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Invited Review

# Emerging concepts in T follicular helper cell responses to malaria

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ABSTRACT

Antibody responses to malaria and candidate malaria vaccines are short-lived in children, leaving them susceptible to repeated malaria episodes. Because T follicular helper ($T_{FH}$) cells provide critical help to B cells to generate long-lived antibody responses, they have become the focus of recent studies of *Plasmodium*-infected mice and humans. The emerging data converge on common themes, namely, that malaria-induced Th1 cytokines are associated with the activation of (i) Th1-like memory $T_{FH}$ cells with impaired B cell helper function, and (ii) pre-$T_{FH}$ cells that acquire Th1-like features (T-bet expression, IFN-γ production), which impede their differentiation into fully functional $T_{FH}$ cells, thus resulting in germinal center dysfunction and suboptimal antibody responses. Deeper knowledge of $T_{FH}$ cells in malaria could illuminate strategies to improve vaccines through modulating $T_{FH}$ cell responses. This review summarizes emerging concepts in $T_{FH}$ cell responses to malaria.



## 1. What are T follicular helper cells?

The induction of long-lasting antibody-mediated immunity relies on the development of germinal center (GC) reactions within secondary lymphoid organs. Following their initial interaction with antigen, naïve B cells can either differentiate into short-lived plasma cells (PCs), that secrete a first wave of low affinity antibodies to rapidly control infection, or can migrate deeper into B cell follicles to establish a GC. Within the GC, activated B cells undergo somatic hypermutation of their Ig genes followed by selective survival of B cells expressing high affinity Ig. B cells then exit GCs as either antibody secreting PCs, many of which migrate to the bone marrow, or memory B cells (MBCs), which circulate until re-exposure to cognate antigen drives them to rapidly differentiate into antibody secreting cells.

GC development and function requires a specialised subset of $CD4^+$ T cells called T follicular helper ($T_{FH}$) cells that express a unique combination of surface and effector molecules. $T_{FH}$ cells express the chemokine receptor CXCR5 (Schaerli et al., 2000), which allows them to migrate into B cell areas of secondary lymphoid organs to provide help to B cells via co-stimulatory molecules and production of the IL-21 cytokine (Vinuesa et al., 2005). $T_{FH}$ cells express high levels of inducible T cell costimulator (ICOS), programmed cell death-1 (PD-1), CD40 ligand (CD40L), B and T lymphocyte attenuator (BTLA), OX40 and CD84 (Crotty, 2014). The transcription factor B cell lymphoma 6 (Bcl-6) is the master regulator of $T_{FH}$ cell differentiation and its expression distinguishes $T_{FH}$ cells from other $CD4^+$ T cell subsets such as $T_{H1}$, $T_{H2}$, $T_{H17}$ and T regulatory ($T_{reg}$) cells (Tangye et al., 2013).

$T_{FH}$ cell differentiation and maintenance largely depends on interactions with dendritic cells (DCs) and B cells. Bcl-6 is rapidly upregulated in $T_{FH}$ cells following priming by DCs in the T cell zone of secondary lymphoid tissues (Baumjohann et al., 2011; Choi et al., 2011; Kerfoot et al., 2011). Bcl-6 upregulation requires ICOS-mediated signalling (Choi et al., 2011) and drives CXCR5 expression which mediates $T_{FH}$ cell migration to the GC. After the establishment of GCs, cognate signals provided by GC B cells sustain the $T_{FH}$ pool by reinforcing their phenotype and promoting their survival. ICOS and CD28, as well as the signalling lymphocytic activating molecule (SLAM) family members such as CD84 and Ly108, have been identified as important factors in the T:B cell engagement that is required to maintain the GC reaction (Ramiscal and Vinuesa, 2013). Under normal conditions, $T_{FH}$ cells express high levels of the SLAM-associated protein (SAP), which plays a central role in GC development by facilitating $CD4^+$ T cell adhesion to B cells (Kageyama et al., 2012).

Similar to other T helper subsets, $T_{FH}$ cell differentiation and function is modulated by cytokines. Whereas IL-6 and IL-21 are positive mediators of $T_{FH}$ cell differentiation (Karnowski et al.,

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2012), high levels of IL-2 appear to be inhibitory (Ballesteros-Tato et al., 2012). Other pro-inflammatory cytokines such as IL-12, type I IFNs and IFN-γ also modulate $T_{FH}$ cell differentiation (Nakayamada et al., 2011; Lee et al., 2012; Oestreich et al., 2012; Ray et al., 2014). $CD4^+$ T cells react to their cytokine environment by signaling through the signal transducer and activator of transcription (STAT) family of proteins to initiate differentiation towards different T helper subsets. STAT1, STAT3, STAT4 and STAT5 have been shown to regulate $T_{FH}$ gene expression and differentiation. STAT3, which binds to Bcl-6 (Oestreich et al., 2012; Ray et al., 2014), is activated by IL-6 and IL-21 and induces naïve $CD4^+$ T cells to secrete IL-21 (Eto et al., 2011; Lu et al., 2011; Ma et al., 2012). On the other hand, the IL-2-STAT5 signalling axis, which results in the induction of the transcription factor Blimp-1, is a strong negative regulator of $T_{FH}$ differentiation. Mounting evidence (Oestreich et al., 2012; Weinmann, 2014) suggests that environmental signals mediated via STAT proteins also modulate the molecular balance between helper T cell lineage-specific transcription factors. Thus, the cytokine environment appears to play an important role in balancing $T_{H1}$ and $T_{FH}$ cell gene programs in response to infection and immunization.

## 2. Why are $T_{FH}$ cells expected to play an important role in malaria?

Malaria remains one the most serious infectious diseases with ~250 million clinical cases annually (WHO, 2015). Most cases of severe malaria are caused by *Plasmodium falciparum*, which is endemic in sub-Saharan Africa and throughout much of the tropics. The blood-stage of *Plasmodium* infections cause the symptoms of malaria that range from an undifferentiated febrile illness to more severe manifestations including respiratory distress, renal failure and coma (White and Ho, 1992). In areas of intense malaria transmission, children under the age of 5 years with low levels of immunity are the most susceptible to severe malaria. It is only after years of repeated infections that non-sterilizing clinical immunity to malaria develops (Baird, 1995; Marsh and Kinyanjui, 2006; Tran et al., 2013). Naturally acquired clinical immunity to malaria targets blood-stage parasites and requires antibodies, as demonstrated by studies in which the transfer of purified IgG from malaria-immune adults to children with symptomatic malaria rapidly reduced parasitemia and fever (Cohen et al., 1961). Consistent with this, various studies support a role for antibodies in inhibiting merozoite invasion of red blood cells (RBCs) (Blackman et al., 1990; Tran et al., 2014; Chiu et al., 2015), blocking cytoadherence of parasitized RBCs to vascular endothelium (Beeson et al., 2013, 2004) and opsonizing parasites for phagocytosis by effector cells such as monocytes and macrophages (Hill et al., 2013; Chiu et al., 2015).

However, a large body of observational data demonstrates that *P. falciparum*-specific antibodies are inefficiently acquired and short-lived, particularly in children (Cavanagh et al., 1998, 2004; Kinyanjui et al., 2007, 2009). Additionally, in areas of seasonal malaria transmission the prevalence and breadth of *Plasmodium*-specific MBCs and antibody responses increase gradually in a step-wise fashion with age/cumulative exposure (Crompton et al., 2010; Weiss et al., 2010; Nogaro et al., 2011). Moreover, chronic malaria exposure is associated with an increase in atypical MBCs (Weiss et al., 2009; Portugal et al., 2012) that express an array of inhibitory receptors and exhibit stunted B cell receptor signaling and impaired proliferation, cytokine production and antibody secretion. (Portugal et al., 2015; Sullivan et al., 2015). In contrast, it has been reported that atypical MBCs isolated from malaria-infected adults may actively secrete antibodies based on the expression of mRNA encoding secretory Ig, although Ig secretion per se was not demonstrated. (Muellenbeck et al., 2013). It remains possible that under certain conditions atypical MBCs secrete antibodies, but what those conditions are remains to be determined.

Together, these observations suggest that B cell memory is compromised during malaria infection. As $T_{FH}$ cells are key components of the GC reaction that is required for the efficient induction of high-affinity MBCs and long-lived PCs, $T_{FH}$ cells have been the focus of recent studies aimed at understanding the cellular processes underlying the inefficient acquisition of humoral immunity to malaria. In the remainder of this review we summarize recent findings on the role of $T_{FH}$ cells during malaria, including experimental infection in murine models as well as human field studies. The factors modulating $T_{FH}$ cell responses to *Plasmodium* infection and the implications of those findings for the induction of clinical immunity to malaria are discussed.

## 3. Evidence from mouse infection models

Murine models of malaria provide valuable mechanistic insights into immunological processes that cannot be deduced from human studies (Brian de Souza and Riley, 2002; Stephens et al., 2012). For the past two decades, most studies aimed at understanding the development of humoral immunity to malaria have used the non-lethal, self-resolving *Plasmodium chabaudi chabaudi* mouse infection model (Stephens et al., 2012). Early studies with this model highlighted the importance of $CD4^+$ T cells in the control of parasitemia. The initial stage of *P. chabaudi* infection is characterised by a strong $T_{H1}$ response followed by a switch to a $T_{H2}$-mediated antibody response, which appeared to be required for control of parasite replication (Langhorne et al., 1989; Taylor-Robinson and Phillips, 1992). Further work revealed that IL-4 deficient mice were still capable of mounting parasite-specific IgG responses that control *P. chabaudi* infection (van der Heyde et al., 1997; Balmer et al., 2000), thus suggesting that a T cell lineage distinct from $T_{H2}$ cells contributes to protective antibody responses. After the identification of $T_{FH}$ cells as the main subset providing help to B cells, (Schaerli et al., 2000) the role of T cell help in malaria was re-evaluated (Perez-Mazliah and Langhorne, 2014). Specifically, recent studies demonstrated that $T_{FH}$ cells are induced by *P. chabaudi* (Pérez-Mazliah et al., 2015) and *Plasmodium yoelii* infections (Butler et al., 2011; Zander et al., 2015). Moreover, functional $T_{FH}$ cells were found to be required for efficient antibody production during non-lethal malaria infections, with IL-21 playing a key role in this process (Pérez-Mazliah et al., 2015). Additionally, expansion of $T_{FH}$ cells following neutralisation of PD-1 and lymphocyte activation gene (LAG)-3-mediated inhibitory signaling (Butler et al., 2011) or boosting of co-stimulatory signals via OX40 receptor ligation (Zander et al., 2015), improved infection outcomes during non-lethal *P. yoelii* infection. Although ICOS-mediated signalling was found to be dispensable for early induction of $T_{FH}$ cells in the non-lethal *P. chabaudi* model, the expression of this co-stimulatory molecule appears to be required for sustained maintenance of high-affinity antibody responses to infection (Wikenheiser et al., 2016). Strategies targeting $T_{FH}$ cells such as PD-1 deletion (Liu et al., 2015) or nanoparticle delivery of *Plasmodium* antigens (Moon et al., 2012) have also been shown to significantly improve GC responses and parasite-specific antibody levels in response to vaccination. Interestingly, a $CD4^+$ population of IL-21 and IFN-γ producing T cells that expresses CXCR5 has been identified during *P. chabaudi* infection. These cells arise in the absence of Bcl-6, suggesting that they do not belong to a classical $T_{FH}$ lineage (Carpio et al., 2015).

Although non-lethal malaria models provided insight into the role of $T_{FH}$ cells in controlling parasite replication, the extent to



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