5 Nematodes and Regulatory T Cells

Katherine A. Smith and Rick M. Maizels
University of Edinburgh, Edinburgh EH9 3JT, UK

Introduction

The survival of nematode organisms in the mammalian host is accompanied by a suite of immunological changes, which render the immune system unable to expel the parasites. Many of these features were recognized some years ago (Ottesen *et al.*, 1977), and indeed a prescient review written in 1976 by Ogilvie and Wilson suggested that immune-suppression following parasite infection may be mediated by suppressor T cells or by soluble factors released by the parasite.

More recently, regulatory T cells (Tregs) have emerged as functional suppressive cells, which act as key controllers of immune responsiveness. Their recognition has in turn provided a major new paradigm for the immunology of infections with nematodes and other helminth pathogens (Maizels *et al.*, 2004). At the same time, there is substantial epidemiological evidence that helminth infections can protect against allergies and other disorders of immune over-reaction (Cooper *et al.*, 2003; Maizels, 2005; Fleming and Cook, 2006; Smits and Yazdanbakhsh, 2007), focusing attention on the immunological interactions between these parasites and their hosts (Hoerauf *et al.*, 2005; Elliott *et al.*, 2007).

Clear evidence of immune down-regulation in nematode infections was first obtained in human lymphatic filariasis and onchocerciasis. In these tissue infections, adult worms lodge in lymphatic vessels or subcutaneous sites, and peripheral blood T cell populations show parasite antigen-specific anergy or hyporesponsiveness (Ottesen *et al.*, 1977; Piessens *et al.*, 1980a). Notably, in *Onchocerca volvulus*-infected individuals there is 'spillover' suppression of immune responsiveness to unrelated ('bystander') antigens such as those from mycobacteria or vaccine components (Greene *et al.*, 1983), and responses to tetanus vaccination are diminished (Cooper *et al.*, 1998). Moreover, children infected with gastrointestinal nematode parasites mount poorer responses to cholera vaccination (Cooper *et al.*, 2001), and are hyporesponsive to both parasite antigens and bacterial ligands (Turner *et al.*, 2008). These systemic effects on both parasite-specific and bystander antigen responses can, however, be restored by curative drug treatment (Greene *et al.*, 1985; Sartono *et al.*, 1995; Cooper *et al.*, 2000). Hence, there is a direct association between the presence of live parasites and the suppression of the host immune response.

Initially, our mechanistic understanding of immune down-modulation in filarial nematode infections was fragmentary. Early studies had indicated a suppressor T cell population (Piessens *et al.*, 1980b), arguing that hyporesponsiveness reflected an active down-regulation of immunity. It was subsequently shown that, *in vitro*, T cell responses could be rescued with neutralizing

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antibodies to TGF-β and IL-10 (King *et al.*, 1993), the two immunosuppressive cytokines most closely associated with regulatory T cells. Human gastrointestinal nematode infections in children show similar features, with for example higher TGF-β and IL-10 outputs in the most heavily infected children (Turner *et al.*, 2008). As discussed below, the appearance of the regulatory T cell paradigm in cellular immunology has provided an attractive conceptual framework to analyse these immune profiles in endemic human populations.

Regulatory T Cells

Regulatory T cells refer to one or more subsets of immune system cells that block or suppress immune reactivity, either as a dedicated lineage or as a phenotype induced through suppressive cytokines and signals (Sakaguchi *et al.*, 2008). Most, but not all, Tregs are CD4+ and express either or both the surface IL-2R-α chain CD25 and the forkhead box transcription factor Foxp3 (Gavin *et al.*, 2007). A high proportion also express two surface proteins involved in co-stimulatory signalling, CTLA-4, which inhibits co-stimulation through CD28 (Alegre *et al.*, 2001) and GITR, a receptor implicated in activation of both Tregs and non-regulatory T cells (Shimizu *et al.*, 2002).

Tregs are found in several phenotypically distinct populations (Fig. 5.1). The most prominent are the committed 'natural' regulatory T cell population, which develops as a result of interactions with self-antigens in the thymus, and emerge into the periphery to enforce self-tolerance; this subset is characterized as CD4+CD25+Foxp3+ and remains committed to this function over time (Rubtsov *et al.*, 2010). In addition, a diverse range of both CD4+ and CD8+ T cells can adopt a regulatory function, in some cases by initiating expression of Foxp3. In particular, 'adaptive' Tregs can convert from CD4+Foxp3– to CD4+Foxp3+, as discussed further below, thereby expanding the range of Treg specificities to exogenous antigens (Bluestone and Abbas, 2003). Crucially, this state is not necessarily permanent, and adaptive Tregs can lose expression of CD25 and Foxp3 under certain conditions.

Distinct from the Foxp3-expressing Tregs are further sets of cells with functional regulatory capacity (Fig. 5.1). For the most part, these cells are suppressive through the production of IL-10; these are termed Tr1, but because IL-10 can also be released by effector cells of the Th2 (IL-4+) and Th1 (IFNγ+; Jankovic *et al.*, 2007) types, the demarcation between different subsets is difficult to define. In any event, such cells are relatively plastic in their cytokine profile and should not be regarded as a single immutable population. Another cytokine associated with both induction and suppressive function of Tregs is IL-35, which is a heterodimer of Ebi3 and p35, the latter chain shared with IL-12. IL-35-producing Tregs may be either Foxp3+ or Foxp3–, but are dependent on IL-10 for their generation (Collison *et al.*, 2010).

Over recent years, Tregs have proven to be instrumental in a wide range of infectious disease systems, including viral, bacterial, protozoal and helminth infections (Belkaid and Tarbell, 2009). In many infection settings, Tregs are essential to minimize immune pathology, as has been shown in murine schistosomiasis (Layland *et al.*, 2007), and their activation may reflect an immune homeostatic mechanism to limit excessive inflammation. However, as described below, certain nematode parasites directly drive Treg expansion in a manner that may represent a crucial part of the pathogen strategy to evade immunity.

A major theme of the effects of regulatory T cells is that they transcend parasite-specificity, and modulate systemic reactions to bystander antigens, which may be of either pathogenic (e.g. bacterial) or non-pathogenic (e.g. innocuous allergen) origin. For example, the response to mycobacterial antigens can be profoundly depressed by co-infection with filaria (Freedman *et al.*, 1991), as are responses to tetanus toxoid vaccination (Nookala *et al.*, 2004), and it seems likely that in part at least, this effect is due to the action of regulatory T cells. Likewise, the intestinal nematode *Heligmosomoides polygyrus* is able to suppress inflammatory reactions to unrelated allergens, an effect that can be transferred between mice by regulatory T cells (Wilson *et al.*, 2005). These studies on filarial parasites, and gastrointestinal nematodes, will be discussed in more detail in the sections that follow.

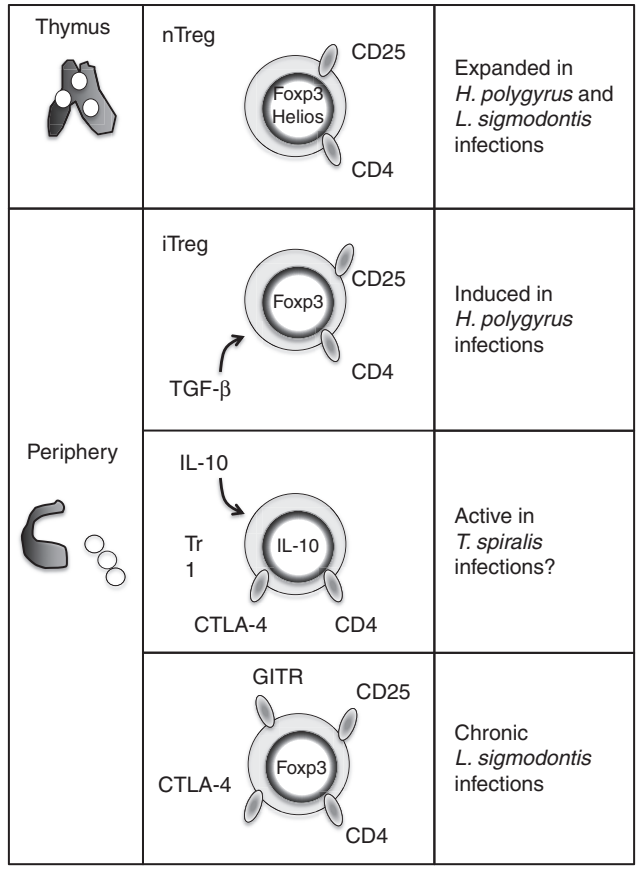


Fig. 5.1. Treg phenotypes in nematode infections. Examples are depicted of Treg subtypes associated with nematode infections in murine models. nTreg denote natural or thymic Tregs expressing CD25. Three sets of peripherally induced Tregs (iTreg) are shown: TGF-β-converted CD4+CD25+ T cells; IL-10-induced CD4+ Tr1 cells; and CD4+CD25+CTLA-4+GITR+ Tregs.

Role of Regulatory T Cells in Mouse Models of Filarial Infection

Several mouse models of filarial infection have been studied for the expression and function of regulatory T cell populations. *Brugia malayi*, the human filaria and the closely-related *Brugia pahangi* cannot complete their life cycles in mice, but the L3, adult and microfilaria stages can be introduced with short- to medium-term survival before a CD4+ T cell-dependent immune response eliminates the parasites (Lawrence, 1996). A more recently established system employs the natural rodent filaria *Litomosoides sigmodontis*, which progress through to microfilaraemia in susceptible strains of mice (Hoffmann *et al.*, 2000).

Shortly following infection of mice with the L3 stage of *B. pahangi*, the CD4+ T cell population was shown to express elevated levels of CD25, CTLA-4 and Foxp3 (Gillan and Devaney, 2005). McSorley *et al*. subsequently showed that live *B. malayi* L3 and adult parasites (but not the equivalent antigenic load from dead parasites) expanded the Foxp3+ Treg population in mice (McSorley *et al.*, 2008), and that Treg numbers subsided as mice developed functional immunity. These authors also showed that bystander T cells (carrying an ovalbumin-specific T cell receptor, DO11.10) were induced into Foxp3 expression in mice carrying either L3 or adult stages of *B. malayi* (McSorley *et al.*, 2008). While establishing the principle that nematode parasites can promote Treg, the limited

life span of *Brugia* parasites in mice makes this a difficult system to test the functional importance of Tregs in the outcome of infection.

To assess whether Tregs are critically important in governing susceptibility to nematode infection, Taylor *et al.* have explored in detail the regulatory network of filarial infections using *L. sigmodontis*. Most Foxp3−, as well as the regulatory Foxp3+, CD4+ T cells from mice infected with this parasite showed long-term elevation of CTLA-4, as well as of GITR (Taylor *et al.*, 2005), suggesting that the effector Th2 population was blocked from responding through expression of these regulatory markers. As *L. sigmodontis* survive in mice for many weeks, it was possible to intervene with depleting antibodies, and the combination of anti-CD25 and anti-GITR (Taylor *et al.*, 2005), or of anti-CD25 and anti-CTLA4 (Taylor *et al.*, 2007), resulted in stronger immunity and loss of established adult worms. These studies were the first to demonstrate that intervention against regulatory T cell populations can successfully result in parasite killing, and indeed were the first to present any form of immunological intervention with a beneficial effect against infection. As will be discussed below, the *L. sigmodontis* system has also provided clear evidence that the stimulation of natural Tregs at the initial stages of infection is crucial to parasite establishment in susceptible mice (Taylor *et al.*, 2009).

L. sigmodontis infection has additionally been shown to raise TGF-β expression in host cells, and (as earlier shown for *H. polygyrus* mentioned above) inhibit airway allergy to a bystander antigen (Dittrich *et al.*, 2008). In the same filarial system, infection of diabetes-prone NOD mice was found to protect them from the development of autoimmune disease, in a manner associated with expansion not only of the Th2 compartment, but of the number of CD4+ CD25+Foxp3+ Tregs (Hübner *et al.*, 2009).

Regulatory T Cells in Intestinal Nematode Infections: The Case of *H. polygyrus*

In parallel with the filarial studies, work on mouse gastrointestinal parasites has principally focused upon *H. polygyrus*. The life cycle of this nematode is restricted to the intestinal tract, initially invading the mucosal wall of the small intestine where larval stage mature, subsequently emerging as adult worms into the intestinal lumen (Monroy and Enriquez, 1992). Immunity to infection is slow to develop, but can act rapidly to reject secondary challenge; in both settings the response is entirely dependent upon CD4+ cells (Urban *et al.*, 1991).

H. polygyrus has attracted attention because of its long pedigree as an immunosuppressive parasite. *H. polygyrus* infection inhibits expulsion of other nematode parasites, such as *Nippostrongylus brasiliensis* (Colwell and Wescott, 1973; Wescott and Colwell, 1980) and *Trichinella spiralis* (Behnke *et al.*, 1978), as well as modulating immune responses to other pathogens, including the bacteria *Helicobacter pylori* (Fox *et al.*, 2000) and *Citrobacter rodentium* (Chen *et al.*, 2005), the protozoan parasites *Plasmodium chabaudi* (Su *et al.*, 2005), *Plasmodium yoelii* (Tetsutani *et al.*, 2009) and *Eimeria falciformis* (Rausch *et al.*, 2010), and the trematode *Schistosoma mansoni* (Bazzone *et al.*, 2008). Not surprisingly then, infection also has a substantial impact on bystander antigen responses (Pritchard *et al.*, 1984), including responses to vaccination (Su *et al.*, 2006; Urban *et al.*, 2007), and is able to suppress immunological pathologies such as colitis (Khan *et al.*, 2002), inflammatory bowel disease (Elliott *et al.*, 2004; Hang *et al.*, 2010), diabetes (Liu *et al.*, 2009) and allergy (Bashir *et al.*, 2002; Wilson *et al.*, 2005).

This system has now established a major role for Tregs in the immunobiology of nematode infections. Soon after *H. polygyrus* infection, Treg numbers expand in the draining lymph nodes of nematode-infected animals (Finney *et al.*, 2007; Rausch *et al.*, 2008). As well as increasing the proportion of Foxp3-expressing cells among the total CD4+ population, infection caused a significant increase in the expression of CD103 (Finney *et al.*, 2007), which some investigators have linked to an 'activation' of regulatory cells allowing them to infiltrate inflamed tissue (Huehn *et al.*, 2004; Suffia *et al.*, 2005). Infection-derived Tregs (defined as CD25+) were able to suppress allergy in uninfected allergic recipients, indicating an effect beyond parasite antigen-specific

immune responses (Wilson *et al.*, 2005). Moreover, as discussed below, the secretions of *H. polygyrus in vitro* can convert naive non-regulatory T cells into Foxp3-expressing cells which, on transfer into allergic hosts, can suppress airway inflammatory responses (Grainger *et al.*, 2010). This reflects a highly significant, and potentially crucial, role of the regulatory T cell population expanded by *H. polygyrus* – that the effects of infection spill over into influencing immune responsiveness to other coincident antigens. The impact of this Treg population may be either beneficial to the host, as in suppression of response to allergens, self-antigens and commensal organisms (Maizels, 2005), or detrimental, as in loss of immunity to malaria in mice co-infected with *H. polygyrus* (Su *et al.*, 2005; Tetsutani *et al.*, 2009).

In *H. polygyrus* infection, there is also expansion of a CD8+ regulatory T cell population in the lamina propria (Metwali *et al.*, 2006). These authors found that infection ameliorated the colitic phenotype of IL-10-deficient mice (Elliott *et al.*, 2004), and protected normal mice from chemically-induced colitis in an IL-10 dependent manner (Setiawan *et al.*, 2007). Colitis also occurs in mice deficient in TGF-β signalling (through transgenic expression of a kinase-dead TGF-β receptor II under a T cell-specific promoter). The inability of *H. polygyrus* to block development of this colitic disease (Ince *et al.*, 2009) indicates that TGF-β as well as IL-10 are important in the regulatory pathway stimulated by this infection. As discussed further below, intestinal colitis is regarded as Th1-dependent, and the importance of IL-10 is likely to be much more readily apparent in suppression of Th1-mediated pathologies.

More recently, experimental manipulation of the regulatory compartment has been tested in the context of altering susceptibility or resistance to infection with *H. polygyrus*. Consistent with a model that confers a determining role on the Treg population, we have found that blocking TGF-β signalling with a chemical kinase inhibitor (SB431542) was able to boost Th2 responsiveness and reduce worm load (Grainger *et al.*, 2010). Perhaps surprisingly, mice with deficient TGF-β signalling (through a dominant negative receptor, as described above) were found to be more susceptible to *H. polygyrus* infection (Ince *et al.*, 2009). However, these animals display highly exaggerated Th1 (IFN-γ) responses, and may be unable to mount the Th2 response necessary for clearance of infection. A further test system has been the DEREG mouse in which Foxp3+ T cells can be ablated through administration of diphtheria toxin (DTx), due to the expression of the DTx receptor under the control of a transgenic Foxp3 promoter (Lahl *et al.*, 2007). In the only published study, Foxp3-depleted mice were found to bear a similar worm burden to controls, despite a heightened Th2 response (Rausch *et al.*, 2009). However, intestinal worms were enumerated relatively early in the infection, at a time at which even genetically resistant mice still harbour significant worm numbers; hence this question remains to be investigated in greater depth.

Tregs in Other Mouse Intestinal Nematodes

CD4+Foxp3+ Tregs have featured in many other mouse models of nematode infections, either increasing in number, as in *Trichinella spiralis*-infected mice (Beiting *et al.*, 2007) and rats (Gruden-Movsesijan *et al.*, 2010), or by constraining immunopathology. For example, following *Trichuris muris* infection, Treg depletion with anti-CD25 and anti-GITR resulted in greater pathological damage (D'Elia *et al.*, 2009); however, anti-GITR also reduced worm survival suggesting that, as found in *L. sigmodontis* infection, Tregs are restraining the host protective immune response. Interestingly, with *T. muris* different strains are able to establish for shorter or longer time periods, and infection with the longest-lived isolate generates the highest level of Foxp3+ Treg frequency (D'Elia *et al.*, 2009). *T. muris* infections have also been reported with high levels of intestinal IL-35-expressing Foxp3+ Tregs (Collison *et al.*, 2010). In *Trichinella spiralis* infection, a rise in local Tregs is seen at the site of tissue infection, but depletion of this population by treatment with anti-CD25 does not affect the overall worm load, despite resulting in a heightened Th2 response. In this instance, the primary counter-inflammatory influence to infection is IL-10 produced by CD4+CD25– T cells

(Beiting *et al.*, 2007). In contrast, anti-CTLA-4 antibody treatment of *Trichinella spiralis*-infected mice results in a more potent and protective Th2 response to infection, which reduces muscle larval establishment (Furze *et al.*, 2006), raising the possibility of a CD25-CTLA4+ Tr1-like population controlling the outcome (Fig. 5.1). Importantly, however, not all nematodes induce Tregs, as for example we have found no increase in Foxp3+ cell numbers or activity in mice infected with the short-lived *N. brasiliensis* (Smith *et al.*, unpublished data).

Many intestinal nematodes have tissue-migratory stages during larval development, or else (like *Trichinella spiralis*) encyst in somatic tissues as part of their transmission strategy. In addition, zoonotic infections can occur with parasites, which only reach adulthood in their definitive host, but need to survive as immature forms in the tissues of an intermediate host. *Toxocara canis* is one example, and infections of mice with this nematode (for which the definitive host is the dog) are accompanied by local expansion of Foxp3+ Tregs around the sites of larval-induced granulomas (Othman *et al.*, 2011), while in the case of *Anisakis simplex* (a parasite of marine mammals), it has been reported that injection of mice with a parasite homologue of the MIF (macrophage migration inhibitory factor) cytokine results in increased numbers of Foxp3+ T cells in the lungs and spleen (Park *et al.*, 2008).

Role of Tr1 and IL-10 in Nematode Infections

While IL-10 was first identified as a Th2 cytokine that inhibited responses of Th1 cells (Fiorentino *et al.*, 1989), it is recognized to be produced by B-cells, macrophages, thymocytes and keratinocytes upon activation (Moore *et al.*, 1993). Specifically, IL-10 is produced by a Foxp3– T cell subset designated Tr1 (Groux *et al.*, 1997) that can mitigate inflammatory bowel disease in SCID mice, as well as Th2 inflammation in a mouse model of allergic asthma (Yssel *et al.*, 2001). However, the role of IL-10 in Th2-dominated helminth infections is both complex and double-edged. While generally IL-10 is seen to act as an anti-inflammatory mediator during chronic infection, it is also very important in the early induction phase for the protective Th2 response to establish itself in the face of incipient Th1/Th17 activity. This principle has been most elegantly demonstrated for *Trichinella spiralis* in which IL-10 is essential for the protective Th2 response to expel adult worms from the intestine, and yet inhibits the tissue response to nematode larvae that have entered the musculature (Helmby and Grencis, 2003; Beiting *et al.*, 2007). Similarly, high IL-10 responses are observed equally during the acute Th2 reaction that expels *N. brasiliensis* (Balic *et al.*, 2006), and the muted response that accompanies persisting *H. polygyrus* infection (Finney *et al.*, 2007; Elliott *et al.*, 2008). The latter parasite does not require host IL-10 for its survival, as shown by its ability to colonize IL-10-deficient mice (Elliott *et al.*, 2004).

Tr1 and IL-10 may play a clearer suppressive role in chronic infections, rather than at the early phase during which the commitment of Th cells to a particular phenotype is established. For example, with *L. sigmodontis*, IL-10 acts to favour persistence of microfilariae while having little effect on earlier life-cycle stages (Hoffmann *et al.*, 2001). In this respect, the extensive literature on regulatory IL-10 in humans is critical, as discussed below.

Natural (Thymic) or Adaptive (Induced) Tregs?

A major issue in Treg biology is whether observed Foxp3+ T cell responses represent activation of pre-existing natural Tregs that adopt this phenotype in the thymus, or the conversion of non-regulatory T cells into adaptive Tregs under the influence of inductive factors in the periphery (Bluestone and Abbas, 2003). We have shown in the *H. polygyrus* system that significant conversion can occur when Foxp3-negative cells, purified by fluorescent cell sorting of T cells from a Foxp3-GFP reporter mouse, are transferred to an infected, non-transgenic recipient. When transferred cells also bear a transgenic ovalbumin-specific TCR (DO11.10), up to 50% switch on Foxp3

expression if mice are administered ovalbumin by the oral route (Grainger *et al.*, 2010). The requirement for specific antigen implies that, *in vivo*, only those naive peripheral T cells that are presented with cognate antigen *in situ* are likely to be converted to Tregs; it is interesting to speculate that these T cells may primarily be specific for antigens released from the parasite. Since TGF-β is also a growth and maintenance factor for differentiated natural Tregs (Marie *et al.*, 2005), the release of a TGF-β-like ligand by *H. polygyrus* might also drive expansion of self-antigen-specific Tregs as a consequence of parasite-induced tissue damage.

Linked to this is the more functional question of whether parasite persistence depends on either or both natural and adaptive subsets of Foxp3+ T cells. In the *L. sigmodontis* system, depletion of natural/thymic Tregs (with anti-CD25 antibody) prior to infection resulted in stronger Th2 responses and poorer worm survival (Taylor *et al.*, 2009). Remarkably, the effects of Treg depletion prior to infection were only evident at later time points, up to 60 days post-infection, when adult worm numbers were reduced and fecundity (judged by production of microfilariae) greatly inhibited. Because of rapid proliferation of Foxp3+ T cells soon after infection of mice with *L. sigmodontis*, it appears that the larval L3 stage is able to drive multiplication of pre-existing natural Tregs which sufficiently influence the early response to result in longer and more productive parasite infections over time. With the recent identification of Helios as an intracellular transcription factor expressed solely by natural/thymic Tregs (Thornton *et al.*, 2010), the relative contributions of the two types of Foxp3+ Tregs can now be evaluated in various nematode infections.

Evidence of Regulatory T Cells in Human Filarial Nematode Infection

Thirty years ago, Piessens *et al*. reported increased numbers of suppressor T cells in the majority of patients with *B. malayi* microfilaraemia and in half of those with symptoms of elephantiasis. The presence of these cells was associated with hyporeactivity to filarial antigen and removal of this population *in vitro* or *in vivo* improved reactivity to filarial antigens (Piessens *et al.*, 1980b, 1982). Over subsequent years, careful analysis of human filariasis patient samples has established a number of parameters, which, in retrospect, can be attributed to an expanded Treg population in nematode infection. Indeed, some of the earlier authors had identified regulatory activities among filarial patients' T cells before the emergence of the Treg paradigm (King *et al.*, 1993). Thus, asymptomatic carriers (those with lowest resistance to infection) had highest CTLA-4 levels (Steel and Nutman, 2003), and the poor IL-5 response of patients' cells was augmented in the presence of anti-CTLA-4 antibody. CTLA-4, together with PD-1 (programmed death-1) has been further implicated in inhibiting Th1 and Th17 responses in human tuberculosis patients who are co-infected with filariasis (Babu *et al.*, 2009a).

The first definitive evidence that Tregs were stimulated in nematode infections was the cloning of T cells from human onchocercomata, the granulomas that form around subcutaneous adult *O. volvulus* (Doetze *et al.*, 2000). Cloned CD4+ T cells from clinically excised nodules proved to have Treg characteristics (Satoguina *et al.*, 2002), although as the Foxp3 marker was not available at the time of these studies, it is possible that these IL-10-producing clones represented Tr1 cells. Most recently, a closer link between Tregs and the evolution of filarial disease has been confirmed, with higher numbers of both natural and adaptive Treg numbers in human filarial infections (Metenou *et al.*, 2010) and Treg deficiency in individuals who progress to immunopathological stages such as lymphoedema and elephantiasis (Babu *et al.*, 2009b). Intriguingly, it has now been reported that type 1 (autoimmune) diabetes is absent or reduced in filariasis patients compared with uninfected controls (Aravindhan *et al.*, 2010a, 2010b).

Human filarial patients show immunological hyporesponsiveness, as described above, which is strongly associated with the onset of patency, or microfilariae (Mf) in the blood (Graham *et al.*, 2001). Since Bosshardt *et al.* (1995) showed that ivermectin-mediated clearance of Mf in gerbils did not reverse suppression, hyporesponsiveness may be induced by mature adult worms. Consistent with this idea,

chemotherapeutic removal of adult worms with diethylcarbamazine (DEC) led to the restoration of T cell proliferation and IFN-γ responsiveness (Sartono *et al.*, 1995). Moreover, in untreated gastrointestinal nematode-infected subjects whose peripheral T cells were hyporesponsive *in vitro* to bystander antigens such as BCG or malaria, depletion of Tregs (marked by high CD25 expression) resulted in the recovery of proliferative and cytokine responses to these antigens (Wammes *et al.*, 2010). It is notable that while IFN-γ responses are sharply downregulated in infected patients, IL-4 remains intact and IL-10 production is enhanced (Mahanty *et al.*, 1996; Ravichandran *et al.*, 1997; Sartono *et al.*, 1997). Moreover, across a cohort of infected subjects, IL-5 and IL-13 responses declined with increasing parasite load (Brattig *et al.*, 2002), although it has not yet been determined if these relationships are directly influenced by Treg activity.

At the serological level, hyporesponsiveness is associated with depression of IgE responses, while IgG4 is sharply elevated. Interestingly, serum IgG4 levels correlate not only with parasite density (Kwan-Lim *et al.*, 1990), but also with the failure to mount effector T responses among peripheral blood cells (Yazdanbakhsh *et al.*, 1993). The exceptionally high levels of IgG4 in the bloodstream of patent filariasis patients can now be mechanistically attributed to the action of Tregs, as *in vitro* switching of B cells to the IgG4 isotype is promoted by IL-10 (Satoguina *et al.*, 2005) as well as TGF-β and GITR ligation, although not CTLA-4 (Satoguina *et al.*, 2008). Conversely, curative drug treatment that also reduces Treg activity results in sharp reductions of IgG4 titres in patient groups (Atmadja *et al.*, 1995). Few studies have yet addressed whether there is any link between Tregs and IgA isotype antibodies, as might be predicted from the finding that TGF-β promotes switching to this antibody class in human B cells (van Vlasselaer *et al.*, 1992).

Tregs in Human Intestinal Nematode Infections

While studies with intestinal nematode infections in humans have not progressed to the same level of detail as those with filarial parasites, a number of studies have provided similar data on Treg activation. In general, infection with intestinal parasites does not appear to elevate proportions of Foxp3+ Tregs, but qualitative changes are nevertheless apparent and may be more functionally significant than simple numbers alone. For example, significantly more CTLA4+ CD4+ T cells are found in children with intestinal helminths than in cases of protozoal infection or in uninfected subjects, echoing the situation in tissue infections with filarial nematodes (García-Hernández *et al.*, 2009). More broadly, in functional terms the response of geohelminth-infected children to malarial and mycobacterial antigens is depressed by the greater activity of Tregs, and can be restored by removal of the CD25 high population of peripheral T cells (Wammes *et al.*, 2010). This finding can explain the depressed bystander responses (most importantly to malaria) in helminth-infected children (Hartgers *et al.*, 2009).

The thesis that helminth infection increases the degree of regulatory cell stimulation is also supported by recent work of Turner and colleagues. Studying human intestinal helminths, these authors reported that heavily infected children in areas hyperendemic for *Ascaris lumbricoides* and *Trichuris suum* display high levels of constitutive IL-10 and TGF-β production among their peripheral blood lymphocytes, inversely correlating with individuals' polyclonal and antigen-specific immune reactivity (Turner *et al.*, 2008). Interestingly, this analysis showed that the statistical effect was most marked when data were combined from the two species, and both regulatory cytokines, indicating a possible parallel between the parasites and a redundancy between the mediators. In a parallel study, *in vitro* leukocyte responses to mitogen were found to be suppressed in Brazilian children harbouring the same two parasites (Figueiredo *et al.*, 2010).

In another human nematode infection, *Strongyloides stercoralis*, a fascinating interaction has been reported in patients co-infected with HTLV-1, who show extraordinarily high levels of peripheral Foxp3+ T cells, amounting to ~18% of all CD4+ T cells compared with 4–7% in uninfected or monoinfected patients (Montes *et al.*, 2009). Notably, these patients display marked suppression of IL-5, in a similar

manner to that noted in filariasis infections (Sartono *et al.*, 1997). Moreover, lower eosinophilia and higher worm burdens were also apparent, consistent with a hypothesis that Tregs act to prolong nematode survival by (in this case) blocking eosinophil activation.

Role of Regulatory IL-10 in Human Infections

In humans, the role of IL-10 seems less ambiguous than in mice, and appears consistently to mediate immunoregulatory effects rather than play any obvious role in favouring Th2 reactivity. Strong IL-10 responses are observed in all chronic infections, and anti-IL-10 antibodies can reverse T cell suppression *in vitro* (Mahanty and Nutman, 1995; Doetze *et al.*, 2000). Elevated IL-10 in filarial infections is associated with poor responses to tetanus vaccination (Cooper *et al.*, 1998), while chemotherapeutic treatment of infected patients lowers IL-10 levels while boosting antigen-specific cytokine responses (Henry *et al.*, 2001). It is possible that the unequivocal regulatory role of IL-10 in humans is in part a matter of timing: in humans one is always studying long-term infections in which the original parasite priming event is long past, and IL-10 acts purely at the homeostatic level. In laboratory mice, the infection is studied in its entirety and so the priming event takes precedence. Interestingly, in one case in which the early recognition of *B. malayi* larvae has been studied *in vitro*, the human response is predominantly pro-inflammatory (Babu and Nutman, 2003), suggesting that, as in mice, IL-10 may be important to forestall heightened Th1/Th17 reactivity from the outset. Moreover, as in rodent models, IL-10 in human filarial infections emanates primarily from Th2-like CD4+CD25– cells (Mitre *et al.*, 2008).

As mentioned above, chronic nematode infections are associated with both higher Treg activity and greatly elevated serum IgG4 titres. A mechanistic link between these observations is suggested by the Hoerauf laboratory, which first reported the induction of IgG4 by B cells exposed to IL-10 (Satoguina *et al.*, 2005), and more recently showed that GITR-GITRL interactions between Tregs and B cells were required to induce the switch to IgG4, which was also dependent on the presence of TGF-β (Satoguina *et al.*, 2008). Hence, it may transpire that circulating IgG4 levels in humans could be a surrogate marker not only for the presence of nematode parasites, but also of elevated Treg activity.

Induction of Regulatory T Cells

The conversion of resting, naive T cells into the Treg phenotype requires both TCR ligation and the appropriate mix of co-stimulation and cytokine activation, most importantly by TGF-β (Zheng *et al.*, 2007). While cytokines such as TGF-β may derive from a range of cell types, dendritic cells (DCs) are essential for antigen presentation through MHC Class II, and the provision of a co-stimulatory signal through CD28. In addition, DCs may produce retinoic acid, which amplifies their ability to induce Tregs (Coombes *et al.*, 2007). The propensity of DCs to generate regulatory T cells may be expanded in nematode infection; for example, *H. polygyrus* ES-pulsed DCs are skewed towards the induction of CD4+CD25+IL-10+Foxp3– T cells, which can suppress T cell proliferation *in vitro* through production of IL-10 (Segura *et al.*, 2007). Likewise, a major protein released by adult hookworm, Ac-TMP-1, also modifies DC function, inducing T cell differentiation into both CD4+ and CD8+ CD25+Foxp3+ T cells that express IL-10. The CD4+ suppressor cells were shown to suppress proliferation of effector T cells *in vitro* through production of TGF-β and in an antigen-specific manner (Cuéllar *et al.*, 2009). It will be interesting to ascertain whether the extensive modulation of human dendritic cells by filarial nematodes (Semnani and Nutman, 2004; Semnani *et al.*, 2010) includes a similar adoption of pro-regulatory capacity, and whether such effects are mediated by defined ES proteins of the filarial parasite.

Most recently, it has been established that a key pathway by which *H. polygyrus* can activate the host immunoregulatory system is by producing a TGF-β-like mimic that stimulates T cells through the TGF-β signalling pathway (Grainger *et al.*, 2010). Exposure of naive murine T cells to *H. polygyrus* adult worm excretory-secretory products (HES), together with TCR ligation, induces expression of the Foxp3 transcription

factor in naive non-regulatory T cells. Stimulation of Foxp3 expression fails in cells expressing the dominant negative TGF-β-RII or co-incubated with a chemical inhibitor of the TGF-β-RI kinase. Moreover, naive T cells that have been converted *in vitro* by HES into Foxp3+ regulatory T cells are equally effective as those induced with mammalian TGF-β at suppressing airway allergy in recipient mice (Grainger *et al.*, 2010). The HES activity is thus a phenocopy of host TGF-β, although representing a structurally distinct entity, as anti-mammalian TGF-β antibodies do not block the effects of HES. The ability of this nematode product to induce functional Tregs through the TGF-β pathway illustrates a fascinating case of convergent evolution in which an ancient signalling cascade has been exploited to promote parasite survival. This molecular strategy, moreover, is not restricted to the murine species, as the sheep parasite *Teladorsagia circumcincta* releases a functionally similar product that induces Tregs *in vitro* (Grainger *et al.*, 2010).

Regulatory T Cell Therapy: The Future?

Great interest has been focused on the question of whether certain nematode infections can confer a therapeutic benefit on patients with immunopathological diseases related to dysfunctional immune regulation (Weinstock *et al.*, 2004; Summers *et al.*, 2005b; see also Chapter 14, this volume). Specifically, introduction of *Trichuris suis* has been reported to benefit patients with inflammatory bowel diseases (Summers *et al.*, 2005a, 2006), although the effects on allergic rhinitis appear less certain (Bager *et al.*, 2010; Hepworth *et al.*, 2010; Summers *et al.*, 2010). In parallel, extensive studies on therapy with *Necator americanus* parasites are also under way (Croese *et al.*, 2006; Mortimer *et al.*, 2006; Feary *et al.*, 2010). The inverse relationship between multiple sclerosis prevalence and the incidence of trichuriasis (Fleming and Cook, 2006), the extended remission of MS patients who had fortuitously acquired helminth infections (Correale and Farez, 2007), and the reduced incidence of diabetes in filariasis patients (Aravindhan *et al.*, 2010a, 2010b), all provide circumstantial evidence to suggest that helminth therapy could also extend to life-threatening autoimmune conditions (Fleming and Fabry, 2007).

At this stage, it remains to be demonstrated that any beneficial effects in humans of helminth therapy are mediated by Tregs. It is more likely that the Treg pathway is one of several parallel or complementary mechanisms by which nematodes are able to manipulate host immunity (and, indirectly, ameliorate immunopathology). One challenge therefore is to define the settings in which helminth-elicited Tregs are most able to act in a favourable capacity. Beyond this lies a greater challenge, which is to disembody the biological effect of live helminths, and isolate the molecular principals produced by parasites that are able to modulate the mammalian immune system. To this end, our laboratory and others are pursuing a combined definition of parasite molecular mediators and host regulatory cell types so as to replicate the pathways used by nematodes to dampen immunity and disable pro-inflammatory cascades (Hewitson *et al.*, 2009). The identification of these individual mediators represents a feasible goal towards developing new pharmaceutical products for therapy of immunopathological diseases (Harnett and Harnett, 2008), in which there is little doubt that regulatory T cells will play a prominent role.

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