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Parasites: what are they good for?

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Abstract

Parasitic diseases caused by helminth and protozoan infections remain one of the largest global public health problems for mankind. While natural immunity in man is rare or slow to develop for many parasites, the immune response is capable of recognizing and responding to infection by utilizing a number of different immunological mechanisms. This special topics journal issue examines many of the key findings in the recent literature regarding the immune response against helminth and protozoan infections, as well as highlighting areas in which our current knowledge falls short. The question of how we can tailor immune responses to prevent or reduce disease burden is a burning question within the field of immunoparasitology.

Keywords

alternatively activated macrophages; B cells; innate lymphoid cells; helminth; parasite; protozoan; T cells; Toll-like receptors

Parasitic diseases are caused by a number of protozoa (e.g. *Plasmodium* spp., *Toxoplasma*, *Trypanosoma* spp., and *Leishmania* spp.), and helminth worm (e.g. nematodes, trematodes, and cestodes) species. These diseases remain a major health burden in tropical and subtropical regions around the globe. There are many contributing factors for this, including the geographic range of the insect vectors that transmit many of these pathogens, access to healthcare, as well as insufficient attempts and funds for eradication campaigns. Millions of people remain infected, leading to chronic debilitating disease, disfigurement, and often death. Drugs are available for treatment or prevention of many parasitic infections, but they come at significant cost. The drugs often produce debilitating side effects, are difficult to attain or afford by the resident population that are most likely to be afflicted, and parasite resistance to effective therapeutics is spreading. Furthermore, commercially available vaccines to prevent parasitic diseases have not been developed due to the complexity of the parasite's lifecycle or multistage development, their multicellular composition (e.g. helminths), and antigenic variability. Despite these issues many scientific inroads have been made over the last decade to understand the unique, complex relationship between parasites and their host. Genomes have been sequenced, novel drug targets have been identified, and progress has been made in the development of vaccines for malaria and leishmaniasis [1–3]. The accompanying review articles will highlight some of these advances with a specific focus on Toll-like receptors (TLRs), innate lymphoid cells (ILCs), alternatively activated

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Conflict of Interest

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macrophages, chemokines, CD8⁺ T cells, T and B cell exhaustion, and memory T cells. The accompanying articles, as well as a few additional topics covered in this introduction, will discuss the scientific breakthroughs that have furthered our understanding of the host immune response against protozoan and helminth parasites.

Parasites gain entry into their host through three major routes: fecal-oral (e.g. roundworms, *Giardia*, *Cryptosporidium*, and *Toxoplasma*), direct penetration of the skin (e.g. schistosomes and hookworms), and transmission into the skin by the bite of an insect vector (*Plasmodium*, *Leishmania*, *Trypanosoma*, and filarial worms). The infection may be localized at the point of entry, or the parasite may disseminate to other organs either directly or indirectly via infection of migrating cell populations (e.g. red blood cells and antigen-presenting cells), resulting in pathology in many tissues, including the gastrointestinal tract, spleen, brain, liver, skin, bladder, heart and lungs. Due to the various routes of entry and sites of dissemination, these pathogens induce a myriad of host immunological responses within multiple tissues, including innate, cellular and humoral responses. The host immune response is capable of recognizing, containing, and controlling parasite replication and tissue damage, yet rarely eliminates or provides long-term protection after a single exposure to the parasite. Understanding successful evasion of immunity by parasites is a major goal that has challenged researchers in the field of immunoparasitology. Parasites can avoid immune recognition by varying the expression of surface antigens (e.g. African trypanosomes and *Plasmodium falciparum*), changing its developmental form or tissue tropism, replicating within leukocytes, or in the case of worms, through expression of surface tegument that prevents antibody or complement from binding.

The immune response to protozoans and helminths involves cells of the innate and adaptive immune system, although the type of immune response activated by each group of parasites is different. A T helper type 1 (T_H1) response characterized by the production of interferon- γ (IFN- γ) is generated against protozoans, while helminth infections induce a T_H2 response resulting in interleukin-4 (IL-4), IL-5 and IL-13 production. Detection of pathogens by the immune system is fostered by a limited number of receptors known as pattern recognition receptors (PRRs) that recognize conserved features common to many pathogens, which are referred to as pathogen-associated molecular patterns (PAMPs) [4]. PRRs are expressed by innate as well as non-hematopoietic cells, and can be localized to the cell surface, or found within the cytoplasm or endosomal vesicles. TLRs, which are a class of PRRs, have been shown to recognize PAMPs of protozoans (Figure 1; Reviewed by Ghosh and Stumhofer in this issue). While parasite-derived PAMPs have been shown to bind select TLRs and induce the production of pro-inflammatory cytokines *in vitro*, defining the role of these TLRs in promoting an inflammatory response *in vivo* has been less straightforward. For instance, infection of mice that are deficient in only a single TLR gene rarely results in a phenotypic change after infection, indicating the participation of multiple PRRs in the recognition of protozoan parasites *in vivo*. Additionally in the case of *Plasmodium* infection, multiple groups have reported contradicting results using the same TLR-deficient mice and *Plasmodium* species [5–9], which have further complicated our understanding of the contribution of PRRs in generating a protective or pathologic immune response during malaria infection.

Recently, there has been substantial progress in identifying parasite-derived PAMPs and the PRRs that recognize them [10–12]. For instance, TLR-11 and -12 have been shown to bind *Toxoplasma gondii* profilin as homo- or heterodimers [10, 13, 14]. While these TLRs are responsible for recognizing *T. gondii* infection in mice, TLR-11 and TLR-12 are not expressed in humans; therefore, other PRRs are involved in detecting this parasite infection in man. In support of this idea, Andrade et al. illustrated that *Toxoplasma*-derived DNA and RNA was capable of inducing pro-inflammatory cytokine production by human peripheral

blood monocytes, suggesting that nucleic-acid sensing TLRs (TLR-7, -8, -9) associated with the endosome are key for sensing this infection in humans [14]. Although significant progress has been made in this area many parasitic PAMPs remain undefined, particularly regarding *Leishmania* and *Trypanosoma* infection. Furthermore, involvement of PRRs in recognizing and generating a T_H2 response against helminths remains a mystery. It is possible that sterile tissue damage provides the trigger for generating T_H2 responses during helminth infection through mechanisms that do not require PRRs.

Coffman and Mosmann initially defined subsets of T helper cells based on their cytokine secretion pattern [15], but recently it has become clear that in addition to T cells ILCs are also important sources of cytokines. ILCs have been subdivided into three subsets – group 1 ILCs (comprising natural killer (NK) cells and ILC1s), group 2 ILCs (comprising ILC2s) and group 3 ILCs (comprising ILC3s and lymphoid tissue inducers) – based on their ability to produce type 1, type 2 or T_H17 -associated cytokines. While some ILCs such as NK cells have been shown to be an important source of IFN- γ in a number of protozoan infections [16–19] the role of additional ILC populations in the immune response to protozoan infections has not been widely explored. On the other hand, the role of ILCs in the immune response to helminth infections is better defined (Reviewed by Antignano and Zaph in this issue). For instance, infection with the worm *Nippostrongylus brasiliensis* resulted in identification of ILC2s in the intestine (Figure 2) [20–22]. These ILC2s were shown to secrete IL-5 and IL-13, and were critical for the induction of a T_H2 response. How ILC2s directly or indirectly promote a T_H2 response remains unclear, indicating that researchers have only begun to scratch the surface of elucidating the function of ILCs not only after parasitic infections, but also after exposure to other inflammatory stimuli or during normal tissue homeostasis.

Another important innate cell type that has recently received significant attention – the macrophage – contributes not only to clearance of pathogens and removal of cellular debris, but also plays a role in tissue homeostasis. Macrophage populations have recently been categorized into different subsets based largely on cell surface marker expression and gene expression profiles. These subsets include classically activated macrophages (CAMacs), alternatively activated macrophages (AAMacs), tumor-associated macrophages and regulatory macrophages [23]. CAMacs, which are derived from monocytes recruited from the blood to the site of infection during an inflammatory T_H1 response exhibit microbicidal properties. These CAMacs play an essential role in the clearance of many intracellular protozoan pathogens, and are often targeted by many parasites (e.g. *Leishmania*, *Toxoplasma*, *Trypanosoma cruzi*) in order to promote their growth and survival within the host.

AAMacs, the counterpart of CAMacs, are induced by T_H2 associated cytokines such as IL-4, IL-13 and IL-33. AAMacs express several signature proteins, including the mannose receptor, Arginase 1, chitinases and RELM α [24], and contribute to the immune response against helminth parasites by promoting wound healing (Reviewed by Jang and Nair in this issue). Interestingly, in a model of *Litomosoides sigmodontis* infection, AAMacs were not found to be derived from the blood in response to infection, but instead accumulated in response to IL-4 mediated proliferation of tissue-resident macrophages [25]. Whether these AAMacs are derived solely through proliferation, or can be recruited and differentiated from blood monocytes is unknown, but under active investigation by numerous groups. While their primary function after helminth infections may be to limit and repair tissue damage caused by the worms, the role of AAMacs in T_H1 responses may differ. For instance, during chronic *T. gondii* infection production of chitinases by AAMacs in the brain serve to lyse chitin-rich cysts, exposing parasites to immune-mediated killing by other effector cells [26].

Alternatively, some protozoan parasites actively promote AAMac differentiation in order to promote their survival [27–29].

A major contribution to the field of immunology from the study of parasitic infections has been their use in defining classical T_H1 and T_H2 responses, and the cytokines associated with these responses (T_H1 : IL-12, IFN- γ ; T_H2 : IL-4, IL-5, IL-13). Moreover, these models continue to yield new information about the function of recently described cytokines such as IL-22, IL-25, IL-27 and IL-33. IL-22, which has primarily been shown to have a protective role in the gut by mediating barrier integrity and production of antimicrobial proteins [30], was also shown to contribute to ileitis associated with oral *T. gondii* infection [31, 32]. IL-25 and IL-33, which are epithelial derived cytokines, are instrumental in promoting a T_H2 response after *Trichuris muris* or *N. brasiliensis* infection. The ability of these cytokines to influence the development of T_H2 polarized $CD4^+$ T cells after these infections lies in their capacity to induce expansion and differentiation of innate cell populations. [20–22, 33, 34].

Another cytokine that has received scrutiny over the last ten years is IL-17, due to its association with immunopathology in chronic diseases [35, 36]. Production of IL-17 is primarily attributed to T_H17 cells and group 3 ILCs; however, a recent study indicated that, following infection with *T. cruzi*, B cells are capable of secreting this cytokine [37]. Interestingly, IL-17 production by B cells occurs independently of B cell receptor signaling during *T. cruzi* infection. Instead, the trans-sialidase protein of the parasite modifies glycoproteins on the B cell surface, including the tyrosine phosphatase CD45, resulting in activation of cell-signaling pathways that lead to IL-17A and IL-17F production. Furthermore, these IL-17 producing B cells were shown to be necessary for control of *T. cruzi* infection in mice. Moreover, IL-17 production has been detected in a number of parasitic infections, and has been found to either contribute to parasite control or promote immune-mediated pathology [38–42].

Parasites primarily induce chronic disease; therefore, these pathogens have served as useful models for understanding immunoregulation, particularly the mediators that dampen inflammation and the cells that produce them. For instance, the IL-6 family member IL-27, which is produced by dendritic cells and M ϕ s, has been shown to be a crucial cytokine involved in limiting T_H1 , T_H2 and T_H17 inflammatory responses after parasitic infections [43–48]. In addition to directly inhibiting cytokine production by T cells, IL-27 can also induce regulatory T cell populations during *T. gondii* infection [49], and promote production of IL-10 by $CD4^+$ T cells after *T. gondii*, *L. major* and *P. chabaudi* infection [44, 50, 51] to limit immunopathology.

In addition to understanding the effector function of T cells, there has been increasing interest in identifying the sites where lymphocytes are primed after parasitic infections, and how lymphocytes are recruited to infected tissues. For example, it was long thought that the liver served as the primary site for activation of *Plasmodium* liver stage-specific $CD8^+$ T cells; however, it is now understood that the skin draining lymph node at the site of *Plasmodium* sporozoite inoculation plays an important role in the priming of $CD8^+$ T cell responses and this priming is adequate to induce protective immunity against sporozoite challenge [52, 53] (Reviewed by Villarino and Schmidt in this issue). Also, the advent of intravital microscopy has provided an opportunity to observe the behavior of T cells and other immune cells *in vivo* after infection. This has been particularly useful in understanding how $CD8^+$ and $CD4^+$ T cells are recruited to and move within infected tissues, such as the liver and brain, as well as how these cells may recognize and kill infected cells [54–58].

One mechanism that the host employs to promote recruitment of immune cells to infected tissues is the use of chemokine gradients (Reviewed by McGovern and Wilson in this issue).

Upon infection chemokine gradients serve to mark paths within tissues, guiding various immune cells to their designated targets. For example, CXCL9 and CXCL10 are upregulated in the brain during chronic *Toxoplasma* infection. These chemokines are involved in recruitment and retention of antigen-specific CD8⁺ T cells in the brain, and enhance the speed with which these cells find the location of their target [55]. CCL21 is also upregulated in the brain during chronic *Toxoplasma* infection and influences the migration of CD4⁺ T cells within this organ, promoting their migration from extraparenchymal sites to the parenchyma [58].

As mentioned, the end result of most parasitic infections is chronic disease, primarily due to the inability of the host to completely resolve the infection during the acute immune response. While the cell-mediated immune response is involved in controlling chronic parasitemia, the humoral immune response plays a critical role in limiting parasite replication during this stage of infection. In B cell deficient mice infected with *Plasmodium* spp., *T. gondii*, *T. brucei*, *T. muris* and *L. major*, the absence of B cells results in a significant increase in parasite burden during the chronic stage of infection, resulting in increased pathology and lethality in some cases [59–66]. Additionally, while B cell deficiency does not result in an increase in egg burden in the liver during *Schistosoma mansoni* infection, the absence of these lymphocytes does result in increased pathology in the liver, and an increase in egg burden and pathology in the lung [67, 68]. The observed phenotypes in B cell deficient mice are primarily due to the absence of antibody production; however, one cannot rule out additional effector functions of B cells in the immune response to these parasites such as activation of CD4⁺ T cells and production of cytokines that promote T_H1, T_H2 or T_H17 responses.

As a consequence of persistent infection, responding T cells begin to exhibit changes in their gene expression profile that lead to their functional impairment, a process known as T cell exhaustion [69]. Currently, most of our understanding of T cell exhaustion is based on the increased expression of co-inhibitory receptors such as PD-1, LAG-3, CD160, BTLA, CTLA-4 and Tim-3. These molecules are normally transiently upregulated on activated T cells after infection. However, in the face of persistent infection, expression of these molecules is sustained on T cells, thus negatively regulating their activity and allowing the infection to persist (Figure 3). There is evidence for T cell exhaustion after *T. gondii*, *T. cruzi*, *L. major*, *S. mansoni* and *Plasmodium* infection [70–77] (Reviewed by Zander and Butler in this issue). For example, simultaneous blockade of LAG3 and PD-1 resulted in enhanced clearance of blood-stage *P. yoelii* infection, which was correlated with an improved T_H1 response and enhanced protective antibody production [75]. T cells express multiple co-inhibitory receptors on their surface, but the interplay of signaling through these receptors is largely unknown as are the molecular signatures of the T cells that are responding to chronic parasitic infection. Moreover, detailed information of the pathways that sustain the expression of these inhibitory receptors on T cells during chronic infection is sparse. Greater insight regarding these processes could yield potential avenues for manipulation of T cells during chronic disease to enhance protection and/or pathogen clearance. However, one has to wonder, particularly in the case of malaria infection, whether persistent antigen or repeated exposure is necessary for maintenance of clinical immunity.

T cells are not the only adaptive immune cells that are linked to a dysfunctional phenotype during chronic parasitic infections, as B cells also display impaired responses (Reviewed by Zander and Butler in this issue). These include polyclonal activation, deletion of B cell subsets and formation of atypical memory B cells (Figure 4). All of these B cell impairments may contribute to the slow development of antibody-mediated immunity, especially in the case of malaria. Polyclonal B cell activation occurs during rodent and human malaria, resulting in hypergammaglobulinemia and depletion of B cell pools. The atypical memory B

cells associated with malaria infection resemble the exhausted memory B cells observed in HIV-infected individuals [78], and are characterized by a hyporesponsive phenotype [79]. However, a recent study suggested that atypical memory B cells isolated from subjects in malaria endemic areas could competently secrete antibodies after stimulation, and neutralize *P. falciparum* infectivity *in vitro* [80], suggesting that these atypical B cells are functional and participate in an anti-*Plasmodium* response. However, whether this phenomenon occurs *in vivo* has yet to be demonstrated.

Although significant progress has been made in our understanding of parasitic diseases and the subsequent immune response, there is still much to be learned. No effective vaccine is available to prevent or reduce disease burden for the major protozoan infections, including malaria, or helminth infections. One of the major hurdles that exist in vaccine development is determining what approach – be it parasite derived antigens, attenuated whole parasites or another method – will be necessary to induce protective CD4⁺ and CD8⁺ T cells and antibody in order to provide complete protection upon challenge (Reviewed by Villarino and Schmidt in this issue). Another barrier that has to be approached in order to develop effective vaccines is determining what type(s) of memory T cell populations are formed during chronic parasitic infections, and whether stable populations of memory T cells can be maintained in the absence of antigen in order to afford protection against these infections (Reviewed by Opat and Stephens in this issue). Vaccine trials in humans and rodents have indicated that immunity wanes with time after vaccination or treatment of infection [81–84], suggesting that continual exposure to antigen is required to maintain protection against malaria, *Leishmania* and other chronic infections [85, 86]. However, in the case of *Leishmania* infection in mice, long-lived central memory CD4⁺ T cells are generated after infection and can persist in the absence of antigen [87], indicating that a vaccine capable of expanding these cells might provide long-term protection. Moreover, our ability to successfully design anti-parasitic vaccines will be greatly aided by our continued efforts to define mechanisms by which parasites undermine the immune response to establish chronic infection. Therefore, a more thorough understanding of the basic immunology of the mouse and human responses to these infections is needed, particularly regarding how protective T and B cell responses are initiated and maintained.

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List of Abbreviations

TLR	Toll-like receptor
ILC	Innate lymphoid cell
PRR	Pattern recognition receptor
PAMP	Pathogen associated molecular pattern
CAMacs	Classically activated macrophages
AAMacs	Alternatively activated macrophages

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Figure 1. Recognition of protozoan PAMPs by host TLRs

Infection of host by the protozoan parasites (Clockwise from the top): *T. gondii*, *T. cruzi*, *Leishmania* spp., and *Plasmodium* spp. results in the detection of distinct parasite derived PAMPs, including GPIs, hemozoin, nucleic acids, etc., by TLRs expressed on the cell membrane and within the endosome. Binding of parasite-derived PAMPs to TLRs results in the activation of MyD88-dependent and -independent signaling pathways that lead to the transcription of pro-inflammatory cytokines, chemokines, type I interferons and anti-microbial proteins. Question mark denotes the undefined TLR-4 ligand of *Leishmania*.

Figure 2. Infection with *N. brasiliensis* results in the production of ILC populations within the intestine

Infection with the roundworm *N. brasiliensis* triggers production of the cytokines IL-25 and IL-33 by epithelial cells in the gut. These cytokines promote the production of type 2 innate lymphoid cells (ILC2s), which are capable of producing the T_H2 cytokines IL-5 and IL-13, leading to the promotion of a T_H2 response. However, it is still unclear as to how and if ILC2s are necessary for the development of a T_H2 response after worm infection.

Additionally, the cytokine IL-25 can promote the development of another innate cell population known as MPP^{type2} cells. These progenitor cells can subsequently differentiate

into a number of leukocyte populations associated with T_H2 responses, including macrophages, mast cells and ILC2s.

Figure 3. T cell exhaustion during parasitic infections leads to a persistent chronic infection that cannot be fully resolved

CD4⁺ and CD8⁺ T cells responding to persistent infections, as is the case for most parasitic infections, exhibit alterations in their transcriptional profile, which leads to functional impairments (i.e. decreased cytokine production) and increased rates of apoptosis as part of a process collectively known as T cell exhaustion. Sustained expression of co-inhibitory receptors such as PD-1 and CTLA-4 by T cells during chronic infection negatively regulates the activity of T cells and promotes pathogen persistence.

A. Polyclonal B-cell Activation

B. Atypical memory B-cell Expansion

Figure 4.

Plasmodium infection leads to polyclonal B cell activation and expansion of an atypical memory B cell population, which contribute to B cell dysfunction

A.) Infection of the host by *Plasmodium* can trigger the production of soluble factors such as BAFF by dendritic cells. BAFF can subsequently promote antigen-independent proliferation of naïve and transitional B cells, which may contribute to immune-pathology. **B.)** *P. falciparum* infection in humans causes the expansion of a population of hypo-responsive memory B cells known as “atypical memory” B cells characterized by the expression of an inhibitory receptor FcRL4. Ligation of the BCR and FcLR4 has been shown to impair B cell activation.