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The regulation of CD4$^+$ T cells during malaria

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Abstract

Malaria is a major global health problem. Despite decades of research, there is still no effective vaccine to prevent disease in the majority of people living in malaria-endemic regions. Additionally, drug treatment options are continually threatened by the emergence of drug-resistant parasites. Immune responses generated against *Plasmodium* parasites that cause malaria are generally not sufficient to prevent the establishment of infection and can even contribute to the development of disease, unless individuals have survived multiple infections. Research conducted in experimental models, controlled human malaria infection studies, and with malaria patients from disease-endemic areas indicate the rapid development of immunoregulatory pathways in response to *Plasmodium* infection. These "imprinted" immune responses limit inflammation, and likely prevent progression to severe disease manifestations. However, they also cause slow acquisition of immunity and possibly hamper the development of vaccine-mediated protection against disease. A major target for and mediator of the immunoregulatory pathways established during malaria are CD4$^+$ T cells that play critical roles in priming phagocytic cells to capture and kill malaria parasites, as well as helping B cells produce functional anti-parasitic antibodies. In this review, we describe mechanisms of CD4$^+$ T cell activation during malaria and discuss the immunoregulatory mechanisms that develop to dampen their anti-parasitic and pathological functions. We also offer some ideas about how host-directed approaches might be applied to modulate CD4$^+$ T cell functions to improve vaccine responses and enhance development of natural immunity.

KEYWORDS
CD4$^+$ T cells, immunoregulation, inflammation, Malaria, Tfh cells, Th1 cells

1 | INTRODUCTION

Malaria remains a major public health concern in tropical and subtropical regions of the world. In 2017, there were an estimated 219 million cases and 435 000 deaths, mainly affecting young children living in sub-Saharan Africa. Alarmingly progress in reducing the number of malaria cases has halted.[1] Infection in mammalian hosts is initiated by inoculation of *Plasmodium* sporozoites into the dermis of the skin by the bite of female *Anopheles* mosquitoes leading to the asymptomatic liver stage of the *Plasmodium* lifecycle. Parasites develop in hepatocytes and differentiate into merozoites that are released to infect red blood cells (RBCs) and establish the blood stage of the lifecycle that is responsible for the clinical symptoms of malaria. This relatively complex and multistaged lifecycle of malaria parasites contributes to the slow development of immunity that rapidly wanes in the absence of continuous exposure to parasites.[2,3]

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Furthermore the delay in developing immunity often results in the failure to achieve sterile cure in a timely manner, thereby increasing the risk of disease.[4,5]

Successful control of blood-stage malaria requires a robust antibody response comprising a diverse repertoire of anti-parasitic antibodies with a range of functions. These include the ability to inhibit parasite invasion of hepatocytes and/or RBCs, attach to antibody binding (Fc) receptors on phagocytic cells and fix complement.[6] The importance of antibody for protection against malaria was conclusively demonstrated in an earlier study showing that the passive transfer of immunoglobulin from immune adults to infected children resulted in an increased parasite clearance and a reduced incidence of clinical malaria.[7] These findings along with subsequent research in this area have led to interest in using passive monoclonal antibody (mAb) transfer to protect vulnerable populations against malaria.[8] This topic and the role of antibodies in protection against malaria will not be discussed further in this review. Similarly, although it is clear that components of the innate immune system play important roles in malaria (reviewed in Ref. [9,10]) and $CD8^+$ T cells can be important in recognizing infected hepatocytes during the liver-stage infection (reviewed in Ref. [11]), our focus will be on the activation and function of $CD4^+$ T cells in malaria, with an emphasis on their roles during the blood stage of the *Plasmodium* lifecycle.

The generation of parasite-specific $CD4^+$ T cells is needed to control malaria parasites. In general, two different types of conventional $CD4^+$ T cells are required to control *Plasmodium* parasites that cause malaria; and the types include IFNγ-producing, $Tbet^+$ $CD4^+$ T helper (Th)1 cells that stimulate phagocytic cells to capture and kill parasites[12] and T follicular helper (Tfh) cells required for the development of antigen-specific B cell populations and production of protective anti-parasitic antibodies.[13] However, the development and maintenance of Th1 and Tfh cell responses can be antagonistic and their activities can become dysregulated and contribute to tissue damage if not appropriately controlled.[13-16] Whether dysregulation is caused by counteracting immunoregulatory networks established to prevent excessive inflammation and tissue damage or whether the latter arises in response to the former is still not clear. Regardless, the balance between regulatory immune mechanisms and inflammation has a major impact on the magnitude and effectiveness of immune responses generated following infection or in response to vaccination.[17]

Our recent work with controlled human malaria infection (CHMI) studies showed the rapid development of immunoregulatory networks following the first exposure to submicroscopic levels of malaria parasites. This finding raised important questions about their influence on the development of anti-parasitic immunity and, in particular, their impact on the performance of vaccines in malaria endemic areas.[18] This was highlighted by the relatively poor performance of vaccines tested in disease endemic regions, compared to when tested in healthy volunteers from non-malaria endemic regions. For example, the RTS,S/AS01 vaccine in children and infants affords 36% and 25% efficacy against clinical malaria, respectively[19] while the efficacy of the same vaccine in healthy volunteers in CHMI studies was 52%.[20] Similarly, despite greater than 90% protection against *Plasmodium falciparum* challenge in malaria-naive subjects in the USA provided by a radiation-attenuated *P falciparum* sporozoite (*Pf*SPZ) vaccine,[21-23] efficacy fell to 29% against naturally transmitted *P falciparum* in Malian adults[24] and 20% in Tanzanian adults after mosquito bite CHMI.[25] Although many reasons may account for this discrepancy, the early establishment or imprinting of potent, pathogen-specific immunoregulatory networks in response to early inflammatory signals may be an important factor contributing to this problem. Hence, a better understanding about the development of $CD4^+$ T cell responses during malaria is needed to develop strategies aimed at improving anti-parasitic immunity. Furthermore, this knowledge may also help identify new host-directed strategies to transiently modulate immune regulation as part of vaccination or drug treatment protocols to allow the generation of robust anti-parasitic immunity.

In this article, we will review the activation and differentiation of $CD4^+$ T cell subsets during malaria and discuss their roles in disease control. Our main focus will be on the blood stage of *Plasmodium* infection. We will discuss the critical roles that dendritic cells (DCs) play in this process. Additionally, we will describe known immunoregulatory networks established after infection and discuss how these might be manipulated to boost the efficacy of vaccines or drug treatment. We will direct our attention on several immunomodulatory pathways that have been examined by our group over the past decade. These include IL-10 production by IFNγ-producing Th1 (type 1 regulatory; Tr1) cells, type I interferons (IFNs) produced in response to *Plasmodium* infection that not only suppress anti-parasitic Th1 cell response but also promote the expansion of Tr1 cells[26,27] and co-inhibitory receptor expression by Th1 and Tr1 cells following *Plasmodium* infection, including the immune checkpoint molecules which suppress anti-parasitic activity.[17,28]

2 | $CD4^+$ T CELLS IN MALARIA

$CD4^+$ T cells play important roles in determining the outcome of experimental (rodent) and human malaria. Their activation status has been positively correlated with protective immunity following natural infection, vaccination and in CHMI studies.[22,29,30] For example, the frequency of peripheral blood, IFNγ-secreting, *P falciparum*-specific $CD4^+$ T cells in humans from The Gambia, West Africa was positively associated with protection from infection and malaria.[31] In naive adults immunized with RTS,S/AS, the magnitude of circumsporozoite protein (CSP)-specific $CD4^+$ T cell response and IFNγ production were correlated with protection following challenge with *P falciparum*-infected mosquitoes in CHMI studies.[32-34] However, data from young children immunized with this vaccine was less clear with one study testing the RTS,S/AS vaccine reporting no association between $CD4^+$ T cell activation and protection,[35] while another study found that TNF-producing $CD4^+$ T cell frequency was positively correlated with protection from malaria in vaccinated children.[36] A number of factors may account for these different outcomes,

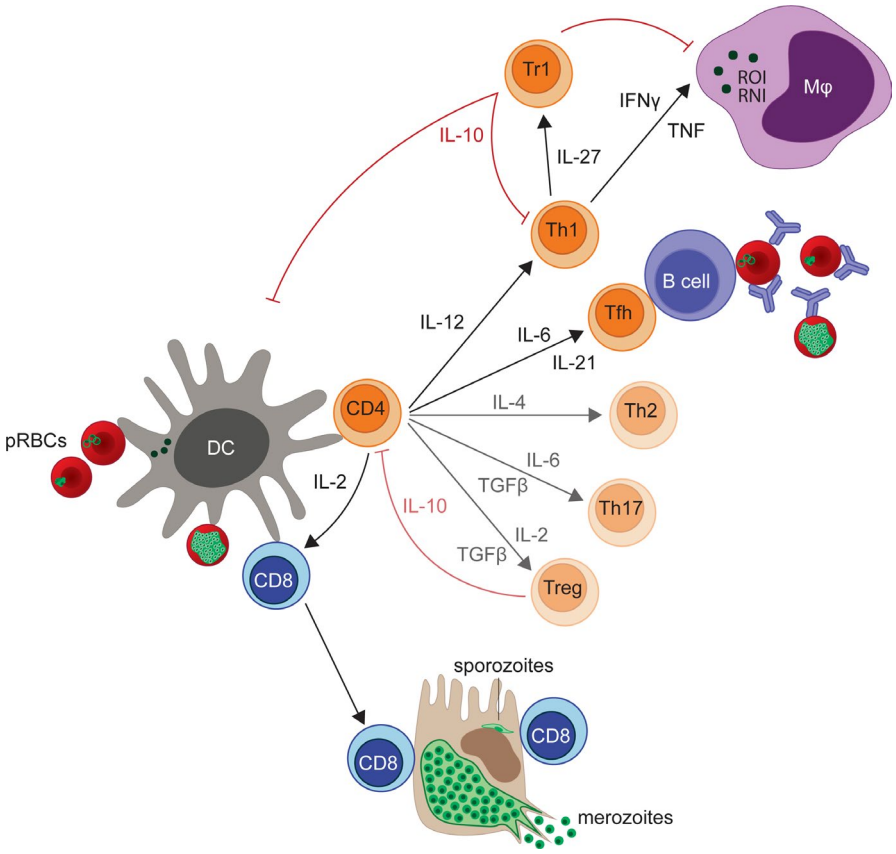

FIGURE 1 The activation and differentiation of $CD4^+$ T cells during malaria. Pattern recognition receptors (PRRs) on dendritic cells (DCs) recognize parasite molecules and stimulate the production of IL-12 or IL-6 and IL-21 that promote the expression of specific transcription factors by $CD4^+$ T cells that result in the development of T helper (Th)1 and T follicular helper (Tfh) cell populations, respectively. Th1 cells produce pro-inflammatory cytokines such as IFNγ and TNF that stimulate production of reactive oxygen and nitrogen intermediates (ROI and RNI, respectively) by macrophages and promote capture and killing of parasitized red blood cells (pRBCs). Th1 cells can also become type 1 regulatory (Tr1) cells that produce IL-10 that dampens pro-inflammatory cytokine production, ROI and RNI, as well as suppressing antigen presentation by DCs. Tfh cell play critical roles in the initiation and maintenance of germinal centre reactions, the selection and maturation of B cells, immunoglobulin class switching and development of high-affinity antibody responses directed against *Plasmodium* molecules. During the pre-erythrocytic stages of malaria, $CD8^+$ T cells can migrate from skin and liver draining lymph nodes to the liver and recognize and kill infected hepatocytes with help from $CD4^+$ T cells. The activation of Th2 and Th17 cells is limited relative to Th1 and Tfh cells in malaria, but they have been reported in experimental models of malaria and human malaria patients. However, their roles are still largely unknown. $FoxP3^+$ $CD4^+$ T regulatory (Treg) cells emerge from the thymus or develop in peripheral tissues in response to TGFβ and IL-2 signaling. They appear to dampen inflammation in the early stages of malaria, before Tr1 cell responses develop

including vaccine formulations, age of study participants, or levels of exposure to malaria parasites. Regardless, there is a reasonable body of evidence indicating that malaria vaccine efficacy is linked to their ability to stimulate robust $CD4^+$ T cell responses.

During *Plasmodium* infection, naive $CD4^+$ T cells are activated following T cell receptor (TCR) recognition of peptide antigen presented by major histocompatibility complex (MHC) II molecules on the surface of DCs. DC-derived costimulatory signals are also required to initiate $CD4^+$ T cell IL-2 production and subsequent expansion.[37] Pattern recognition receptors (PRRs) on DCs are critical to the recognition of parasite molecules and stimulating the selective production of cytokines that promote the expression of specific transcription factors (TFs) by $CD4^+$ T cells that determine their Th cell lineage and thus functions (Figure 1). For example, IL-12 produced by DCs in response to activation of PRRs stimulates expression of Tbet by $CD4^+$ T cells and their subsequent development into Th1 cells that produce pro-inflammatory cytokines such as IFNγ and TNF.[38]

3 | ACTIVATION OF $CD4^+$ T CELLS BY DENDRITIC CELLS

DCs are highly specialized antigen presenting cells (APC) required to initiate adaptive immune response against many pathogens including *Plasmodium*.[39-43] Unlike other APCs such as B cells and macrophages, DCs are primarily responsible for presenting antigen to naive $CD4^+$ T cells in specialized niches of secondary lymphoid tissues and driving their expansion into appropriate Th cell subsets to combat invading pathogens.[39] Two major DC subpopulations found in blood and secondary lymphoid tissues have

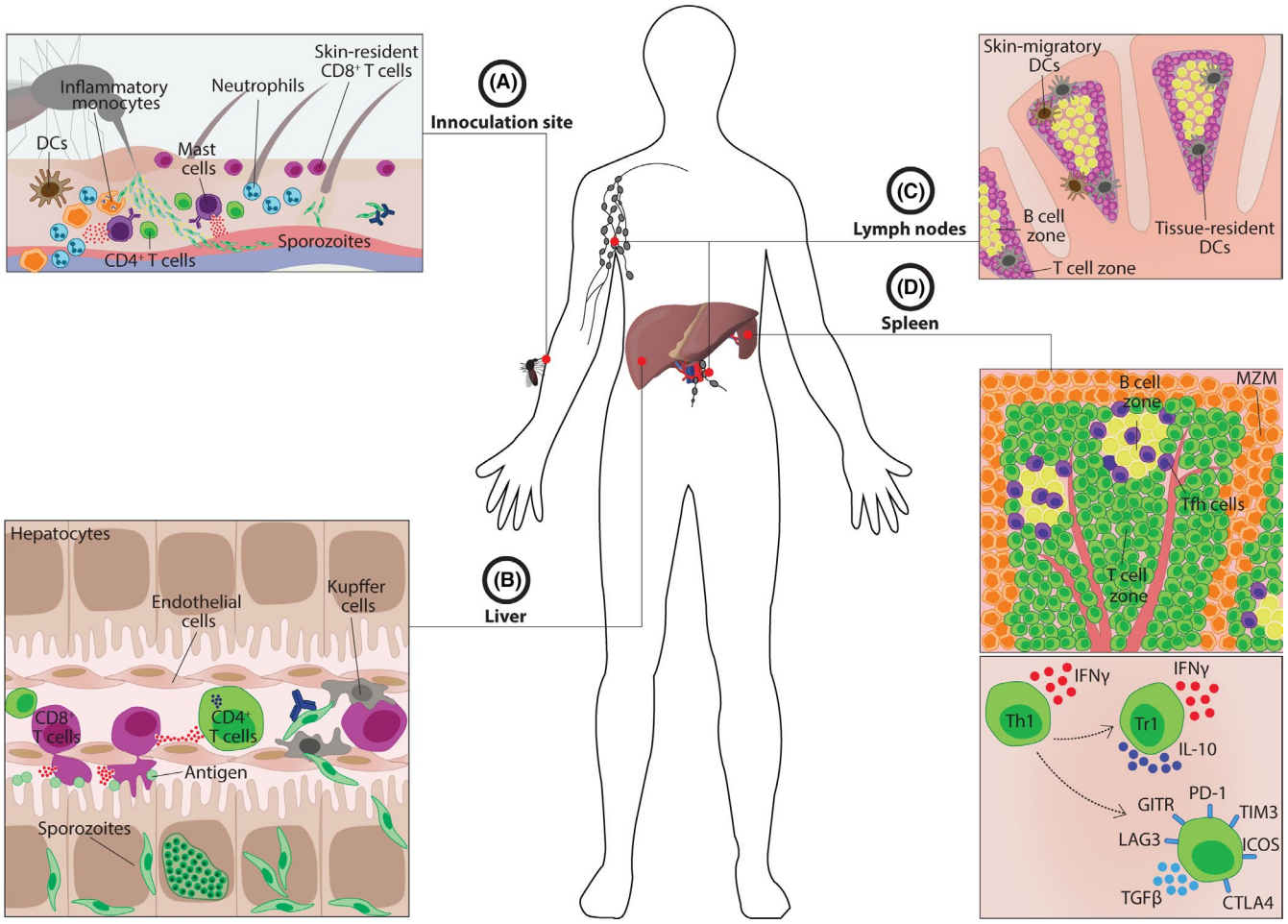

FIGURE 2 The host immune response to *Plasmodium* parasites. A, Following the injection of sporozoites into the skin when a female *Anopheles* mosquito takes a blood meal, parasite products cause inflammation leading to recruitment and activation of various inflammatory cell populations. Sporozoites can enter the blood and migrate to the liver or enter lymphatics, while dendritic cells (DCs) also capture parasite antigen in the skin and transport it to local draining lymph nodes. B, Sporozoites enter the liver and traverse Kupffer cells and enter hepatocytes where they develop into pre-erythrocytic parasite stages. C, DCs in skin- or liver-draining lymph nodes directly capture parasite antigen or receive parasite antigen from skin-migratory DCs that allows them to activate antigen-specific $CD4^+$ and $CD8^+$ T cells. T follicular helper (Tfh) $CD4^+$ T cells move to B cell zones and provide key signals for anti-parasitic antibody production by B cells. This antibody can immobilize sporozoites in the skin during future infections. In addition, antigen-specific $CD4^+$ and $CD8^+$ T cells can migrate from skin and liver draining lymph nodes. Antigen-specific $CD8^+$ T cells can recognize and kill infected hepatocytes with help from $CD4^+$ T cells. If hepatic parasites escape immune detection, then mature and are released into the circulation, they initiate the blood stage of infection. D, Parasitized red blood cells (pRBCs) are captured by DCs or marginal zone macrophages (MZM) in the spleen and DCs can again activate antigen-specific $CD4^+$ T cells. Parasite-specific Tfh cells help B cells produce antibody in splenic B cells follicles (upper panel in D). These antibodies mediate compliment-mediated killing or opsonization of pRBCs by phagocytic cells, as well as blocking merozoite invasion of RBC. $CD4^+$ T cells can also develop a regulatory phenotype, including the induction of IL-10 production by type 1 regulatory (Tr1) cells and increased expression of co-inhibitory receptors, following infection (lower panel in D)

been identified in humans and mice—conventional DCs (cDCs) and plasmacytoid DCs (pDCs).[44] In mice, DCs expressing B220, PDCA-1, and intermediate levels of CD11c are defined as pDCs, while DCs expressing high levels of CD11c and MHCII are identified as cDCs. These latter cells can be subdivided into $CD8\alpha^+$ $CD11b^-$ (cDC1) and $CD8\alpha^-$ $CD11b^+$ (cDC2) lymphoid tissue-resident DCs, and migratory $CD103^+$ $CD11b^-$, $CD103^+$ $CD11b^+$, and $CD103^-$ $CD11b^+$ DCs.[45] Additionally, Langerhans cells and other skin-resident DCs play important roles in pathogen sensing, as well as antigen capture and delivery to draining lymph nodes,[46] while monocyte-derived DCs (mDCs) develop in inflamed tissue from recruited monocytes and can serve to amplify antigen-specific $CD4^+$ T cell responses locally.[47] In humans, HLA-DR^+ $CD11c^+$ cDCs can be subdivided into two subsets—cDC1 and cDC2—based on the expression of CD141 (BDCA3) and CD1c (BDCA1), respectively, while pDCs are classified as HLA-DR^+ $CD11c^+$ CD303 $(BDCA2)^+$ CD304 $(BDCA4)^+$ cells.[48-50] Additionally, $CD1a^+$ and $CD14^+$ dermal DC along with Langerhans cells comprise three major skin DC subsets, and mDCs can also develop from monocytes in appropriate tissue microenvironments.[46]

The role of DCs in malaria has been investigated in both humans and experimental models. After encounter with malaria parasites and recognition via PRRs, DCs undergo maturation and migrate to the T cell zones of secondary lymphoid organs. If the DC has migrated from a peripheral tissue site, as would be the case for skin-resident DCs after capturing sporozoite antigens, the captured antigen is transferred to lymphoid-resident cDCs for presentations to naive T cells.[42] Alternatively, parasitized RBCs (pRBCs) may be captured by macrophages or DCs lining the subcapsular sinus of lymph nodes or marginal sinus of the spleen during blood-stage infection.[51] The removal of pRBCs by tissue-resident macrophages again requires antigen transfer to lymphoid-resident cDCs, but these cDCs themselves may also directly capture, process, and then present parasite peptides following migration from areas of sentinel activity into T cell zones (Figure 2). It should be remembered the behavior of APCs in tissue is derived from studies in mice, and while similar APC populations have been identified in humans, they are not identical and it is likely that there are species differences in the cellular and molecular mechanisms of antigen presentation to $CD4^+$ T cells.

4 | DC ACTIVATION

The development of Th cell subsets is heavily influenced by the PRR cues DCs receive following encounter with pathogen molecules and the additional cellular contacts received on their journey to the T cell zones of secondary lymphoid tissues.[52-54] Several *Plasmodium* molecules, including hemozoin, glycosylphosphatidylinositol (GPI) anchors and immunostimulatory nucleic acid motifs can stimulate DCs through PRRs.[55-57] Additionally, endogenous danger-associated molecular patterns (PAMPs) such as uric acid that accumulates within *P falciparum* and *P vivax* pRBCs are released following cell rupture and can promote the maturation of human dendritic cells in vitro.[58]

5 | TLRS AND *PLASMODIUM*

Innate recognition of *Plasmodium* is critical to the induction of appropriate immune responses.[9,59] Changes in TLR expression on peripheral blood mononuclear cells during *Plasmodium* infection suggest a role for TLRs in recognition of malaria parasites.[60,61] TLR2, 4, and 9 are known to be activated by *Plasmodium* products.[9,59] *P falciparum*-derived GPI can promote TNF production in human peripheral blood monocytes via TLR2 or TLR1/6 through a myeloid differentiation primary response 88 (Myd88)-dependent signaling pathway.[55] Furthermore, *Plasmodium* 2-Cys peroxiredoxin (Prx), an important enzyme involved in regulating redox homeostasis in the parasite cytoplasm (reviewed in Ref. [62]), has been identified as an additional TLR4 ligand in *P berghei* ANKA.[63] Prx activates mouse macrophages and mast cells via a TLR4-dependent activation pathway to stimulate TNF production.[63] TLR9 stimulation via Myd88 activation by *P falciparum*-purified schizonts or schizont extracts is essential for human pDC and mouse monocyte-derived DC activation in vitro.[54] In humans, a *Plasmodium* protein-DNA complex activates TLR9.[56,64] In mice, *Plasmodium*-derived RNA can activate TLR7,[65,66] but a human TLR7 agonist has not yet been identified.

6 | MOUSE DCS AND MALARIA

DCs have been extensively studied in rodent malaria models. Studies in mice show that DC function is compromised during lethal *Plasmodium* infection yet, in non-lethal malaria models, functional DCs were present.[42] Studies on the pre-erythrocytic stages of infection shows the priming of sporozoite-specific $CD8^+$ T cells is dependent on $CD11c^+$ DCs which present sporozoite antigen in either the liver-draining lymph nodes or spleen.[67] Experiments with CD11c-diphtheria toxin receptor mice in which cells expressing high levels of CD11c were depleted show that $CD11c^+$ cells were essential for generating protective immunity against *P yoelli* pre-erythrocytic lifecycle stages,[68] but also promoted the development of experimental cerebral malaria in mice infected with blood-stage *P berghei* ANKA.[69] Antigen presentation in tissue-draining lymph nodes was almost exclusively mediated by $CD8\alpha^+$ DC.[70] In *P chabaudi* infection, loss of $CD8\alpha^+$ $CD103^+$ DC, which is acutely responsive to Flt3 ligand, resulted in impaired $CD8^+$ T cell activation.[71]

Studies with blood-stage *P chabaudi* AS-infected mice showed that following pRBC uptake by splenic $CD11c^+$ DCs, they underwent maturation, started producing IL-12 and primed $CD4^+$ T cell proliferation and IFN-γ production.[72] Similar results were reported in mice infected with *P yoelli* 17NL, whereby following an encounter with pRBCs, DCs expressed high levels of IL-12, the costimulatory molecules CD40 and CD80, as well as MHC class II, thereby supporting $CD4^+$ T cell activation, including IL-2, IFNγ, and TNF production.[73] In other DC adoptive transfer studies, DCs presenting *P yoelii* sporozoite antigens were able to activate antigen-specific $CD4^+$ and $CD8^+$ T cells and initiated protective immune responses against malaria in recipient mice.[43] Similarly, activation of DCs during *P chabaudi* infection led to increased Th1 cell responses.[74] In another study, in vivo depletion of phagocytes showed that DCs were crucial for protection against blood stages of experimental malaria caused by *P berghei*, *P yoelli*, or *P chabaudi* sporozoites.[75] Taken together, there is a significant body of research from experimental malaria models in mice showing critical roles for DCs in $CD4^+$ T cell activation following *Plasmodium* infection.

7 | HUMAN DCS AND MALARIA

Results from studies on human DCs have been less consistent, possibly reflecting the restriction of investigating DCs in blood and a propensity to try and interpret results to fit paradigms established from studies using tissues from disease models. Despite these caveats, seminal studies using in vitro-generated monocyte-derived DC show DC maturation is inhibited by contact dependent[76] and contact-independent[77] mechanisms mediated by the parasite. More

recently, in vitro stimulation of isolated classical blood DC subsets (excluding CD16$^+$ DCs) with pRBCs revealed atypical DC activation, whereby there was increased DC maturation marker expression, but a failure to produce cytokines.[78] Hence, DC activation and function during human malaria are often impaired.

8 | PLASMACYTOID DCS AND MALARIA

Human blood pDCs express TLR7 and TLR9 allowing them to recognize single-stranded RNA[79] and DNA,[80,81] respectively. *Plasmodium*-derived RNA can trigger type I IFN responses in a TLR7-dependent[65] and -independent fashion[66] in mice. In humans, *Plasmodium* protein-DNA complexes are thought to activate TLR9.[56,82] Indeed, simulation of human pDC in vitro with *P falciparum* schizonts upregulated the costimulatory marker CD86 and increased type I IFN production.[54] However, in *P falciparum* CHMI studies, pDCs were only minor contributors to the early type I IFN production during blood-stage infection.[83]

9 | CLASSICAL DCS AND MALARIA

The majority of human DC malarial studies have assessed total myeloid DC function.[84-86] However, there are two distinct human myeloid DC subsets with unique phenotypic and gene expression profiles,[87-89] and as such it is important to understand their specific roles in human malaria. Human cDC1 (CD141$^+$ DC) constitute around 3% of circulating blood DC and are the most efficient cross-presenting DC subset.[90,91] This ability is facilitated by the high expression of CLEC9A, a C-type lectin receptor integral to cross presentation of necrotic antigens.[91] The importance of cDC1 antigen presentation in malaria has been shown in two mouse studies in which cDC1 (CD8α^+ DC) were shown to be the exclusively APCs in draining lymph nodes[70] and impaired CD8$^+$ T cell activation occurred when cDC1 were lost.[71] CLEC9A antigen targeting of cDC1 promoted the generation of antibody development in mice.[92] In humans, CLEC9A facilitated antigen presentation by cDC1 to CD4$^+$ and CD8$^+$ T cells.[93] However, despite an increased frequency of peripheral blood cDC1 in African children with severe malaria, possibly caused by increased levels of Flt3 ligand,[71] HLA-DR expression was reduced on this DC subset,[94] suggesting a reduced ability to present antigen to CD4$^+$ T cells in this disease context. In adults with uncomplicated malaria, cDC1 numbers were reduced, relative to healthy controls,[84,95] but it was not clear whether the loss of cDC1 was a result of this DC subset migrating into tissues such as the spleen. Despite the difficulties studying this relatively rare DC subset, their critical roles in CD4$^+$ and CD8$^+$ T cell activation makes them an important cell populations for further investigation.

Human cDC2 (CD1c$^+$ DC) expresses more HLA-DR than other peripheral blood DC subsets,[96,97] indicating an important role in antigen uptake and presentation to CD4$^+$ T cells. In *P falciparum* CHMI studies, HLA-DR expression on cDC2 was directly associated with capacity for particulate antigen uptake.[96] Similarly, phagocytosis of *P falciparum* pRBCs was more efficient in DC with higher levels of HLA-DR in vitro.[78] However, HLA-DR expression was consistently reduced in children with severe malaria[94] and Papuan adults with uncomplicated malaria,[95] suggesting *Plasmodium* may inhibit cDC2 antigen uptake during malaria. In contrast, in adults with asymptomatic infection and Malian children considered genetically more resistant to malaria than neighboring ethnic groups,[98] HLA-DR expression on cDC2 was increased.[95] Together, these studies suggest that by retaining cDC2 antigen uptake and presentation functions, *Plasmodium* infection may be better controlled.

In addition to reduced HLA-DR expression on cDC2 in malaria,[94,95] HLA-DR and CD86 expression were also decreased on *Plasmodium*-experienced cDC2 after in vitro TLR stimulation,[96] indicating a reduced capacity to respond to PAMPs. This was also found in mouse studies, where pre-incubation of DC with intact *P yoelii* pRBCs inhibited DC maturation following TLR stimulation.[99] Inhibition of human cDC2 maturation following TLR activation with a broad range of agonists during submicroscopic *P falciparum* infection[96] suggests that *Plasmodium* may affect downstream signaling cascades common to different TLR activation pathways.[59] cDC2 express a broad range of TLRs, including TLRs1-7,[100] indicating an extensive pathogen recognition repertoire. Upon in vitro TLR stimulation, naive cDC2 produce a diverse range of cytokines including IL-10,[101] IL-12,[102] IL-8,[100] TNF,[91] IL-6,[91,100] and IL-1β.[91] Following *P falciparum* infection, cDC2 increased TNF, but not IL-12 production.[96] Increased TLR2 and TLR4 expression by myeloid DC and monocytes during acute malaria has previously been reported and may explain the increased TNF production observed.[60,103] Similarly, increased TNF production by PBMCs in response to TLR1, 2, or 4 stimulation has also been reported following *P falciparum* infection,[61] although the specific cell sources of cytokine were not identified. TNF can promote DC maturation and survival in vitro,[104,105] but is not sufficient for full functional maturation,[100] defined as the DCs ability to induce effector T cell responses.[106]

Reduced cDC2 subset maturation and altered cytokine production during *Plasmodium* infection is likely to impact T cell differentiation, activation, and proliferation. The functional properties of DC are dependent on their activation; Th1 cells are induced after stimulation with mature DC. In contrast, immature DC promotes the development of IL-10 producing regulatory CD4$^+$ T cells.[107] Impaired HLA-DR expression, increased TNF and limited IL-12 production in subpatent malaria,[96] suggests cDC2 have reduced ability to prime effector T cell responses and may instead be more likely drive regulatory CD4$^+$ T cell responses.[107] We have previously reported dysfunctional cDC2 in clinical malaria[84,95] and also in primary *Plasmodium* infection.[96,108] The disarmament of cDC2 during *Plasmodium* infection may provide increased opportunity for possible co-infections, such as bacteremia[109] during malaria. Indeed, the importance of functional peripheral blood cDC2 is highlighted in *Staphylococcus aureus* infection,[110] in which cDC2 are the sole DC subset to respond to *S aureus*. cDC2 increase HLA-DR expression alongside enhanced phagocytosis, consequently inducing

appropriate T cell activation and proliferation.[110] The *Plasmodium* parasite-induced impairment of cDC2 function may provide some insights into the exacerbated clinical outcome of children with *Plasmodium* and bacteremia co-infections.[109]

10 | CD4$^+$ T CELL SUBSETS IN MALARIA

The recognition of parasite peptide expressed on DC MHC II by an antigen-specific TCR on CD4$^+$ T cells in secondary lymphoid tissue results in the formation of an ordered immunological synapse.[111] In addition to TCR-peptide/MHC class II interactions (signal 1), the synapse also comprises costimulatory molecules, integrins, and other cell surface signaling molecules expressed by interacting DC and CD4$^+$ T cell (Reviewed in Ref. [112]). The affinity and avidity of interactions in signal 1, as well as integrin composition in the synapse, determines the interaction time between the DC and CD4$^+$ T cell, that in-turn, establishes the strength of signal transmitted into the CD4$^+$ T cells. This strength of signal is further amplified and shaped by costimulatory signals (signal 2), most notably those received by bidirectional signaling from CD28 and CD40 ligand (CD40L) on CD4$^+$ T cells following engagement with CD80/CD86 or CD40, respectively, on DCs. The former interaction stimulates IL-2 production, expression of IL-2 receptors, and the initiation of cell proliferation, while the latter enables the CD4$^+$ T cell to differentiate into a *bona fide* Th cell subset (Reviewed in [[113]). Signals 1 and 2 activate key signaling pathways in CD4$^+$ T cells that mobilize and activate molecules able to enter the cell nucleus and start the process of modifying the genomic landscape to allow transcription factors to access gene promoters. The third critical signal (signal 3) that determines CD4$^+$ T cell fate comes from cytokines produced by cDC presenting antigen or other proximal APC that have been activated by parasite molecules (Figure 1). These PRR and DAMP signals received by the APC during an encounter with pathogens and their products determine the cytokines produced.[114,115]

11 | TH1 CELLS

As previously discussed, encounters between blood-stage malaria parasites and DCs stimulates IL-12 production, which in-turn, promotes polarization of CD4$^+$ T cells into Th1 cells (Figure 1). In mice infected with *P chabaudi*, this development pathway was reinforced following ATP released by pRBCs binding P2X7 receptors on CD4$^+$ T cells, but to the detriment of Tfh cell development.[38] Th1 cells are thought to be critical to control of blood-stage malaria. However, direct evidence for this is lacking, and this conclusion is based on the assumption that CD4$^+$ T cells are the main source of IFNγ following *Plasmodium* infection. Although this is generally correct when IFNγ is measured in parasite antigen re-stimulation assays, other cellular sources of IFNγ, including CD8$^+$ T cells, NK cells, and $\gamma\delta$ T cells have also been widely reported following infection.[116] In fact, frequencies of IFNγ-producing $\gamma\delta$ T cells were correlated with protection against blood-stage malaria,[117] and primate *Pf*SPZ vaccine studies revealed an accumulation of liver tissue-resident, *Pf*-specific IFNγ-producing CD8$^+$ T cells following vaccination in protected animals.[22] NK cells also rapidly produce IFNγ following contact with *Pf* pRBCs in culture,[118] as well as in CHMI studies,[119] but it is not clear whether this contributes to anti-parasitic immunity in vivo. Instead, recent evidence points to an important role for NK cells in malaria in antibody-dependent cellular cytotoxicity, rather than as a major cellular source of IFNγ.[120,121]

Nevertheless, an important role for IFNγ in controlling the growth of *Plasmodium* has been reported in many studies. Mice receiving neutralizing anti-IFNγ mAbs were much less efficient at controlling the growth of lethal[122] and non-lethal[123] rodent *Plasmodium* species, and IFNγ blockade in mice with established protective immunity resulted in less efficient control of parasite growth following a second parasite challenge.[124] These results were supported by experiments with IFNγ-deficient mice showing diminished control of non-lethal *Plasmodium* species.[125,126] In mice, IFNγ has been shown to promote the activation of macrophages following encounters with pRBC and enhance the production of anti-microbial reactive oxygen and nitrogen intermediates.[125,127] Additionally, IFNγ-deficient mice infected with *P chabaudi* AS produced less parasite-specific IgM, IgG2a, and IgG3, but more IgG1, compared to wildtype control mice.[125] In CHMI studies with *P falciparum*, there was a strong association between parasite biomass measured by estimating the area under the blood parasitemia curve, and the amounts of IFNγ produced by PBMCs in response to parasite antigen.[13,125,128-130] It should be noted that the majority of IFNγ produced in the latter assays comes from antigen-specific CD4$^+$ T cells (ie, Th1 cells). Finally, the magnitude of early IFNγ production in these assays was found to be a correlate of natural malaria immunity in the Fulani people of West Africa, an ethnic group with increased resistance to *P falciparum* infection, relative to other ethnic groups in this part of the continent.[131]

Th1 cells also produce other pro-inflammatory molecules that contribute to their functions. These include IL-2 and TNF, the former being important for driving lymphocyte expansion during infection, as well as for maintaining FOXP3$^+$ natural regulatory (Treg) cell populations.[113] TNF was originally shown to inhibit the growth of *P vinckei* in mice, although the cellular source of TNF in this study was attributed to macrophages.[132] In several RTS,S vaccine studies, the frequency of CSP-specific IL-2-producing CD4$^+$ T cells increased after vaccination,[33,36,133,134] and in two of these studies, CSP-specific IL-2$^+$ CD4$^+$ T cells and TNF$^+$ CD4$^+$ T cells were associated with protection against malaria.[33,36] IL-2 from antigen-specific CD4$^+$ T cells also appears to be important for the rapid activation of NK cells in individuals immunized with RTS,S/AS01.[135]

The anti-parasitic functions of Th1 cells and their cytokine products are important for protection against malaria, but an unchecked Th1 cell response or the activity of Th1 cell cytokines in sensitive tissues can also contribute to the development of severe malaria syndromes. In particular, IFNγ and TNF stimulate vascular endothelium to increase the expression of a range of integrins and other adhesion molecules that enable pRBCs to sequester and/or

accumulate. This in turn results in the recruitment of immune cells, production of pro-inflammatory mediators that cause collateral tissue damage associated with malaria complications such as cerebral malaria and respiratory distress syndrome (reviewed in Ref. [136-138]). Additionally, Th1 cell cytokines can also impact other anti-parasitic immune responses. For example, IFNγ produced by Th1 cells can stimulate high Tbet expression by atypical memory B cells in *P falciparum*-exposed children in Africa, resulting in reduced B cell receptor signaling and skewing toward the production of IgG3 antibodies, although this latter outcome may not be detrimental for anti-parasitic immune responses.[139] In mice, Tbet-dependent Th1 cell development helps control blood parasitemia, but also promotes the development of experimental cerebral malaria following *P berghei* ANKA infection.[140] Additionally, Th1 cell-derived IFNγ and TNF can suppress B cell responses by inhibiting Tfh cell differentiation and stimulating the expression of Th1 cell molecules such as Tbet and CXCR3 during Tfh cell development.[15] There have also been reports of IFNγ promoting the depletion of antigen-specific CD4$^+$ T cells in lethal and non-lethal rodent *Plasmodium* infections, as well as shaping the magnitude and tissue migration properties of these cells.[141,142] Thus, although Th1 cell cytokines play important roles in preventing malaria, they may also contribute to disease pathogenesis if not appropriately regulated.

12 | TH2 AND TH17 CELLS

Less is known about the role of Th2 and Th17 cells in *Plasmodium* infection than Th1 cells. The dominant polarization of CD4$^+$ T cells into Th1 cells during *Plasmodium* infection may limit the generation of other Th cell subsets. For example, mice infected with *H polygyrus* normally generate a strong anti-helminth Th2 response, but this was suppressed and converted into a dominant Th1 cell response following co-infection with *P chabaudi*.[143] Initially it was presumed that *P chabaudi* infection in mice was characterized by a predominant Th1 cell response during the acute/early phase of infection, but then Th2 cells dominated during the later chronic phase of infection and helped B cells produce parasite-specific antibodies.[144,145] This was supported by studies on *P falciparum* immune individuals in which there was a positive correlation between *Pf*155/RESA-specific CD4$^+$ T cell IL-4 production and serum anti-*Pf*155/RESA antibodies. IL-4 was important for immunoglobulin class switching in mice infected with *P chabaudi*,[146] but the absence of IL-4 had little impact on blood parasitemia.[147] However, it is most likely that these studies were describing what we now define as Tfh cells and their influence on antibody production. Nevertheless, IL-4 produced by Th2 cells has been reported to provide help for CD8$^+$ T cell responses during liver-stage malaria, as well as for the maintenance of memory CD8$^+$ T cells.[148] In addition, Th2 cell-derived IL-4 can suppress the ability of human macrophages to kill *P falciparum*.[149]

There are few reports on Th17 cells in malaria. However, they were elevated in the blood of people infected with *P vivax*.[150] CD4$^+$ T cells co-expressing IFNγ and IL-17 were also observed in African children with *P falciparum*-induced anemia, but IL-17 levels were not associated with clinical disease, whereas IFNγ levels were associated with anemia.[151] Studies in mice have shown the development of Th17 cells in acute *P berghei* ANKA and *P yoelii* infections,[152] but again there was no association with the development of severe disease.[153] Thus, although there is evidence for the emergence of Th17 cells as part of the inflammatory response associated with malaria, there is limited evidence for any anti-parasitic or pathogenic role for these cells.

13 | TFH CELLS

The production of IL-6 and IL-21 by APCs following encounter with malaria parasites promotes the development of Tfh cells (Figure 1). These cells express high levels of CXCR5, programmed cell death protein 1 (PD-1), and the transcription factor Bcl6. They are essential for the initiation and maintenance of germinal centre (GC) reactions, the selection and maturation of B cells, immunoglobulin class switching and development of high-affinity antibody responses.[154] As mentioned earlier, antibody responses are essential for mediating protection against malaria by suppressing parasite replication and sequestration, thus preventing disease. Antibody responses to both pre-eyrthrocytic and erythrocytic antigens are associated with protection against infection and disease.[155] Furthermore, these responses are also associated with efficacy of the RTS,S vaccine,[19,156,157] and experimental models of malaria shows a strong correlation between antibody response and protective immunity to re-infection in mice.[158]

In both experimental mouse models and human *Plasmodium* infection, Tfh responses have important roles in driving the production of protective antibodies required for protection.[13,15,16,159,160] Tfh cell differentiation is stimulated by multiple DC subsets with IL-6 and ICOSL driving upregulation of Bcl6 during early Tfh development. PD-1 is required for optimal GC localization of Tfh cells, where activation of B cells occurs via multiple Tfh cell costimulator markers (including ICOS, CD40L, and CD28) and cytokine production (including IL-21, IL-4, and IL-6) (reviewed in Ref. [154]). After providing help to B cells, Tfh cells downregulate Bcl6 expression, convert to a memory phenotype and re-circulate in the blood.[161] In humans, blood circulating Tfh cells share phenotypic, functional, and transcriptional profiles of lymphoid Tfh cells, allowing for a general understanding of human Tfh cell responses during infection from readily available blood samples.[162,163] Circulating human Tfh cells can be differentiated into Tfh cell subsets based on chemokine receptor expression into Th1- (CXCR3$^+$ CCR6$^-$), Th2- (CXCR3$^-$ CCR6$^-$), Th17- (CXCR3$^-$ CCR6$^+$) and Th1/17-like (CXCR3$^+$ CCR6$^+$) populations, and share transcription factor and cytokine profiles of their conventional CD4$^+$ T cell counterparts.[163] The transcriptional profile of CXCR3$^-$ circulating Tfh cells (Th2- and Th17-like) is most closely related to tonsil Tfh cells,[162] and these Th2- and Th17-like subsets have the greatest capacity to activate naive B cells.[163]

We recently reported that CD4$^+$ T cell activation during *P chabaudi* AS infection in mice results in bifurcation of cells into Tfh and Th1 cell linages.[164] Tfh cell development and function in rodent malaria models was dependent on IL-6.[165] Although ICOS was dispensable for Tfh cell differentiation in early infection, it was needed for sustained responses and high-affinity antibody development.[166] ICOS-driven Tfh cell responses were hampered by cell extrinsic type I IFN signaling[167] and negatively regulated by cell intrinsic IRF-3.[168] In humans, there is preferential induction of Th1-like Tfh cell subsets which have reduced capacity to activate naive and memory B cells in both *P falciparum* infection in Malian children,[13] and *P vivax* infection in Brazilian adults.[160] The development of Th1-like Tfh cells in malaria is thought to be mediated by IFNγ and other inflammatory cytokines, and in mice, IFNγ signaling results in increased Tbet and CXCR3 expression in Tfh cells, associated with reduced GC reaction and impaired B cell responses.[15] In humans, Th1-like Tfh cells promoted Tbet expression in naive B cells and Tbet expression was higher in atypical memory B cells which are thought to be dysregulated memory B cells.[139] Taken together, current data suggest that inflammatory responses caused by malaria are detrimental to robust Tfh cell activation, and consequently, anti-parasitic antibody generation.

14 | IMMUNOREGULATORY NETWORKS ESTABLISHED DURING MALARIA

14.1 | Tr1 cells

The inflammatory cytokines produced by Th1 cells following *Plasmodium* infection can damage tissues, and as such, these CD4$^+$ T cell responses need to be tightly regulated. Thymus-derived FoxP3$^+$ regulatory T (Treg) cells were thought to play important regulatory roles during malaria, but Th1 cells that initiate an IL-10 production program (Tr1 cells) have now been recognized as major regulators of inflammation during malaria, as well as many other infectious diseases.[17,169] Tr1 cells develop from Th1 cells that acquire regulatory functions following exposure to inflammatory conditions.[170,171] This transition to a regulatory phenotype requires the transcription factors Blimp-1 and Tbet, and results in the co-expression of IFNγ and IL-10.[172] Although the precise mechanism of Tr1 cell development during infection has not been fully elucidated, recent studies indicate that type I IFN signaling is critical to their development. In humans participating in *P falciparum* CHMI studies, stimulation of PBMCs by pRBCs stimulated type I IFN production by multiple immune cell populations, which in turn promoted Tr1 cell development and suppression of inflammatory cytokines, including IFNγ, TNF, IL-1β, IL-17, and IL-6.[18] In mice infected with *P yoelii*, type I IFNs were also shown to be important for the development of Tr1 cells and suppression of humoral immunity.[173] In addition to suppressing inflammation and antibody responses, IL-27-dependent Tr1 cell development was also shown to be important for protection against immune-mediated tissue pathology in mice infected with *P chabaudi* AS.[174] DC-derived IL-10 has also been reported to be required for Tr1 cell development during *P yoelii* infection, suggesting an autocrine signaling loop whereby APC-derived IL-10 reinforces IL-10 production by Tr1 cells in malaria.[175]

Tr1 cells have been identified as a dominant antigen-specific CD4$^+$ T cell population in African children with *P falciparum* malaria,[176-178] as well as in healthy volunteers participating in CHMI studies, as described above.[18] A study in children living in eastern Uganda showed that the frequency of Tr1 cells in young children was positively correlated with high parasite burden, but significantly, also associated with a reduced risk of developing clinical malaria after infection.[179] Remarkably, a relatively high frequency of antigen-specific Tr1 cells was also found in neonates whose mothers had active placental malaria during pregnancy,[180] suggesting that these cells influence anti-parasitic immunity from very early in life.[181] Thus, IL-10 production by Tr1 cells is important to minimize host tissue damage caused by inflammation during malaria, but may also contribute to the slow development of natural immunity, as well as poor responses to vaccination with *Plasmodium* antigens.

14.2 | Treg cells

Increased frequencies of thymus-derived FoxP3$^+$ Treg cells have been reported in many infectious diseases and following vaccination.[182] Treg cells exert their suppressive functions following antigen-specific stimulation through the TCR.[183] During infection, Treg cells limit immune-mediated pathology, but may also facilitate pathogen persistence and associated chronic infection by suppressing inflammation.[184] They inhibit CD4$^+$ T cell proliferation and IL-2 production via the expression of immune checkpoint molecules such as CTLA-4, production of anti-inflammatory cytokines such as IL-10 and TGFβ or by their high expression of IL-2 receptors to consume local IL-2 supplies and depriving other T cells of this vital growth factor.[183,185-187] Studies on Treg cells in malaria have reported conflicting results.[188,189] We reported a detrimental role for Treg cells in experimental cerebral malaria caused by *P berghei* ANKA infection by showing that depletion of these cells using an anti-CD25 mAb prior to infection protected mice from disease and increased antigen-specific CD4$^+$ T cell responses.[190] These results were supported by the findings of others;[191] however, another study using the same model found no protection from disease after Treg cell depletion.[192] We also reported that expansion of Treg cells using IL-2 provided protection against experimental cerebral malaria via a CTLA-4-dependent mechanism,[193] highlighting the potential of these cells in protecting against disease. Depletion of Treg cells in mice infected with a lethal strain of *P yoelii* protected mice from death and increased antigen-specific T cell responses.[194] However, others have reported minimal impact of Treg cells on disease outcome in this model, and instead, showed that Tr1 cells had a greater influence on immune responses and survival.[195] These inconsistencies in experimental models of malaria can be attributed to several factors, including incomplete characterization of regulatory T cell populations at the time studies were conducted and limitations in the methodologies used to manipulate

Treg cells in vivo, as well as differences in the reagents and infection models examined.[137]

Increased Treg cell frequencies have been associated with high parasitemia in malaria patients. Experimental sporozoite infection of human volunteers via mosquito bite showed a rapid expansion of Treg cells associated with a burst of TGF-production, increased blood-stage parasite growth, but decreased pro-inflammatory cytokine production and antigen-specific T cell responses.[196] Studies in Gambian children showed increased frequencies of Treg cells in severe and uncomplicated malaria during convalescence. This was associated with increased parasitemia, but negatively associated with the magnitude of Th1 cell responses.[197] A study in Brazil found individuals with uncomplicated malaria caused by *P falciparum* and *P vivax* had an increased frequency of Treg cells expressing CTLA-4 that was positively associated with blood parasitemia.[85] Adults with uncomplicated and severe malaria living in a malaria-endemic region of Indonesia also showed elevated Treg cell frequencies, relative to exposed, asymptomatic controls, and again Treg cell frequency correlated positively with blood parasitemia and total parasite biomass in patients with severe malaria.[198] In the same study, the authors reported the presence of a TNFRII$^+$ Treg cell subset that was associated with severe malaria and hyper-parasitemia in adults. This same Treg cell subset has also been shown to emerge at peak of parasitemia in a CHMI study with *P falciparum*.[199] In a malaria-endemic region of Thailand, an increased frequency of Treg cells was reported in patients with acute *P vivax* infection, compared to endemic and immune controls.[86] In children residing in a malaria-endemic region of Uganda, both the frequency and absolute numbers of Treg cells in peripheral blood declined with increased malaria exposure.[200] Together, these findings support a role for Treg cells for controlling parasite-mediated inflammation and protecting host tissue during early encounters with malaria parasites. However, their influence declines as alternative regulatory mechanisms such as Tr1 cells emerge in response to specific types of pro-inflammatory responses.

15 | EXPRESSION OF CO-INHIBITORY RECEPTORS BY CD4$^+$ T CELLS

Co-inhibitory receptors such as CTLA-4, LAG-3, TIM-3, and PD-1 are often found on T cell populations following chronic antigen exposure, including in experimental malaria models and malaria patients.[17] A high percentage of CTLA-4 expressing CD4$^+$ T cells were found in mice infected with *P berghei* ANKA, and these restricted parasite-specific T cell responses, but also prevented immune-mediated pathology.[201] Another study in BALB/c mice-infected *P berghei* ANKA showed higher expression of CTLA-4 and PD-1 on CD4$^+$ T cells. Blockade of CTLA-4 or PD-1/PD-L1 resulted in increased T cell activation, but caused mice to develop experimental cerebral malaria, despite BALB/c mice normally being resistant to this disease manifestation.[202] CTLA-4 expression on CD4$^+$ T cells has also been reported in humans infected with *P falciparum* and *P vivax*.[203] Patients with acute *P falciparum* malaria showed increased expression of CTLA-4 and PD1 on CD4$^+$ T cells, relative to healthy control subjects, and patients with cerebral malaria had higher frequencies of CTLA-4$^+$ CD4$^+$ T cells than patients with uncomplicated malaria.[204] The authors of this study also reported that CD4$^+$ T cells expressing both CTLA-4 and PD-1 were the main antigen-specific cell populations following stimulation with pRBCs, and co-produced IFNγ and IL-10. Another study in Malian children showed that *P falciparum* infection was associated with PD-1 expression on CD4$^+$ T cells in individuals presenting with clinical malaria.[159] Additional work from this study in mice infected with *P yoelii* found prolonged infection led to enhanced expression of PD-1 and LAG-3 on antigen-specific CD4$^+$ T cells, and only blockade of both PD-L1 and LAG-3 led to improved control of parasite growth.[159] In *P falciparum* CHMI studies, Tr1 cells expressing several different immunoregulatory molecules rapidly developed after anti-parasitic drug treatment, but only PD-1 blockade altered CD4$^+$ T cell responses to parasite antigen ex vivo, and in this instance both IFNγ and IL-10 production increased.[28] These data suggest that blockade of one immunoregulatory pathway may result in compensatory expansion of other regulatory pathways.

A study from Ghana reported an increased frequency of CD4$^+$ T cells expressing CTLA-4, PD-1, LAG-3, and TIM-3 in children with uncomplicated malaria, compared to afebrile, healthy children, and higher frequencies of CTLA-4$^+$ or PD-1$^+$ CD4$^+$ T cells in children with severe malaria, compared to those with uncomplicated malaria.[205] In Kenyan children living in rural areas with persistent *P falciparum* exposure, there was an increased frequency of CD4$^+$ T cells expressing PD-1 and LAG-3, which negatively correlated with frequencies of activated and classical memory B cells, suggesting that *P falciparum*-associated expression of co-inhibitory receptors on CD4$^+$ T cells impacts the development of B cell responses.[206] These data were consistent with findings in *P yoelii*-infected mice showing that blockade of PD-L1 and LAG-3 not only restored CD4$^+$ T cell functions, but also increased Tfh cell numbers and germinal center B cell responses, resulting in rapid parasite clearance.[159]

TIM-3 expression was upregulated in the spleen of *P berghei* ANKA-infected mice,[207] as well as on CD4$^+$ T cells from *P falciparum*-infected individuals.[208] TIM-3 blockade improved lymphocyte activity and accelerated parasite clearance in infected mice, while in vitro blockade of TIM-3 in PBMC cultures from *P falciparum* patients reduced cell apoptosis and increased IFNγ, TNF, IL-4, and IL-10 gene transcription,[208] suggesting that TIM-3 blockade may enhance both inflammatory and anti-inflammatory responses. Taken together, the co-inhibitory receptors expressed by CD4$^+$ T cells during malaria have a profound influence on their functional capacities.

16 | TFH CELL REGULATION

Similar to Th1 cells, Tfh cell activation is heavily influenced by immunoregulatory pathways. For example, FoxP3$^+$ Tfh (Tfr) cells with regulatory functions, including IL-10 production[209] have

been reported in mice and humans.[210] In mice infected with *P yoelii*, CTLA-4$^+$ Tfr cells suppress GC B cell responses.[211] To date, there have been no reports of Tfr cells in human malaria and their influence on antibody production during infection remains unknown. Given the central role of Tfh cells in antibody production, and therefore protective immune responses against malaria, it has been proposed that optimizing Tfh cell activation may help boost vaccination efficacy.[155] Indeed, multiple host targets for modulation to improve Tfh cell responses have been identified, including CTLA-4 discussed above,[211] as well as PD-L1 and LAG-3.[159] However, care needs to be taken with these approaches because they can lead to adverse outcomes. For example, simultaneous activation of OX40 and blocking of PD-1 signaling in mice infected with *P yoelii* increased Th1 cell responses, but restricted Tfh cell responses and the generation of anti-parasitic antibodies.[16] Similarly, combining a viral vector vaccine with RTS,S resulted in a diminished RTS,S-mediated antibody response caused by the strong Th1 cell response induced by the viral vector vaccine.[212] Nevertheless, changing vaccine composition by different adjuvants can overcome some of these problems and expand parasite-specific Tfh cell responses.[213] Taken together, these studies identify possible pathways to manipulate Tfh cells in humans to improve the efficacy of vaccines targeting malaria, but also highlight some of the potential pitfalls.

17 | TARGETING CD4$^+$ T-CELL MEDIATED REGULATORY NETWORK FOR HOST-DIRECTED THERAPY

Attempts to block co-inhibitory receptor activity for clinical advantage will need to proceed with caution. The emergence of compensatory immunoregulatory mechanisms that are even more potent than those already established should be avoided, as well as preventing the development of inflammation that causes disease. Careful consideration will need to be given to the modality of drugs employed for host-directed therapies. For example, monoclonal antibodies (mAbs) have relatively long half-lives and if adverse reactions are encountered may be difficult to reverse. In contrast, small molecule inhibitors often have shorter half-lives, but thought needs to be given to practical dosing frequency. A common theme emerging in the malaria field is that despite increased expression of co-inhibitory receptors on T cell subsets during malaria, blockade of any single molecule often has variable efficacy at modulating immune responses among human populations. This is not that surprising given similar experiences in targeting these molecules in cancer therapy.[214] Although combining blockade of different co-inhibitory receptors is proving a promising approach in cancer,[215] such an approach for tropical diseases may prove more challenging given the resource constraints, as well as heterogeneity among human populations and the malaria parasites responsible for disease.

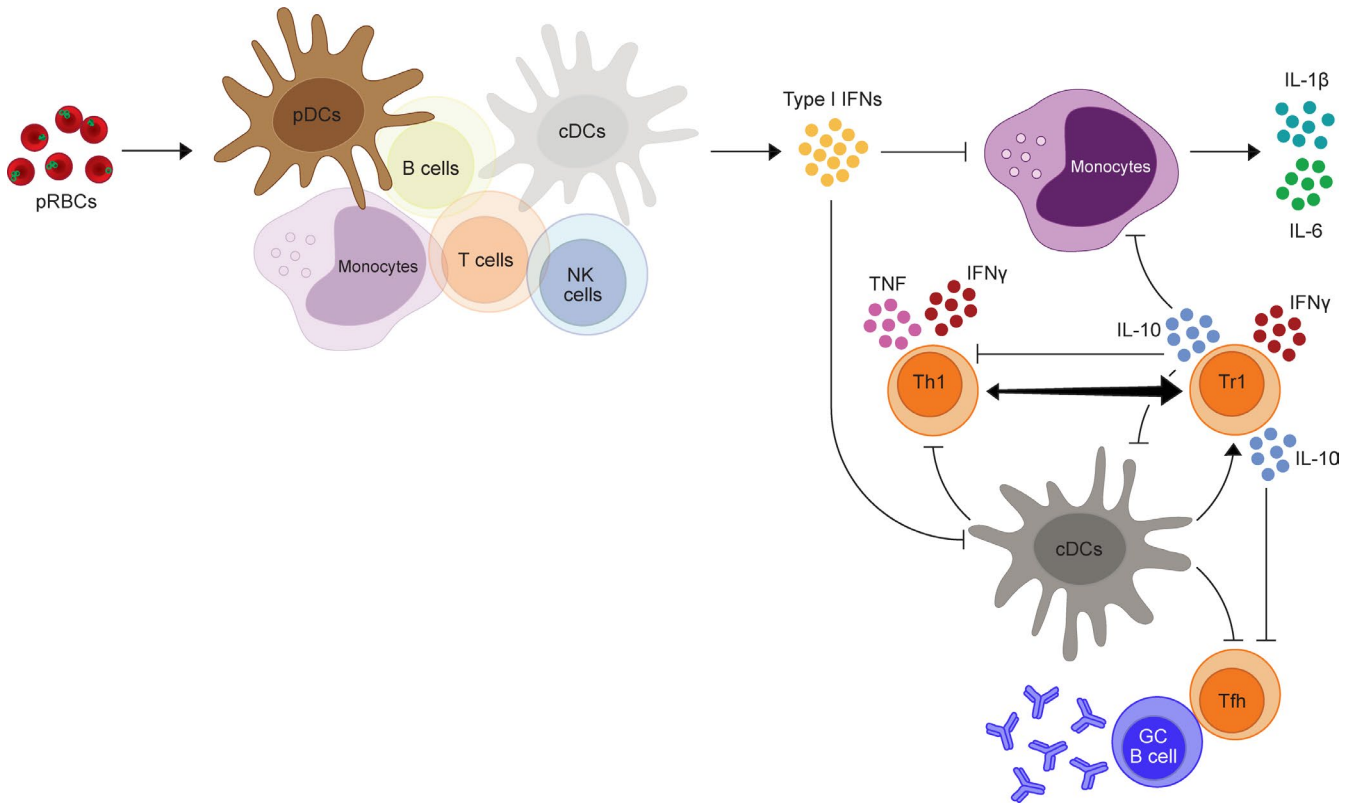

FIGURE 3 Type I interferons (IFNs) are important immunoregulators during malaria. Type I IFNs are produced by many different cell types following encounter with *Plasmodium* molecules. They suppress the ability of dendritic cells (DCs) to present antigen to T cells. They also promote the development of IL-10-producing type 1 regulatory T (Tr1) cells from T helper (Th)1 cells. The IL-10 produced by these cells inhibits pro-inflammatory cytokine production, and T follicular helper (Tfh) cell expansion, thereby limiting anti-parasitic antibody production

A different approach could involve targeting pathways initiated early after infection that promote the expression of co-inhibitory receptors and other immunoregulatory molecules. For example, type I IFN production by host cells following exposure to *Plasmodium* products impairs the ability of cDC2 to drive Th1 cell expansion,[216,217] as well as restricting Tfh cell development and suppressing parasite-specific antibody production.[173] Furthermore, type I IFNs promote the development of Tr1 cells that not only hamper Th1 and Tfh cell responses during human and rodent malaria, but also constrain pro-inflammatory cytokine production by phagocytic cells[18,167,173] (Figure 3). Type I IFNs bind to a cell surface IFNα receptor (IFNαR) comprising IFNαR1 and IFNαR2 chains, making targeting this signaling pathway with antibodies directed against one of the receptor chains relatively straight forward. However, as mentioned previously, the long half-life of antibodies may make this approach risky given the likelihood of latent viruses residing in many people living in malaria-endemic areas. An alternative way to modulate this pathway could be to target downstream signaling molecules. For example, several Janus Activated Kinase (JAK) inhibitors have recently been licensed as orally available drugs with relatively short half-lives.[218] These drugs have been used to treat children with a type I interferonopathies associated with gain of function mutations in *TMEM173* (encoding STING).[219] In addition, they have been reported to reduce serum type I IFN levels and interferon-inducible gene scores in dermatomyositis patients.[220] Thus, these types of drugs may be one way to target the type I IFN signaling pathway in a transient manner, and given the growing safety profiles of these treatments, they could potentially be incorporated into vaccination programs or mass drug administration strategies. This latter approach may be especially attractive given recent findings in intermittent preventative treatment trials with anti-parasitic drugs in Ugandan children that showed those receiving drug at 4 weekly intervals had reduced incidence of malaria, compared to children receiving drug every 12 weeks, suggestive of more rapid development of immunity to malaria in the former group.[221] Therefore, host-directed treatments to enhance this effect may have significant clinical impact.

18 | CONCLUDING REMARKS

Results from malaria vaccine trials in the past decade have highlighted the need for a new and significant change in approach. The rapid imprinting of anti-inflammatory pathways in people following exposure to *Plasmodium* parasites appears to not only protect them from developing severe forms of disease, but also makes responses to malaria vaccines poor. Our increased understanding about the immunoregulatory pathways established during malaria provides opportunities to try and manipulate these responses to our advantage. Key to these approaches will be directing $CD4^+$ T cell responses to effectively activate phagocytic cells to capture and kill pRBCs and help B cells produce the types of antibody responses that are effective and long-lived without contributing to disease. We are seeing great strides being made in the cancer and other inflammatory diseases, and there is no reason why the malaria field cannot make similar inroads over the next decade.

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CONFLICT OF INTEREST

All authors have declared that no conflict of interest exist.

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