Potential functions of atypical memory B cells in *Plasmodium*-exposed individuals

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Abstract

Naturally acquired immunity against clinical malaria is slow to develop, taking years of repeated exposure to parasites to acquire sufficiently broad and potent antibody responses. Increasing evidence suggests that *Plasmodium* infection and the resulting immune stimulation contribute to changes in the B cell compartment. In particular, accumulation of atypical memory B cells (atMBCs) is common in *Plasmodium*-exposed individuals. Similarities to B cell subsets present in other acute and chronic disease settings have provided insight into the development and potential function of these cells; however, their contribution to protection against malaria is still poorly understood. Here, we discuss recent findings that have increased our understanding of atMBCs and outline outstanding questions related to their function and development in the protective immune response to malaria.

Keywords

1. Introduction

Malaria is a deadly disease mainly affecting children in southern and southeastern Asia and sub-Saharan Africa. Despite major progress in reducing morbidity and mortality globally, an estimated 228 million cases occurred in 2018, resulting in 405,000 deaths (WHO, 2019). Malaria, caused mainly by the parasites *Plasmodium falciparum* and *Plasmodium vivax*, remains a major public health challenge and global financial burden (Shretta et al., 2016; Gunda et al., 2017; Hailu et al., 2017; Tang et al., 2017; WHO, 2019). The development of an effective vaccine therefore has high priority in the fight to eradicate this disease.

Naturally acquired immunity to malaria relies on the development of memory B cells (MBCs) and long-lived plasma cells that serve as the source of antibody responses that inhibit parasite replication and survival (Fig. 1) (Cohen et al., 1961; Riley et al., 1992; Osier et al., 2008). However, these responses are initially short-lived and require repeated parasite

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exposure over an extended period of time to broaden and develop into a protective response (Baird, 1998; Griffin et al., 2015; Rodriguez-Barraquer et al., 2016, 2018). Over the course of several years, protection against disease is acquired, but sterilising immunity is never reached (Reyburn et al., 2005; Doolan et al., 2009; Roca-Feltrer et al., 2010; Rodriguez-Barraquer et al., 2016). One of the reasons for the slow development of protective immune responses may be the effects of the malaria parasite on the host's immune system. Increasing evidence suggests *P. falciparum* infection alters the composition of the B cell compartment in ways that may impair the immune response. A common feature of circulating B cells in *Plasmodium*-exposed individuals is the accumulation of atypical MBCs (atMBCs). However, it remains unclear whether this expansion is indicative of a productive role in the immune response or a malfunction of the immune system during chronic antigen exposure. Recent efforts have uncovered several potential drivers of atMBC development and provided new insight into their potential role or lack thereof in the immune response to *Plasmodium*. However, the heterogeneity of this B cell population, coupled with the use of different markers, models, and experimental conditions, has hampered interpretation of these sometimes conflicting studies. Here, we will discuss recent findings and present outstanding questions with respect to the origin and function of atMBCs, their similarities and differences to atypical B cell subsets in other diseases, and their potential role in immunity against malaria.

2. Definition of atMBCs

2.1. AtMBCs in malaria

In the broadest terms, human atMBCs are defined as B cells lacking CD21 and CD27 surface expression (CD19$^+$CD21$^-$CD27$^-$). High expression of the pan-B cell marker CD19, as well as CD20, is also seen in the atMBC population (Sundling et al., 2019). Cells falling into this category frequently have altered expression (compared with other B cell populations) of other surface proteins including CD11c, CXCR3, and CXCR5, as well as transcription factors such as T-bet (Weiss et al., 2010; Muellenbeck et al., 2013; Portugal et al., 2015; Sullivan et al., 2015). Generally, these cells also demonstrate increased expression of inhibitory receptors such as CD72, CD85j, FcRL3, and FcRL5 (Portugal et al., 2015; Sullivan et al., 2015). However, expression of these markers on atMBCs is heterogeneous, and division based on marker status often reveals subpopulations of atMBCs with potentially distinct functions. For example, division based on FcRL5 status reveals two populations with distinct B cell receptor (BCR) signalling and antibody secretion phenotypes (Sullivan et al., 2015). Heterogeneity in surface marker expression among CD21$^-$CD27$^-$ B cells may indicate differences in time from antigen exposure, developmental path, or functional status. The general definition of atMBCs as CD21$^-$-CD27$^-$ B cells also fails to exclude smaller subsets such as CD21$^-$CD27$^-$CD20$^-$CD38^{hi} plasmablasts that spontaneously secrete antibodies ex vivo (Sullivan et al., 2015) and are likely distinct from true atMBCs. However, it is starting to become clear that atypical B cell markers are not confined to the CD21$^-$CD27$^-$ B cell population. While CD11c can be regarded as a marker of an atypical phenotype, Sundling et al. (2019) showed that, following malaria, CD11c expression is not strictly limited to atMBCs, and the expression of surface markers associated with atMBCs is dynamic (Sundling et al., 2019). In addition, FcRL5 was also found to be expressed on a

subset of CD21$^+$CD27$^+$ memory B cells in individuals exposed to *Plasmodium* (Sullivan et al., 2015). In more recent studies, atMBCs are therefore often defined according to different definitions such as CD19^{hi}, CD20^{hi}, and/or expression of CD11c, T-bet, CXCR3, or inhibitory receptors.

Differences between markers used to identify atMBCs may contribute to the varied functional phenotypes observed for these cells, and as more becomes known about the phenotype and characteristics of CD21$^-$CD27$^-$ B cells, the definition of atMBCs will require refinement to ensure selective analysis of these cells. Here, unless otherwise specified, the term atMBC refers to the CD21$^-$-CD27$^-$ B cell population. We would like to note that it has recently been proposed that the term atMBC may be misleading, since these cells may be part of a normal immune response and might not functionally serve as memory cells (Sanz et al., 2019). In addition, the distinction between unswitched (IgM$^+$/IgD$^+$) and switched atMBCs is important as these cells might have different origins and functions.

2.2.   Atypical B cells in other contexts

CD21$^-$CD27$^-$ B cells are not unique to *Plasmodium*-exposed individuals. Similarities to atMBCs associated with other chronic infectious and autoimmune diseases may offer clues to the function of atMBCs in the immune response to *Plasmodium*. CD21$^{lo/-}$ B cells are enriched in autoimmune diseases (Warnatz et al., 2002; Wehr et al., 2004; Isnardi et al., 2010) and share similarities with a population of FcRL4$^+$ B cells identified by Ehrhardt et al. (2005) as tissue-based MBCs that express high levels of CD20, low levels of CD21, and mostly lacked CD27 (Ehrhardt et al., 2005). In HIV-infected individuals, a similar population of CD27$^-$CD21^{lo} B cells expressing FcRL4 was identified (Moir et al., 2008) and referred to as tissue-like MBCs due to their similarity to the tissue-based MBCs discovered by Ehrhardt et al. (2005). Some groups have classified these cells as 'exhausted' or 'anergic' based on their hypo-responsiveness to BCR stimulation and upregulation of inhibitory receptors (Moir et al., 2008; Isnardi et al., 2010). CD21$^-$CD27$^-$ B cells have since been identified in individuals with hepatitis C (HCV), tuberculosis (TB), and autoimmune disorders (Wei et al., 2007; Oliviero et al., 2015; Joosten et al., 2016). CD21$^-$CD27$^-$ atMBCs also share features with an atypical B cell population expanded in aged mice (Hao et al., 2011; Rubtsov et al., 2011; Wang et al., 2018).

In the context of HIV infection, CD21$^-$CD27$^-$ B cells frequently upregulate the transcription factor T-bet and exhibit reduced antibody production (Moir et al., 2008; Knox et al., 2017). In contrast, T-bet$^+$ atMBCs described in autoimmune diseases, including common variable immunodeficiency (CVID) and systemic lupus erythematosus (SLE), seem to produce inflammatory cytokines and autoantibodies. In SLE, a population of IgD$^-$CD21$^-$CD27$^-$T-bet$^+$CD11c$^+$CXCR5$^-$ cells, termed DN2 cells, expands and their abundance is associated with disease severity (Jenks et al., 2018; Wang et al., 2018). These SLE-associated DN2 B cells can localise to sites of inflammation and contribute to autoreactive antibody production (Wang et al., 2018). In these cells, T-bet expression is associated with autoreactivity (Liu et al., 2017; Wang et al., 2018). Additionally, SLE-associated DN2 B cells upregulate FcRL5, although it is unclear if this expression is associated with activating or inhibitory effects (Jenks et al., 2018; Wang et al., 2018). Expression of CD11c, FcRL5, and T-bet is a shared

feature between autoimmune-associated atMBCs and malaria-associated atMBCs, but while autoimmune-associated atMBCs appear to play a primary role in the generation of autoantibodies, the role of malaria-associated atMBCs remains elusive.

While malaria-associated atMBCs share characteristics with HIV, HCV, TB, and autoimmune-associated atMBCs, phenotypic distinctions between these populations, such as differences in the expression of specific inhibitory receptors (e.g., FcRL4 versus FcRL5) and responsiveness to different Toll-like receptor (TLR) ligands (e.g., TLR7/8 versus TLR9), suggest their development and function may be impacted by the infectious agent and the resulting immune environment. Nevertheless, insights from studies on atMBCs found in other settings may provide direction for further study of malaria-associated atMBCs.

Finally, while healthy human adults harbour a small population of CD21⁻CD27⁻ B cells, it is unknown whether atMBCs observed in disease are derived from this population. The function of these cells in healthy individuals remains unknown, although it has been suggested they may act as early responders to viral infections (Knox et al., 2017).

2.3. Atypical B cells in mice

In mice, B cells with characteristics of human atMBCs have been identified in the contexts of autoimmunity, ageing, and disease. Aged female mice have a population of CD21⁻CD11b⁺CD11c⁺ B cells, termed age-associated B cells (ABCs), that are associated with the response to viral infections and the development of autoimmunity (Rubtsov et al., 2011; Rubtsova et al.,2013). This population of B cells arises during the peak of viral infection and produces high titers of IgG2a antibodies (Rubtsova et al., 2013). In a model of SLE, these CD11b⁺CD11c⁺ B cells were the major contributors to autoantibody production (Rubtsov et al., 2011). These cells express genes involved in plasma cell differentiation, including *Blimp1* and *Xbp1*, as well as transcription factors used to define human atMBCs, such as T-bet (Rubtsov et al., 2011). In the context of malaria, Kim et al. (2019) identified a population of atypical mouse B cells bearing similarity to human atMBCs (Kim et al., 2019). Defined by expression of FcRL5, this B cell subset transiently expanded following *Plasmodium chabaudi* infection in mice (Kim et al., 2019). Transcriptional profiling of these cells revealed similarities to human atMBCs, including increased expression of receptors and adaptors with inhibitory functions (Kim et al., 2019). FcRL5⁺ B cells also increased expression of T-bet, markers defining ABCs (CD11b, CD11c), and markers upregulated on human atMBCs (CD86, CD40) (Kim et al., 2019). Perez-Mazliah et al. (2018) also characterised a population of CD21ˡᵒCD11b⁺CD11c⁺FcRL5ʰⁱ B cells which increased following *P. chabaudi* infection in a mouse model of MSP1₂₁-specific B cells (Perez-Mazliah et al., 2018). Within this population, cells expressed inhibitory markers and markers of antigen exposure also seen in human atMBCs (Perez-Mazliah et al., 2018). Mouse atypical B cells expressed genes associated with human atMBCs including *Cxcr3, Tbx21*, and *Fcrl5* (Perez-Mazliah et al., 2018). Mouse atypical B cells also upregulated *Blimp1* and had low expression of *Cxcr5*, suggesting these cells could represent a population of pre-plasmablasts (Perez-Mazliah et al., 2018). While human atMBCs in *Plasmodium*-exposed individuals showed a modest increase in *BLIMP1* expression compared with classical MBCs, much larger upregulation was observed for the BLIMP-1 repressor *BCL6* (Sullivan

et al., 2015). Despite similarities in transcriptional profiles between mouse atypical B cells and human atMBCs, subtle differences in gene expression between these cell populations suggest that mouse atypical B cells might not be functionally identical to human atMBCs. Since mice do not harbour a B cell marker equivalent to human CD27, it is difficult to infer whether this population is truly representative of the CD21⁻CD27⁻ atMBCs seen in humans. Moreover, it has been proposed that FcRL5 serves as a memory marker in mice (Perez-Mazliah et al., 2018) and is thus not functionally equivalent to FcRL5 in humans. Due to the difficulties in comparing results about atMBCs from mouse and human studies, we focus this review on atMBCs in humans, unless specifically mentioned otherwise.

2.4. T-bet as the defining transcription factor for atypical B cells

First identified as a regulator of CD4$^+$ T cell differentiation, T-bet is a transcription factor expressed in certain populations of B cells where it promotes class switching to IgG3 in humans and IgG2a/IgG2c in mice (Zhang et al., 2012; Obeng-Adjei et al., 2017). Recently, Stone et al. (2019) uncovered a role for T-bet in IFNγ-stimulated B cells in mice (Stone et al., 2019). In these cells, T-bet functions to repress IFNγ-stimulated gene programmes that are incompatible with antibody-secreting cell (ASC) formation. In this way, T-bet promotes ASC development by preventing B cells from acquiring an inflammatory effector cell fate.

IFNγ signalling through the IFNγ receptor can also stimulate expression of T-bet in human B cells (Fig. 2) (Ambegaonkar et al., 2019; Zumaquero et al., 2019). In naïve B cells derived from human tonsil or peripheral blood, IFNγ signalling, BCR crosslinking, and TLR7 or TLR9 engagement all contributed to the induction of high levels of T-bet expression (Fig. 2) (Ambegaonkar et al., 2019; Zumaquero et al., 2019). Under these in vitro conditions, T-bet$^+$ cells also upregulated additional surface markers associated with an atypical phenotype, but expression of these markers appears to be independent of T-bet expression and may be driven by other processes. For example, CD11c expression was shown to be dependent on IL-21 stimulation in both human and mouse T-bet$^+$ B cells (Naradikian et al., 2016; Wang et al., 2018) and did not increase in T-bet$^+$ B cells in the absence of IL-21 stimulation (Ambegaonkar et al., 2019), suggesting IL-21 is a driver of CD11c expression in atMBCs. Other atypical surface markers may also require specific stimulatory conditions to reach maximum expression.

While T-bet is generally seen as a hallmark of atMBCs in both humans and mice, the atypical phenotype is not dependent on the induction of T-bet. In a mouse model of SLE, the elimination of T-bet from B cells did not prevent disease, although the generation of auto-antibodies and resulting pathology was delayed (Rubtsova et al., 2017). In mice with T-bet-deficient B cells, *Ehrlichia muris* infection or *Plasmodium* infection gave rise to B cells with atypical characteristics, including FcRL5 or CD11c expression, at the same frequencies as seen in the presence of T-bet (Kim et al., 2019; Levack et al., 2020). These results indicate that T-bet is not the defining transcription factor of this lineage. Rather, the main function of T-bet seems to be the promotion of appropriate classswitching (Levack et al., 2020). In addition, with the discovery of other (non-atMBC) subsets of human B cells that express atypical markers, such as FcRL5 and CD11c, it has become clear that these markers are not confined to one subpopulation and that the function of subsets with atypical markers may be

highly context-specific. T-bet should thus be used in combination with other markers to distinguish atMBCs.

3. Expansion and contraction of atMBC populations

On average, atMBCs account for up to 30% of the circulating mature B cell population of individuals living in malaria-endemic regions, while this is less than 10% in malaria-naïve adults (Weiss et al., 2009, 2011; Portugal et al., 2012, 2015). As detailed below, recent studies suggest *Plasmodium* parasites stimulate the immune system in ways that may promote the development of atMBCs. In particular, the increased levels of pro-inflammatory cytokines induced by *Plasmodium* infection may contribute to the observed atMBC expansion through several distinct but complementary mechanisms (Fig. 1). In subjects infected with either *P. falciparum* or *P. vivax*, atMBCs showed higher levels of proliferation than classical MBCs in vivo (Scholzen et al., 2014; Changrob et al., 2018). Furthermore, in people who experienced a single episode of *P. falciparum* malaria, the atMBC population expanded and peaked 10 days after malaria diagnosis, whereas the fraction of classical MBCs did not increase until 1 month after diagnosis (Sundling et al., 2019). The atMBC compartment showed greater expansion in individuals who had previously experienced *Plasmodium* infections than in subjects with primary infections (Sundling et al., 2019), suggesting that atMBCs may be derived from both activated naïve and memory B cells.

Additional observations in humans also suggest that the atMBC population contracts after clearance of infection. Following peak levels of atMBCs during natural *P. falciparum* infection, the compartment gradually contracted to background levels with an estimated half-life of 295 days (Sundling et al., 2019). Similarly, Ayieko et al. (2013) showed that in the absence of infection, the percentage of class-switched atMBCs in malaria-exposed adults in Kenya decreased from an average of 7.7% to 2.8% over the course of 1 year (Ayieko et al., 2013). However, despite the gradual decline of atMBC numbers over time, elevated fractions of atMBCs following natural *P. vivax* infection have been observed 3 years postinfection (Changrob et al., 2018). Taken together, these results suggest that the atMBC population expands during infection and that the maintenance of this population is dependent on continued exposure to antigen, but that a population of long-lived atMBCs can persist for years in the absence of antigen exposure.

3.1. Drivers of atMBC expansion

Plasmodium infection can selectively induce a pro-inflammatory, Th-1 polarised, immune response resulting in the activation and differentiation of specific cell types. When activated by *Plasmodium*-infected erythrocytes, dendritic cells can induce CD4^{+} T cells to differentiate into Th-1 cells (Fig. 2) (Gotz et al., 2017). The infection also selectively induces the differentiation of CD4^{+} T cells into Th-1 polarised T follicular helper cells (Tfh-1) (Figueiredo et al., 2017). In the germinal centre, B cells require help from Tfh cells during the process of affinity maturation (Fig. 1). Th-2 polarised Tfh cells provide superior B cell help compared with Tfh-1 cells in the form of survival signals (Morita et al., 2011; He et al., 2013; Yap et al., 2019). However, during *Plasmodium* infection, high levels of the pro-inflammatory cytokine IFNγ, together with higher levels of IL-10 and IL-6, promote the

development of Tfh-1 cells, which are poor B cell helpers and contribute to impaired germinal centre responses (Figueiredo et al., 2017). This combination of abnormal dendritic cell activation, resulting in the stimulation of a Th-1 polarised response, and reduced B cell help from Tfh-1 cells, may promote the expansion of atMBCs (Fig. 2).

In *Plasmodium*-exposed individuals, B cells upregulate T-bet and display an atMBC-like phenotype including increased surface expression of FcRL5 and CD11c (Obeng-Adjei et al., 2017). These T-bet[+] atMBCs demonstrate reduced BCR signalling compared with naïve and MBCs, which has been interpreted as an impaired capacity to produce antibodies (Obeng-Adjei et al., 2017). Repeated parasite exposure seems to drive the upregulation of T-bet, resulting in an increase in T-bet[hi] atMBCs in children who have experienced five or more episodes of malaria (Obeng-Adjei et al., 2017). In vitro, this phenotype was recapitulated by stimulating tonsillar or peripheral naïve B cells with supernatants of peripheral blood mononuclear cells (PBMCs) co-cultured with infected erythrocytes, in combination with anti-IgM (Obeng-Adjei et al., 2017). This activation of naïve B cells could be inhibited by neutralising IFNγ in the supernatant or by blocking the IFNγ receptor, supporting the essential role of IFNγ in this process.

Parasite-specific B cells have been detected among atMBCs from *Plasmodium*-exposed individuals (Muellenbeck et al., 2013; Krishnamurty et al., 2016; Aye et al., 2020), indicating that antigen exposure is a factor in the expansion of these cells. However, Aye et al. (2020) recently reported increased levels of tetanus toxoid-specific atMBCs in individuals with continuous, high exposure to *Plasmodium* compared with individuals with previously high exposure but current low exposure, suggesting that the inflammatory environment of a *Plasmodium* infection can drive bystander B cell activation and the development of atMBCs (Aye et al., 2020). Collectively, these findings suggest that both antigen exposure and prolonged stimulation within the highly inflammatory environment of a *Plasmodium* infection contribute to the development of an atypical phenotype in B cells.

4. Potential functions of atMBCs

Studies of atMBC function in immunity to malaria are conflicting, and their exact role remains a point of debate. In general, there are two main theories regarding the role of atMBCs. First, expression of inhibitory receptors and reduced in vitro proliferation and antibody secretion suggest atMBCs may be a population of B cells expanded and hyper-activated as a result of chronic immune stimulation that have upregulated inhibitory receptors as a mechanism to downregulate this immune activation. Conversely, atMBCs may have a role as a class of functional B cells that actively contribute to the immune response during chronic infection, potentially as antigen-presenting cells or pre-antibody secreting cells. These two theories are not necessarily mutually exclusive, and we will explore recent evidence in support of both, as well as several other proposed functions of atMBCs, by comparing the phenotypes of atMBCs associated with malaria with those of related subsets found in other contexts (Table 1).

4.1. Regulators of immune responses

As discussed in section 3, atMBCs expand in response to *Plasmodium* infection, show signs of hyper-activation and have more recently undergone proliferation than classical MBCs in vivo. However, these cells are resistant to further stimulation in vitro. Downregulation of the PI3K, phospholipase C, and BCR signalling pathways, all necessary for orchestrating B cell responses (Kurosaki et al., 2010; Pieper et al., 2013), suggests these cells are less readily activated in response to BCR signalling (Portugal et al., 2015). Antigen-stimulated proliferation and cytokine production are also reduced in atMBCs compared with other B cell populations (Portugal et al., 2015).

The expression of inhibitory receptors such as FcRL3, FcRL5, and CD85j by atMBCs has been proposed to be a means to downregulate the hyper-activated state of these cells. In this scenario, FcRL5 may function as an IgG receptor, binding all subclasses of IgG with varying affinities (Wilson et al., 2012; Franco et al., 2013). During *Plasmodium* infection, polyclonal B cell activation in response to blood-stage parasites leads to hypergammaglobulinemia (Gilles and McGregor, 1959; McGregor and Gilles, 1960). In the presence of high quantities of IgG, crosslinking of BCR and FcRL5 by immune complexes may lead to reduced BCR signalling. FcRL5 expression by atMBCs may represent a regulatory mechanism aimed at reducing plasma antibody levels by inhibiting BCR signalling in the presence of high quantities of IgG (Sullivan et al., 2015). Given that atMBCs derived from individuals with different chronic infections express different surface receptors, the selective expression of specific FcRL proteins could be dependent on the antibody response stimulated by each pathogen and reflective of an attempt by the immune system to limit antibody responses during chronic immune activation.

Finally, atMBCs that express T-bet have been shown to upregulate markers – including HLA-DR, ICOS-L, and CD86 – that play important roles in antigen presentation to CD4^{+} T cells (Muellenbeck et al., 2013; Obeng-Adjei et al., 2017; Ambegaonkar et al., 2019). This indicates that interactions with T cells may be important for atMBC development or their function. *Plasmodium* infection drives a Th-1 polarised cytokine response which promotes the expansion of Tfh-1 cells with reduced helper activity compared with Tfh-2 cells (Obeng-Adjei et al., 2015). The development of an atypical phenotype could be partially driven by a lack of T cell help at crucial developmental stages. It has been proposed that atMBCs regulate these "impaired" Tfh cells to reduce their functionality over the course of infection (Ambegaonkar et al., 2019).

4.2. Dysfunctional B cells as a result of chronic immune activation

As discussed in Section 4.1, atMBCs show signs of hyper-activation in vivo and are resistant to further activation in vitro. An alternative explanation for the less responsive state of atMBCs in vitro is that these cells have become exhausted as a result of overstimulation by antigen exposure or the inflammatory environment during *Plasmodium* infections, as has also been suggested for phenotypically similar B cell subsets in the context of HIV infection and autoimmunity (Moir et al., 2008). A potential mechanism driving atMBCs into this less responsive state may be related to the function of T-bet in IFNγ-stimulated cells. It was shown in mice that T-bet plays an essential role in repressing the inflammatory gene

programme that is induced by IFNγ signalling in B cells (Stone et al., 2019). Cells that were unable to downregulate this inflammatory gene programme, for example as a result of excessive TLR7 or TLR9 stimulation, remained effector B cells and did not differentiate into antibody-secreting cells (Stone et al., 2019). Given that *Plasmodium* DNA can serve as a TLR9 ligand (Rivera-Correa et al., 2017), it is conceivable that a subset of B cells can get stuck in the pre-antibody secreting stage during *Plasmodium* infection and become dysfunctional or poor contributors to humoral immunity.

Recently, Ly et al. (2019) demonstrated that T-bet expression in germinal centre B cells during *Plasmodium berghei* infection in mice drives localization to the dark zone where these cells undergo affinity maturation (Ly et al., 2019). T-bet expression appears to prevent premature exit of germinal centre B cells, helping to drive the development of high affinity antibodies (Ly et al., 2019). In the setting of primary *Plasmodium* infection, T-bet may thus be important for the development of a potent humoral immune response. In contrast, chronic immune activation and antigen exposure may interfere with the normal role of T-bet in germinal centre reactions. In HIV-infected individuals, CD19^{hi}T-bet^{hi} B cells were mainly extrafollicular, but shared high clonal overlap with germinal centre B cells, which suggests these two groups may share a common precursor (Austin et al., 2019). The CD19^{hi}T-bet^{hi} B cells in HIV-infected individuals may represent recent germinal centre emigrants or a population of cells that remain outside the germinal centre during the immune response, fitting with the low expression of the germinal centre homing receptor CXCR5 on these T-bet^{+} B cells. High frequencies of CD19^{hi} B cells in lymph nodes were associated with low serum neutralising activity against HIV, suggesting that the abundant presence of these cells hampered the development of high-affinity B cells in the germinal centre. These contrasting findings may represent the difference between the important function of T-bet in B cell development during an optimal immune response and an impaired response of T-bet^{+} B cells in conditions of chronic immune activation.

4.3. Activated B cells as part of the normal immune response

The atMBC compartment expands during and shortly after infection, followed by a long contraction phase (Sundling et al., 2019). Andrews et al. (2019) identified three populations of activated memory B cells that transiently arise in response to influenza vaccination (Andrews et al., 2019). These populations were defined by low expression of CD21 and high expression of FcRL5, and could be distinguished from each other by the expression of CD85j, CD62L and CD27 (Andrews et al., 2019). The AM3 population (CD21^{lo}CD27^{-}FcRL5^{+}CD11c^{+}CD85j^{hi}CD62L^{lo}T-bet^{+}) was the most phenotypically similar to atMBCs identified in *Plasmodium*-exposed individuals, with the notable exception that AM3 cells did not express CXCR3. Of the three activated memory B cell populations, AM3 cells were the last to peak in frequency, approximately 28 days after vaccination, followed by a return to baseline over the course of several months (Andrews et al., 2019). AM3 cells almost exclusively recognised epitopes present in the vaccine that individuals had not previously been exposed to. Based on these observations, the authors proposed these cells represent a population of new antigen-specific cells that were distinct from B cells activated as part of the recall response (Andrews et al., 2019). These observations suggest that atMBCs are part of the normal B cell response to vaccination, and possibly infection, and

that these cells decline in numbers over the course of several months in the absence of antigen or other stimuli, similar to the kinetics observed for malaria-associated atMBCs.

The AM1 and AM2 cell populations described by Andrews et al. (2019) shared similarities with CD21^{lo} B cell subsets described by others (Ellebedy et al., 2016; Lau et al., 2017; Kim et al., 2019). Lau et al. (2017) defined a population of CD21^{lo}CD27$^+$ B cells with elevated T-bet, CD11c and FcRL5 expression. Based on the upregulation of key genes related to plasma cell differentiation (including *BLIMP1*), the authors proposed that these cells represent a transitional stage of recent germinal centre emigrants that are primed to become long-lived plasma cells (Lau et al., 2017). Kim et al. (2019) found that B cells expressing both FcRL5 and CD11c were enriched among B cells specific for tetanus toxoid C fragment (TTCF), suggesting these markers delineate long-lived, functional antigen-specific MBCs. However, despite FcRL5 and CD11c expression, TTCF-specific B cells were almost exclusively found in the classical (CD21$^+$CD27$^+$) and activated (CD21$^-$CD27$^+$) memory B cell compartments, not among atypical (CD21$^-$CD27$^-$) MBCs (Kim et al., 2019). This could be indicative of functional differences between antigen-specific MBCs and atMBCs, possibly as a consequence of different stimuli that mediate B cell activation upon vaccination versus during chronic infection. These studies point out that the 'atypical' B cell markers T-bet, CD11c, and FcRL5 are not sufficient to distinguish atMBCs and highlight the need for careful phenotyping of B cell subsets. Future studies will have to determine whether different T-bet$^+$FcRL5$^+$CD11C$^+$ B cell subsets have different functions and origins, and to what extent these subsets are clonally related.

4.4. Pre-antibody secreting cells

AtMBCs identified in *Plasmodium*-exposed individuals share phenotypic and functional similarities with a population of double negative (IgD$^-$CD27$^-$) B cells that lack CXCR5 expression but do express CD11c and T-bet, termed DN2 cells (Jenks et al., 2018; Scharer et al., 2019). Similar to malaria-associated atMBCs, DN2 cells also express FcRL5, but not FcRL4 (Jenks et al., 2018). Furthermore, DN2 cells and atMBCs share key transcriptional features including expression of the BLIMP-1 repressor *BCL6,* the cytokine receptor IL21R, and the inhibitory protein CD72 (Sullivan et al., 2015; Jenks et al., 2018).

It has been proposed that atMBCs are dysfunctional as a result of decreased B cell signalling and limited antibody secretion upon stimulation compared with classical MBCs. In both DN2 and atMBCs, BCR crosslinking induced an approximately 1.5-fold increase in phosphorylated BLNK (pBLNK) compared with unstimulated cells, which was significantly lower than the increase in pBLNK in classical MBCs (Portugal et al., 2015; Jenks et al., 2018; Ambegaonkar et al., 2019). In the case of DN2 cells, the higher level of pBLNK compared with unstimulated cells has been interpreted as a sign of functionality (Jenks et al., 2018), while the reduced level of pBLNK in atMBCs compared with classical MBCs led to the conclusion that this represents a lack of responsiveness (Portugal et al., 2015; Ambegaonkar et al., 2019). In line with limited BCR signalling, atMBCs failed to differentiate into antibody-secreting cells in vitro upon stimulation by BCR crosslinking and TLR9 engagement (Portugal et al., 2015; Sullivan et al., 2015). Similarly, DN2 cells generated in vitro by BCR crosslinking, TLR7 engagement, and cytokine (IFNγ, IL-2,

IL-21, and BAFF) stimulation of naïve B cells did not continue to differentiate into antibody-secreting cells by prolonged incubation under the same conditions (Zumaquero et al., 2019). However, DN2 cells efficiently differentiated into antibody-secreting cells in the absence of BCR crosslinking, indicating that transient BCR engagement is key to optimal stimulation and differentiation of these cells (Zumaquero et al., 2019). In line with the thought that atMBCs may be preantibody-secreting cells similar to DN2 cells, Muellenbeck et al. (2013) observed secretory Ig transcripts by PCR in atMBCs but not classical MBCs. It remains to be determined whether atMBCs can be induced to secrete antibodies under the same conditions used for DN2 differentiation, but based on these recent findings it may be too early to conclude that atMBCs are dysfunctional.

4.5. Autoreactive cells

DN2 cells are predominantly expanded in SLE patients of African American descent with active disease (Jenks et al., 2018). Stimulation and subsequent differentiation of DN2 cells from these SLE patients into antibody-secreting cells resulted in the production of autoantibodies, suggesting that DN2 cells are precursors of pathogenic plasma cells (Jenks et al., 2018). Interestingly, antibodies isolated from malaria-associated atMBCs also showed increased self-and polyreactivity compared with classical MBCs (Muellenbeck et al., 2013). In addition, autoreactive T-bet^{+}CD11c^{+} cells expanded in response to *Plasmodium yoelii* infection in mice (Rivera-Correa et al., 2017). These cells contributed to the development of *Plasmodium*-induced anaemia by generating autoantibodies targeting phospholipids on the erythrocyte surface, in particular phosphatidylserine (Rivera-Correa et al., 2017). Erythrocyte-reactive cells were also induced in vitro by stimulation of human PBMCs with *P. falciparum* lysate (Rivera-Correa et al., 2017). The abundance of atMBCs in *Plasmodium*-infected individuals was associated with levels of anti-phosphatidylserine IgG in plasma and the development of anaemia (Rivera-Correa et al., 2019). The presence of phosphatidylserine in the outer membrane leaflet is increased in *Plasmodium*-infected erythrocytes (Schwartz et al., 1987). The production of phosphatidylserine-specific autoantibodies by T-bet^{+}CD11c^{+} cells may thus be beneficial during *Plasmodium* infections to reduce the population of infected erythrocytes, but inadvertently also target uninfected erythrocytes with this phospholipid exposed on their surface. Although future studies are necessary to determine the extent of overlap between DN2 cells and the atMBC population, the many parallels in phenotype and functionality suggest that similar drivers may underlie the formation of these cell populations in conditions of chronic inflammation.

4.6. A model for the role of atMBCs in health and disease

Based on the evidence to date, we propose a model in which atMBCs function as a normal part of the immune response. The expansion of these cells shortly after exposure to antigen, and their contraction following the clearance of infection, suggests they are activated cells that respond to antigenic stimulation. In the inflammatory conditions characteristic of chronic infections and autoimmunity, continuous antigen exposure and TLR signalling may disrupt the normal development of these cells. DN2 cells develop into ASCs only in the absence of BCR crosslinking (Zumaquero et al., 2019), suggesting continuous BCR engagement by antigen could prevent these cells from developing correctly. Similarly, TLR7/9 engagement prevents cells from repressing an inflammatory gene programme which

can inhibit their development into ASCs (Stone et al., 2019). As a result, atMBCs may accumulate and become dysfunctional. During chronic or repetitive infections, it is likely that both normal and dysfunctional atMBCs are present, which could potentially explain some of the seemingly discrepant results that have been obtained.

5. Concluding remarks

AtMBCs remain an incompletely understood B cell population in the immune response to *Plasmodium.* There is evidence for a functional role of atMBCs in modulating the immune response, as well as for contributing to parasite antigen-specific responses and malaria-associated anaemia. As such, the precise function(s) of atMBCs remains elusive. To complicate matters, the true heterogeneity of the B cell compartment is becoming increasingly appreciated as new subpopulations of B cells with distinct phenotypes are identified. Within the atMBC population are subpopulations, defined by differences in surface marker expression, that likely also differ in function.

Conflicting reports on atMBC functionality highlight the need for more in-depth functional studies focused on further defining the role of atMBCs in the immune response to infection. Focus on *P. falciparum*-specific B cells and methods to isolate these cells remain a priority as atMBCs and classical MBCs in this population may behave differently in response to infection than corresponding non-*P. falciparum*-specific B cells. Additionally, increased efforts to characterise the similarities and differences between human and mouse atMBCs will be invaluable in the study of these cells. Until the relationship between these populations is understood, correlating murine and human study data will be difficult.

Future studies investigating the factors that stimulate alterations in the transcriptional and surface marker profiles of atMBCs will assist in teasing out their developmental pathway during infection. In this vein, epigenetic studies probing for differences in gene expression and regulation may provide valuable insight into the evolutionary path of atMBCs relative to naïve and classical MBCs, similar to studies on the development of memory CD8^{+} T cells (Dogra et al., 2016; Akondy et al., 2017). Investigation of atMBC plasticity and methods to either reverse loss of function or stimulate the development of atMBCs will be valuable in vaccine development. Understanding how natural immunity to malaria is acquired will help direct efforts to design effective vaccines. As the potential positive or negative impacts of atMBCs in the development of natural immunity remain disputed, it is important to focus efforts on understanding how these cells can be harnessed to improve vaccine efficacy.

Acknowledgements

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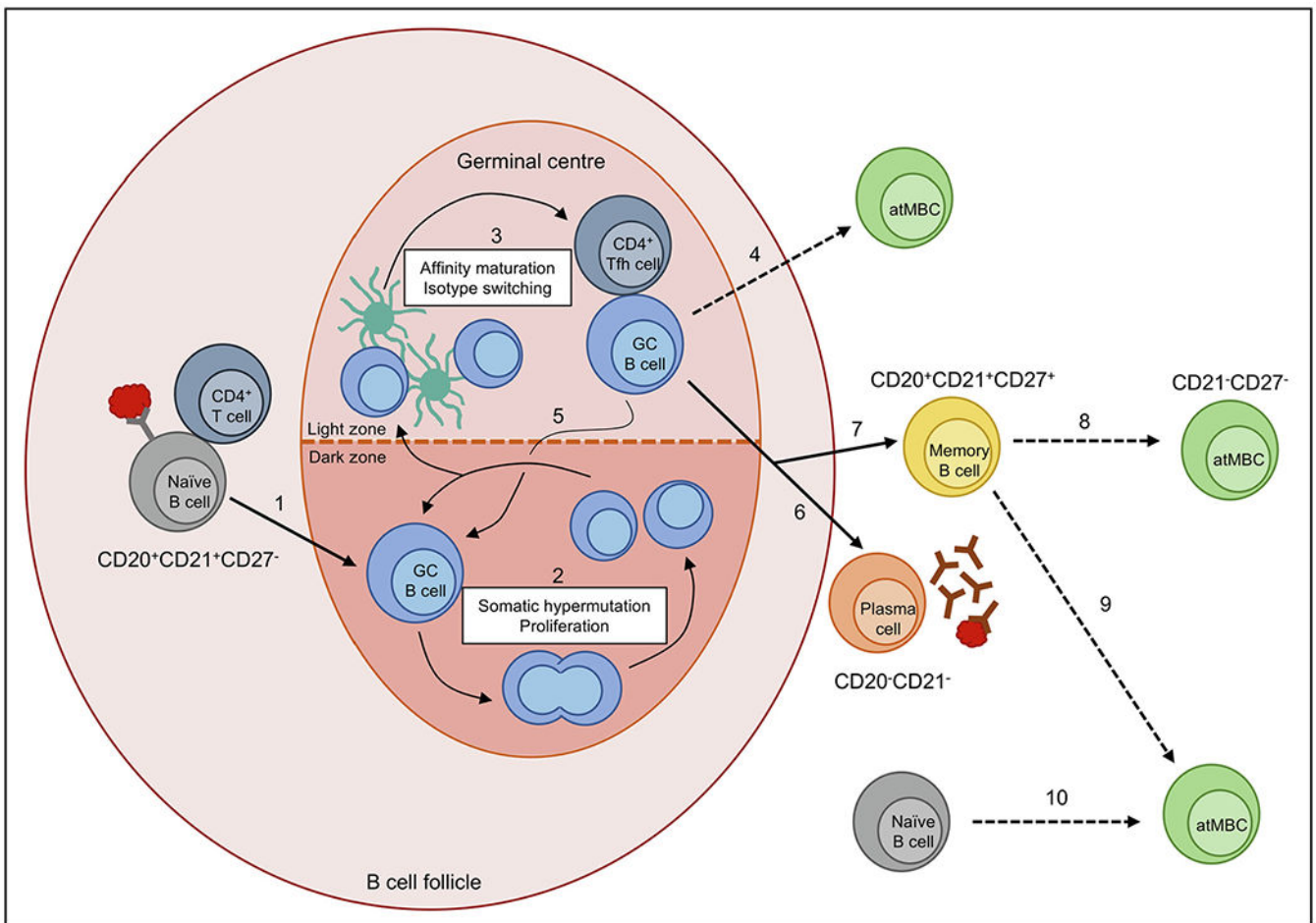
Fig. 1.
Potential pathways of atypical memory B cell (atMBC) development. In response to *Plasmodium* infection, antigen-recognising naïve B cells interact with antigen through B cell receptors (BCRs) and receive survival signals from CD4$^+$ T cells, which drive naïve B cells to enter germinal centres (GCs) (1). In the GC, these B cells undergo proliferation and somatically hypermutate the BCR to increase antigen affinity (2). In the light zone of the GC, B cells with high affinity are selected through interactions with antigen-presenting follicular dendritic cells and CD4$^+$ T follicular helper (Tfh) cells before undergoing class switch recombination (3). Poor Tfh cell help during this step may drive B cell differentiation into atMBCs (4). B cells can undergo multiple rounds of affinity maturation (5) before exiting the GC. Outside of the GC, B cells can differentiate into plasma cells (6) which produce high affinity antibodies to control the infection, or differentiate into long-lived memory B cells (7) which remain in circulation to respond to subsequent infections. Upregulation of inhibitory receptors and reduced BCR signalling driven by poorly understood mechanisms may promote the development of atMBCs from classical MBCs (8), representing an abnormal immune response. Alternatively, atMBCs could arise during activation of MBCs in a normal response to infection via an unknown mechanism (9). Finally, similar to DN2 cells in lupus, atMBCs may represent a population of pre-antibody secreting cells originating from the extra-follicular activation of naïve B cells (10).
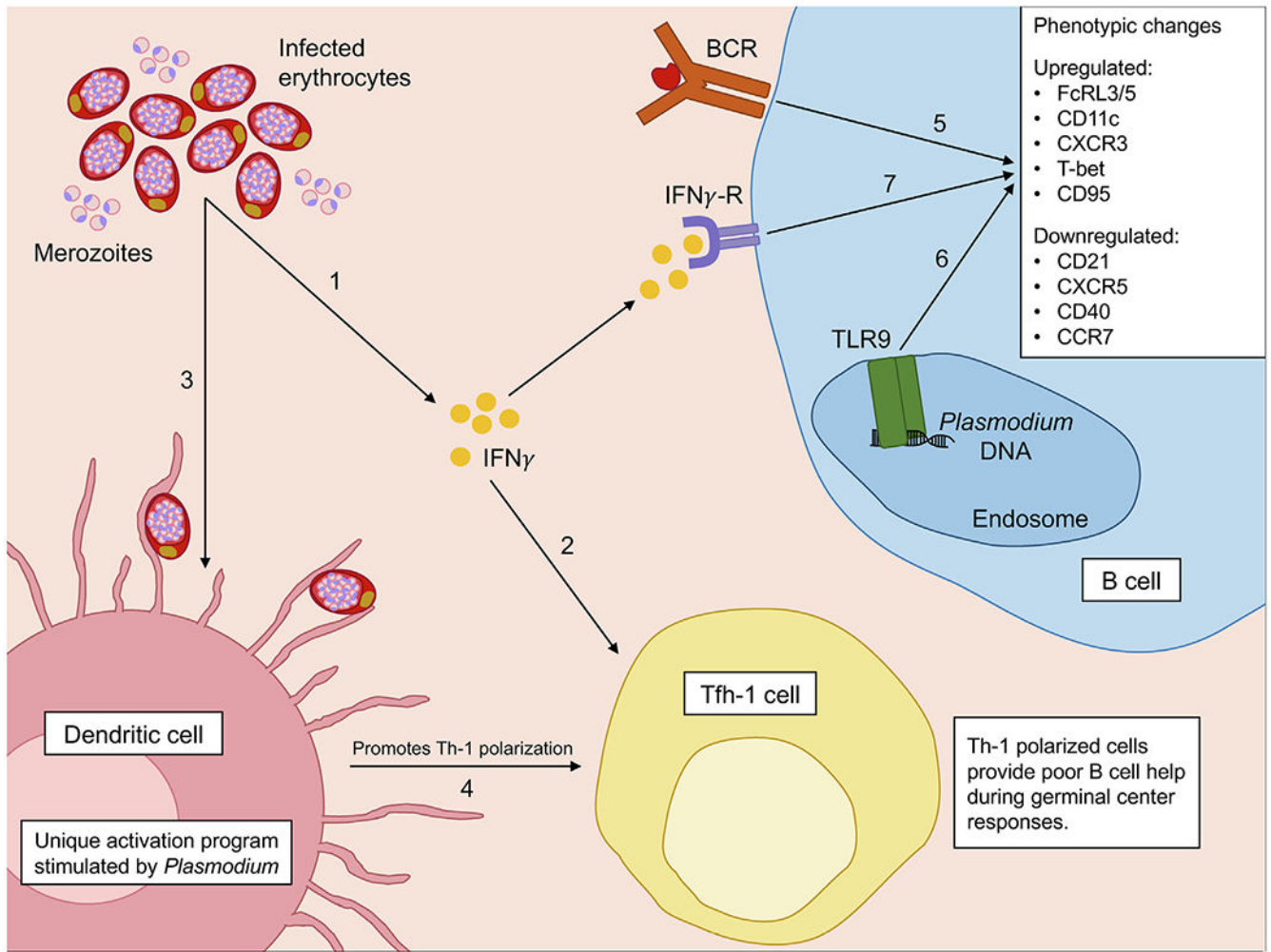
Fig. 2.
Potential drivers of the atypical memory B cell (atMBC) phenotype in response to
Plasmodium infection. *Plasmodium* infection activates the immune system in several
distinct, yet complimentary, ways which seem to promote the development of atMBCs.
Infected erythrocytes stimulate increased IFNγ production by innate immune cells (1). High
serum levels of IFNγ promote the development of Th-1 polarised T follicular helper (Tfh-1)
cells which provide poor B cell help during germinal centre reactions (2). In response to
infected erythrocytes, dendritic cells undergo a unique activation programme (3) also
resulting in the promotion of Th-1 polarised Tfh-1 cells (4). Crosslinking of the B cell
receptor (BCR) by antigen (5) and activation of Toll-like receptor-9 (TLR9) by *Plasmodium*
DNA (6) both contribute to the upregulation of transcription factor T-bet, impaired BCR
signalling, and development of an atypical phenotype. T-bet expression in B cells can also be
induced by IFNγ signalling through the IFNγ receptor (IFNγ-R) (7).

Table 1

Potential roles of atypical memory B cells in the immune response to *Plasmodium*.

Potential Role	Experimental Observations in Human Studies Supporting Potential Role	Antigen-specific	AtMBC [a] Definition	Reference
Immune Regulators	BCR crosslinking by FcRL5 and immune complexes may reduce BCR signalling	No	CD19+CD10+CD21−CD27−IgD−IgG+	Sullivan et al., 2015
	T-bet+ atMBCs upregulated markers important for CD4+ T cell interactions	No	CD19+CD21−CD27−Tbet^hi	Obeng-Adjei et al., 2017
		No	CD19+Tbet+	Ambegaonkar et al., 2019
Dysfunctional B Cells	Reduced antibody production in vitro	No	CD19+CD21−CD27−	Portugal et al., 2015
		No	CD19+CD10−CD21−CD27−IgD−IgG+	Sullivan et al., 2015
	Downregulation of BCR signalling pathways compared with cMBCs	No	CD19+CD21−CD27−	Portugal et al., 2015
		No	CD19+CD21−CD27−Tbet^hi	Obeng-Adjei et al., 2017
		No	CD19+Tbet+	Ambegaonkar et al., 2019
	Reduced proliferation	No	CD19+CD21−CD27−	Portugal et al., 2015
	Reduced cytokine production	No	CD19+CD21−CD27−	Portugal et al., 2015
	High frequency of extrafollicular cells in lymph node associated with low HIV-1 serum neutralisation	No	CD19^hiT-bet^hi	Austin et al., 2019
Activated B Cells	CD11c+ B cell expansion and contraction observed in malaria patients	No	CD19+CD21−CD27−	Sundling et al., 2019
	Atypical markers induced in vitro by stimulation with IFNγ	No	CD19+Tbet+	Ambegaonkar et al., 2019
		No	IgD−CD27−CD11c+CXCR5−	Zumaquero et al., 2019
	AtMBCs transiently arise in response to influenza vaccination	No	CD21^loCD27−FcRL5+CD85j^hiCD62L^lo	Andrews et al., 2019
Pre-antibody Secreting Cells	pBLNK increased compared with unstimulated cells following BCR engagement	No	IgD−CD21−CD27−CXCR5−CD11c+IgA−	Jenks et al., 2018
		No	CD19+CD21−CD27−Tbet^hi	Obeng-Adjei et al., 2017
		No	CD19+Tbet+	Ambegaonkar et al., 2019
	DN2 B cells differentiated into ASCs in the absence of BCR crosslinking	No	IgD−CD27−CD11c+CXCR5−	Zumaquero et al., 2019
	Secretory Ig transcripts found in atMBCs but not in cMBCs	No	CD19+CD21−CD27−Ig+	Muellenbeck et al., 2013
Autoreactive B Cells	Stimulated DN2 B cells differentiated into autoreactive plasma cells	No	IgD−CD21−CD27−CXCR5−CD11c+	Jenks et al., 2018
	Antibodies from malaria-associated atMBCs have increased self- and polyreactivity	GMZ2	CD19+CD21−CD27−Ig+	Muellenbeck et al., 2013
	atMBCs secrete autoantibodies upon in vitro stimulation	No	CD19+FcRL5+T-bet+	Rivera-Correa et al., 2019

atMBCs, atypical memory B cells; BCR, B cell receptor; cMBCs, classical memory B cell; ASCs, antibody-secreting cells.