are conducted in the western world and its effectiveness in developing countries could be even further reduced due to concurrent helminth infections. Interestingly, a study using schistosome-infected mice, with a pre-existent Th2 immune background, demonstrated that CpG immunostimulatory sequences co-administered with inactivated, gp120-depleted HIV-1 viral particles lead to potent Th1 anti-HIV-1 immune responses overcoming the Th2 bias. In contrast, schistosome-infected mice immunized with HIV-1 immunogen in incomplete Freund's adjuvant, induced Th2 anti-HIV-1 immune responses [39]. These findings suggest that an effective vaccine for those living in helminth endemic areas might need a modified adjuvant compared to a vaccine for use in the western world.

Helminths and allergic diseases

Infection patterns have been changing enormously in the westernised countries over the last few decades. Infections are declining but other diseases, like allergies and autoimmune diseases, are emerging. One of the questions arising is whether or not there is a correlation between these two phenomena, as together with an improved hygiene other environmental factors changed as well in the same time frame. The possibility of a direct correlation between allergies and helminth infections has been intensively studied, and results often show negative associations, suggesting that helminth infections could protect from allergic responses, although some studies find the opposite to be true. An overview of these studies is given in table 1.1A. The inconsistencies might be explained by different factors:

the age and genetic background of the study population, as well as the helminth species studied. However, the intensity of infection is thought to be the most important factor, as chronic, heavy helminth infections are increasingly associated with a regulatory response and protection from allergies, whereas acute, low level infections are thought to potentiate allergic reactions by increasing the Th2 response, without inducing regulation [40, 41].
Whether or not there is a causal relationship between helminths and allergy can only be investigated by assessing the effects of introducing or removing helminths. Some of these studies have been conducted, showing that treatment of schistosomiasis or intestinal helminths in humans indeed increases skin reactivity to house dust mite [42]. However, a recent, large scale study has shown that one year anti-helminth treatment had no effect on SPT reactivity in Ecuador [43] (table 1.1B). Differences between the outcomes of antihelminth treatments may be explained by differences in length and frequency of treatment given [44].

In murine models introduction of helminths can be studied. For example the gastrointestinal nematode *H. polygyrus* was shown to suppress inflammation in a model of allergic airway infection induced by either OVA or Derp1 [45]. In addition, in a model of chronic schistosome infection it was found that allergic reactions in response to OVA challenge were reduced, as measured by a decrease in airway responsiveness and eosinophilia in the lungs [46]. Other murine intervention models that have been studied are described in table 1.1B, all showing a protective effect of helminths on allergy.

The cellular mechanisms that mediate suppression of allergic immune responses by helminths was studied by Wilson and colleagues, who showed that adoptive transfer of T cells from mice infected with a nematode, *H. polygyrus*, could suppress allergic inflammation in recipient mice. $CD4^+CD25^+$ cells were shown to be the most potent cells in inducing suppression. While elevated levels of IL-10 and TGF-β and increased foxp3 expression were found, $CD4^+CD25^+$ cells from IL-10 deficient mice could also transfer suppression of allergic inflammation. These experiments indicate that regulatory T cells are important in helminth induced inhibition of allergic inflammation but that IL-10 was not required for the suppressive actions of regulatory T cells in this model [45]. Also other studies on helminth infections have shown the induction of regulatory T cells. These cells were heterogeneous with respect to production of regulatory cytokines and expression of regulation-associated markers such as IL-10, TGF-β, CTLA4 [47], GITR or foxp3 [48](reviewed in [49]).

Table 1.1. Studies that have examined the relationship between helminths and allergies. **A.** Correlations between helminth infections and allergies.

Study area	Population (age range)	Helminth	Outcome	Ref.
Allergy: Human association studies				
Reported negative associations				
Venezuela	children	Geohelminhths	Reduced skin reactivity to environmental and ascaris antigen	[51]
Brazil	175 subjects	*Schistosoma mansoni*	Reduced skin response to aeroallergens	[52]
Gabon	520 children (5-14)	*Schistosoma haematobium*	Reduced skin reactivity to mite	[53, 54]
Ethiopia	604 adults (>16)	Hookworm	Reduced risk of wheeze	[55]
Gambia	448 adults (>15)	Geohelminhths	Protection from skin reactivity	[56]
Ecuador	2865 children 5-19 years	Geohelminths	Reduced skin reactivity to allergens	[57]
Brazil	84 asthma patients (6-35)	*Schistosoma mansoni*	Reduced course of asthma	[58]
Ethiopia	563 children (1-4)	*Ascaris lumbricoides*, hookworm	Reduced wheezing	[59]
Uganda	62 infants	Maternal helminthiasis (filariasis and hookworm) at delivery	Protection against infantile eczema	[60]
Reported; no association				
Ecuador	4433 children (5-18)	Geohelminths	No association with allergic symptoms	[61]
Ethiopia	7649 (>5)	Geohelminths	No association with weeze/asthma	[62]
Reported positive associations				
The Netherlands	1379 children (4-12)	*Toxocara* spp.[1]	Increase in allergic manifestations	[63]
China	2164 children (8-18)	*Ascaris lumbricoides*	Increased sensitization to aeroallergens, increased risk of asthma	[64]
South Africa	359 children (6-14)	*Ascaris lumbricoides* (ascaris-specific IgE)	Increased SPT positivity to aeroallergens	[65]

[1] *Intestinal parasite of cats and dogs, human is a non-compatible host*

Table 1.1B. Intervention studies in human (treatment) and mouse (infection).

Study area Population (Age range) (*mouse*:strain)	*	Helminth	Intervention strategy	Outcome	Ref.
Allergy: Intervention					
Human studies					
Gabon 317 children (5-13)	+	Geohelminth	Treatment; Praziquantel Mebendazole every 3 months for 30 months	Increased atopic reactivity to mite	[42]
Ecuador 1632 school children	0	Geohelminths	Treatment; Albendazole; 2-monthly for one year	No effect on atopy or clinical allergy	[43]
Brazil 33 asthma patients (6-39)	+	Geohelminths *Schistosoma mansoni*	Treatment; Albendazole and oxamniquine once	Less production of Derp1 specific IL-10	[66]
Venezuela 89 asthma patients	-	*Trichuris trichiura, Ascaris lumbricoides*	Treatment; Albendazole; monthly for 1 year	Decreased numbers of asthmatic crises and need for asthma medication	[67]
Mouse studies					
BALB/c and C57BL/6	+	*Schistosoma mansoni*	Infection	Suppression of asthma symptoms	[46]
BALB/c and C57BL/6	+	*Heligmosomoides polygyrus*	Infection	Suppression of allergic airway inflammation	[45]
B10.A and C57BL/6	+	*Ascaris suum*	Treatment with worm extract	Suppression of lung inflammation	[68]
BALB/c	+	*Ascaris suum*	Treatment with PAS-1 protein	Suppression of allergic responses induced by an allergic protein of *A. suum* (APAS-3)	[69]
BALB/c and C57BL/6	+	*Schistosoma mansoni*	Infection	Inhibition of anaphylaxis	[50]
C3H/HeJ	+	*Heligmosomoides polygyrus*	Infection	Inhibition of production of allergen-specific IgE and anaphylaxis	[70]

**Effect of helminths is beneficial for the host with respect to the effect on allergy (+)*
Effect of helminths is detrimental for the host with respect to the effect on allergy (-)
No effect of helminths on allergy (0)

These molecules may contribute differentially to the regulatory response mediated by these cells in various models of allergic inflammation. In addition, cells other than regulatory T cells could play a role in the suppression of allergies by helminth infections. For example, adult *S. mansoni* worms could protect mice in an experimental model of fatal allergic anaphylaxis. In this model $CD4^+CD25^+$ T-cells and IL-10 produced by these cells did not seem to be involved in protection from anaphylaxis, but it was found that IL-10 and B cells were involved in this protection as transfer of IL-10 producing B-cells from infected IL-4 deficient mice ameliorated disease [50]. In summary, although epidemiological studies suggest that helminth infections can suppress allergic inflammation, solid evidence comes from animal models, where chronic nematode and trematode infections suppress allergic inflammation. Cell transfer experiments support the notion that several cell subsets are involved in the protective mechanisms.

Helminths and auto-immune diseases

The hygiene hypothesis proposes that the lack of serious childhood infections impairs development of an appropriately educated immune system. Although the rise of autoimmune diseases in the western world is correlated with improved hygiene, causal proofs are difficult to find, as most auto-immune diseases are influenced by multiple genetic and environmental factors [71] and, unlike allergic disorders, are less prevalent and therefore more difficult to study.

In murine models of autoimmune diseases, the presence of helminth infections have been shown to have a protective effect. Infection with *S. mansoni*, exposure to its eggs or to soluble extracts from either adult worms or eggs all inhibit development of type1 diabetes in NOD mice. The T cells of the protected mice could not transfer diabetes to NOD-SCID mice, whereas T cells from non-infected diabetic mice did [72, 73].

In addition, helminths were found to be protective in several models of inflammatory bowel disease (IBD), where the disease and the parasites are co-localized. A concurrent *S. mansoni* infection in a semi-permissive rat model attenuated the course of colitis [74] and the intestinal nematode *Trichinella spiralis* suppressed macroscopic and histological symptoms of colitis in mice [75]. Currently, *Trichuris suis* is used to treat patients with ulcerative colitis and Chrohn's disease and initial results look very promising [76, 77].

Furthermore, *S. mansoni* was shown to be protective in EAE, a murine model for multiple sclerosis [78]. Interestingly the reduction of EAE severity by *S. mansoni* infection was shown to be mediated by STAT6, indicating a role for this molecule in the induction of a Th2 environment.

Table 1.2. Studies that have examined the relationship between helminths and auto-immune diseases.

Model	*	Helminth	Antigen	Outcome	Ref.
Auto-immune diseases and helminths					
Human					
Human	+	*Trichuris suis*	Infection	Amelioration of Crohn's disease	[77]
Human	+	*Trichuris suis*	Infection	Reduced ulcerative colitis	[79]
Rat					
Wistar	+	*Schistosoma mansoni*	Infection	Reduced duration of colitis	[74]
Mouse					
NOD	+	*Schistosoma mansoni*	Infection of ova treatment	Inhibition of development of type I diabetes	[72]
NOD	+	*Schistosoma mansoni*	Egg treatment or egg or worm derived proteins	Inhibition of development of type I diabetes	[73]
C57BL/6J	+	*Schistosoma mansoni*	Infection	Suppression of symptoms of type I diabetes	[80]
NOD	+	*Dirofilaria immitis*	Recombinant antigen	Inhibition of development of type I diabetes	[81]
IL-10 deficient C57BL/6	+	*Heligmosomoides polygyrus*	Infection or transfer of MLN from infected mice	Suppression of established colitis	[82]
BALB/c	+	*Schistosoma mansoni*	Egg treatment	Inhibition of development of colitis	[83]
BALB/c	+	*Hymenolepis diminuta*	Infection	Suppression of symptoms of colitis	[84]
C57BL/6	+	*Trichinella spiralis*	Infection	Reduction of severity of colitis	[75]
C57BL/6J	+	*Schistosoma mansoni*	Infection (but not ova treatment)	Reduced induction and progression of EAE	[85]
SJL,C57BL/6	+	*Schistosoma mansoni*	Ova treatment	Reduction of severity of EAE	[85]
BALB/c	+	*Schistosoma mansoni*	Infection (but also αGalCer)	Graves' hyperthyroidism	[86]
DBA/1	+	*Acanthocheilonema viteae*	Secreted protein ES-62	Reduced initiation, progression and severity of collagen induced arthritis	[87]

** effect of helminths is beneficial for the host with respect to the effect on the auto-immune disease (+)*

In general the effects of helminths on autoimmune diseases are consistent (table 1.2), showing that these parasites can protect a host from developing autoimmune disease and/or can relieve symptoms of established autoimmune inflammation. In the modulation of autoimmune diseases by helminths both the generation of a Th2 environment as well as the immunomodulation might be beneficial.

Molecular mechanisms of immune modulation by helminths

Introduction

Effects of parasitic helminths on the host immune response have been studied extensively as described above. In several models, it has been shown that these infections lead to the generation of Th2 responses as well as anti-inflammatory/regulatory responses. However, the molecular immunological pathways that induce these Th2 or regulatory responses are still being characterized and in this section we will discuss what is known about cell characteristics and molecules involved in this particular immunological cross talk.

Instructions for the development of specific immune responses are largely mediated by dendritic cells, which are present in peripheral tissues as sentinel cells and upon activation migrate to draining lymph nodes to activate naïve T cells, not only by presenting antigen but also by providing signals that determine polarization of T cell development towards a Th1, Th2 or regulatory T cell phenotype [88]. In this way dendritic cells play a central role in providing information on the nature of the invading/residing pathogen by integrating signals received and conveying them to T cells via expressing a variety of factors that will affect T cell differentiation into polarized subsets. Although several lineages of DCs have been identified both in man and mouse [89] the paucity of them has restricted their extensive use. However, the generation of large numbers of DCs has become feasible through the *in vitro* culturing of monocyte derived DCs from peripheral blood mononuclear cells in man or bone marrow derived DCs in mice, which has enabled experimentation on these cells to understand how they sense the presence of viruses, bacteria or parasites in their microenvironment. Encountering various stimuli can change the maturation status of these cells, an important step in whether these cells participate in immune activation or not. The characteristics of dendritic cells in terms of expression of co-stimulatory molecules or released cytokines, are determined by the receptors that are engaged and the downstream signalling that follows. This is an intense area of research with some surprising findings regarding the activity of helminth derived molecules.

In the following sections, there will be a focus on what is known about the modulation of dendritic cell function by helminths and helminth derived molecules, which may be involved in triggering of Th2 and/or regulatory T cell responses by these cells. Helminths and their products can modify DCs in different ways ranging from influencing the DC maturation status to affecting the downstream signalling within the DCs.

Helminths and maturation status of dendritic cells

One of the differences found between DCs that induce a Th1 response (DC1), DCs inducing a Th2 response (DC2) and DCs inducing tolerance or regulatory T cells (DCreg) is their activation status. In DC1, there is often a high expression of the maturation marker, CD83, as well as increased expression of co-stimulatory molecules such as CD80 and CD86. When these DCs encounter naïve T cells, they induce strong Th1 responses. However, when stimuli that are associated with a Th2 response are considered, they are often less strong inducers of DC maturation. For example, soluble egg antigens of *S. mansoni* (SEA), strong inducers of Th2 responses *in vivo*, when incubated with murine DCs *in vitro*, do not lead to strong maturation of these cells, but do lead to the development of Th2 responses when they encounter T cells [90]. Partial upregulation of some markers associated with maturation, have been seen with excretory/secretory proteins of a parasitic nematode, *Nippostrongylus brasiliensis* (NES) an infection associated with a Th2 immune skewing. Here CD86, CD40 and OX40L were upregulated compared to medium, but no increase in CD80 or MHC class II molecules was found [91]. Similarly, products released by schistosome larvae did upregulate MHC class II, CD40 and CD86, but to a lower extent than a Th1 stimulus, and in addition CD80 and OX40L were not upregulated [92]. In human DC experiments, *in vitro* maturation of DCs in the presence of some Th2 inducing stimuli, such as an ascaris derived phosphatidylserine fraction (ascaris PS), results in a partial reduction of CD83 expression compared to DCs matured in the absence of ascaris PS, suggesting that this Th2 stimulus may inhibit DC maturation (Van Riet *et al.*, unpublished results).

Next to these antigen mixtures also little upregulation of DC maturation markers was seen in DCs stimulated with the glycoconjugate lacto-N-fucopentaose III (LNFPIII), expressed on molecules present in SEA [93]. When injected into mice, LNFPIII stimulates an immune response dominated by Th2. Another well characterized helminth derived molecule, the filarial PC-containing secreted glycoprotein ES-62 [94], when incubated with bone marrow derived DCs, resulted in the upregulation of some of the maturation markers but to a lower degree than what is seen with stimuli that lead to the development of DC1 and thereafter Th1 responses. The partially activated status was confirmed when SEA [95] or NES [91] stimulated murine DCs were analysed by microarrays and only a limited

set of genes was found to be upregulated in comparison to fully mature DC1.

Thus, DC2 usually do not reach a fully activated status, although some maturation markers are upregulated. However, the question arises whether the partial maturity of DCs is responsible for driving Th2 responses? When human DCs are exposed to helminth derived molecules during their maturation (induced by factors such as LPS or TNF-α plus IL-1), they mature and when co-cultured with T cells, they are still able to stimulate Th2 responses. This would suggest that fully mature DCs can also trigger Th2. An important point to consider is what exactly is a mature phenotype; in human DCs a high expression of CD83 only? Or is it important to also have CD80 or CD86 upregulated or both? For example, although human DCs exposed to SEA or schistosome PS, in the presence of maturation factors, can show high expression of CD83 [96], they might be defective in the expression of other maturation markers. For example, SEA was found to reduce CD86 expression induced by LPS in human monocyte derived DCs (Van Riet *et al.*, unpublished results).

With respect to regulatory responses, it is known that immature DCs can induce a tolerogenic immune response and may stimulate naïve T cells to become regulatory T cells [97]. Although LNFPIII as well as ES-62, under some experimental conditions, induce an anti-inflammatory response [98, 99], so far only lyso-phosphatidylserine from *S. mansoni* (lysoPS) when cultured with DCs during their maturation process has been shown to stimulate IL-10 producing T cells with regulatory activity. The dendritic cells that induced this regulatory response were fully mature, with respect to CD83 [96].

Activation of Toll-like receptors by helminth derived molecules

Microbial products have been shown to interact with specific Pattern Recognition Receptors (PRRs) on, among others, dendritic cells and initiate a cascade of responses that could lead to generation of Th1, Th2 or regulatory T cell responses. The main groups of PRRs are Toll like receptors (TLRs), scavenger receptors and C-type lectin receptors (CLRs)[100], which are located the surface of the cell or on the surface of endosomal or lysosomal membranes. In addition, cytoplasmic PPR, including the “Nod like receptors” (NLRs) and “RIG-like receptors” (RLRs), can recognize ligands that are present in the cytosol [101, 102]. In general, it is thought that TLRs expressed on the surface of the cell might be particularly suitable for interaction with extracellular pathogens, whereas those expressed internally, would be specialized to interact with intracellular pathogens [103]. Helminth derived molecules have been shown to be involved in TLR signalling via different TLRs, including TLR2, 3 and 4 (figure 1.1). Schistosomal and ascaris derived lipids were found to

signal via TLR2 [96] (described in Chapter 5), dsRNA from schistosomal eggs can activate TLR3 [104] and the glycoconjugate lacto-N-fucopentaose III (LNFPIII) as well as the filarial PC-containing secreted glycoprotein ES-62 were both shown to act in a TLR4 dependent fashion (figure 1.1). Both LNFP-III and ES-62, stimulate TLR4 and act on dendritic cells to induce a Th2 response [93, 105]. These results might be surprising in the light of the fact that TLR-signalling is mostly associated with a Th1 response. There are several possible mechanisms that could be responsible for the difference in responses between the most intensively studied TLR4 ligand, LPS, and the two helminth derived TLR4 activating antigens; LNFPIII and ES-62. For example, different co-receptors may be involved that activate different downstream signalling or interfere with normal TLR4 signalling. A profound effect of co-receptor usage on TLR mediated effects is seen with TLR2 ligands. The TLR2 ligand zymosan does not induce a Th1 response, and it engages both TLR2 and Dectin-1 to activate DCs such that they prime IL-10 producing T cells [106]. Also for schistosome lysoPS, which activates TLR2 on dendritic cells and mediates induction of regulatory T cells, it is possible that, like zymosan, it engages co-receptors in combination with TLR2 and therefore results in DCs with capacity to induce regulatory T cells.

Regarding TLR4 activation by helminth derived molecules that have the capacity to trigger Th2 skewing, it has to be noted that TLR4 can either activate the MyD88 dependent or the TRIF dependent pathways. For helminth derived TLR4 ligands it is not yet known which adaptor molecule is involved in downstream TLR signalling. In any case the use of HeJ mice that have a point mutation in the TIR domain of TLR4 has shown that there are differences in how the mutant receptor responds to helminth derived TLR4 ligands and LPS. The HeJ mice do not produce cytokines in response to LPS whereas no differences are seen in responses to ES-62 in HeJ mice compared to WT mice, indicating that ES-62 engages and triggers TLR4 differently than LPS and possibly signals via a co-receptor in HeJ mice.

One interesting consequence of TLR engagement has come to light recently in studies of intracellular compartmentalization and this might have implications for the development of Th1, Th2 or regulatory T cell responses. A role for the TLR4 ligand LPS was found in the selection of antigenic cargo for MHCII presentation in dendritic cells. MHCII presentation of OVA (ovalbumin) and HEL (hen egg lysozyme) antigens was compared when these antigens were either conjugated to LPS bearing microspheres or to LPS free microspheres [107]. When conjugated to LPS-microspheres, the antigens were preferentially processed and presented in the context of MHC class II on the surface of DCs leading to T cell activation. Related to this, one elegant study has investigated the compartmentalization in dendritic cells of soluble egg antigen preparation

(SEA) from *S. mansoni* which induces a Th2 response, compared to *Proprionebacterium acnes* (Pa), which triggers a strong Th1 response [90]. It was found that Pa was colocalised with transferrin within DCs, whereas SEA was not. In addition, Pa was processed in LAMP2 positive antigen processing compartments, and SEA was either localised in LAMP2 negative or –dull compartments when administered alone, or in LAMP2 dull compartments when DCs were cultured with both Pa and SEA. These results indicate that processing of SEA might be less extensive than that of Pa, leading to a reduced antigen presentation that might in turn affect T cell polarization. Therefore, it is important to consider the possible consequences of TLR engagement in compartmentalization of antigens within antigen presenting cells and the subsequent effects this may have on the type of immune responses induced.

Downstream effects of helminth derived molecules: MAP kinases

It has been noted that the activation of TLRs by helminth derived molecules leads to different downstream activation of kinases involved in intracellular signalling compared to TLR activation by Th1 stimuli. Although the TLR4 ligand LPS has been found to strongly activate the MAP-kinases p38, JNK and ERK, the molecule LNFPIII that, as already discussed above, acts via TLR4, phosphorylates only ERK. Similarly, ES-62 that like LNFPIII activates TLR4 but in contrast to LPS leads to Th2 responses, suppresses activation of p38 and JNK, but induces ERK dependent inhibition of IL-12 production (figure 1.1). These results support the notion that helminth derived molecules with Th2 inducing capacity can engage TLR4 but obviously in a manner that is distinct from LPS which has no Th2 inducing activity. With respect to TLR2, PAM3CSK, a TLR2 activating molecule that can induce Th2 responses, was shown to shift the balance of p38 and ERK in favor of ERK [108], leading to stabilization of c-Fos and subsequently to a suppression of IL-12 production. Interestingly we found that schistosome and ascaris derived phospholipids also decrease the balance of *p*-p38 / *p*-ERK, like PAM3CSK, but in contrast to PAM3CSK these lipids do not increase the duration and magnitude of ERK phosphorylation, but instead, they seem to inhibit LPS-induced phosphorylation of p38 (described in Chapter 5).

Taken together, the results from studies of intracellular signalling suggest that helminth derived molecules that act via TLRs and very likely also by engaging a co-receptor such as DC-SIGN or Dectin-1, interfere with TLR signalling, thereby changing T cell skewing. A recent study has shown that engagement of DC-SIGN by antibodies can lead to activation of ERK and phosphorylation of Akt without p38 activation [109]. So a more general characteristic of molecules that lead to Th2 skewing, might indeed be the specific induction of dominance of ERK over p38. The question of how Th2 inducing and regulatory T cell inducing molecules differ in their signalling is yet unanswered and a target for future studies.

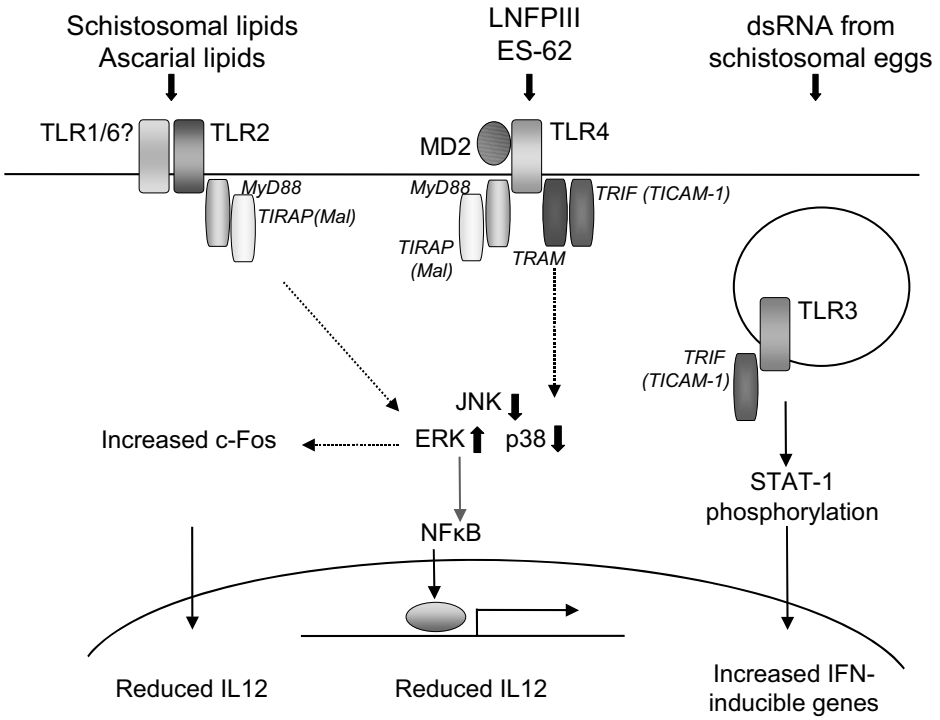

Figure 1.1. Toll-like receptor signalling in dendritic cells by helminth derived molecules. dsRNA from schistosomal eggs induces IFN-inducible genes via TLR3, whereas schistosomal and ascarial lipids as well as LNFPIII and ES-62 from schistosomes and filarial worms, induce a moderate Th2 response or immunomodulation via TLR2 or 4. For the TLR4 ligands LNFPIII and ES-62 this Th2 response was shown to be induced via increased phosphorylation of ERK. The lipids increased c-Fos expression in DCs, which has been shown to be stabilized by ERK, indicating that the Th2 inducing helminth antigens might signal via a similar pathway, although via different TLRs. Both lipids were shown to activate TLR2 and to be dependent on MyD88 and TIRAP, however, for the ascarial lipids it has not yet been shown that the reduced IL-12 and increased c-Fos is related directly to TLR2 signalling.

The remainder of this thesis "Helminth infections and immunomodulation; consequences and mechanisms" will describe the following:
In **Chapter 2** antibody profiles to glycolipids and (glyco)proteins of *Ascaris lumbricoides* were studied, as these different classes of compounds may have distinct roles in shaping of and interacting with humoral immune responses. In **Chapter 3** and **Chapter 4** examples of the consequences of helminth infections on vaccination efficacy were studied; the effect of influenza and tetanus vaccination on children living in a helminth endemic area of Gabon are described, respectively. Finally, to learn about the mechanisms involved in building immune responses upon helminth infections, human monocyte derived dendritic cells exposed to helminth derived molecules were studied at the molecular level and these results are portrayed in **Chapter 5**.