



# T<sub>H</sub>1-Polarized T<sub>FH</sub> Cells Delay Naturally-Acquired Immunity to Malaria

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## OPEN ACCESS

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equally to this work

### Specialty section:

This article was submitted to  
Microbial Immunology,  
a section of the journal  
Frontiers in Immunology

**Received:** 26 December 2018

**Accepted:** 30 April 2019

**Published:** 17 May 2019

### Citation:

Yap XZ, Hustin LSP and  
Sauerwein RW (2019) T<sub>H</sub>1-Polarized  
T<sub>FH</sub> Cells Delay Naturally-Acquired  
Immunity to Malaria.  
*Front. Immunol.* 10:1096.  
doi: 10.3389/fimmu.2019.01096

Humoral immunity is a critical effector arm for protection against malaria but develops only slowly after repeated infections. T cell-mediated regulatory dynamics affect the development of antibody responses to *Plasmodium* parasites. Here, we hypothesize that T follicular helper cell (T<sub>FH</sub>) polarization generated by repeated *Plasmodium* asexual blood-stage infections delays the onset of protective humoral responses. IFN- $\gamma$  production promotes polarization toward T<sub>FH</sub>1 and increased generation of regulatory follicular helper cells (T<sub>FR</sub>). Delineating the mechanisms that drive T<sub>H</sub>1 polarization will provide clues for appropriate induction of lasting, protective immunity against malaria.

**Keywords:** T<sub>H</sub>1, T<sub>FH</sub>1, IFN- $\gamma$ , follicular T helper cells, B cells, malaria, humoral immunity

## NATURALLY-ACQUIRED IMMUNITY IN MALARIA

Only after years of continued exposure to *Plasmodium* parasites do individuals from malaria endemic regions develop clinical immunity (CI), that protects against clinical disease but not from parasitaemia (1). This protection is mediated through both cellular and humoral immune effector mechanisms. In particular, humoral immunity (HI) apparently plays a pivotal role against blood-stages, which are responsible for pathology and disease. Seminal findings demonstrate that IgG transfer from malaria-immune adults to children with acute malaria can indeed reduce symptoms and parasite load (2).

Effective HI induction requires B cells to be activated by antigen-presenting cells (APCs), predominantly dendritic cells (DCs). Sustained “help” from cognate CD4<sup>+</sup> T cells is subsequently required for B cell proliferation, affinity maturation, and Ig class-switching. T follicular helper cells (T<sub>FH</sub>), which co-localize with B cells in the germinal centers (GCs), are crucial for both naïve B cell activation during primary infections and reactivation of memory B cells (MBC) in secondary infections. T<sub>FH</sub> and other CD4<sup>+</sup> helper T cells (T<sub>H</sub>) can drive naïve B cells to differentiate into high-antibody-producing plasma cells (PC) or MBC, which rapidly reactivate and produce specific Abs during secondary infections.

While typically taking a number of years to develop fully, clinical malaria immunity is of relatively short duration and rapidly wanes in the absence of re-infection (3, 4). Antibody efficacy and specific MBC counts increase gradually with age and cumulative exposure, resulting in a strong T<sub>H</sub>1 (IFN- $\gamma$ -producing) immune response (5–9). The origins of the relatively slow acquisition of clinical immunity, however, remain elusive.

Here we hypothesize that T cell responses generated by repeated blood-stage malaria infection may in fact delay the onset of potent humoral responses. We contextualize the role of T<sub>H</sub> and T<sub>FH</sub> polarization surrounding the B cell response in malaria, and suggest that excessive polarization toward the IFN- $\gamma$  producing T<sub>H</sub>1 phenotype reduces the longevity of antibody responses.

## B-CELLS AND PLASMA CELLS ARE DEREGULATED IN MALARIA

Potent humoral responses are characterized by the generation of specific and high-affinity long-lived PCs (LLPCs) and MBCs in the GCs. Yet both adults and children in malaria endemic areas show a delay in the development of MBC and short-lived antibodies targeting *P. falciparum* blood-stage antigens (10). Accordingly, antibodies generated during one acute malaria season are undetectable by the next (10). Similar delays in CI onset are found in malaria-naïve immigrants to Papua New Guinea (11).

Sustained parasitaemia may be a key factor affecting B cell differentiation. Recent studies have provided valuable insights into B cell subset dynamics and antibody kinetics in the context of *Plasmodium* infection. While it is clear that IgG<sup>+</sup> MBCs are key effectors in long-term memory, high levels of non-IgG<sup>+</sup> anti-*P. falciparum* MBCs may have a role in early protection (12). Frequent exposure to asexual parasites, as experienced in highly malaria-endemic regions, is associated with the development of MBCs with reduced memory function, known as atypical memory B-cells (AMBC). While the presence of AMBCs may contribute to the delayed and short-lived nature of HI to malaria (13), their presence may also be symptomatic of a more broadly deregulated humoral response.

Frequent parasite exposure seems to be a driving factor in AMBC development. AMBC frequency increases proportionate to transmission intensity, age, and cumulative malaria exposure (13–19), and AMBC proportions increase after each acute malaria episode (20). Conversely, the percentage of AMBCs declines in the absence of parasite exposure, inducing stable populations of malaria-specific classical MBCs (17, 19, 21, 22). This may be the result of direct B cell interactions with *Plasmodium* parasites, or indirectly generated by the pro-inflammatory environment (23, 24), or by a combination of the two, i.e., AMBCs as a product of persistent antigen engagement by B cells within a highly inflammatory environment of chronic malaria exposure, driven by T<sub>H</sub>1 cells (25).

Hence, inappropriate IFN- $\gamma$  production may be a reflection of inadequate T cell help caused by frequent exposure to blood-stage *P. falciparum*.

## BLOOD-STAGE INFECTION INDUCES CHANGES IN T CELL PHENOTYPES AND POPULATIONS

Malaria parasites typically induce human T cells with high surface expression of PD-1 and LAG3 and high production of both IFN- $\gamma$  and IL-10 (26–28). Hence, CD4<sup>+</sup> T cells in the malarial environment frequently display a phenotype associated with immunosuppression. Furthermore, the malarial environment polarizes CD4<sup>+</sup> T cells toward the IFN- $\gamma$ -producing T<sub>H</sub>1-like phenotype, consequently reducing B-cell responses by suppressing antibody-inducing T<sub>H</sub>2 and T<sub>FH</sub> lineages. While this may be beneficial for containing parasite-mediated pathology, it may contribute to immunopathology and limit reactivation of

long-lived MBC. Modeling analyses by Lonnberg et al indicate that monocytes in particular have a role in regulating the T cell response, producing cytokines which skew naïve cells away from the T<sub>FH</sub> lineage and toward a T<sub>H</sub>1 phenotype (26).

## THE IMPACT OF T<sub>FH</sub> CELLS ON HUMORAL IMMUNITY

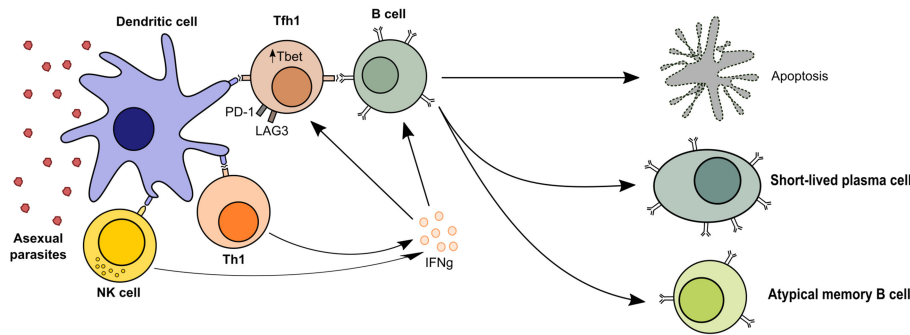
The T<sub>FH</sub> subset is particularly crucial for B cell development in the GC and the subsequent generation of a functional memory B cell compartment. T<sub>FH</sub> responses are widely hypothesized to be disrupted in malaria, as reflected by the relatively high frequency of autoreactive AMBCs and classical MBCs (29).

Due to the challenges of obtaining secondary lymphoid tissue, human research on T<sub>FH</sub> cells has primarily concentrated on circulatory CD4<sup>+</sup>CXCR5<sup>+</sup> T<sub>FH</sub> (30). These circulatory T<sub>FH</sub> cells share functional characteristics with GC T<sub>FH</sub> cells including IL-21 production and the ability to induce B cell differentiation *in vitro* (31). They also have properties of a central memory-like T<sub>FH</sub> population (26, 31–34). In contrast to GC-resident T<sub>FH</sub>, however, circulatory T<sub>FH</sub> cells lack BCL6 expression, which is required for survival and induction of secondary antibody responses (31, 35–38). BCL6 re-expression can be induced by re-challenge with cognate MBC (39), indicating that sustained antigen presence is required for T<sub>FH</sub> function.

In the last decade, circulatory T<sub>FH</sub> subsets equivalent to T<sub>H</sub>1, T<sub>H</sub>2, T<sub>H</sub>17, and T<sub>REG</sub> have been characterized in mice and humans (40, 41). T<sub>H</sub>1-like T<sub>FH</sub> cells (T<sub>FH</sub>1) show reduced potential to provide adequate help during antibody maturation *ex vivo* compared to T<sub>H</sub>2-like T<sub>FH</sub> cells (T<sub>FH</sub>2) (33, 35, 42). The concept that T<sub>FH</sub> subset imbalance may affect development of antimalarial immunity has gained more traction due to T<sub>FH</sub> subsets' potential roles in other chronic diseases, such as HIV (43). In parallel, polarization toward T<sub>H</sub>1-like responses has been well-documented in malaria and causes fundamental changes in multiple cell subtypes, such as induction of Th1-like regulatory cells (T<sub>REG</sub>1) (6, 28, 44).

Thus, dysfunctional GC processes and inappropriate T<sub>FH</sub> reactions are a likely consequence of malaria infection. Indeed, polarization of T<sub>FH</sub> is observed in Malian children, with more activated T<sub>FH</sub>1, more T<sub>H</sub>1-like cytokine responses, and less prominent T<sub>H</sub>2 polarization (26, 34, 45–47). This T<sub>H</sub>1-like cytokine response may lead to decreased GC reactions and therefore reduced generation and reactivation of T cell-dependent antibody responses (Figure 1).

Murine data suggest that circulatory T<sub>FH</sub> may represent pre-T<sub>FH</sub> generated from partly committed T<sub>FH</sub> lineage cells rather than mature memory GC-derived T<sub>FH</sub> cells (45). In murine malaria models, frequency of pre-T<sub>FH</sub> expressing the T<sub>H</sub>1-associated transcription factor Tbet increases after a single *P. berghei* ANKA infection (46). It will be important to clarify whether malaria-induced circulating T<sub>FH</sub>1 are simply pre-T<sub>FH</sub> generated in the periphery after a single exposure without entering the GC, and if circulating T<sub>FH</sub>2 therefore represent the mature T<sub>FH</sub> memory pool. This may explain the differential functionality of these two T<sub>FH</sub> subtypes in malaria. A proper



**FIGURE 1 |** T<sub>H</sub>1-like T cell responses in malaria. Follicular T helper cells are required for B cell activation and the generation of humoral immunity, but malaria profoundly affects T cell polarization and leads to short-lived antibody responses. The presence of asexual parasitaemia promotes activation of T<sub>H</sub>1 and NK cells, which produce high levels of IFN- $\gamma$ . This microenvironment promotes cellular upregulation of exhaustion markers like LAG3 and PD-1 and T<sub>FH</sub> differentiation into T<sub>FH</sub>1 cells, which are less effective at activating B cells. Quality of the T cell help in malaria-driven inflammation is therefore reduced, leading to B cell apoptosis or differentiation into short-lived plasma cells and atypical memory B cells, which are poor contributors to the long-term maintenance of humoral immunity.

understanding of the relationship between circulating- and GC T<sub>FH</sub> will be essential to delineate their particular role in the development of HI.

## HOW IS THE T<sub>H</sub>1-LIKE SIGNATURE AND T<sub>FH</sub>1-LIKE POLARIZATION REALIZED?

Studies with transgenic murine *P. yoelii* parasites suggest a positive feedback loop induced by Type I interferon and IL-2; T<sub>H</sub>1 cytokines secreted during *Plasmodium* infection increase CD4<sup>+</sup> T cell responsiveness by up-regulating Tbet and BLIMP-1 (44, 47). Consequently, CD4<sup>+</sup> T cells gain an increased predisposition to become T<sub>H</sub>1 cells.

Deregulation of humoral malaria immunity may be the result of an increased T<sub>FH</sub>1:T<sub>FH</sub>2 ratio in combination with the efficacy of the individual responses. Sustained polarization toward a T<sub>FH</sub>1 response after a single infection may affect an individual's ability to respond to subsequent malaria episodes. Frequencies of CXCR3<sup>+</sup>CCR6<sup>-</sup> T<sub>FH</sub>1s increase transiently but significantly during acute malaria, while CXCR3<sup>-</sup>CCR6<sup>-</sup> T<sub>FH</sub>2 frequencies decrease long-term in response to multiple malaria parasite exposures (48). In addition, T cell co-receptors may play a role in regulating T<sub>FH</sub> activation, as shown in *P. yoelii*-infected mice, where activation of OX40 leads to up-regulation of IFN- $\gamma$  (49), resulting in activation of the inhibitory PD-1 pathway. Consequently, T<sub>FH</sub> help will shut down, resulting in dysfunctional B cell responses including the generation of AMBCs (25) and decreased parasite clearance due to lower specific IgM and IgG titres (49, 50). Therefore, CXCR3<sup>+</sup> over-activation may be an important albeit not exclusive factor that limits T cell-dependent antibody responses to *Plasmodium*.

Co-infection with other pathogens can also impact humoral immunity to malaria. Multiple murine studies demonstrated that co-infection with murine Epstein-Barr virus analog MHV68 during *P. yoelii* XNL infection led to very high mortality from symptoms of malaria (51, 52). The latter study indicated that mortality was due to loss of humoral immunity by the MHV68

virus via induction of host IL-10 (52). Host factors involved in parasite sensing can also have a role: humanized mice engineered to express a single MHCII haplotype, HLA-DR4 (0401), had higher rates of parasitaemia and morbidity to *P. yoelii* 17XNL infection than mice engineered to express alternate haplotypes. The loss of parasite control was due to downregulation of humoral immunity by overproliferating T<sub>REG</sub>s (53).

## OTHER CHECKPOINT FACTORS INFLUENCING T CELL DIFFERENTIATION IN MALARIA

Regulatory T cell subtypes are likely key modulators of HI. The recently characterized regulatory follicular helper T cell (T<sub>FR</sub>) subset is especially relevant for HI regulation. Contrary to T<sub>R</sub>1, which arise from T<sub>H</sub>1, T<sub>FR</sub> are a FOXP3<sup>+</sup> subclass derived directly from T<sub>REG</sub> which express both BCL-6 and BLIMP-1 (54). Crucially, T<sub>FR</sub> can directly suppress both T<sub>FH</sub> and B cells in GC reactions and therefore directly affect GC formation (55–59).

T<sub>FR</sub> have not yet been studied in the context of malaria, even though their importance is indicated by their key role in controlling antibody production in HIV (60). T<sub>FR</sub> cell functionality is assumed to be determined by their ratio with T<sub>FH</sub>. As the proportion of T<sub>FR</sub> increases with age, similarly to T<sub>REG</sub>s (57), we hypothesize that T<sub>FR</sub> have the potential to play a role in the delayed onset of NAI. Murine studies show that the T<sub>FR</sub> fraction increases with age while the T<sub>FH</sub> proportion remains constant (60). T<sub>FR</sub> may therefore progressively regulate the T<sub>H</sub>1 driven over-activation of DCs, T cells and B-cells.

Conversely, a higher T<sub>FR</sub>:T<sub>FH</sub> ratio may inhibit T<sub>FH</sub> activation and proliferation, as suggested by T<sub>FR</sub>-induced downregulation of the proliferation marker Ki67 in T<sub>FH</sub> cells *in vitro*, dampening T<sub>FH</sub>1 activation (61, 62). However, T<sub>FR</sub> also downregulate the T<sub>H</sub>2-associated cytokines IL-21 and IL-4 in *in vitro* murine studies, potentially leading to marked defects in GC formation, and B cell affinity maturation (61, 63–65). Changes in the T<sub>FR</sub>:T<sub>FH</sub> ratio may therefore redirect GC B cells

toward becoming extra-follicular MBCs and short-lived PCs, therefore further decreasing generation of long-lived high-affinity antibodies (58, 62).

## SUMMARY, CONCLUSIONS, AND OUTLOOK

Malaria infection induces T<sub>H</sub>1 polarization characterized by the production of IFN- $\gamma$ . Overproduction of IFN- $\gamma$  may be central to poor acquisition of HI by polarizing T<sub>FH</sub> toward T<sub>FH</sub>1 and causing a positive feedback loop of T<sub>H</sub>1 polarization. It will be crucial to understand the specific parasite components responsible for T<sub>H</sub>1 polarization so that we can better target parasite antigens which catalyze T<sub>H</sub>1 polarization.

Malaria-naïve adults and children from low-transmission regions tend to generate strong pro-inflammatory responses: T<sub>H</sub>1 cytokines IFN- $\gamma$  and TNF $\alpha$ , and other pro-inflammatory cytokines such as IL-1 $\beta$  and IL-6, are produced, which may favor generation of T<sub>H</sub>1-like responses. However, children with sustained parasitaemia develop a cytokine signature consisting of IFN- $\gamma$ , Type I IFN, and regulatory cytokines IL-10 and TGF- $\beta$  (9, 66, 67). It is unclear whether this is related to parasite density, incidence of infections, or both. Parasite burden and transmission intensity could affect T<sub>FH</sub> polarization through systemic cytokine-mediated effects.

Dendritic cells and NK cells may be responsible for maintaining T<sub>H</sub>1 polarization. Malaria could affect early T cell polarization by disrupting dendritic cell function (68, 69), and DCs co-incubated with blood-stage parasites *in vitro* are shown to polarize naïve T cells toward a T<sub>H</sub>1-like phenotype that produces IFN- $\gamma$  and TNF $\alpha$  (70, 71). Furthermore, DCs are required for NK cell activation to blood-stage parasites (72). NK cells are major producers of IFN- $\gamma$ , and rapid reactivation of NK cells in response to blood-stage infection could lead to the formation of a T<sub>H</sub>1 cytokine signature, thereby inhibiting development of positive HI-forming responses. The presence of memory-like responses (trained immunity) from NK cells upon re-encountering pRBCs *in vitro* (73) suggests that NK cell activation in response to malaria may occur rapidly after the first infection, increasing early tendencies toward Th1-like responses. Moreover, NK cell cross-talk with dendritic cells is

important for CD4 T cell priming in murine malaria models (74, 75), suggesting that NK cells may bias T<sub>H</sub>1 polarization through multiple pathways.

However, it is unclear whether the blood-derived T<sub>FH</sub> differ functionally from their GC counterparts. Better models of T<sub>FH</sub> will be required to study these differences and assess the functional relationship between T<sub>FH</sub> subsets and the generation of humoral immunity more thoroughly: what phenotypes are generated by B-cells co-stimulated by T<sub>FH</sub>1, the quality of the antibody response, and whether their ability to differentiate into LLPCs or classical MBCs is impacted by malaria-generated T<sub>FH</sub>1s. A culture system to induce T<sub>FH</sub> or novel systems such as humanized mice which could generate larger quantities of T<sub>FH</sub> and even allow for isolation of tissue-resident T<sub>FH</sub> would permit further, in-depth study of these cells. This would also permit mechanistic studies into how T<sub>FH</sub>1 polarization occurs.

In summary, malaria infection, especially repeated infection with high parasitaemia, may generate “inappropriate” T<sub>H</sub>1-like T cell responses that fail to provide the adequate environment for long-lasting HI. This may be due to (i) compromised T<sub>FH</sub> help, reducing the generation of functional GC and development of typical memory B-cells, leading to a loss of HI longevity; (ii) increased proliferation of regulatory subsets such as T<sub>FR</sub> which may further inhibit HI by decreasing T<sub>FH</sub> activation and proliferation; (iii) a strong T<sub>H</sub>1-like immune signature characterized by high production of IFN- $\gamma$ , illustrated by the increased fraction of T<sub>H</sub>1 and other T<sub>H</sub>1-like cells, including the T<sub>FH</sub>1 subset. To break the cycle, we need improved methods to study T<sub>FH</sub> and understand the underlying mechanisms of T<sub>H</sub>1 polarization in malaria.

## AUTHOR CONTRIBUTIONS

XZY and LH wrote the first draft of the manuscript, which was reviewed by RWS. All authors have approved the publication of the final manuscript.

## FUNDING

XZY is supported by funding from the Bill and Melinda Gates Foundation (Grant numbers: OPP1091355 and OPP1080385).

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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