Review

How malaria modulates memory: activation and dysregulation of B cells in *Plasmodium* infection

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Humoral immune responses play a major role in naturally acquired immunity to malaria, but are slow to develop and ineffectively maintained. Although this may be partially due to the complex nature of *Plasmodium* parasites and the high degree of antigenic variation, there is evidence that the parasite also actively alters B cell function. We integrate recent findings on the effect of *Plasmodium falciparum* (*Pf*) on B cells and the association of parasite exposure with altered B cell proportions, such as the expansion of atypical memory B cells. We propose a model of how the parasite may mediate these effects by direct interaction with B cells via the cysteine-rich interdomain region 1 α (CIDRα) of the erythrocyte membrane protein 1 (*Pf*EMP1) and modulation of the host B cell activating factor (BAFF) immune pathway, and how this may compromise protective immune memory by interfering with B cell differentiation.

Humoral immunity in malaria

Malaria caused by protozoan *Plasmodium* parasites remains one of the most widespread and mortality-causing human infectious diseases worldwide [1]. Currently, there is no evidence that functional sterile immunity towards the malaria parasite is achieved on a large scale in malaria-endemic areas. Instead, adults acquire a state of clinical immunity (see Glossary) towards severe disease, in which they control and tolerate infection [2]. Through constant reinfection they can thus become asymptomatic carriers of the asexual blood-stage, as well as infective, sexual gametocyte-stages. Antibodies play a key role in conferring this clinical immunity, as demonstrated in a seminal study by passive transfer of antibodies from clinically immune adults to acutely infected children, controlling blood-stage parasitemia and alleviating disease [3]. Clinical immunity, however, develops only slowly after multiple exposures to the parasite [2]. Moreover, there is a notion that clinically protective humoral responses to malaria may be short-lived and can be lost in the absence of regular exposure [2,4]. It remains controversial whether these phenomena are indeed due to insufficient immune memory in malaria

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Glossary

Anti-parasite immunity: leads to a reduced burden of blood-stage parasite densities, controlling parasite multiplication by interfering with erythrocyte invasion or by enhancing parasite clearance. Anti-parasite immunity is considered to be long-lived [2].

BAFF receptor (BAFF-R): expressed on all B cell subsets except terminally differentiated plasma cells, BAFF-R has the highest affinity for trimeric BAFF [40].

B cell activating factor (BAFF): type II transmembrane protein of the tumor necrosis factor ligand superfamily that mediates B cell proliferation, differentiation, and survival. BAFF is biologically active as a cell-surface anchored protein or in soluble trimeric or 60-mer forms. It is recognized by three different receptors (BAFF-R, TACI, BCMA) which are differentially expressed during B cell development [40,85].

Clinical immunity: also referred to as anti-disease immunity. Clinical immunity reduces disease symptoms at a given parasite density and/or severe or complicated clinical disease. In highly malaria-endemic areas, clinical immunity is acquired during early childhood, whereas in (very) low endemic areas it may be delayed or even never attained. Clinical immunity is often acquired in parallel with anti-parasite immunity, but in contrast to anti-parasite immunity appears to be short-lived [2].

Cysteine-rich interdomain region 1 α (CIDR1α): extracellular domain of the variant surface antigen *Plasmodium falciparum* erythrocyte membrane protein 1. Binding of CIDRα to B cells occurs via the variant and constant chains of the B cell receptor in an antigen-nonspecific manner as well as via host-cell receptors including PECAM1 (CD31) and CD36 [11,22].

Humoral immunity: is mediated by molecules in body fluids as opposed to cells. Humoral immunity usually refers to immune responses mediated by antibodies produced by B cells.

Hypergammaglobulinemia: a state of elevated plasma levels of immunoglobulin (antibodies).

Immune memory: defined as the ability of the immune system, after initial priming in a first infection, to respond more rapidly and effectively in a subsequent exposure to the same pathogen. Immune memory is a characteristic of the adaptive immune system (B cells and T cells).

Memory B cells (MBCs): defined by their ability to respond rapidly following reexposure. This is aided by both their greater precursor frequency compared to unexpanded naïve B cells, strategic location, and enhanced ability for proliferation and differentiation that further enlarges the MBC and plasma cell pool.

Plasma cells (PCs): generated either in the primary germinal reaction or during a recall response from MBCs. PCs exist both as short-lived cells immediately after antigen exposure, or as a long-lived form in so-called plasma-cell niches such as the bone marrow. They are the producers of antibodies, the effector molecules of the humoral immune response.

Polyclonal B cell activation (PBA): characterized by generalized proliferation and differentiation of B cells into antibody-secreting cells, regardless of their antigen specificity. PBA results in hypergammaglobulinemia [17].

Sterile immunity: is distinct from anti-parasite immunity because it prevents rather than controls blood-stage infection. Sterile immunity in malaria would target either the sporozoite-stage (precluding liver-stage infection in the first place) or the infected hepatocyte (preventing progression to blood-stage infection) [2].

Transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI): upregulated upon B cell activation and expressed on memory and plasma B cells, TACI has low affinity for trimeric BAFF and instead preferentially binds BAFF in its 60-mer or membrane-bound forms [40].

Box 1. Malaria-specific plasma and memory B cell responses

Analysis of the circulating MBC repertoire of individuals in malaria-endemic areas has demonstrated that prevalence, breadth, and magnitude of malaria antigen-specific MBC responses, similarly to plasma antibodies, increase with age [6,62,86,87]. This is independent of the level of malaria endemicity and is consistent with a gradual, albeit slow and potentially inefficient [62], acquisition of humoral memory upon repeated exposure. The magnitude of malaria-specific MBC responses in naturally exposed individuals is in the same range as childhood vaccination-induced MBCs in the same population [6,62,86–88].

The overall proportion of individuals with detectable MBCs specific for individual malaria antigens, however, appears to be lower than for vaccination-induced MBCs; despite multiple exposures throughout their lives, the proportion of adults with detectable MBC for any given blood-stage malaria antigen ranges from 30–60% in low- and high-endemic areas [6,62,86,87]. By contrast, prevalence of MBCs induced by vaccination or natural virus infection, even after several decades, reaches 60–100% [6,62,86,87,89,90]. This low prevalence of malaria-specific compared to childhood vaccination-induced MBCs is particularly evident in individuals that are seropositive for the respective antigens [6,86,87]. Moreover, in individuals with antibody responses towards routine childhood vaccination antigens such as diphtheria toxoid, the detectability of corresponding vaccination antigen-specific MBCs increases with age, whereas the opposite is true for malaria-specific MBCs [6]. This indicates that there may indeed be a defect in either the induction or maintenance of long-lived malaria-specific MBC responses [86].

Only a few studies have addressed the issue of MBC maintenance in malaria. In low-endemic Thailand, no statistically significant decay was found when using the last reported clinical episode and longitudinal MBC data collected over 1 year [87]. By contrast, prevalence and magnitude of malaria-specific MBCs were considerably lower in adults without clinical episodes over 6 years, compared to those with one to three infections [87]. Moreover, whereas MBC prevalence in Kenyan children was clearly less affected by prolonged non-exposure (7 years) than were plasma antibody levels, malaria antigen-specific but not tetanus-specific MBC frequencies were higher in persistently exposed children compared to those who were previously exposed [91]. Whether this is simply due to the lack of boosting in the temporarily unexposed cohort or a specific defect in maintenance of malaria-induced MBCs remains to be determined.

[4]. Humoral immune memory is conferred by long-lived antibody-producing plasma cells (PCs) and quiescent memory B cells (MBCs). The latter are characterized by their ability to respond, proliferate, and differentiate more rapidly into PCs in subsequent infections than are antigen-inexperienced naïve B cells [5]. Recent data on the prevalence of malaria antigen-specific MBC responses in naturally exposed individuals indeed suggest that these are acquired and maintained less efficiently than childhood vaccination-induced MBCs (Box 1).

Possible explanations for the slow and incomplete induction of humoral immunity and clinical protection, as well as the absence of sterile protection, include the relatively complex antigen mixture provided by the malaria parasite [6] and antigenic variation between different parasite strains [7]. In addition, there is now increasing evidence that the malaria parasite also actively interferes with B cell function, similar to the direct modulation of antigen-presenting cell function and T cell regulation during malaria [8,9]. Herein, we review the most recent findings on B cell modulation mediated by *Plasmodium* parasites, also in the context of B cell modulation in other diseases. We propose a model on how dysregulation of B cells at different levels of development, both by direct interaction with the malaria parasite and by targeting host immune-regulation pathways, may translate into impaired humoral immune memory.

Mediators of parasite-induced B cell activation and modulation

The earliest observed indication for modulation of B cell function by the parasite is hypergammaglobulinemia during acute *Plasmodium* infection, resulting from polyclonal B cell activation (PBA) mediated by the blood-stage of the parasite [10,11]. The role of PBA in malaria immunity is controversial: immunoglobulins induced upon PBA are not only parasite specific but are also reactive towards unrelated antigens including self-antigens [12,13]. At least some of these induced auto-reactive antibodies might target crossreactive parasite antigens or neo-antigens expressed by infected erythrocytes [12,13]. PBA may thus be directly involved in the generation of protective anti-malarial responses [12–15], and possibly also their maintenance, as shown for polyclonal/bystander B cell stimulation in other disease and standard vaccination settings [16,17]. Other data suggest that PBA may represent a mechanism of immune modulation by altering B cell receptiveness and function, and might thus contribute to B cell exhaustion [18]. To date, two mechanisms potentially underlying PBA have been uncovered.

The CIDRα domain expressed on the parasite-infected red blood cell surface induces activation and altered responsiveness of B cells

Early studies raised the question as to whether the malaria parasite (similar to *Trypanosoma*) contains a B cell mitogen inducing PBA via direct parasite:B cell interaction [19]. Indeed, the CIDR1α region of the variant (*var*) surface antigen *Pf*EMP1 has been identified as a key mediator of PBA [11]. In *in vitro* experiments, CIDRα causes adherence of intact *P. falciparum*-infected red blood cells (*Pf*iRBC) to human B cells from non-immune donors and induces B cell activation, proliferation, and cytokine production [11,20]. Because phenotypic changes in these experiments were largely confined to $CD27^+$ B cells, it was concluded that MBCs are the key B cell subset affected by CIDRα [20]. Studies with purified B cell subsets would help to substantiate this interpretation.

Interaction of B cells with CIDR1α *in vitro* leads to signaling through the mitogen-activated protein kinase (MAPK) and the nuclear factor κB (NF-κB) pathways, both of which are also activated by B cell receptor (BCR) crosslinking [18,20]. Nevertheless, the effect of CIDR1α on B cells is not identical to BCR crosslinking because CIDR1α does not lead to phosphorylation of the tyrosine kinases Lyn and Syk, the first kinases in the BCR signaling pathway [18]. Accordingly, the mRNA expression profile in CIDRα-treated B cells is also clearly distinct from BCR crosslinked B cells [20]. Although there was no inhibitory effect of CIDR1α on BCR signaling *in vitro*, the authors