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Parasites and immunoregulatory T cells

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Surviving a parasitic infection requires the generation of a controlled immune response. Failure to establish or to maintain homeostatic conditions usually causes disease. Investigation of the immunoregulatory network as the response to the parasitic process or induced by the parasite promises enormous therapeutic benefits for the control of parasitic diseases. Recent findings implicate various populations of regulatory T cells in this homeostatic regulation.

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Introduction

Surviving a parasitic infection requires the generation of a controlled immune response that recognizes the invading pathogen and that limits a potentially harmful host response. From the point of view of the parasite, microbes have to avoid elimination by the host immune response and sustain their lifecycle while at the same time delay or prevent host destruction. Thus, during parasitic infection, immune regulation can arise as the downstream effect of host response to the parasitic process and/or can be actively induced by the parasite as a survival strategy. The modulation of host response by parasites is achieved through complex adjustment of innate and acquired immune responses of the host — a process that tends to restore or to maintain a 'homeostatic' environment. Parasites have developed sophisticated and redundant strategies to do this; these include evasion of humoral and cellular immunity by antigenic variation, interference with antigen processing or presentation, and subversion of phagocytosis and killing by cells of the innate immune system (reviewed in [1]).

T helper (Th)1 cells, which produce interferon (IFN)-γ, are crucial for the eradication of intracellular parasites, whereas Th2 cells, which produce interleukin (IL)-4, IL-5 and IL-13, are important for the regulation of the immune response to helminthes. Over the past two decades, seminal studies have shown that these cells can reciprocally regulate each other during infection (reviewed in [2]). Recent reports also implicated IL-27 as an important regulator of both Th1 and Th2 polarized immune responses during parasitic infection [3,4].

Another layer of this regulation is a common strategy used by parasites: the induction of regulatory responses normally associated with the termination of effector immune responses of the host. This can be achieved through the direct induction of host immune regulatory cytokines such as IL-10 and transforming growth factor β (TGF-β), which are produced by innate immune cells in response to pathogen-derived molecules or indirectly through the generation of regulatory cells. Although it has long been recognized that T cells with suppressive or anergic activity or IL-10-producing T cells could be generated *in vivo* during parasitic infection [5,6], it is only recently that the concept has emerged that specialized subsets of regulatory T cells (T_{reg}) contribute to this regulatory network.

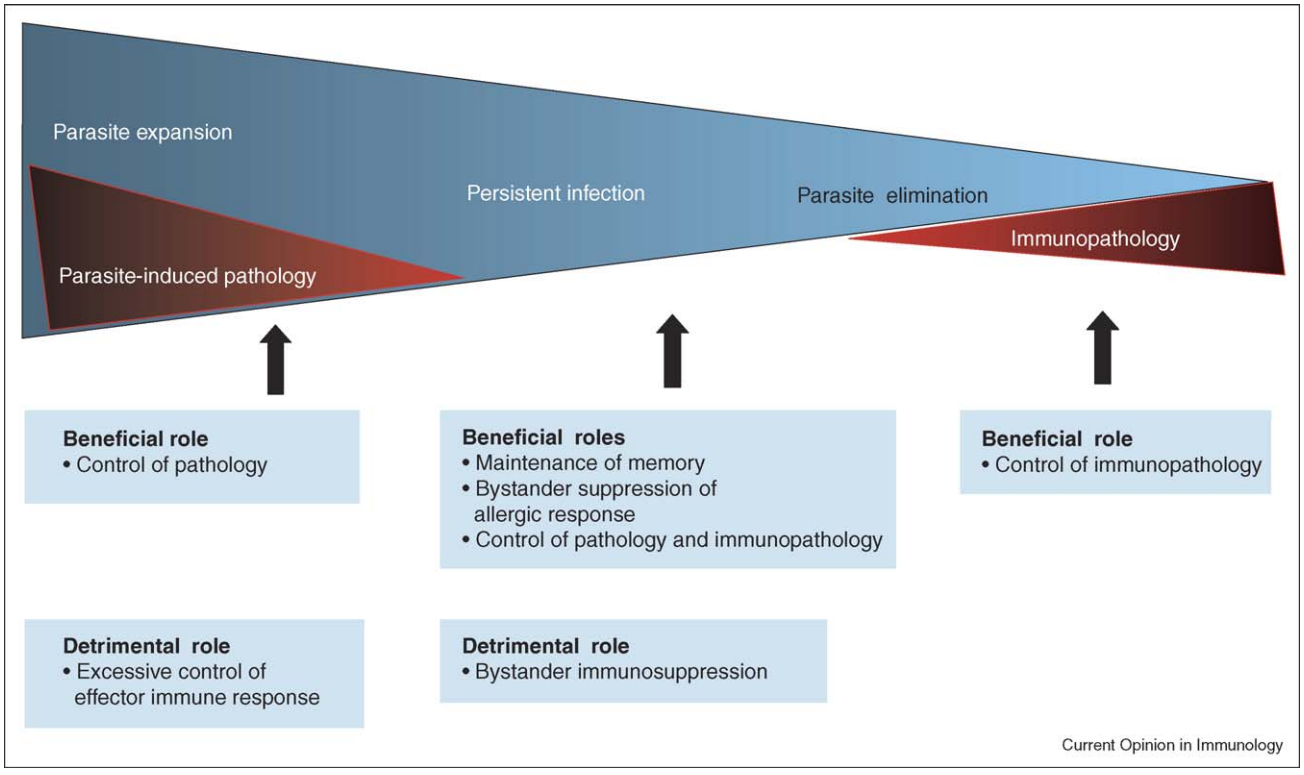
Several T_{reg} types have been described based on their origin, generation and mechanism of action, with two main subsets identified: 'natural' $Foxp3^+CD4^+CD25^+$ T_{reg}, which develop in the thymus and regulate self-reactive T cells in the periphery, and 'inducible' T_{reg} (e.g. Tr1 or Th3 cells), which can develop in the periphery from conventional $CD4^+$ T cells. Both types of T_{reg}, by virtue of their capacity to control the intensity of effector responses, have been shown to play a major role in the control of various parasitic infections.

In this review, we will focus on recent findings regarding the role of T_{reg} during parasitic infection and the two main cytokines associated with their function — IL-10 and TGF-β.

Roles of interleukin 10 in immune regulation during parasitic infection

The role of IL-10 as an immunoregulatory cytokine during parasitic infection has been well documented [7]. IL-10 can strongly inhibit the immune responses (both Th1 and Th2) to many parasites [8–10]. The role of IL-10 in blunting immune responses to specific antigens was also demonstrated in human parasitic infection such as filariasis, leishmaniasis and schistosomiasis [11–13]. One major role of IL-10 is the control of immunopathology and inflammation (Figure 1). The most

Figure 1



Positive and negative roles of immune regulation during parasitic infections. In the figure, the triangle shape refers to the number of microbes in the host, and the red triangles represent disease expression. The interactions between host and parasite range from uncontrolled parasite growth to sterile elimination. Immune regulation (e.g. natural T_{reg}, Tr1, IL-10 and TGF-β) can play a role at both extremes of the host–parasite interaction. Excessive control of effector immune responses can lead to uncontrolled growth of the parasite and to eventual death of the host. By contrast, immune regulation might control parasite-induced pathology. At the other extreme of the host–parasite interaction, effector immune responses can efficiently eliminate the parasite. This situation can lead to unleashed effector immune responses and immunopathology. At the extreme of this scenario, the host can die from uncontrolled immune responses. In this case, immune regulation might be beneficial by controlling immunopathology. Persistent infections are in the middle of this range. In these situations, immune regulation might be beneficial in controlling pathology and immunopathology, maintaining long-term immunity against reinfection, and providing bystander control of allergic responses. By contrast, bystander suppression might be detrimental, inducing systemic immunosupression.

spectacular example of this control is illustrated by its vital role during acute *Toxoplasma gondii* infection. In the absence of IL-10, mice will control parasite numbers but die from unleashed effector immune responses [14]. IL-10 is also a survival factor in several other parasitic infections [10,15,16]. IL-10 can be produced by natural T_{reg} and, in some cases, is associated with their function; however, this cytokine is also produced by many hematopoietic cells including dendritic cells (DCs), B cells and conventional T cells. Parasites themselves can induce production of IL-10 by the cells they infect or contact. Recently it was shown that macrophages activated in the presence of immune complexes secrete high levels of IL-10. Such events account for the enhanced susceptibility to *Leishmania major* infection in mice [17].

Tr1 (IL-10 producing cells) are also a potential source of this cytokine during parasitic infection. These cells can be generated during various infections [18] and develop from conventional T cells in the periphery after encounter with specific signals (reviewed in [19]). For instance, exposure to deactivated or immature antigen-presenting cells or repetitive exposure to antigen or IL-10 itself has been associated with the emergence of this population (reviewed in [20]). Of note, all these conditions also prevail during parasitic infections, in which antigen-presenting cell functions are often targeted and the immune system is chronically exposed to parasitic antigens. The presence of microbial products can also favor the generation of these cells. For instance, Tr1 cells can be generated *in vitro* by DC stimulated with phosphatidylserine from *Schistosoma mansoni* [21].

Natural T_{reg} control tissue damage during parasitic infection

Some of the earliest studies on natural T_{reg} emphasized that such cells control the extent of immune-mediated pathology in infectious diseases. Activated natural T_{reg} efficiently control pathogenic T cells and innate responses in a model of murine colitis, allowing minimized collateral

tissue damage [22,23]. Similar to the situation in gut tissue, during chronic parasitic infection the host must maintain constant immune pressure. Natural T_{reg} seem to be necessary to monitor this response and to prevent detrimental tissue damage. In particular, protection of sensitive tissues or tissues that have highly specialized functions — such as liver — requires T_{reg}-mediated control of immunopathology.

Chronic infection with *S. mansoni*, which creates extensive damage of liver, illustrates such control. Only tight control of the egg-induced immune response allows survival of the infected host [8]. Recently it was shown that immunosuppressive $CD4^+CD25^+$ T_{reg} isolated from hepatic granulomas and from lymphoid tissues are a main producer of IL-10 in schistosome-infected mice [24,25]. In the non-healing model of *L. major* infection, pathology is also held in check by natural T_{reg} [26,27]. *Leishmania amazonensis* infection in mice is characterized by the accumulation of natural T_{reg} at sites of infection that transiently downregulate immunopathology [28].

Another potential benefit of T_{reg} responses to the host during parasitic infections is the consequence of their bystander effects. A few years ago, the concept of a hygiene hypothesis emerged; this stated that increasing rates of allergy and asthma in Western countries are a consequence of reduced infectious stressors during early childhood [29]. The mechanistic explanations appear to be associated with a 'counterregulatory' model that involves induction of various T_{reg} populations during infections. Experimental work has lent strong support for this hypothesis [30]. During gastrointestinal infection, helminth-driven natural T_{reg} suppression of effector function is responsible for protection from airway inflammation [31••]. Thus, microbial pressure in the gut or other peripheral tissues could lead to the maintenance of a pool of activated Treg (both natural and inducible) that would maintain host immune homeostasis and enhance the threshold required for immune responses.

T_{reg} can mediate a mutually beneficial relationship for the host and the parasite

Even when natural T_{reg} successfully preserve host homeostasis by controlling excessive immune responses, one consequence of such control is enhanced pathogen survival and, in some cases, long-term pathogen persistence. In a resistant murine model, mice remain chronically infected at the site of primary infection [32]. Natural T_{reg} accumulate at the site of infection and control, through IL-10-dependent and -independent mechanisms, the local function of effector cells. This ensures the long-term survival of the parasite in the immune host [33].

Parasite persistence (and therefore immune suppression by natural T_{reg}) also provides a major benefit to the host by maintaining life-long immunity to reinfection. This model illustrates the fine balance that can be established between pathogens and hosts and how this equilibrium can, in some instances, become mutually beneficial.

Detrimental roles of natural T_{reg} to the host during parasitic infection

In some cases, regulatory control is excessive; this allows parasite to expand in an uncontrolled manner and thus fails to secure host survival. In a murine model of malaria, for example, depletion of natural T_{reg} protected mice from death caused by the lethal strain of *Plasmodium yoelii* by restoring a vigorous effector immune response, which eradicated the parasites [34].

Filaria nematode infection is associated with a profound downregulation of the host immune system. When mice are infected with *Brugia pahagi*, natural T_{reg} expand and suppress excessive Th2 responses [35]. In a model of murine filarial infection, the infection and subsequent immunosuppression is associated with accumulation of T_{reg} in the thoracic cavity [36]. Similarly, in humans infected with *Plasmodium falciparum* — a causative agent of malaria — removal of T_{reg} enhances peripheral blood mononuclear cell proliferative and IFN-γ responses to malaria antigen [37••]. The non-healing lesions caused by a specific strain of *L. major* in mice were associated with enhanced IL-10 production and T_{reg} presence at the site of infection [38••]. Enhancement in the number of natural T_{reg} in mice chronically infected with *L. major* was sufficient to trigger disease reactivation and to inhibit the effector memory response [39]. Thus, overexpression of T_{reg} regulatory function, either from the endogenous pool or induced by the infection, can clearly become detrimental to the host by favoring excessive parasite expansion.

Manipulation of T_{reg} by parasites

Because T_{reg} offer an opportunity for parasites to generate favorable conditions for persistence, it is conceivable that their induction and survival can also be manipulated by parasites. The anti-inflammatory cytokine TGF-β, which is produced at high levels during chronic infections, is also an important factor for the local survival and function of T_{reg} [40]. This cytokine is highly expressed in tissues such as the gut, the skin or the lung, which are in constant contact with microbial antigen. During infection, the downstream effects of inflammatory responses are also often associated with anti-inflammatory processes including TGF-β production. In a murine model of *Toxoplasma* infection, intraepithelial lymphocytes secrete TGF-β to prevent the development of lethal ileitis [41]. Several pathogens can directly trigger the production of this cytokine by the cells they infect or contact. Compelling data in a murine model of malaria suggest that TGF-β and T_{reg} are central regulators of immunopathology and parasite expansion [34,42–45]. Following experimental malaria infection of human

volunteers, enhanced TGF-β and Foxp3$^+$ T_{reg} responses in peripheral blood mononuclear cells correlate with a faster parasitic growth rate [37••]. Although in all of the above-described infections there is compelling evidence for a role of natural T_{reg}, Foxp3 expression might not always correlate with these cells. Activated conventional CD4$^+$ T cells exposed to high levels of TGF-β can become suppressive Foxp3$^+$ cells [46–48].

One mechanism by which parasites might manipulate T_{reg} function is by the creation of an environment that favors T_{reg} retention. In experimental parasitic models, natural T_{reg} preferentially accumulate at the infected site [28,33,36]. The integrin $\alpha_E\beta_7$ — expression of which is positively modulated by TGF-β — has been shown to favor T_{reg} retention at sites of parasitic infection [49]. Of interest, exposure of T cells to *L. major*-infected DCs also enhances the expression of this integrin, which suggests that the parasite itself manipulates its environment to favor T_{reg} retention. In addition to their encounters with specific antigens, whether host- or pathogen-derived, natural T_{reg} can also clearly respond to microbial products independently of TCRs. Natural T_{reg} selectively express TLR-4, -5, -7 and -8. Some TLR ligands can directly control the expansion and function of natural T_{reg} both *in vivo* and *in vitro* [50,51]. Parasites can also express some TLR ligands such as TLR-2 or TLR-11 [52,53]. This feature of natural T_{reg} to respond to microbial products could offer some parasites an opportunity to enhance immunosuppression. Finally, mature DCs are more efficient at inducing proliferation of transgenic natural T_{reg} than immature cells are [54]. Therefore, parasite-associated DC maturation, stimulation of TLR or other pattern-recognition receptors, induction of cytokine production, and release of factors and antigens from pathogen-mediated tissue damage could all favor T_{reg} activation and thereby support survival of the parasite.

Antigen specificity of natural T_{reg} and control of parasitic infection

Although the antigen specificity of inducible T_{reg} (IL-10-producing T_{reg} or Th3 cells) is associated with parasitic antigens, the nature of the antigen recognized by natural T_{reg} is less clear. Natural T_{reg} are believed to recognize a wide array of self antigens [55]. The hypothesis that natural T_{reg} only recognize self-antigen is compelling during the onset of acute disease, because tissue damage is also associated with enhanced presentation of self antigens; however, most systems for which there is evidence that natural T_{reg} recognize microbial antigens produce chronic infections [24,25,33,56]. In a murine model of *Leishmania* infection, natural T_{reg} that accumulate at the site of infection are able to recognize parasitic antigen [57••]. In addition, far from being anergic, as *in vitro* experiments suggested, natural T_{reg} proliferate vigorously when they encounter their specific microbial antigens [57••]. Notably, these cells were restricted to the site of infection and were strictly dependent on antigen for their survival, which suggests that natural T_{reg} do not develop as 'memory cells'. Several crucial points might condition the antigenic specificity of natural T_{reg} during a given infection. The target of natural T_{reg} control as well as their antigenic specificity might be dependent on the duration of the host–parasite interaction. In acute infection, natural T_{reg} can respond essentially to self-antigen, whereas during chronic infection natural T_{reg} respond preferentially to microbial antigens.

The importance of understanding the nature of the antigens recognized by natural T_{reg} was made clear in a recent study [58••]. Previous reports clearly demonstrated that the *Leishmania* antigen of mammalian RACK1 (LACK) can protect mice [59]. Surprisingly, in some conditions, vaccination of mice with LACK antigen can also favor emergence of IL-10-producing T_{reg} [58••]. These cells were a predictor of vaccination failure. Removal of CD25$^+$ cells abrogated IL-10 production and restored protection by the vaccine [58••]. Those results highlight the necessity to address the potentiality of each parasitic antigen to trigger T_{reg} following vaccination.

Controlling regulatory functions to favor parasite control

The capacity of a host to mount an effective immune response is limited by the pre-existence of counterregulatory elements. Targeting the molecules involved in regulatory cell activity *in vivo* such as CTLA-4, TGF-β or IL-10 alone or in combination has often proved effective to control a large number of chronic infections [60–62]. Many mechanisms that boost immune responses and that favor the control of pathogens also abrogate T_{reg} functions [63–65]; the main target of this control appears to be activation of effector T cells that become unresponsive to natural T_{reg} suppression. Far from being switched off by activation, natural T_{reg} proliferate and their suppressive functions are boosted by encounters with activating signals [50,54].

Strategies to manipulate natural T_{reg} function or number clearly have high therapeutic potential. In a large number of infections in both mice and humans, depletion of natural T_{reg} (based on expression of CD25) has resulted in enhanced effector immune responses [39,66–68]. Targeting CD25 might not always correlate with T_{reg} neutralization. For example, some natural T_{reg} express the transcription factor Foxp3 but do not express CD25. Also, CD25 is transiently expressed on effector T cells. Targeting glucocorticoid-induced TNF family-related receptor (GITR), which is constitutively expressed by natural T_{reg} and is induced on activated T cells *in vivo*, has also shown significant results [36,69]. Because T_{reg} are central to the control of host homeostasis, systemic strategies might not be applicable *in vivo* in humans. Such strategies could bear the risk of triggering autoimmune disorders or

uncontrolled pathological immune responses. Thus, there is clearly a need to develop strategies to control T_{reg} function targeted at sites of infection.

Controlling T_{reg} function to enhance memory responses

To date, no vaccines are available against parasitic diseases. The enhancement of immune regulatory elements by parasites might contribute to this failure. T_{reg} can clearly control the intensity of secondary responses to infections [39]. Likewise, natural T_{reg} are able to hamper the efficacy of vaccines against infectious agents [70,71]. In a model of vaccination against murine malaria, depletion of natural T_{reg} can induce a more durable immunity and better control of the parasite burden compared with vaccine alone [72•]. Notably, such depletion also allowed an enhanced T cell response to subdominant epitopes [72•]. This point might be particularly crucial for vaccines against parasitic infection, because parasites usually have few, if any, dominant antigens. Another way in which to manipulate T_{reg} priming at the site of vaccination might be to induce an environment unfavorable to T_{reg} priming. Thus, in *Leishmania* infection, the site of infection conditions the quality of recall responses [73].

Controlling T_{reg} function to prevent immunopathology

Most pathologies are the consequence of uncontrolled immune responses. Induction or activation of regulatory elements represents a therapeutic objective when tissue damage is excessive. In a murine model of colitis, the transfer of natural T_{reg} was sufficient to control established inflammatory disease [74]. Increasing natural T_{reg} function or number could potentially be achieved by providing a cytokine milieu that favors natural T_{reg} activity or survival, such as the presence of IL-2 or TGF-β. In a model of murine schistosome infection, retroviral transfer of the Foxp3 gene at the onset of granuloma formation enhances Foxp3 expression in the granuloma and strongly suppresses granuloma development [75•]. A similar approach performed on antigen-specific cells might represent a powerful way to generate a large number of antigen-specific T_{reg} that could target sites of infection and tissue damage.

Conclusions

Although T_{reg} populations have become a major focus of attention over the past few years, it is important to remember that virtually all populations of cells can acquire regulatory properties. The challenge of the next few years will be to decipher their relative dependence and contribution to the regulatory responses against parasitic infections.

In conclusion, natural and inducible T_{reg} participate in the immune responses to many — and perhaps all — parasitic diseases. We have clearly just begun to understand their specific role in various infections. Because parasites have coevolved with their hosts, they have evolved to manipulate the most central elements of the regulatory network of their host. Thus, they might represent a powerful tool with which to decipher the mechanisms that favor T_{reg} functions. Learning the mechanisms by which T_{reg} are mobilized and activated and the nature of the antigens they recognize will be the next steps in the design of rational approaches to achieve the appropriate balance between protection and pathology during parasitic infections.

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