Trends in Parasitology

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Review

Regulatory T cells in parasite infections: susceptibility, specificity and specialisation

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Regulatory T cells (Tregs) are essential to control immune system responses to innocuous self-specificities, intestinal and environmental antigens. However, they may also interfere with immunity to parasites, particularly in chronic infection. Susceptibility to many parasite infections is, to a greater or lesser extent, controlled by Tregs, but often they play a more prominent role in moderating the immunopathological consequences of parasitism, and dampening bystander reactions in an antigen-nonspecific manner. More recently, Treg subtypes have been defined which may preferentially act in different contexts; we also discuss the degree to which this specialisation is now being mapped onto how Tregs maintain the delicate balance between tolerance, immunity, and pathology in infection.

Highlights

Many, but not all, parasite infections are sustained by regulatory T cell (Treg) suppression of protective immunity.

Interfering with Treg inhibitory mechanisms, including suppressive cytokines, may alter the outcome of infection.

Tregs are important in protecting against immune pathology in nearly all infections so far studied.

Treg subsets coexpressing transcription factors shared with effector subsets (Th1, Th2, or Th17) may be most effective in mitigating pathology in the tissues.

Parasites and Tregs: emergence of an effective partnership

Tregs are a subset of $CD4^+$ T cells which prevent autoimmune and allergic responses, and rein in overzealous reactions to infection which can cause damage to the host [1]. Although first defined as controllers of errant effector T cells, the functions of Tregs have emerged to be remarkably multidimensional, impacting metabolism and tissue homeostasis, controlling innate as well as adaptive immune cells, and driving T cell memory [2]. Hence the role of Tregs in infection is highly context-dependent, with their influence depending on phase of parasite life cycle and tissue location, and according to the host or pathogen genotype [3]. Understanding these nuances is integral to the biology of each specific infection, and for rational management of pathology and infection control [3–5]. In this review, we discuss recent advances in Treg biology across the range of parasitic infections, and how new knowledge of their phenotypes can be harnessed towards immune intervention to drive protective immunity.

While regulatory T cells modulate all immune responses, three fundamental questions arise in the context of infections. (i) Susceptibility: do Tregs dictate the outcome of infection, for example by suppressing protective immunity or protecting from pathology? (ii) Specificity: are Tregs directly activated by the pathogen, potentially recognising parasite antigens, and/or are they driven by self-antigens to fulfil a physiological role in restoring homeostasis? (iii) Specialisation: are specific subsets of Tregs responsible for the clinical states observed in infection, and can selective manipulation of key subsets improve the outcome?

As discussed in the following section, the answers to these questions vary widely according to the nature of the parasite, and also of the host. Understanding when (and which) Tregs can be detrimental or beneficial is critical in designing therapeutic interventions which will favour clearance without collateral damage, promoting immunity to challenge infection and preventing parasite transmission.

An important new perspective has been established in charting the remarkable diversity of phenotype and function within the Treg compartment, ranging from origin, through tissue

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location, to gene expression [6–8]. For example, tissue regulatory cells impacting metabolism and repair in adipose and other nonlymphoid niches [9], differ from CXCR5$^+$ follicular Tregs (Tfr) controlling B cell responses within the lymph nodes [10]. Tregs are also found with differing levels of activation markers reflecting their suppressive potency and survival; while the functional borderlines between these types of regulatory T cells are still being defined, they will differentially impact parasite infections in specific tissue or intestinal niches, as detailed in the following section.

Treg subsets: a hierarchy of divisions

The regulatory T cell population compartment encompasses both **Forkhead Box p3 transcription factor (Foxp3$^+$)** (see Glossary) and Foxp3$^-$ populations, the latter exerting important down-modulatory effects through expression of interleukin (IL)-10, in the case of Tr1 [11], and IL-35 from Tr35, T cells [12]. Within the classical Foxp3$^+$ Treg population, an ontogenetic dichotomy is seen (Figure 1) between **thymic regulatory T cells (tTregs)** (thymic, previously known as natural) which differentiate in the thymus, primarily with self-antigen specificity and previously classified as Helios$^+$ Tregs; and **peripherally induced regulatory T cell (pTregs)** which are induced from peripheral Th cells in a **transforming growth factor-β (TGF-β)**-dependent manner [6].

A second level of heterogeneity within Foxp3$^+$ Tregs is through expression of key transcription factors originally associated with T effector subtypes, including Tbox expressed in T cells (T-bet), **GATA binding protein 3 (GATA3)** and **ROR orphan receptor γt transcription factor (RORγt)** corresponding to Th1, Th2, and Th17 respectively [2]. Whether these represent stable Treg subsets, or even an intermediate effector/regulatory population, is often debated; functionally however, they are fine-tuned for suppressing their corresponding Th subset in local tissues, due to shared trafficking via common chemokine receptors [2]. Interestingly, as described later, **T-box transcription factor 21 (T-bet$^+$)** Tregs have a higher profile in protozoal parasite infections, while GATA3$^+$ (and to some extent RORγt$^+$) Tregs are more prominent in **helminth** infections.

Thirdly, in diverse settings, Tregs invoke multiple regulatory mechanisms including the release of IL-10 and TGF-β, surface-mediated inhibition through the CTLA-4 and **programmed cell death 1 (PD-1)** pathways, which modify or block target cell activation, and the ectoenzymes CD39 and CD73 which release anti-inflammatory adenosine [2]. Treg CD25 may deprive effector cells of essential IL-2, while additional interactions are mediated by the surface proteins **glucocorticoid-induced TNFR-related protein (GITR)**, **inducible T cell costimulator (ICOS)**, **lymphocyte activating gene 3 (LAG-3)** and TIGIT, each of which may be suitable targets for therapies aimed at neutralising Treg activity, as discussed in the following section.

In vivo manipulation of regulatory T cell responses

Formal evidence that Tregs block immunity to parasites is derived from deletion and depletion experiments, using either depleting antibodies or genetic means (Figure 2). Anti-CD25 antibodies achieve partial Treg depletion but may also inactivate effector CD4$^+$ T cells which express CD25; antibody given prior to infection mitigates this problem to some extent, and where anti-CD25 treatment boosts immunity this cannot be attributed to loss of effector cells. A more targeted route to Treg depletion is in transgenic mice expressing the **diphtheria toxin (DTx)** receptor under the control of the Foxp3 promoter, such as in the **DEpletion of REGulatory T cells (DEREG) mouse**; DTx administration results in a profound ablation of the Foxp3$^+$ Treg compartment at any chosen time during infection. In addition, as discussed in the following section, individual genes can be deleted within the Foxp3$^+$ compartment by using conditional knockouts coupled with a Foxp3-Cre construct.

Glossary

Apicomplexan: protozoal parasites with apical complex required for cell invasion; CTLA-4, CD152, cytotoxic T-lymphocyte-associated protein 4, a coinhibitory receptor in the CD28 superfamily which competes with CD28 for binding of CD80/86 and gives an inhibitory signal.
DEpletion of REGulatory T cells (DEREG) mouse: transgenic mouse strain in which the diphtheria toxin receptor is expressed under control of the Foxp3 promoter, allowing selective depletion of Tregs at various time points of infection.
Diphtheria toxin (DTx): administered to DEREG mice to induce apoptosis of Tregs.
Forkhead Box p3 transcription factor (Foxp3): the canonical Treg transcription factor.
GATA binding protein 3 (GATA3): the canonical transcription factor for Th2 cells, also required for T cell development.
Glucocorticoid-induced TNFR-related protein (GITR): a costimulatory receptor which increases proliferation, activation and survival of T cells when interacting with its ligand.
Helminth: a parasitic worm from the Nematode or Platyhelminth phyla.
Inducible T cell costimulator (ICOS): a member of the CD28 superfamily which increases proliferation, activation, and survival of T cells when interacting with its ligand.
Lymphocyte activating gene 3 (LAG-3): an inhibitory receptor which inhibits T cell activation when interacting with the peptide:MHC II complex.
Peripherally induced regulatory T cell (pTreg): Tregs induced from peripheral CD4$^+$ T cells.
Programmed cell death 1 (PD-1): an inhibitory receptor which provides an inhibitory signal to immune cells when it engages with its ligand.
Retinol orphan receptor γt transcription factor (RORγt): the canonical Th17 transcription factor.
T-box transcription factor 21 (T-bet): the canonical Th1 transcription factor.
T-cell immunoglobulin and mucin-domain–containing molecule 3 (Tim-3): a coinhibitory molecule that has been shown to be important to the Th1 and Treg response.
Thymic regulatory T cell (tTreg): previously natural regulatory T cell, Tregs which are induced in the thymus as part

Role(s) of Tregs in apicomplexan parasite infections

Apicomplexan parasites are protozoa which invade host cells through a specialised invasion structure (the apical complex) and which are obligate intracellular pathogens; two of the most well-studied are the vector-borne *Plasmodium* species causing malaria [13] and the cosmopolitan pathogen *Toxoplasma gondii* that can cross the placenta to infect the unborn child [14]. A summary of recent studies on these and other protozoal parasites is presented in Table 1.

In malaria, the role of Tregs has been controversial for more than a decade, with some but not all reports indicating Treg suppression of protective immunity, clinical disease and/or long-term memory [15]. In human *Plasmodium falciparum* malaria for example, early work found elevated FOXP3^{+} Treg numbers, in proportion to parasite burden and clinical severity [16], but subsequent studies found a greater influence of Tr1-like FOXP3-negative T cells producing IL-10 [17] while expressing Treg-like inhibitory receptors such as CTLA-4, LAG3, and TIM3 [17,18].

These findings are mirrored in mouse model systems; for example *Plasmodium yoelii* infection increases Foxp3^{+} Treg numbers, primarily within the natural/thymic rather than peripheral/induced compartment [19]. In some models, Tregs primarily repress protective immunity rather than

of thymic selection and often have self reactivity.

Transforming growth factor-β (TGF-β): the cytokine important for Treg induction and function.

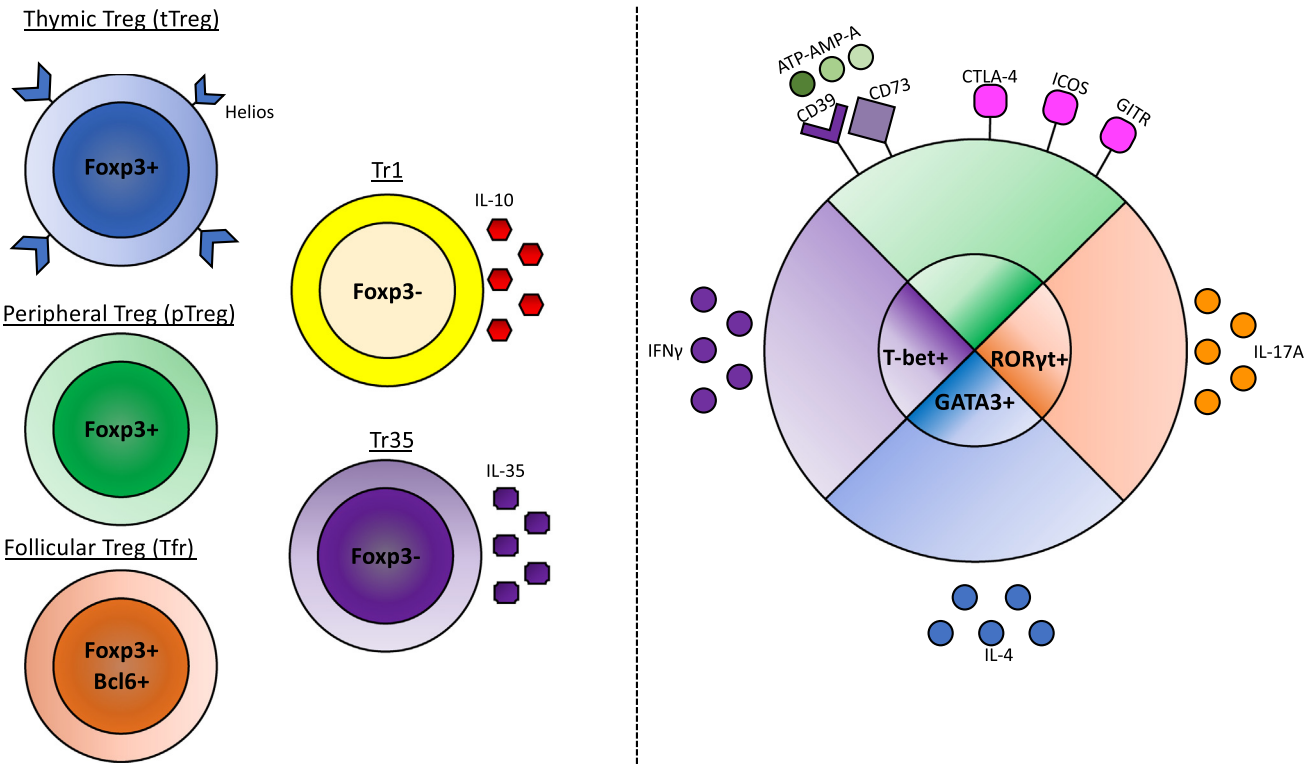

Figure 1. Subsets of regulatory CD4^{+} T cells. The left panel shows the three primary subtypes of Forkhead Box p3 transcription factor (Foxp3^{+}) regulatory T cells (Tregs), including thymic Tregs which preferentially express Helios, peripherally induced Helios-negative pTregs, and follicular Tregs (Tfrs) which express Bcl6. Also in the left panel are regulatory cells which do not express Foxp3 but can produce interleukin (IL)-10 (Tr1 cells) or IL-35 (Tr35 cells). The right panel shows key Treg suppressive mechanisms known to be important during parasitic infection, including expression of coinhibitory receptors (among them PD-1, ICOS, and GITR), and ectoenzymes CD39 and CD73 which degrade proinflammatory ATP to adenosine. Also depicted are the specialised subsets of Tregs coexpressing transcription factors more associated with Th1 (T-bet), Th2 (GATA3) or Th17 (RORγt). Abbreviations: GATA3, GATA binding protein 3; GITR, glucocorticoid-induced TNFR-related protein; ICOS, inducible T cell costimulator; PD-1, programmed cell death 1; RORγt, ROR orphan receptor γt transcription factor; T-bet, T-box expressed in T cells.

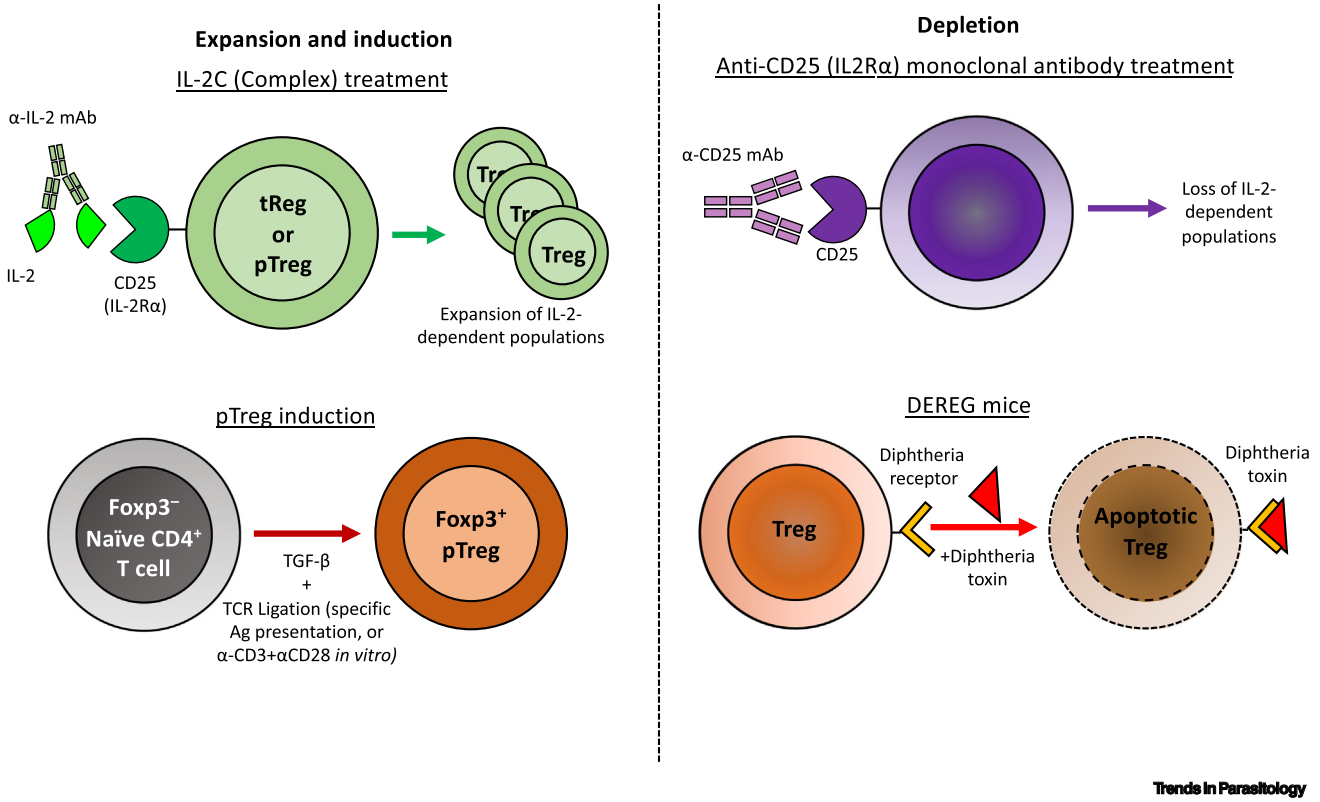

Figure 2. *In vivo* regulatory T cell (Treg) interference protocols in murine models. The left panel shows the methods used to increase Treg numbers *in vivo*, including IL-2 complex treatment in which IL-2 complexed with α-IL-2 monoclonal antibody is coadministered to increase Treg activation. Alternatively, transforming growth factor-β (TGF-β) can also be administered *in vitro* to induce Treg differentiation from naïve CD4$^+$ T cells. The right panel shows two approaches to deplete Tregs in murine models. These include the use of monoclonal antibodies to deplete CD25-expressing cells (most but not all Tregs) and DEpletion of REGulatory T cell (DEREG) mice, which express the diphtheria toxin receptor in Tregs under the Forkhead Box p3 transcription factor (Foxp3) promoter, allowing cell-specific ablation of Tregs by administration of diphtheria toxin. Abbreviations: mAb, monoclonal antibody; pTreg, peripherally induced regulatory T cell; TCR, T-cell receptor; tReg, thymic Treg.

ameliorate inflammation, as infection of transgenic Foxp3-overexpressing mice, with *P. chabaudi* leads to increased disease severity, as did boosting the Treg compartment by administration of an IL-2:anti-IL-2 complex (IL-2C) [20]. However, in *Plasmodium berghei*-infected mice, IL-2C-mediated expansion of Tregs ameliorated disease [21]. Tregs during *Plasmodium chabaudi* infections of mice also upregulate CXCR3 and T-bet, and although the functional importance of the latter has not been established, these data indicate the emergence of specialised Treg subtypes in malaria infection [22]. Similarly, in human *Plasmodium vivax* malaria, a T-bet$^+$ subtype is found with altered functionality, in this case subdued suppressive capacity during acute infection [23].

Blocking CD25 using a monoclonal antibody protected mice from lethal infection with *P. yoelii* [24], in particular reducing IL-10 production from Tregs [25], as did depletion of Tregs in DEREG mice, demonstrating that in some settings at least, Tregs suppress the antiparasite response during malaria infection [26].

Indeed, there is no better illustration of the context-dependency of Foxp3$^+$ Tregs than in this model of malaria. Using the specific nonlethal strain 17XNL, Treg depletion in DEREG mice during the first 2 days of infection exacerbated infection with a lethal outcome, but if delayed to days 9–11 of infection produced a sharp increase in effector T cells and elimination of *P. yoelii* [15]. At this later

Table 1. Selected recent studies exploring Treg phenotypes and function during protozoal infection[a]

Parasite species	Host species	Major points (expansion, immunity, pathology)	Refs
Leishmania spp.			
Leishmania braziliensis	Human	Peripheral blood Tregs upregulate CD25 and CTLA-4	[116]
Leishmania donovani	Human	Tregs in asymptomatic infections express amphiregulin	[117]
	Mouse	Tregs limit pathology but do not suppress anti-parasite responses	[39]
Leishmania infantum	Human	Peripheral blood Tregs express elevated CD73	[118]
Plasmodium spp.			
Plasmodium berghei, P. chabaudi, P. yoelii	Mouse	Lethal (*P.b., P.y.* XL) infections, but not non-lethal (*P.c., P. y.* XNL), associated with ICOS$^+$ Foxp3$^+$ Tregs	[119]
Plasmodium berghei, P. falciparum	Mouse, human	IL-10 producing Tr1 cells may control resistance versus susceptibility	[17]
Plasmodium chabaudi	Mouse	Expansion of T-bet$^+$CXCR3$^+$ Tregs	[22]
Plasmodium falciparum	Human	Treg frequencies and symptomatic malaria decline after repeated infection	[120]
Trypanosoma spp.			
Trypanosoma cruzi	Mouse	Thymic atrophy and disruption of both tTreg and pTreg	[43]
	Mouse	Reduced frequency of Tregs allows protective CD8$^+$ T cell expansion	[44]

[a]Earlier reviews provide more complete information on studies prior to 2015 [3,13,121,122].

time Tregs switch towards higher expression of CTLA-4, which, as discussed later, could prove to be a functional target for antimalarial immunotherapy. The same *P. yoelii* model has also recapitulated aspects of human infection in demonstrating immune downregulation by Foxp3$^-$ IL-10-producing Tr1 cells [27]. Overall, in both mouse and human malaria, regulatory T cells play an unquestionably key role in the outcome of infection, but one which varies at different stages of infection and encompasses both Foxp3$^+$ and Foxp3$^-$ populations [13].

Within the Foxp3$^-$ compartment, a Th1-like Tr1 population induced by infection may be instrumental to controlling infection as well as the loss of vaccine efficacy in malaria infected patients [28,29]. During experimental human *P. falciparum* infection, IFNγ$^+$IL-10$^+$ Tr1 cells, induced by type I interferon signalling through cMAF and BLIMP-1, express multiple coinhibitory receptors including CTLA-4, LAG3, and PD-1 [17,29]. Such cells are implicated in protection of children from severe malaria in endemic regions by production of IFNγ to enhance parasite clearance, but most significantly IL-10 to regulate immunopathology and avoid clinical disease [30]. This profile is reflected in murine infection with *P. chabaudi*, in which IL-10$^+$ Tr1 cell induction is dependent on Blimp-1, which if deleted from T cells results in lethal neurological pathology alongside reduced parasitaemia, illustrating the double-edged nature of Tr1 cells during *Plasmodium* infection [29]. Immunoregulatory cells, both Foxp3$^+$ Tregs and Foxp3$^-$ Tr1 cells are critical to controlling the immune response to *Plasmodium* infection and the prevention of severe immunopathology associated with infection.

Turning to *Toxoplasma gondii* infection, insights have primarily come from mouse models; for example, following oral infection of C57BL/6 mice, there is a collapse in Treg numbers and function leading to a fatal outcome [31]. A similar outcome occurs following Treg depletion in *T. gondii* infection of genetically resistant BALB/c mice [32]. The possibility that Foxp3$^+$ Tregs moderate immunopathology without compromising protection was also supported by the finding that IL-2C

administration protected mice against a lethal strain of *T. gondii* [33], while adoptive transfer of GFP-Foxp3^{+} Tregs into infected mice led to lower weight loss, reduced intestinal pathology and delayed mortality despite higher parasite burdens [34]. In considering how Tregs may contribute to protective immunity, as well as moderated pathology, it is interesting to note elevated Foxp3^{+}T-bet^{+} co-expression in this infection [31], with unrestrained pathology and mortality when T-bet$^{fl/fl}$Foxp3^{Cre} mice are infected with *T. gondii* [35].

It has been suggested that reduced Treg numbers and functionality also underpin the detrimental effects of the parasite on the foetus in pregnancy [36]. Indeed, adoptive transfer of Tregs from the foetal-maternal interface of naïve pregnant mice into dams infected with *Toxoplasma gondii* leads to an increase in both placental and foetal weights, with higher levels of key mediators such as IL-10 and TGF-β [37]. In toxoplasmosis, therefore, Tregs play a critical role in host survival and well-being, but primarily through moderating immunopathology, even at the cost of higher levels of infection, while the consequence of removing Treg suppression is lethality rather than protective immunity.

Tregs in kinetoplastid infections – *Leishmania* and *Trypanosoma*

A further example of the double-edged nature of Tregs is found in *Leishmania major* infections, in which these cells not only render mice more susceptible, but, by permitting the survival of a residual parasite population, ensure that immunity to challenge infection is maintained [38]. However, in other models, such as *Leishmania donovani,* Tregs were found to be less critical than the overall levels of IL-10, which primarily emanated from CD4^{+}Foxp3^{-} Tr1-like cells and myeloid sources [39].

In New World species causing cutaneous leishmaniasis, several lines of evidence support a protective role for Tregs, not only in restraining inflammatory pathology but also, counter-intuitively, reducing parasite burdens. In the case of *L. (Viannia) panamensis*, adoptive transfer of Tregs to infected mice or administration of IL-2C to boost Treg numbers, each led to reduced cytokine responses, attenuated pathology and lower parasite burden [40]. Conversely, DTx-treated DEREG mice present with increased production of proinflammatory cytokines IL-17 and interferon gamma (IFNγ), exacerbated lesions and increased parasite numbers. Notably, *L. panamensis* infection leads to an increase in T-bet^{+} Tregs which may allow them to contribute to tissue parasite control as well as restraining inflammatory outcomes [40].

During murine infection with *Leishmania infantum*, a cause of visceral leishmaniasis, both Foxp3^{+} Tregs and Foxp3^{-} Tr1 cells produce IL-10 that is responsible for increased parasite burdens [41]. Foxp3^{-} Tr1 cells are also the main source of IL-10 during human cutaneous *Leishmania braziliensis* infection, in both the circulating peripheral blood mononuclear cell (PBMC) population and tissue lesions [42]. Thus, in these latter species, there is a more balanced scenario in which both conventional Tregs and Tr1 cells both protect the host and impact parasite burden via their production of IL-10.

In other protozoal infections, also, Tregs appear primarily to control pathology. Murine infections with the intracellular protozoan *Trypanosoma cruzi* result in decreased frequencies of Tregs [43], and the resulting deficiency is associated with severe immunopathology that can be rescued by transfer of *in vitro* induced Tregs [44]. T-bet and CXCR3 are also upregulated in Tregs at day 20 post-infection with *T. cruzi*, to levels comparable to those in conventional T cell compartments, which may again limit the capacity of Tregs to ameliorate pathology [44]. In human Chagas' Disease, caused by *T. cruzi*, Tregs show an activated phenotype which differs according to clinical presentation, with less advanced disease cases having high numbers of IL-10 producing Tregs while those with cardiac morbidity have increased CTLA-4^{+} Tregs alongside an inflammatory cytokine environment, showing that context and phenotype are important to determining clinical outcome in Chagas' disease [45].

African trypanosomes differ from their American congeners by following an extracellular lifestyle. Adoptive transfer of CD25$^+$ 'Tregs' into mice infected with *Trypanosoma congolense* leads to increased parasitaemia despite raised serum IFNγ, IL-6, MCP1 and TNF [46]. Conversely, blocking CD25 using a monoclonal antibody also reduced susceptibility to the same parasite [47]. Hence, the impact of Tregs in the extracellular African trypanosome setting is a more one-dimensional inhibition of resistance, but in infections with the intracellular American species, Tregs of different functional properties interface with both susceptibility and pathology in a more complex manner.

Tregs in helminth parasite infections

Across a broad range of helminth infections, Tregs generally act to break down immune resistance while also mitigating immune pathology, so that their role is seen to be more pivotal to the outcome of infection than is apparent in protozoal infection (Table 2) [48,49]. Nevertheless, as with protozoal parasitism, Tregs can act to the benefit or detriment of the host depending on many specific factors. Arguably, in chronic human infections such as filariasis, Tregs may establish a tolerant asymptomatic carrier state that advantages both host and parasite, by permitting ongoing transmission while causing minimal pathological harm [50]. Indeed, when PBMCs

Table 2. Selected recent studies exploring Treg phenotypes and function during helminth infection[a]

Parasite species	Host species	Major points (expansion, immunity, pathology)	Refs
Cestodes			
Echinococcus multilocularis	Mouse	Treg depletion raises Th1/Th17 and reduces parasite load	[61]
	Mouse	Treg depleted mice have smaller lesions in early stage infections	[62]
Taenia crassiceps	Mouse	Greater Treg numbers in susceptible strain, anti-CD25 reduces parasite numbers	[68]
Nematodes			
Ascaris suum	Mouse	Tregs expand in mice upon infection	[123]
Heligmosomoides polygyrus	Mouse	Tregs determine outcome of infection	[55]
	Mouse	Increase in adipose Tregs expressing LAP and CD134	[124]
Hookworm (*Ancylostoma duodenale* and *Necator americanus*)	Human	Higher peripheral Tregs and plasma IL-10 levels in infected children	[125]
	Human	Higher peripheral ICOS$^+$Tregs in infection, reduces with anthelmintic treatment	[126]
Mansonella perstans	Human	Increased CD25$^+$ Tregs, depressed cytokine responses	[127]
Onchocerca volvulus	Human	Decreased Tregs associated with hyper-reactive disease	[128]
Strongyloides stercoralis	Human	Duodenal Tregs increased adjacent to the parasites	[129]
Wuchereria bancrofti	Human	Higher Foxp3$^+$ Treg frequency regardless of pathological state	[130]
Trematodes			
Fasciola hepatica	Sheep	Infection increases Foxp3+Tregs	[131]
Schistosoma haematobium	Human	Induces Tregs which maintain cytokine output after clearance	[132]

[a]Earlier reviews provide more complete information on studies prior to 2015 [3,49].

were tested from subjects with lymphatic filariasis, those with high levels of circulating microfilariae were the only ones to lack parasite-specific T cell responses. However, if PBMCs were first depleted of $CD4^+CD25^{(high)}$ cells, responsiveness was restored [51].

Among mouse models, a critical role for Tregs has now been demonstrated in multiple systems. Most work has been conducted on the intestinal nematode *Heligmosomoides polygyrus*, in which infection of mice drives activation and expansion of $Foxp3^+$ Tregs in the mesenteric lymph nodes (MLNs) [52,53], and small intestinal Peyer's patches in close proximity to the parasites [54]. Tregs from *H. polygyrus*-infected mice express higher levels of proliferative markers [55] and are more suppressive than those from naïve mice [52,53,56]**.** Expansion of Tregs with IL-2C rendered genetically resistant BALB/c mice susceptible to chronic infection; while anti-CD25 depletion immediately before infection allowed susceptible C57BL/6 to clear the infection [55]. Unexpectedly, while DTx-mediated depletion of $Foxp3^+$ Tregs increased $CD4^+$ Th activation and cytokine production, it also increased the worm burden and caused severe weight loss. These effects were attributed to 'immunological chaos' with unrestrained IFN-γ production. Hence, Tregs not only maintain susceptibility to *H. polygyrus*, but protect animals against immunopathological consequences of intestinal barrier breach [55].

A similar scenario of parallel boosting of immunity and pathology in Treg depletion was also seen in reduced egg burdens but accelerated granuloma formation in *Schistosoma mansoni*-infected mice following anti-CD25 treatment [57], while adoptive transfer of $CD4^+CD25^+$ Tregs from infected mice has been shown to reduce granulomatous pathology in recipient mice, albeit without affecting egg burdens [58].

In mice infected with *Strongyloides ratti*, a model for human strongyloidiasis, there is a similar expansion of $Foxp3^+$ Tregs [59] with DTx treatment of DEREG mice leading to increased worm expulsion and higher type 2 responses, showing that escape from Treg suppression promotes immunity [60]. In DEREG mice infected with the cestode tapeworm *Echinococcus multilocularis,* DTx treatment similarly reduced worm burden, alongside enhanced Th1/Th17 responses and smaller lesion sizes [61,62]. In contrast, DTx treatment of *Trichuris muris*-infected DEREG mice was first reported to have no effect on worm burden [63], with a subsequent study finding a modest reduction only if DTx treatment was given at early time-points, indicating a change in Treg roles during the course of infection [64].

Although Treg depletion may unleash a protective immune response, in chronic helminth infection the effector T cell population may be anergised or exhausted. Experiments with the filarial parasite *Litomosoides sigmodontis* showed that clearance required treatment not only with anti-CD25, but also anti-GITR or anti-CTLA-4 as discussed in the following section [65,66]. Likewise, in *Schistosoma japonicum* infection, increased parasite killing with anti-CD25 treatment is enhanced when anti-CTLA-4 blocking antibodies are also given [67]. In several other settings, anti-CD25 alone is sufficient to rebalance the response in favour of immune protection, as seen with *S. mansoni* [57,58] and *Taenia crassiceps* tapeworms [68]. However, there are also cases in which anti-CD25 treatment does not change worm burdens, as reported for *T. muris* and *Trichinella spiralis* infections [64,69,70].

$Foxp3^-$ Tr1 cells are also important in many helminth infections, although in general less prominent than in protozoal parasitism. Filariasis in humans caused by nematodes such as *Onchocerca volvulus* or *Wuchereria bancrofti* causes an expansion of Tr1 cells which produce the majority of IL-10 during infection [71]. Tr1 cells (and Tregs) are also important in class switching to IgG4 antibody production during helminthiases, promoting an anti-inflammatory

isotype [72]. In summary, regulatory T cells are pivotal to parasite establishment, and maintenance of a tolerant state, during helminth infection.

Treg subsets in helminth infections

Recent reports have analysed both GATA3^{+} and RORγt^{+} Treg subsets in helminth parasitism. While GATA3 is classically associated both with early development of T cells, and within the committed Th2 subtype, if Tregs are unable to express it (as in Foxp3^{Cre} GATA3$^{fl/fl}$ mice), they cannot effectively regulate inflammation at barrier sites [73]. Like T-bet^{+} Tregs, GATA3^{+} Tregs can also play inflammatory roles, producing IL-4 in human asthma [74]. Nevertheless, in *S. ratti*-infected mice, Tregs lacking *Rbpj* predominantly express GATA3 and type 2 cytokines, resulting in more effective worm expulsion [75]; thus either *Rbpj* is essential for Treg control of Th2 responses, and/or GATA3 expression within Tregs is normally held in check by this factor.

Tregs coexpressing the Th17-associated transcription factor RORγt upregulate many products such as CTLA-4, IRF4, GITR and ICOS, suggesting that they are more suppressive than conventional Tregs [76]. RORγt^{+} Tregs have also been implicated as major players in the balance between resistance and susceptibility to *H. polygyrus,* as Foxp3^{Cre}RORγt$^{fl/fl}$ mice show greater resistance, implicating RORγt^{+} Tregs as key suppressors of the anti-helminth type 2 response in the gut [76]. These studies also show an interesting mutual suppression as there is a doubling in both GATA3^{+} Th2 cells and GATA3^{+} Tregs in Foxp3 ^{Cre} RORγt$^{fl/fl}$ [76]. The opposite is true for Foxp3^{Cre} GATA3$^{fl/fl}$ mice which show a twofold expansion of RORγ^{+}+ Tregs and a threefold increase in IL-17A expression in Tregs [77]. Only in the absence of STAT3 (activated by cytokines such as IL-6 and IL-27) is there coexpression of GATA3 and RORγt [76].

Evidence that infection preferentially induces certain Treg subsets in humans was provided by an Indonesian study, in which CTLA-4^{+} Tregs were enhanced in rural, soil-transmitted helminth-exposed residents compared to either European controls, or Indonesian city dwellers [78].

Antigen specificity of Tregs in parasite infection

Currently, the antigen specificities of Tregs active during parasite infections are, for the most part, unknown, raising questions of whether natural self-reactive cells are stimulated, or if peripheral T cells with parasite antigen specificity are induced to adopt a regulatory phenotype. In one of the few studies to address this question, Tregs from *L. major*-infected mice were shown to recognise antigens from *Leishmania*-infected dendritic cells (DCs); by using a transfer model, the *Leishmania*-specific Tregs were those expressing CD25 in a naïve donor, indicating their tTreg status [79].

More recently, the antigen-specific response to *S. ratti* has been studied using the *Hulk* model, in which larvae transgenically express the peptide 2W1S tagged with GFP. When *Hulk* larvae are used to infect mice and the antigen-specific response tracked using a tetramer, both 2W1S-specific Th2 and Treg cells were found, confirming the generation of cells recognising a larval encoded epitope [80]. If parasite antigen-specific Tregs are generated, it is likely that they may persist as a memory population in challenge infections, in a manner similar to the reported inhibition of antiviral responses many months after initial infection with murine hepatitis virus [81].

While otherwise the antigens recognised during parasitic infection are largely unknown, there are many examples of bystander Treg suppression of immune responses unrelated to parasitic infection in mice [82,83], alongside restoration of bystander responses following anthelmintic treatment in humans [84]. Importantly, Tregs from infected mice are able to suppress allergen-specific airway inflammation when transferred from donors that have not experienced the allergen

itself [82]. However, with this bystander suppression, there can be heightened risk of tumours; in fact, *H. polygyrus* infection causes an induction of Tregs which increase susceptibility to colon cancer and promote tumour development [85].

From susceptibility to resistance: protection by counteracting Treg mechanisms

Treg expression of coinhibitory surface ligands, including CTLA-4 and PD-1, as well as negative regulators such as ICOS, GITR, LAG3, and TIGIT, interfere with costimulatory signals to prevent T effector cell activation and induce tolerance [86]. These molecules are also commonly upregulated in Tregs during parasite infection and can play important roles in mediating immune suppression [49]. In parallel with the successful introduction of co-inhibitory (checkpoint) blockade in cancer therapy, similar approaches have been tested across a number of infection settings [87].

CTLA-4 (cytotoxic T-lymphocyte-associated protein-4, CD154) competes with CD28 for the CD80 and CD86 ligands, thereby blocking the CD28-dependent coactivating Signal 2 from antigen-presenting cells (APCs) required for effector T cell activation [86]. CTLA-4 not only binds CD80 and CD86 with higher affinity than CD28, but can strip these stimulatory ligands from the APC surface by trogocytosis (transendocytosis), in particular targeting DCs [88]. Anti-CTLA-4 antibodies reverse Treg suppression of the response to blood stage *P. yoelii* infection [15] while also abolishing the protective effect of Tregs in *P. berghei*-induced cerebral malaria [21]. As noted in the preceding text, however, Foxp3-negative CTLA-4^{+} T cells form an important part of the regulatory network in human malaria [18].

CTLA-4 is also upregulated on Tregs in many different helminth infections including *Brugia malayi* [89] and *L. sigmodontis* [66]. In the latter case, helminth-responsive Th2 cells become dysfunctional but their responsiveness can be restored using an α-CTLA-4 blocking antibody [66]. Furthermore, when combined with α-CD25 blocking antibody, *L. sigmodontis* parasites are cleared [66]. Similarly, combined α-CTLA-4 and α-CD25 administration increased killing of *S. japonicum*, although this was at the cost of increased pathology [67]. In the first week of *H. polygyrus* infection, Treg suppression is can be blocked with anti-CTLA-4 antibody; however by day 21 Tregs from infected mice were unaffected by α-CTLA-4 treatment indicating a switch in pathways during the course of infection [56].

PD-1 (programmed cell death 1, CD279) is another member of the CD28 superfamily which, like CTLA-4, delivers an inhibitory signal to T cells when it engages with its ligands PD-L1/PD-L2 and leads to cell cycle arrest [86]. Blockade of PD-1 clears blood-stage malaria from mice [90], but exacerbates IFNγ responses in cerebral malaria [91]. However, if PD-1 blockade is combined with OX40 agonism, to promote maximal effector responses, uncontrolled IFNγ production can confound protective immunity to *P. yoelii* and result in higher parasite loads [92]. In this system, PD-L2 competes with PD-1 so that PD-L2 blockade led to increased levels of IL-10 and Tregs, while soluble PD-L2 enhanced Th1 responses and rescued mice from fatal outcome of *P. yoelii* infection [93]. In human malaria, PD-L2 was found to be more highly expressed in APC from subjects with lower parasitaemia, supporting the hypothesis that it may counteract PD-1/PD-L1-mediated suppression [93]. There is also upregulation of PD-1 on Tregs from helminth-infected hosts including those infected with *L. sigmodontis* [66] and *S. mansoni* [94]. Interestingly, PD-1 was also upregulated on effector Th2 cells in helminth infection, indicating an anergic or dysfunctional state, and T cell responsiveness could be recovered by blocking PD-L2 [95]. PD-1 does not always play a critical role, however, as *S. japonicum* infected PD-1 deficient mice show similar parasite burdens, as a result of a compensatory increase in Treg immunosuppression with elevated CD39 and CD73 expression [96].

Among other functional receptors on Tregs, ICOS (CD278) acts as costimulatory receptor in Treg activation, response to antigen and function [97]. ICOS knock-out mice have reduced Treg induction upon infection with *H. polygyrus* and *S. mansoni* alongside elevated type 2 cytokines, showing that ICOS is important to the Treg response to helminths [97]. GITR plays a more nuanced role; it can act as a costimulatory molecule for T helper cells but when expressed on Tregs and binding its ligand (GITRL) on APCs, Treg suppressive function is lost, making it an enticing therapeutic target [98]. Naïve Tregs treated with GITR blocking monoclonal antibody lose their suppressive capacity *in vitro.* However, Tregs from *P. yoelii* infection with a lethal strain of the parasite are unable to be inhibited using α-GITR antibody, suggesting this molecule is not required for Treg mediated suppression during this parasite infection [99]. Conversely, during filarial nematode infection with *L. sigmodontis* the expression of GITR on $CD4^+$ T cells by day 40 post-infection is doubled, and dual blockade of GITR and CD25 using monoclonal antibodies leads to increased worm clearance associated with increased type 2 cytokines [65].

Lymphocyte-activating gene 3 (LAG3) is a CD4 homologue which interferes with MHC Class II binding; blockade of LAG-3 accelerates clearance of murine *Plasmodium* species *in vivo*, if combined with either anti-PD-L1 [90] or anti-PD-1 [100]. While the enhancement of effector cytokines occurred in these studies without any change in Treg numbers, the data are consistent with functional inhibition of Treg suppressive pathways.

T-cell immunoglobulin and mucin-domain–containing molecule 3 (Tim-3) is another coinhibitory receptor expressed by Tregs as well as type 1 lymphocytes. Tim-3 is upregulated on T cells in human and mouse malaria, and blockade of Tim-3 using a blocking monoclonal antibody during *P. berghei* infection led to decreased parasitaemia and increased survival compared to controls [101].

Another surface receptor on Tregs likely to impact on parasite infection is ST2, the receptor for the alarmin cytokine IL-33, which is expressed on colonic Tregs [102], thereby linking innate immune signals with the adaptive Treg population. In many parasitic infections, especially intestinal helminthiases, damage to the epithelial wall stimulates multiple alarmins like IL-33 and IL-25, and Treg activation may accompany stimulation of type 2 responses. However, Treg responses to *H. polygyrus* in $ST2^{-/-}$ mice are similar to their wildtype counterparts, despite their increased susceptibility to the parasite [103]. In contrast, IL-33 administration to C57BL/6 mice infected with *L. donovani* led to increased Treg activation and Foxp3 expression, while IL-33-deficient animals better controlled the infection, suggesting that the efficiency of Tregs is impaired by lack of IL-33 signalling [104]. Taken together, these results suggest that the relationship between IL-33-activated Tregs can have an impact on host response and parasite burden, but that this requires further investigation in each specific helminth and protozoan infection.

Targeting regulatory cytokines: IL-10, IL-35, and TGF-β

Another approach to combat the action of Tregs is to interfere with the three key immunosuppressive cytokines they produce, namely IL-10, IL-35 and TGF-β. In the case of malaria, IL-10 appears to be essential to avert immunopathology as *P. yoelii* infection of IL-10-deficient mice increased parasite clearance but at the cost of aggravated hepatic pathology [27]. Likewise, anti-IL-10R blockade in *P. berghei* infection increased both T cell responses and incidence of cerebral malaria [105]. Neutralisation of IL-10 in *Leishmania* infection does have clearer benefits, with fewer *L. major* lesions in IL-10-deficient mice [106]. IL-10R blockade in mice led to sterile immunity, likely due to the elimination of the latent phase of infection [107] and protected dogs from *L. infantum* infection, preventing their risk as a potential reservoir for human infection [108].

However, it is important to note that in each of these studies all sources of IL-10, not only from Tregs, will be negated.

IL-35 is a member of the IL-12 superfamily which also has regulatory effects during inflammation and is important to tTreg mediated suppression [12]; expression of IL-35 in intestinal, but not splenic $CD4^+Foxp3^+$ T cells has been reported in *T. muris*-infected mice [109]. A recent report on a cohort of multiple sclerosis patients adventiously infected with intestinal helminths showed heightened production of IL-35 alongside dampened disease pathology, although cytokine production was associated with Breg (regulatory B cell) [110]. It will be interesting if future studies block this regulatory cytokine during the parasite infections discussed in the preceding text.

Transforming growth factor β (TGF-β) is a pleiotropic cytokine which plays many roles during homeostasis and inflammation across a wide range of infection settings [111]. TGF-β has proven a successful therapeutic target in *Leishmania* infection and in helminth infection. Treatment with a monoclonal blocking antibody protected both resistant and susceptible mice against leishmaniasis [112]. During infection with the helminth *H. polygyrus*, TGF-β receptor I inhibitor SB431542 decreases adult worm burden, showing the importance of TGF-β signalling during helminth infection [113]. As with IL-10 and IL-35 however, it must be noted that to date, Treg-specific deletion of immunosuppressive cytokines has not been examined in the context of helminth infections.

Concluding remarks

Tregs are universally essential to a healthy outcome of parasite infection, whether protecting the host from excessive immune pathology during infection, or ensuring the balance and coordination of an effective immune response. As we increasingly appreciate the many forms and functions of Tregs, we anticipate a clearer definition of the phenotype and specialisation of Tregs most appropriate to the parasite species and niche in question. Many of the original studies used relatively blunt tools, and revisiting the contribution of the distinct subsets would be timely and informative. This in turn should illuminate a path to modulating Tregs for the optimal result. There is nevertheless a notable contrast between the helminth systems, in which enhanced Treg activity seems generally to be the rule, and protozoal infections, in which Tregs play more nuanced roles, albeit often crucial in minimising pathology. In both spheres, however, there is frequently a Goldilocks rule in which either too little or too much Treg activity is detrimental, posing a problem for immune intervention that needs to be carefully calibrated on a case-by-case basis (Figure 3).

Major questions which remain (see Outstanding questions) centre around the antigen-specificity of Tregs, and their provenance as natural or induced, that may indicate how parasite antigens are recognised and why in some cases Treg populations are activated. Our insights are often limited snapshots of long-term chronic infections from which it can be difficult to identify key early events; in this respect the introduction of controlled human infection systems is likely to be most transformative [114]. At the other end of the time scale, many parasite infections establish a new, stable homeostatic set-point, often with immune hyporesponsiveness to both parasite and bystander antigens; our ability to modulate Treg activity in this relationship is likely to be of increasing importance. While Treg cell subtypes become better defined by transcription factor and co-inhibitory marker profiles, we remain less informed about the upstream signals and cues which may direct Tregs to commit to different pathways and to migrate to different sites; understanding these is likely to allow us to manipulate and channel the Treg population in the most favourable manner. An interesting corollary is whether parasites are ahead of us in controlling Treg behaviour, as for example in the ability of some helminths to activate TGF-β signalling [115]; in this sense, we still have much to learn from parasites.

Outstanding questions

Do GATA3, T-bet and/or RORγt-expressing Tregs play specialised roles in regulating the response to parasite infection? Can selective manipulation of these subsets relieve immune suppression and/or ameliorate immune pathology?

What are the signals that drive Treg specialisation in parasite infections?

Are Tregs in infection recognising specific parasite antigens, and if so, can those antigens be exploited to activate or inhibit Treg suppression?

Can we calibrate Treg activity to maximise disease tolerance without increasing susceptibility?

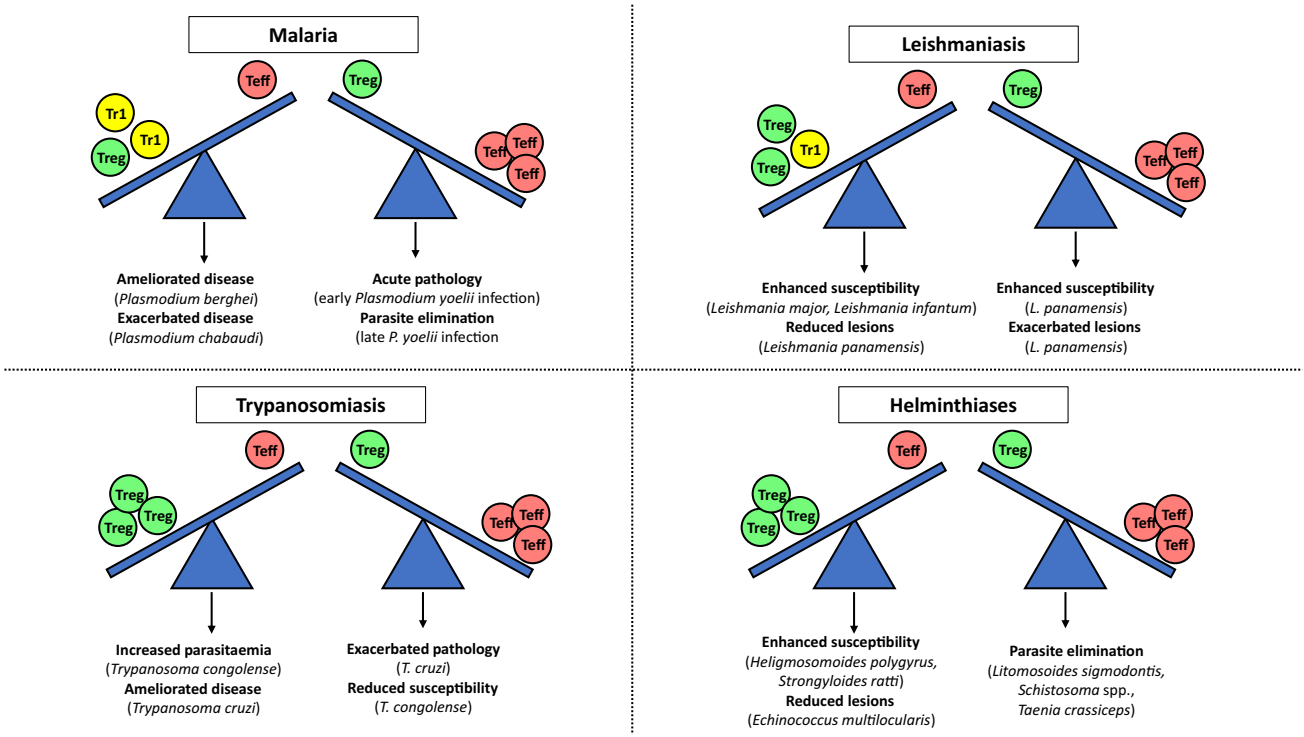

Figure 3. Context-dependent impact of regulatory T cells (Tregs) in different parasite infections. In the different mouse models of malaria, leishmaniasis, trypanosomiasis, and helminthiases, Tregs may suppress protective immunity and exacerbate infections, or they may play a beneficial role in dampening immune pathology. Contrasting roles can be found in different but related species, or even in the same species when evaluated at different time points, emphasising the multifaceted roles of Tregs depending on the precise infection setting.

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Declaration of interests

The authors declare no competing interests.

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