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Dysfunctional adaptive immunity during parasitic infections

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Abstract

Parasite-driven dysfunctional adaptive immunity represents an emerging hypothesis to explain the chronic or persistent nature of parasitic infections, as well as the observation that repeated exposure to most parasitic organisms fails to engender sterilizing immunity. This review discusses recent examples from clinical studies and experimental models of parasitic infection that substantiate the role for immune dysfunction in the inefficient generation and maintenance of potent anti-parasitic immunity. Better understanding of the complex interplay between parasites, host adaptive immunity, and relevant negative regulatory circuits will inform efforts to enhance resistance to chronic parasitic infections through vaccination or immunotherapy.

Keywords

B cell; chronic infection; exhaustion; helminth; T cell; parasite; protozoan

INTRODUCTION

Chronic parasitic infections remain an enormous public health burden, with parasites of the genera *Schistosoma*, *Leishmania*, *Trypanosoma*, *Toxoplasma* and *Plasmodium* accounting for substantial disease morbidity and mortality throughout the world. The protozoan species *Plasmodium* alone is responsible for >215 million new cases of malarial disease and > 700,000 deaths annually [1]. Although sterilizing anti-*Plasmodium* immunity fails to develop [2], evidence suggests that clinical immunity against malarial disease develops with time and following repeated exposure. Similarly, both humans and mice that recover from acute *Leishmania* infection display less severe clinical disease manifestations upon subsequent exposures to the parasite [3]. These findings suggest that exposure to parasites and their encoded antigens can induce a degree of clinical immunity and provide the rationale and incentive for pursuing the development of anti-parasitic vaccines. Furthermore, while the advent of novel anti-parasitic drugs have successfully led to a decrease in the

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CONFLICTS OF INTEREST

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incidence rates of parasitic infections, they have also aided in positively selecting for both resistant parasites and their vectors [4, 5]. Thus, alternative immune-based approaches and effective vaccines against *Plasmodium* and other protozoan and helminth parasites are desperately needed.

Despite considerable effort, no licensed vaccines for human parasitic infections exist. The lack of licensed anti-parasitic vaccines can be in part attributed to the highly complex life cycles of these organisms in which antigenic variation may differ significantly between the various growth stages of the parasite [6, 7], including the parasite's inhabitation of its intermediate or definitive host. Other challenges in parasite vaccine development include the apparent poor immunogenicity of individual parasite antigens and our incomplete understanding of which parasitic antigens should be used to elicit the most appropriate or potent immunity. Finally, some of the parasites that cause the most severe disease have evolved to either evade or directly impede the generation of long-lasting, sterilizing immunity.

In this review we highlight the critical roles of T cell- and B cell-mediated immunity in parasite control, provide illustrations of how several parasitic infections impair the development of cellular and humoral immunity, and outline several outstanding questions regarding the processes by which parasitic infections trigger dysfunctional immune responses. Although immune modulation, immune evasion, and the induction of dysfunctional immunity have likely evolved to prevent host mortality and increase parasite transmission to new hosts, understanding the molecular and cellular pathways through which parasitic infections impair or modulate adaptive immunity will identify novel points of therapeutic intervention as well as inform efforts to develop next-generation anti-parasite vaccines.

EFFICACIOUS, PARASITE-SPECIFIC ADAPTIVE IMMUNITY

Efficient control of parasitic infections generally depends on the coordinated activity of multiple adaptive immune cells types, including CD8 and CD4 T cells and antibody secreting B cells. Cytotoxic CD8 T cells, discussed in detail in this issue by Villarino and Schmidt, are essential for the recognition and elimination of intracellular pathogens, including obligate intracellular protozoa of the genera *Toxoplasma*, *Leishmania* and *Plasmodium*. CD8 T cell-mediated protection requires cognate interactions between the T cell receptor (TCR) on the surface of the CD8 T cell and pathogen-derived peptides (epitopes) presented on the surface of infected cells in association with host class I major histocompatibility complex (MHC) molecules. Functional recognition of this ligand (MHC-peptide) by the TCR triggers signaling cascades that culminate in the secretion of anti-microbial cytokines or degranulation of the CD8 T cell, which results in release of cytotoxic effector proteins that trigger apoptotic pathways within target cells [8]. Large bodies of experimental evidence and clinical correlates show that, with the possible exception of *Babesia* and blood-stage *Plasmodium* parasites, effective control of protozoan parasitic infections and liver stage malaria depends on the induction and maintenance of efficacious, parasite-specific CD8 T cell responses [9–11]. *Babesia* and blood-stage *Plasmodium* parasites replicate exclusively in mammalian red blood cells (RBC), which lack functional

MHC expression. Thus, parasite-infected RBC are not major targets of cytotoxic CD8 T cells, and control of parasites replicating within erythrocytes requires the induction and activity of anti-parasite antibody responses (discussed below). *Toxoplasma* is competent to infect virtually all nucleated mammalian cell types, yet this parasite is most commonly transmitted to humans via oral ingestion of tissue cysts in undercooked meats or oocysts shed by cats, the parasite's definitive host. Thus, infection and replication of *Toxoplasma* parasites generally begins in gut epithelial cells [12]. Unless host immunity eliminates all infected cells and viable parasites, *Toxoplasma* will rapidly breach the gut epithelium and disseminate systemically. Following dissemination, *Toxoplasma* establishes chronic infections, generally within immune privileged tissues of the central nervous system including the eye and the brain [12]. In these tissues, *Toxoplasma*-specific CD8 T cells are essential for controlling parasite replication and limiting systemic reactivation. *Leishmania* parasites are phagocytized by neutrophils and macrophages following deposition in dermal tissues by the sandfly vector [13]. The antimicrobial activity of these phagocytic cells, which generally relies on interferon-gamma (IFN- γ -induced oxidative burst pathways, is associated with control of leishmaniasis. In experimental rodent models, depletion of CD8 T cells results in uncontrolled parasite replication and fatal leishmaniasis [14–16], demonstrating that *Leishmania*-infected phagocytic cells can serve as functional targets for parasite-specific CD8 T cells.

CD4 T cells are also important regulators of efficacious anti-parasite immunity. As noted, inflammatory cytokines like tumor necrosis factor (TNF) and IFN- γ function to activate phagocytic cells to enhance killing of protozoan parasites, and parasite-specific Type 1 helper (T_H1) CD4 T cells are an important source of these cytokines. By contrast, after infection by some helminths, CD4 T cells are stimulated to adopt a unique Type 2 helper (T_H2) functional profile, which includes the secretion of specific cytokines like interleukin 4 (IL-4) and IL-5 that activate B cells and mast cells, which in turn promotes killing or expulsion of worms. In addition to these anti-parasitic effector pathways, CD4 T cells also are important regulators of CD8 T cell differentiation and survival. For example, in the absence of parasite-specific CD4 T cell responses, liver-stage *Plasmodium*-specific CD8 T cells fail to form sizable memory populations [17], possibly as a consequence of reduced availability of IL-4 or the essential T cell growth factor IL-2. Similarly, during Trypanosome infection, CD4 T cells contribute to stimulating protective parasite-specific CD8 T cell responses, although they are not absolutely essential [18]. Thus, CD4 T cells are centrally important regulators of anti-parasite immunity via their secretion of cytokines, provision of help for CD8 T cell and B cell responses, and possibly through direct cytotoxic killing of parasite-infected cells [19–21].

In contrast to intracellular parasites, control of extracellular parasites, or parasites that replicate in host RBC, critically depends on the induction of high-affinity antibodies. This is particularly true for parasites such as *Trichinella*, *Trypanosoma*, and *Schistosoma*, as well as for protozoan parasites like *Babesia* and *Plasmodium* that replicate within host RBC. Extracellular parasites and parasite-infected RBC are not direct functional targets for cytotoxic CD8 T cells. For malaria, several clinical studies have shown clear correlations between high *P. falciparum*-specific antibody titers and resistance to severe disease [22, 23].

Most strikingly, the demonstration that passive transfer of immunoglobulin (Ig) from semi-immune adults results in improved clinical outcomes in *P. falciparum* infected children firmly established a causal relationship between parasite-specific antibodies and protection [24]. Collectively, these studies highlight the critical role for effective antibody-secreting B cell responses during several parasitic infections. Thus, understanding how or whether parasite infections induce potent antibody responses, and how we might intervene to augment such responses, remain important lines of investigation.

Although B cell responses and the induction of secreted antibodies can occur independently of T cells following infection or vaccination [25], the most potent antibody responses require specific participation of a functionally distinct subset of CD4 T cells known as T follicular helper (T_{FH}) cells. T_{FH} cells provide critical signals to antigen-specific B cells that result in the affinity maturation of antibodies and the differentiation of B cells into either long-lived antibody-secreting plasma cells or long-lived memory B cells [26]. In rodent models of helminth and protozoan parasitic infections, antibody-secreting B cells are essential for controlling infection and survival of the host. For example, in mice genetically deficient in B cells or deficient in antibody secretion, *Toxoplasma* infection is lethal [27]. Infections with the helminth *Heligmosomoides polygyrus* are lethal to activation-induced cytidine deaminase (AID)-deficient mice, which harbor B cells that cannot isotype switch or undergo affinity maturation [28, 29]. Finally, the absence of B cells or functional antibody secretion also results in lethal *Plasmodium* infections. Of note, the transfer of parasite-specific IgG or parasite-specific mature B cells rescues these B cell deficient mice from otherwise lethal helminth, *Plasmodium* or *Toxoplasma* infections [28–33], which illustrates the critical role for antibodies in resistance to these parasitic infections. Because the orchestration of anti-parasitic immunity generally involves both potent T_H1-mediated IFN- γ secretion and antibody responses, understanding how T_H1 and T_{FH} CD4 T cell subsets are induced and maintained during chronic parasitic infections remains an important goal.

The examples cited above highlight the essential roles of adaptive cellular and humoral immunity in controlling helminth and protozoan parasite infections and underscore the importance of understanding the cellular and molecular basis for the induction of durable, anti-parasitic immunity. However, a large body of evidence shows that parasites generally establish chronic infections and are poor inducers of immunity. Several factors likely contribute to the chronic nature of parasitic infections, including their complex lifecycles, which includes shifting tissue/cellular tropism during development, as well as their propensity to exhibit antigenic variation. As discussed below, another hypothesis to explain the persistent nature of these infections is that parasites have a propensity to trigger immune dysfunction or exhaustion (Table 1), which impairs the development of durable, sterilizing immunity.

T CELL EXHAUSTION DURING PARASITIC INFECTIONS

Following acute infection or vaccination, antigen-specific T cells undergo a program of proliferative expansion and acquire anti-microbial effector functions that include cytotoxic potential and cytokine secretion. As discussed by Stephens *et al.* in this issue, T cells that acquire and exert multiple antimicrobial functions are referred to as polyfunctional, a

property of memory T cells that is commonly associated with effective resistance to reinfection [34]. Similarly, T cells responding to chronic infections in which the pathogen is not efficiently eliminated also undergo similar phases of expansion and contraction. However, unlike scenarios of acute infection, T cells responding to persistent infection exhibit specific alterations in gene expression profiles that are associated with functional impairments, including loss of anti-microbial activity and increased rates of apoptosis. These phenomena are collectively known as T cell exhaustion [35].

T cell exhaustion was first described more than 15 years ago, and much of what we currently know about T cell exhaustion comes from clinical examples of chronic HIV or HCV infection [35], as well as rodent models of persistent lymphocytic choriomeningitis virus infection [36, 37]. During T cell exhaustion, loss of function appears to occur in a step-wise manner; CD8 T cells undergoing exhaustion first lose the capacity for IL-2 secretion followed by marked decreases in their potential for proliferative expansion and ability to exert cytotoxic function. In extreme cases, exhausted CD8 T cells lose the capacity to secrete TNF, followed by IFN- γ , and are ultimately deleted from the host [38, 39]. Importantly, these progressive declines in T cell activity and survival translate in to deficiencies in pathogen control [35].

Our current understanding of the molecular basis for T cell exhaustion is ever expanding. For example, one class of molecules whose enhanced expression patterns are associated with T cell exhaustion is co-inhibitory receptors. Co-inhibitory receptors are critical components of immune homeostasis. At the peak of effector T cell expansion following acute infection, T cells up regulate expression of multiple co-inhibitory receptors, which act to dampen activation and limit immune-mediated pathology [40]. There are many molecules that have been characterized as co-inhibitory receptors of adaptive immune cells, including programmed cell death-1 (PD-1), lymphocyte activation gene 3 (LAG-3), 2B4, CD160, B- and T-lymphocyte attenuator (BTLA), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and T cell membrane protein 3 (Tim-3) (Table 2). Because these molecules largely function to prevent over exuberant T cell activation, their essential role in preventing immunopathology becomes apparent in mice made genetically deficient in one or more co-inhibitory receptors, as autoimmunity is common among these strains [41–43]. Moreover, mice that are resistant to development of acute immunopathology in a model of *Plasmodium*-induced cerebral malaria can be made susceptible following *in vivo* disruption of PD-1 and CTLA-4 negative regulatory pathways during the acute phase of the T cell response [44]. Although T cells responding to acute infection are well known to transiently up-regulate co-inhibitory receptors, a critical distinction is observed during chronic infection; expression of these molecules is sustained on CD8 and CD4 T cells responding to persistent infection or continued antigenic stimulation, which further negatively regulates the activity of T cells and facilitates pathogen persistence.

Recent work examining the contribution of T cell exhaustion to facilitating establishment of chronic parasitic infections underscores the generalizable nature of this phenomenon. Much of what is currently known about T cell dysfunction during parasitic infection comes from studies of experimental toxoplasmosis. Despite apparent control of parasite replication during the acute phases of *Toxoplasma* infection, parasite-specific CD8 T cells exhibit signs

of functional exhaustion during the chronic phases of infection, characterized by increased expression of PD-1 [45], enhanced rates of apoptosis and loss of cytokine expression [46–49]. Loss of CD8 T cell-mediated control during the late phases of chronic infection enables *Toxoplasma* parasites to transition from slow- to fast-replicating forms, ultimately causing the death of the host. The *in vivo* relevance of PD-1 expression by exhausted CD8 T cells after *Toxoplasma* infection was shown by *in vivo* administration of anti-PD-L1 monoclonal antibodies (mAb) that disrupt interactions between PD-1 and its major ligand, PD-L1. Indeed, survival, proliferation and function of parasite-specific CD8 T cells were enhanced following administration of anti-PD-L1 mAb in mice chronically infected with *Toxoplasma*, which rescued these mice from an otherwise lethal infection [48]. Collectively these data show that chronic toxoplasmosis is linked to the erosion of functional CD8 T cell immunity. In the future it will be of interest to examine the potential contribution of additional co-inhibitory receptors or secreted factors (Table 2) to dysfunctional *Toxoplasma*-specific T cell responses. Little is currently known about the combinatorial expression of multiple co-inhibitory receptors on *Toxoplasma*-specific CD8 T cells, and even less is known about the status of CD4 T cells responding to chronic toxoplasmosis. A more complete understanding of the relative contribution of these numerous pathways of immune exhaustion should aid in the development of novel strategies to enhance immunity against this parasite.

Evidence for T cell exhaustion can also be found in examples of experimental and clinical leishmaniasis, trypanosomiasis and malaria infection. Cutaneous leishmaniasis in humans has been linked to exhaustion of PD-1+ parasite-specific CD8 T cells, which could be functionally improved *in vitro* through the addition of toll like receptor 2 (TLR2) agonists [50]. Exhausted CD4 T cells have also been described in the context of experimental *Leishmania* infection [51]. *L. major* parasites lacking arginase, a virulence factor that impedes macrophage activation, are able to establish chronic infection in mice. In this scenario, parasite-specific CD4 T cells exhibit impaired proliferation and INF- γ expression, similar to CD8 T cells responding to cutaneous leishmaniasis, chronic toxoplasmosis or chronic viral infection. Importantly, the biological relevance of PD-1 during chronic *Leishmania* infection was also revealed by *in vivo* studies in which anti-PD-1 mAb effectively restored parasite-specific CD4 T cell function and resolved chronic infections in rodents [51].

Additional evidence for exhaustion of CD4 T cells during parasitic infections comes from studies of clinical and experimental trypanosomiasis. Exhausted or dysfunctional T cells have been observed in rodent models of *T. cruzi* infection [52]. In heart biopsy tissues collected from patients chronically infected with *Trypanosoma cruzi*, parasite-specific CD4 T cells expressed multiple co-inhibitory receptors, including CTLA-4 and the leukocyte immunoglobulin like receptor 1 (LIR-1) [53]. Similarly, studies focused on CD8 T cells collected from the peripheral blood of patients with Chagas' disease linked clinical severity to declines in the number and function of *T. cruzi*-specific CD8 T cells [54]. Although T cells responding to *T. cruzi* infection exhibit phenotypic alterations that are associated with immune exhaustion, the *in vivo* relevance of co-inhibitory receptor expression on *T. cruzi*-specific T cells remains to be explored.

During prolonged or chronic *Plasmodium* infection, the role and biological relevance of several co-inhibitory pathways have been established. For example, compared to wild type mice, mice genetically deficient in BTLA are resistant to prolonged non-lethal blood stage *P. yoelii* infection [55]. Resistance in BTLA-deficient animals was linked to enhanced CD4 T cell-intrinsic pro-inflammatory cytokine expression and augmented anti-parasite-specific antibody responses. These data suggest that targeting BTLA or its ligand, herpesvirus entry mediator (HVEM), could prove useful for enhancing the activity of parasite-specific T and B cells. More recently, accelerated clearance of blood-stage *P. yoelii* infection was demonstrated following simultaneous blockade of both PD-L1 and LAG-3 [56]. In this experimental model, accelerated blood-stage *P. yoelii* clearance was correlated with enhanced CD4 T_H1, T_{FH}, and protective antibody responses [56]. Interestingly, parasite-specific CD8 T cell function was also markedly improved following PD-L1 and LAG-3 blockade, although the precise contribution of cytotoxic T cells to resolution of blood-stage *Plasmodium* infections remains an open question.

Although functional evidence establishing a direct link between co-inhibitory receptors and T cell exhaustion in *P. falciparum*-infected humans is currently lacking, phenotypic evidence of exhaustion during malaria is accumulating [56, 57]. Thus, in the future it will be of significant interest to examine whether the activity of *P. falciparum*-specific T cells can be enhanced *in vitro* through disruption of co-inhibitory pathways. If the data support operational roles for these pathways in suppressing anti-parasitic function of T cells in humans, these results would provide clear rationale for targeting co-inhibitory receptors during chronic *P. falciparum* infection with the aim of restoring or improving parasite-specific T cell function.

Evidence for T cell exhaustion or dysregulation during helminth infections has also been reported. T cells isolated from individuals persistently or repeatedly infected with *Wuchereria bancrofti* exhibit signs of diminished cytokine expression in response to antigen restimulation, which is associated with increased CTLA-4 expression on both CD4 and CD8 T cell subsets [58]. Notably, *in vitro* blockade of CTLA-4 on peripheral blood mononuclear cells from patients with filarial disease increased IL-5 expression, with concomitant decreases in IFN- γ , suggesting filarial infections can alter the T_H1/T_H2 balance [58]. The effectiveness of neutralizing CTLA-4 *in vivo* has also been shown in a rodent model of *Litomosoides sigmodontis* filarial infection in which blocking CTLA-4 in combination with CD25⁺ T regulatory cell (Treg) depletion resulted in enhanced parasite clearance [59]. Hyporesponsiveness among parasite-specific T_H2 cells after *L. sigmodontis* infection was also recently linked to T cell expression of the co-inhibitory receptor PD-1 [60]. In that report, T_H2 cells progressively lost proliferative potential and the capacity to express key cytokines IL-4, IL-5 and IL-2 following chronic helminth infection, a phenotype that could be reversed *in vivo* by disrupting interactions between the co-inhibitory receptor PD-1 and its alternative ligand, PD-L2. Notably, blocking the PD-1:PD-L2 pathway also improved parasite control.

Additional recent evidence for T_H2 cell exhaustion during helminth infections comes from studies of schistosomiasis. In mice chronically infected with *Schistosoma mansoni*, T_H2 cells progressively lose function, but can be restored by interfering with expression of gene

related to anergy in lymphocytes (GRAIL), an E3-ubiquitin ligase [61]. It is worth noting that hyporesponsive, parasite-specific T_H2 cells are also associated with decreased hepatic fibrosis, suggesting that a balance between potent anti-parasitic effector T cell responses and collateral, immune-mediated tissue damage may be established during chronic helminth infections. Indeed, the immunomodulatory properties of helminths and helminth-derived antigens are under evaluation for their ability to modulate immunopathologic diseases (reviewed in [62]).

Although several of the studies described above establish the biological relevance of co-inhibitory receptor expression during chronic parasitic infections, several important issues remain to be explored, including a dissection of the potentially complex interplay between multiple co-inhibitory receptors. Consistent with this, the synergistic effects of targeting multiple co-inhibitory pathways have been described in both virus-specific human T cells [63, 64] and rodent models of *Plasmodium* infection [56]. On the other hand, simultaneously targeting co-inhibitory and co-stimulatory pathways may also enhance the function and activity of pathogen-specific T cells. For example, during *Plasmodium* infection HVEM could interact with both co-stimulatory (LIGHT) and co-inhibitory (CD160 or BTLA) receptors [65], and evidence for a critical link between activating and inhibitory circuits has already been established in T cells responding to chronic *Toxoplasma* infection [66]. Finally, in addition to targeting co-inhibitory receptor-ligand interactions, the administration of specific soluble growth factors has also been shown to improve the activity of exhausted T cells. For example, recombinant IL-21 or IL-2 when co-administered during PD-1 receptor blockade has been shown to synergistically restore T cell function [67, 68]. Collectively these studies highlight both the utility and potential complexity of co-inhibitory receptor blockade and underscore the need for additional studies to dissect the relative role and contribution of each towards regulating activation, proliferation and survival of parasite-specific T cells.

B CELL DYSFUNCTION DURING PARASITIC INFECTIONS

In addition to T cell exhaustion described above, a substantial body of evidence supports that chronic parasitic infections are also linked to dysfunctional B cell responses. Given the critical role for B cells and secreted antibody in controlling helminth and hematogenous protozoan infections, understanding the impact of parasitic infections on the B cell compartment is an important area of research. Chronic parasitic infections have been shown to impact B cells in at least three key ways: polyclonal B cell activation, atypical memory B cell expansion and deletion of specific B cell subsets.

After blood stage *Plasmodium* infection, polyclonal activation of B cells has been reported to occur as a consequence of interactions between *P. falciparum*-infected RBC and B cells. These interactions appear to be largely mediated by the cysteine-rich interdomain 1 α (CIDR1 α) of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which has been shown to drive the polyclonal activation of CD27⁺ (memory) B cells [69]. The phenotypic and functional changes in these CIDR1 α -activated B cells included the activation of MAPK and NF κ B pathways, and altered gene expression profiles, which are distinct from B cell receptor (BCR) induced changes [70]. It was originally believed that CIDR1 α -induced

activation of B cells renders those cells hypo-responsive to subsequent activation and signaling through the BCR. However, experimental evidence shows that B cells activated by CIDR1 α are equally responsive to BCR crosslinking as resting CD27+ B cells [69]. Although direct proof that these interactions occur *in vivo* is lacking, it is possible that B cell interaction with CIDR1 α (or related parasite proteins) effectively lowers the threshold of activation for B cells. As a consequence, these B cells might be subsequently rendered hyper-responsive to BCR crosslinking, which may potentiate their development into terminally differentiated cells that have lost function through pathways of senescence or direct inhibition via regulatory feedback circuits. Polyclonal B cell activation has also been reported during helminth infection. Experimental infection with *H. polygyrus* revealed profound infection-induced expansion of bystander B cells [28], and strong negative correlations have been identified between clinical helminth infection and B cells/antibody responses against bacterial and viral vaccine antigens [71–73].

In contrast to direct physical contact between parasites and B cells, recent data also support the notion that parasite infection can result in the release of soluble factors that indirectly trigger the activation, proliferation and differentiation of B cells. For example, *Plasmodium*-infected RBC have been shown to trigger dendritic cells to express B cell activating factor (BAFF). Moreover, expression of the anti-inflammatory cytokine IL-10 and the pro-inflammatory cytokine IFN- γ is commonly associated with parasitic infections and are also known to drive BAFF expression. BAFF drives antigen-independent proliferation of naïve B cells and transitional B cells, and in murine models is known to influence the differentiation of IL-10 secreting regulatory B cell subsets. Therefore, the effects of BAFF could manifest as dramatic changes in either the subset composition, potential for maturation, differentiation or the functional capacity of B cells [74]. As a consequence, marked shifts may occur in the balance between protective and pathogenic B cell responses during chronic parasitic infections.

In addition to driving the polyclonal expansion of B cells subsets, *Plasmodium* has been reported to cause the specific functional impairment of B cells. Nearly five years ago, Weiss *et al.* reported that prolonged or repeated exposure to *P. falciparum* in humans living in endemic areas was associated with the appearance of a distinct population of hypo-responsive memory B cells [75], a population that was first reported in patients chronically infected with HIV [76]. Initial experiments revealed that these “atypical” memory B cells expressed high levels of at least one inhibitory receptor, FcR-like protein 4 (FcRL4). Indeed, *in vitro* studies demonstrated that the co-ligation of the BCR and FcRL4 effectively abrogated B cell activation [77, 78]. Further molecular profiling studies demonstrated that these atypical FcRL4+ B cells expressed gene signatures similar to CD8 T cells undergoing exhaustion during persistent virus infection [79]. Finally a study by Illingsworth *et al.* reported numerical expansions of atypical MBC in populations repeatedly exposed to *P. falciparum*, compared to naïve subjects and individuals living in areas where transmission had ceased 5 years prior to the study [57].

Although these initial studies posited a mechanistically attractive explanation of the failure of *P. falciparum* infection to trigger sterilizing humoral immunity against reinfection, more recent data are reshaping this view. In an elegant study Muellenbeck *et al.* examined the

function of cells that fit the phenotypic definition of atypical MBC [80]. In this report, atypical MBC were sort purified from the peripheral blood of subjects from *P. falciparum* endemic regions and subjected to various activation triggers *in vitro*. The study revealed that atypical MBC were fully competent to secrete high affinity antibodies. The authors went on to show that the secreted antibodies from these cells were effective at neutralizing *P. falciparum* infectivity in a system of human RBC culture [80]. Collectively, these recent data suggest that atypical MBC may be functional participants in the anti-*Plasmodium* humoral immune response. These new data also provide critical insight into the functional capability of these cells, however many issues still need to be resolved. For example, although these cells can secrete neutralizing antibodies *in vitro*, their relative contribution to serum antibody responses *in vivo* remains a key question.

Finally, in addition to the impact of parasitic infections on B cell activation or development described above, other processes have also been reported to impact B cell survival and activity during parasitic infections. Both *Trypanosoma* and *Plasmodium* infections have been associated with clonal deletion of B cells, with or without B cell polyclonal activation and proliferation [81–84]. Thus, another potential explanation for the lack of durable, sterilizing protective immunity following chronic or repeated parasitic infections relates to the loss of both antigen-specific and bystander B cells.

ALTERED ACTIVITY OF REGULATORY IMMUNE CELL SUBSETS

Although the essential protective roles of effector and memory T cell subsets in combating parasitic infections is well established, less is known about the relative contribution of specific regulatory cell subsets, including T regulatory cells (Tregs) and B regulatory cells (B10 cells). Tregs are a functionally distinct CD4 T cell subset capable of exerting governance of immune homeostasis and suppressive control of multiple immune cell types, including T cells, B cells and dendritic cells [85]. During acute infections, Tregs prevent overt immunopathology by dampening effector immune cell activation, either through the release of soluble factors such as IL-10, the sequestration of T cell growth factors such as IL-2, or through direct contact with antigen presenting cells, which reduces surface expression of T cell co-stimulatory ligands [85, 86]. Although much has been learned about the function of Tregs during scenarios of acute infection, autoimmunity and the maintenance of homeostasis, it is not clear whether aberrant expansion and suppressive function of Tregs contributes to chronic parasitic infections. For example, Treg expression of soluble factors, such as IL-10 and TGF- β (Table 2), or direct cell contact between Tregs and antigen presenting cells could result in reduced expression of co-stimulatory molecules CD80/CD86 that are required for the full program of T cell activation, differentiation and survival [87]. High and sustained levels of expression of the high affinity IL-2 receptor, CD25 also characterizes Tregs [85, 86]. Thus, an additional mechanism by which aberrant activity of Treg cells could regulate the activation and proliferation of T cells is through the sequestration of IL-2, a critical T cell growth factor. For both *Toxoplasma* and *Plasmodium* infections, the appearance of Tregs have been described, but the specific role and contribution of this particular subset of cells remains controversial. For example, *P. falciparum* infection in Gambian children with severe malaria was linked to the appearance of higher frequencies of circulating Tregs, relative to effector T cells, and *in vitro* studies

demonstrated the ability of these Tregs to suppress the proliferation of autologous T cells activated with parasite antigen [88]. Conversely, numerical expansions of Tregs also been shown to correlate with enhanced clearance of *Plasmodium*-infected RBC and protection against pathologic inflammation [88, 89]. During experimental toxoplasmosis, depletion of Tregs has revealed a limited role for their ability to modulate immunity or pathogenic responses [90], whereas adoptive transfer of specific Treg subsets impedes the induction of immunity against *Toxoplasma* [91]. Despite these seemingly paradoxical results, whether and how Tregs specifically suppress parasite-specific T cell proliferation or activation *in vivo* remains an open and important question.

Similar to Tregs, IL-10 secreting B regulatory (B10) cells have been shown to dramatically suppress auto-reactive CD4 T cells during experimental, chronic autoimmune disease and effector T_H1 responses during acute infections. Notably, new data show that B10 cells numerically expand and modulate adaptive immunity during chronic parasitic infections, including babesiosis and schistosomiasis [92, 93], and during acute malarial disease [94]. Thus, B10 cells may be underappreciated negative regulators of immunity against other parasitic infections such as *Toxoplasma*, *Leishmania* and chronic *Plasmodium*. In the future it will be of interest to determine whether the establishment of chronic parasitic infections is in part regulated by aberrantly suppressive activities of IL-10 secreting B10 cells.

CONCLUSIONS, PERSPECTIVES AND OUTSTANDING QUESTIONS

The development of novel immune-based interventions to improve resistance to chronic parasitic infections will require a deeper understanding of how parasites impair host immunity. Recent findings have illustrated that certain parasites have an unprecedented ability to manipulate the host's immune system and cause dysfunctional humoral and cell-mediated responses. Indeed, the chronic nature of these infections indicates that most helminth and protozoan parasites are poor inducers of long-term, sterilizing immunity. Our understanding of the immunological pathways and mechanisms that contribute to chronic parasitic infections remains incomplete, and several outstanding questions and lines of investigation warrant further exploration:

What is the relative role and contribution of CD4 T cell exhaustion to chronic parasitic infection?

Recent studies have identified clear parallels between CD8 T cells responding to chronic viral infections and chronic parasitic infections. However, much less is known about CD4 T cells. In contrast to CD8 T cells, pathogen-specific CD4 T cells can develop into a number of functionally distinct subsets including T_H1, T_H2, T_H9, T_H17, T_{FFH} and Treg cells. Indeed, aside from T_H1 and T_H2 subsets discussed above, the propensity for other functionally distinct subsets to undergo exhaustion during chronic parasitic infection is unknown. Such information could reveal novel interventional strategies to enhance the activity of these T helper cells. Moreover, specific subsets of T helper cells express multiple co-inhibitory receptors, most notably PD-1 and CTLA-4. CTLA-4 is widely expressed on Tregs and PD-1 is routinely used as a marker to identify T_{FFH} cells, yet whether blocking PD-1 or PD-L1 *in vivo* acts directly on these critical CD4 T cell subsets is unclear. Determining the mechanisms by which parasitic infections impact CD4 T cell function, and delineating how

this impacts B cell function, will be important for developing next-generation immune-based interventional strategies.

What are the molecular signatures of CD4 T cells responding to chronic parasitic infection?

Understanding how persistent antigen impacts the differentiation and maintenance of parasite specific CD4 T cells, including T_{FH} cells, remains an important goal. In the case of *Plasmodium* infection, continued exposure to blood-stage parasites appears necessary to maintain parasite-specific antibody responses and protect against severe disease [95]. Unlike conventional memory T cells, the maintenance of exhausted T cells is known to depend on persisting antigen [35], and attrition of *Plasmodium*-specific T cells may explain the loss of clinical immunity in individuals who move away from malaria-endemic areas. Therefore, chronic parasitic infections may be a double-edged sword: persistent antigen or repeated parasite exposure may be necessary to maintain clinical immunity, but repeated or persistent infections may concomitantly erode the functional attributes of parasite-specific immune cells. Thus, it will also be of significant interest to understand the maintenance and define the molecular signatures of these cells in longitudinal studies as chronic infection progresses, or as repeated exposures occur. Such information could potentially identify new molecular targets for enhancing parasite-specific CD4 T cell function using novel drugs or small molecule inhibitors.

It is becoming clear that parasites have a distinct capacity to negatively impact host immunity through a diverse array of mechanisms. More detailed understanding of these regulatory pathways will be useful for identifying novel interventions (including vaccines) against parasitic infections. Defining the specific cellular and molecular circuits that underlie the ability of parasites to negatively impact immunity and establish chronic infection will shape future efforts to enhance resistance to these infections.

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LIST OF ABBREVIATIONS

BAFF	B cell activating factor
BAFF-R	B cell activating factor receptor
BTLA	B- and T-lymphocyte attenuator
CTLA-4	Cytotoxic T-lymphocyte antigen 4
CIDR1a	Cysteine-rich interdomain 1alpha
FCRL4	FcR-like protein 4
HVEM	Herpesvirus entry mediator

LAG-3	Lymphocyte activation gene 3
MBC	Memory B cell
PD-1	Programmed cell death 1
PD-L1	Programmed cell death 1 ligand 1
PD-L2	Programmed cell death 1 ligand 2
PfEMP1	<i>P. falciparum</i> erythrocyte membrane protein 1
Tim-3	T cell immunoglobulin and mucin protein 3

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Table 1

Parasitic infections associated with immune dysfunction or exhaustion.

Parasite	Cell type impacted	Phenotype	References
<i>Plasmodium</i>	B cells	Polyclonal activation, deletion, anergy, reduced class switching	[56] [55, 56, 69, 75, 81]
	CD8 T cells	Loss of pro- inflammatory cytokine expression	[56]
	CD4 T cells	Apoptosis, deletion, loss of pro-inflammatory cytokine expression	[56, 96, 97] [55]
	Dendritic cells	Apoptosis, inefficient antigen presentation	[98–101]
<i>Toxoplasma</i>	CD8 T cells	Deletion, anergy, loss of effector function (cytolytic activity, cytokine expression)	[46–49]
<i>Leishmania</i>	CD8 T cells	Deletion, anergy, loss of effector function (cytolytic activity, cytokine expression)	[50, 102]
	CD4 T cells	Impaired proliferation, cytokine expression	[51]
	B cells	IL-10 expression from B regulatory cells	[103]
<i>Trypanosoma</i>	B cells	Polyclonal activation, deletion, anergy	[82]
	CD8 T cells	Apoptosis, loss of effector function	[54, 104, 105]
	CD4 T cells	Anergy, loss of effector function	[53, 106]

Table 2

Cell surface expressed and soluble factors that modulate functional adaptive immunity during parasitic infections.

Cell surface expressed	Inhibitory Receptor	Cellular Expression	Known Ligand(s)	Parasitic Infection (References)
	PD-1	CD4 and CD8 T cells, B cell	PD-L1, PD-L2	[51, 56, 107]
	LAG-3	CD4, CD8, and subsets of $\gamma\delta$ T cells, NK cells	MHC II	<i>Plasmodium</i> [56]
	2B4 ^a	Monocytes, $\gamma\delta$ and CD8 T cells, NK cells	CD48	None reported to date
	CTLA-4	CD4 and CD8 T cells	CD80, CD86	<i>T. Cruzi</i> , <i>Plasmodium</i> [44, 53]
	Tim-3	T _H 1 cells, dendritic cells	Galectin-9	<i>Plasmodium</i> , <i>Toxoplasma</i> [108–110]
	CD160 ^a	NK cells, subsets of CD8 T cells, and minor populations of CD4 T cells	MHC I	None reported to date
	BTLA	CD11c+ and CD11b+ cells, T and B cells	HVEM	<i>Plasmodium</i> [55]

Soluble	Protein	Target cell(s)	Impact	Parasite Infection (References)
	IL-10	Dendritic cells, macrophages, T cells, B cells	Downregulates MHCII and co- stimulatory molecule expression; decreases pro- inflammatory cytokine production in dendritic cells and macrophages; inhibits phagocytosis, inhibits cytokine production and proliferation of CD4 T cells; suppresses CD8 T cell response; affects B cell differentiation and isotype switching	<i>Babesia</i> , <i>Schistosoma</i> , <i>Plasmodium</i> , <i>Leishmania</i> [92, 93, 98, 103, 111–114]
	TGF- β	Dendritic cells, macrophages, T and B cells	Inhibits the function of pro- inflammatory cells; inhibits lymphocyte proliferation; inhibits T _H 1 and T _H 2 responses and NK cell activity; promotes Treg expansion	<i>T. cruzi</i> [115, 116]
	TNF	Dendritic cells	Systemic activation of dendritic cells resulting in reduced T cell priming	<i>Plasmodium</i> [101]

^aBiological roles for 2B4 and CD160 during parasitic infections have not been reported