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Atypical memory B cells in human chronic infectious diseases: an interim report

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Abstract

Immunological memory is a remarkable phenomenon in which survival of an initial infection by a pathogen leads to life-long protection from disease upon subsequent exposure to that same pathogen. For many infectious diseases, long-lived protective humoral immunity is induced after only a single infection in a process that depends on the generation of memory B cells (MBCs) and long-lived plasma cells. However, over the past decade it has become increasingly evident that many chronic human infectious diseases to which immunity is not readily established, including HIV-AIDS, malaria and TB, are associated with fundamental alterations in the composition and functionality of MBC compartments. A common feature of these diseases appears to be a large expansion of what have been termed exhausted B cells, tissue-like memory B cells or atypical memory B cells (aMBCs) that, for simplicity's sake, we refer to here as aMBCs. It has been suggested that chronic immune activation and inflammation drive the expansion of aMBCs and that in some way aMBCs contribute to deficiencies in the acquisition of immunity in chronic infectious diseases. Although aMBCs are heterogeneous both within individuals and between diseases, they have several features in common including low expression of the cell surface markers that define classical MBCs in humans including CD21 and CD27 and high expression of genes not usually expressed by classical MBCs including T-bet, CD11c and a variety of inhibitory receptors, notably members of the FcRL family. Another distinguishing feature is their greatly diminished ability to be stimulated through their B cell receptors to proliferate, secrete cytokines or produce antibodies. In this review, we describe our current understanding of the phenotypic markers of aMBCs, their specificity in relation to the disease-causing pathogen, their functionality, the drivers of their expansion in chronic infections and their life span. We briefly summarize the

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features of aMBCs in healthy individuals and in autoimmune disease. We also comment on the possible relationship of human aMBCs and T-bet^{+}, CD11c^{+} age/autoimmune-associated B cells, also a topic of this review volume.

INTRODUCTION

Throughout recorded history one of the most feared causes of death was infectious diseases that in epidemic proportions have the power to decimate entire societies. From the writings of the historian Thucydides describing the plague of Athens in 430 B.C. it is clear that it was appreciated even at that time that individuals who survive an infection are subsequently protected from future infections. Thucydides wrote: “Yet it was with those who had recovered from the disease that the sick and the dying found most compassion. These had no fear for themselves, for the same man was never attacked twice – never at least fatally” (1). We now understand that Thucydides was describing the acquisition and function of immunological memory and indeed, for most infectious diseases, those that survive are immune to disease upon re-exposure to the same pathogens. However, not all pathogens induce protective immunity, and by definition, cause chronic infectious diseases including HIV-AIDS, malaria, TB and hepatitis viruses. Although these are complex diseases and we do not in any case fully understand the cellular or molecular basis of the failure to rapidly develop immunologic memory, it is now clear that many chronic infections are associated with fundamental differences in the composition of the memory B cell (MBC) compartment. It has been postulated that such alterations in immune cell populations may contribute to the poor acquisition of immunity to such diseases. In this review we focus on a phenomena that appears to be in common between HIV, *Plasmodium falciparum*, Mycobacterium tuberculosis (Mtb) and Hepatitis C infections, namely, the large expansion of a subpopulation of B cells (up to 50% of all circulating B cells) that normally represents only a small percent (approximately 3–5%) of peripheral blood B cells in healthy individuals. These B cells have several characteristics of MBCs and are referred to variously as exhausted MBCs, tissue-like memory (TLM) B cells in HIV-AIDS or atypical MBCs (aMBCs) in malaria. Here we refer to these cells as aMBCs for simplicity’s sake. It is becoming increasingly clear that aMBCs are not a homogenous population of B cells, but rather show significant heterogeneity both within an individual for a given disease as well as between different chronic infections. It is possible that aMBCs contain a variety of subsets with specialized functions that differentially expand under different conditions. HIV-AIDS, malaria and TB, that together cause more than five million deaths a year, continue to elude conventional vaccine development. Thus, it is a public health priority to understand the cellular and molecular basis of the function of aMBCs and the drivers of their differentiation, to improve our chances of developing effective, life-saving vaccines for these deadly diseases.

We now understand that MBCs develop in secondary lymphoid organs such as the spleen and lymph nodes (2–5) (Figure 1). Naïve B cells encounter antigens in the follicles in many cases as immune complexes or complement fixed antigens bound to Fc receptors, complement receptors or scavenger receptors on the surface of dendritic cells (DC). B cells engage antigen through their B cell receptors (BCRs) that initiates signaling cascades that

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induce the transcription of a variety of genes associated with B cell activation. The BCR bound antigen is then internalized into specialized compartments where the antigen is processed and then presented on MHC class II molecules on the B cell surface. At the B-T cell border of the follicle, B cells engage $CD4^+$ T cells that have been activated by antigen processed and presented by DCs to initiate differentiation toward T follicular helper (Tfh) cells. If the quality of the T-B interaction is high, the T cells further differentiate into Tfh cells and the Tfh cells and B cells proliferate and enter germinal centers (GCs). If the interaction is in some way inadequate, B cells are not induced to enter the GCs but rather proliferate and differentiate into short-lived antibody secreting plasma cells (PCs) and what have been termed GC-independent MBCs (3). B cells that enter the GC first enter the GC dark zone (DZ) where they clonally expand by proliferation and undergo class switching and somatic hypermutation (SHM). B cells exit the DZ to enter the light zone (LZ) where B cells with the highest affinity for antigen are ultimately selected by the Tfh cells to differentiate into long-lived MBCs and PCs, in a process that depends on the ability of B cells to capture antigen from follicular DC (fDC) and present it to Tfh cells.

In this review we describe the characteristics of aMBCs, the role of pathogen-derived antigens in their expansion, the functionality of these cells, their longevity and cellular and molecular mechanisms that drive their differentiation. We briefly comment on aMBCs in autoimmune disease and in healthy individuals and the similarities between aMBCs and age-associated B cells (ABCs) in mouse models. Lastly, we propose a model for the expansion of aMBCs during chronic infectious diseases in the framework of the emerging view of the normal development of B cell memory.

HIV-AIDS

In HIV-AIDS there are striking changes in both the MBC compartment and in the dynamics and quality of the peripheral blood plasmablasts. HIV-associated aMBCs were first identified in 2008 by Moir *et al.* (6) as an abnormally expanded mature B cell population in the blood of HIV-viremic patients. Moir *et al.* used two B cell surface markers, namely CD21 and CD27, to identify three circulating MBC populations in HIV-infected individuals with persistent viremia. Conventional MBCs, also referred to as resting MBCs, that represent the majority of MBCs in the peripheral blood of healthy individuals, express both CD21 and CD27. In contrast two populations of B cells that are not present in substantial numbers in healthy individuals are either $CD27^+$ $CD21^{Lo}$, referred to as activated MBCs or $CD27^-$ $CD21^{Lo}$, identifying aMBCs. Of note, both activated MBCs and aMBC express higher levels of the pan B cell marker CD20 than other B cells populations that circulate in the peripheral blood. The HIV-associated aMBCs also express markers that distinguish them from other B cell populations, namely CD11c, T-bet and several inhibitory receptors (7, 8). The aMBCs identified in the peripheral blood of HIV-viremic individuals were initially named tissue-like memory B cells to reflect their similarity to a population of MBCs described by Cooper and colleagues that are resident within tonsil tissue (9). This population was defined by the expression of FcRL4, a member of the Fc receptor homologue family that has the potential to inhibit BCR signaling. Tonsil B cells that expressed FcRL4 appeared to represent a novel subpopulation of MBCs that lacked the classical CD27 marker for MBCs in humans and were $CD20^{Hi}$ $CD21^{Lo}$. $FcRL4^+$ MBCs had a distinctive morphology,

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function and tissue distribution and had undergone similar levels of isotype switching and SHM as compared to conventional $CD27^+$ MBCs. $FcRL4^+$ MBCs failed to proliferate in response to BCR crosslinking but secreted Ig in response to T cell cytokines. The aMBCs in HIV-viremic individuals also expressed FcRL4 and shared other properties of $FcRL4^+$ tonsil MBCs including refractoriness to stimulation through their BCRs. Moir *et al.* (6) also reported that aMBCs expressed patterns of inhibitory receptors and markers associated with homing to sites of inflammation similar to those described for antigen-specific T cell exhaustion (10) and thus used the term 'exhausted MBCs'. Notably, siRNA-mediated knock-down of FcRL4 and other known or putative inhibitory receptors, including Siglec-6, CD32b, LAIR1, CD85, CD72, CD22 and PD-1, led to enhanced BCR-mediated proliferation and increased differentiation to HIV-specific antibody secreting cells (11).

Particularly informative were studies comparing the HIV-specific antibody repertoires in MBCs of HIV-viremic individuals. Within HIV-infected individuals, HIV-specific B cells were enriched in activated memory and aMBC populations in contrast to B cells specific for pathogens other than HIV, including influenza and tetanus, that were more concentrated in resting MBC compartments (12). This finding provided the first evidence that the HIV virus *per se* was a driver of aMBC expansion in infected individuals. Indeed, consistent with these data is the observation that the frequency of aMBCs decreased significantly in HIV-infected individuals in the months following antiretroviral treatment and reduction in HIV viremia. A number of longitudinal and cross-sectional studies have confirmed these findings (7, 13–17). We can also infer from these data that the life span of aMBCs in the absence of HIV viremia is on the order of months, although the longevity of aMBCs in viremic individuals is not known and full normalization of aMBC is in the order of years (18). A recent Ig repertoire analysis provided evidence that resting MBCs and aMBCs share clonal families of VH suggesting a plasticity between MBC populations (19). These studies also showed that HIV-specific mAbs from aMBCs have lower frequencies of SHM as compared to resting MBCs despite having undergone a greater number of cell divisions and that the degree of SHM correlated with HIV-neutralizing activity. Thus, HIV neutralization was significantly higher in the antibodies expressed by resting MBCs as compared to aMBCs, suggesting that aMBC expansion may contribute to inadequate antibody responses in HIV viremic individuals.

Malaria

Malaria is a deadly disease caused by parasites of the *Plasmodium* species, the deadliest of which, *P. falciparum*, accounts for approximately 400,000 deaths each year, mostly among young African children (20). Antibodies play a key role in naturally acquired immunity to malaria as demonstrated by the passive transfer of antibodies from malaria immune adults to children with clinical malaria, resulting in the reduction of both parasitemia and fever in these children (21). This study also provided evidence that adults living in malaria-endemic areas acquire protective antibodies and we now understand from longitudinal studies of children in malaria endemic Africa that this process is remarkably slow requiring years of repeated *P. falciparum* infections (22). The acquisition of resistance to clinical malaria with age is accompanied by an increase in both malaria-specific classical MBCs and long-lived antibody responses (23, 24). However, age is also accompanied by a large expansion of what were termed aMBCs defined by flow cytometry immune-phenotyping as $CD10^-$ $CD19^+$

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$CD20^{+}$ $CD21^{Lo}$ $CD27^{-}$ $CD11c^{Hi}$ (25, 26). These aMBCs also express an array of inhibitory receptors including CD85, CD32B and CD22 as well as CD45 and the chemokine receptor CXCR3 and decreased expression of CD35, CD40, CXCR5 and CCR7. As compared to naïve B cells, aMBCs express less of the inhibitory receptor LAIR1 which is intriguing given the recent surprising finding of a LAIR1 insertion into broadly-reactive *P. falciparum*-specific antibodies in malaria exposed individuals (27). Both aMBCs and classical MBCs have similar percentages of cells expressing unswitched (30%) versus IgG switched (40–70%) BCRs (25). Atypical MBCs are a minority of MBCs in healthy U.S. adults (perhaps 3–6%) but in children and adults in malaria endemic areas these can represent over 50% of all circulating B cells (25).

By genome-wide expression profiling of purified B cells from Mali (26) and a cohort of individuals living in an area of high malaria transmission in Uganda (28), aMBCs were shown to have a transcriptional profile that distinguished them from naïve and classical MBCs. aMBCs showed differentially reduced expression of genes in the BCR signaling pathway and costimulatory molecules and increased expression of inhibitory receptors as well as suppressed expression of genes encoding pro-apoptotic factors. Of interest is the observation of a high degree of overlap in the gene expression patterns of aMBCs from malaria exposed individuals and HIV-associated aMBCs with functional overlap in most categories with the exception of apoptosis (28). In contrast to HIV-associated aMBCs that express FcRL4, aMBCs from malaria exposed cohorts show differentially increased expression of FcRL5 (26, 28). Using serological reagents available at the time it was first reported that malaria-associated aMBCs expressed FcRL4 (25) but with improved reagents and molecular analysis it is now clear that aMBCs in malaria express FcRL5 (and FcRL3) and not FcRL4 (26, 28). This observation is of interest as it suggests that HIV-associated aMBCs and aMBCs in malaria, despite sharing several phenotypic and functional features have differentiated to express different members of the FcRL family of receptors. It remains to be determined if differential expression of FcRL4 versus FcRL5 influences the function of these two subpopulations. To this point, we observed heterogeneity in the expression of FcRL5 within the aMBC population in malaria exposed individuals (26) and Sullivan *et al.* (28) reported that $FcRL5^{+}$ aMBCs were significantly more deficient in their ability to secrete antibody in response to BCR crosslinking and CpG stimulation as compared to $FcRL5^{-}$ aMBCs. These results suggest the possibility of a functional heterogeneity among aMBCs differentiating within an individual. Relevant to these observations, Li *et al.* (29) reported that FcRL5 expression distinguished two subsets of aMBCs in healthy individuals that have distinct gene expression profiles. $FcRL5^{+}$ aMBCs expressed more CD11c and inhibitory receptors as compared to $FcRL5^{-}$ aMBCs and responded poorly to multiple stimuli. Analysis of genome-wide expression data showed that aMBCs from the peripheral blood of adults with lifelong malaria exposure also expressed T-bet which distinguished aMBCs from classical MBCs (Obeng-Adjei *et al.* unpublished observations). In these studies, T-bet was also shown to be up-regulated in aMBCs in malaria exposed children which showed a bi-nodal distribution of T-bet expression (T-bet intermediate and T-bet high) with $T\text{-}bet^{Hi}$ expression correlating with the expression of aMBC markers.

Immune repertoire next generation sequencing of the VH and VL in naïve, classical MBCs and aMBCs from malaria-exposed individuals showed no significant differences in their

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SHM rate (26, 30). Both populations had undergone a similar number of cell divisions, had similar clonal expansion profiles and shared approximately 10% of their VH clones. These data suggest that aMBCs and classical MBCs are related and exhibit the plasticity noted for HIV-associated aMBCs. In contrast, Wardemann and colleagues (31) came to the conclusion that aMBCs and classical MBCs developed from different precursors based on an VH, VL gene repertoire analysis of approximately 200 *P. falciparum*-specific Ig sequences from adults in a highly endemic area of Gabon. They observed differences in the levels of SHM and little clonal relationship between aMBCs and classic MBCs. It will be of interest to learn if an analysis of a larger sample size confirms these results.

Several observations suggest that *P. falciparum* infection *per se* drives the expansion of aMBCs including a positive correlation between aMBC expansion and intensity of *P. falciparum* transmission in studies primarily carried out in Africa including Mali (25, 26, 32), Uganda (28, 33, 34), Kenya (35, 36), the Gambia (37) and Ghana (38). Of interest, in HIV-malaria coinfected Rwandan adults the expansion of aMBC was greater as compared to that in individuals infected with malaria alone (39). Moreover, Frosch *et al.* (40) showed that in HIV-infected Kenyan adults the proportion of *P. falciparum*-specific aMBCs in peripheral blood increased as a result of the loss of naïve and resting MBCs. A differential expansion of aMBC was observed in age-matched children living in similar villages in rural Kenya, with the exception of *P. falciparum* exposure that occurred only in the village in which children showed expansion of aMBCs (35). In addition, aMBCs have been reported in the peripheral blood of healthy European adults following experimental *P. falciparum* infection (41). Wardemann and colleagues (31) first showed that the VH and VL genes isolated from single *P. falciparum* antigen-binding aMBCs from malaria exposed individuals in Gabon encoded antibodies specific for the *P. falciparum* recombinant antigen, GMZ2. Most recently, using two different B cell tetramers containing the C-terminal region of the *P. falciparum*-encoded proteins, merozoite surface protein 1 (MSP1) and apical membrane protein 1 (AMA1), approximately 20% of MSP1- and AMA1-specific B cells were found in the aMBC compartment (42). Together these last two studies provide the most direct evidence that *P. falciparum* antigens play a role in the expansion of aMBCs in malaria.

Concerning the functionality of aMBCs, Muellenbeck *et al.* (31) first provided evidence that *P. falciparum* antigen-specific aMBCs spontaneously secreted *P. falciparum* specific Abs. However, the evidence for antibody secretion was based on the presence of Ig transcripts and mass spec data of circulating antibodies not on direct detection of secreted antibodies from aMBCs. In contrast, Portugal *et al.* (26) provided evidence that aMBCs obtained from uninfected adults living in a malaria endemic area had lost two key features of adaptive immune B cell functions, namely the ability to respond to BCR crosslinking by phosphorylating key BCR signaling molecules to initiate signaling cascades and to proliferate and differentiate to antibody or cytokine secreting cells following a variety stimuli including BCR crosslinking in combination with exposure to CD40 agonists, a Toll-like receptor 9 agonist and T cell cytokines. Sullivan *et al.* (28) reported that aMBCs were poor spontaneous producers of antibody and that higher surface expression of FcRL5 defined a distinct subset of aMBCs that failed to secrete antibody upon stimulation. In addition, in recent studies the level of T-bet expression correlated inversely with the phosphorylation of B cell signaling molecules (Obeng-Adjei *et al.* unpublished

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observations). Taken together these findings suggest that aMBC differentiation may involve the altered expression of T-bet that drives inhibitory receptor expression resulting in the loss of BCR signaling. Concerning the different results by Muellenbeck *et al.*, Portugal *et al.* and Sullivan *et al.*, it may be that under some conditions aMBCs maintain the ability to secrete antibodies, possibly reflecting heterogeneity in aMBC populations in individuals from different malaria endemic areas or who have different malaria exposure histories.

Recent studies have begun to explore the possible mechanisms underlying the expansion of aMBCs in malaria focusing on the role of inflammation and Tfh cells in this process (43) (Figure 2). Several recent observations bear on this issue. aMBCs that expressed T-bet at intermediate or high levels were shown to be more likely to express surface IgG3 as compared to T-bet-negative B cells which skewed toward IgG1 expression (Obeng-Adjei *et al.* unpublished observations). Moreover, total serum IgG3 had the greatest fold increase among IgG subclasses during acute malaria, which correlated with serum levels of the inflammatory cytokine IFN-γ. Based on these data we speculate that in the context of pediatric malaria, intermediate T-bet expression may contribute to IgG3 class switching, while T-bet 'overexpression' may play a role in aMBC differentiation.

These observations raise the question of the link between inflammation and aMBC expansion. A common theme emerging from recent studies is one in which malaria-induced TH1 inflammatory cytokines drive the activation of Tfh cells impaired in B cell helper function and the acquisition of TH1-like features in Tfh cells, resulting in GC dysfunction and suboptimal antibody responses (43). Because malaria can induce IFN-γ production in multiple cell types, it remains possible that IFN-γ from sources other than Tfh cells could drive T-bet expression in B cells *in vivo*. However, because of the proximity of Tfh cells and B cells in secondary lymphoid tissue, it seems plausible that Tfh-derived IFN-γ plays a greater role in driving T-bet expression in B cells. To this point, one BCR function that appears to be intact in aMBCs is the ability to internalize bound antigen into the cell and traffic it to acidic antigen processing compartments (S.K. Pierce, unpublished observations) indicating that aMBCs may have the potential to interact with Tfh cells. Obeng-Adjei *et al.* (44) recently provided evidence that Malian children have a population of resting Tfh cells (PD-1^+ $CXCR5^+$ $CD4^+$) in circulation that contain both $CXCR3^-$ Tfh cells that are superior in providing help to B cells and TH1-polarized $CXCR3^+$ Tfh cells that are less effective in B cell help. In longitudinal studies they observed that acute febrile malaria, that is accompanied by increases in TH1 inflammatory cytokines, resulted in the preferential activation of $CXCR3^+$ Tfh. These authors proposed that $CXCR3^+$ Tfh may play a role in the slow acquisition of antibody-mediated immunity in malaria and perhaps even play a role in driving aMBC expansion. Indeed, recent studies provided evidence that IFN-γ, produced by activated $CXCR3^+$ Tfh cells in response to *P. falciparum*-infected red blood cell lysates, plays an essential role, along with BCR crosslinking, in the generation of B cells *in vitro* that phenotypically and functionally resemble aMBCs (Obeng-Adjei *et al.* unpublished observations).

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Hepatitis C virus

Hepatitis C virus (HCV) chronically infects over 170 million people worldwide (45) and over 70% of HCV-infected individuals develop hepatitis and 20–30% of these individuals progress to liver cirrhosis (46). HCV infections are characterized by persistent B cell activation and profound hypergammaglobulinemia consisting of non-HCV specific antibodies (47). Recent studies have suggested that B cell populations similar to aMBCs are expanded in chronic HCV infections. Oliviero *et al.* (48) observed an increase in aMBCs (characterized as $CD10^{-}$, $CD19^{+}$ $CD21^{Lo}$, $CD27^{-}$) in chronic HCV-infected individuals as compared to healthy controls and a correlation between the proportion of aMBCs that expressed FcRL4 and the degree of liver inflammation. In both cirrhotic and non-cirrhotic HCV infections, Doi *et al.* (49) observed an expansion of aMBCs ($CD19^{+}$ $CD27^{-}$ $CD21^{-}$) relative to healthy controls. The aMBCs proliferated poorly in response to BCR and CD40 crosslinking in the presence of CpG, IL-2 and IL-10 but secreted isotype-switched antibodies in response to CD40 crosslinking alone in the presence of CpG, IL-2 and IL-10. Subsequently, these researchers found that HCV-infected individuals with nonfibrotic liver disease, cirrhosis and liver cancer had expanded populations of aMBCs that expressed T-bet (50). The maintenance of these T-bet^{+} aMBCs depended on HCV viremia as effective antiviral therapy resulted in a marked reduction in the number of T-bet^{+} aMBCs.

Up to 50% of HCV infections are accompanied by the B cell lymphoproliferative disorder, mixed cryoglobulinemia (MC), characterized by the aberrant production of rheumatoid factor (RF)-containing immune complexes and a biased use of the RF-encoding VH1-69. It has been proposed that RF bearing B cells are activated by persistently high levels of HCV-containing immune complexes (51). Abnormal B cell lymphoproliferation is dependent on the presence of HCV, as elimination of HCV results in resolution of MC (52). Charles *et al.* (53) characterized VH1-69 expressing B cells from HCV-infected MC patients and determined that these were predominantly $CD20^{Hi}$ $CD10^{-}$ $CD21^{Lo}$ $CD27^{+}$ $CD11c^{+}$ $FcRL4^{Hi}$ IL-$4R^{Lo}$. $CD21^{Lo}$ B cells whether from healthy individuals or HCV-infected MC individuals were deficient in their ability to respond to BCR crosslinking by mobilizing Ca^{2+}. In addition, $CD27^{+}$ $CD21^{Lo}$, VH1-69-expressing B cell failed to differentiate into antibody-secreting plasmablasts in response to CD40L, IL-2 and IL-10. Kong *et al.* (54) observed an expansion of aMBCs ($CD27^{-}$ $CD21^{-}$) in both chronic HCV-infected individuals and HCV-related MC patients that was accompanied by an increase in the frequencies of several T cell subpopulations including Tfh cells. In addition, HCV-associated MC was shown to be accompanied by an increase in $CD21^{-}$ marginal zone (MZ) B cells that expressed FcRL5 (55). Visentini *et al.* (56) reported that $CD21^{-}$ VH1-69-expressing B cells that were functionally exhausted, failing to respond to either BCR or TLR stimulation, were also expanded in MC. These authors proposed that these cells may have differentiated from MZ B cells under the conditions of chronic HCV infection.

TB

The impact of chronic intracellular bacterial infections including that of *Mycobacterium tuberculosis* (Mtb), on B cells has not been studied in detail in part because there was little evidence for a direct role for antibodies in immunity to these pathogens (57). However, interest in the B cell biology of Mtb infections increased with the demonstration that B cells

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play key roles in the regulation of T cell responses in TB. Joosten *et al.* (58) analyzed the phenotype and function of B cells in individuals with latent TB infection (LTBI) or active TB and in individuals successfully treated for TB. Both individuals with TB and LTBI showed expansion of B cells with an aMBC phenotype, namely $CD21^{-}$ $CD27^{-}$ or IgD^{-} $CD27^{-}$, and a large portion of the $CD21^{-}$ $CD27^{-}$ B cells in some individuals with TB and LTBI expressed FcRL4, CD85, and CD22. Overall, $CD19^{+}$ B cells from TB and LTBI patients were refractory to stimulation by CD40 and BCR crosslinking. These alterations in B cell populations appear to be dependent on the presence of Mtb and disappeared upon successful treatment of the infection.

aMBCs in healthy individuals

In general, little is known about T-bet^{+} B cells in healthy individuals (59). T-bet has been detected in MBCs and plasmablasts in healthy adults, but at lower levels than other T-bet^{+} lymphocytes (60). T-bet expression in circulating $CD21^{-}CD27^{-}$ has been described in healthy adults, in whom $CD21^{-}CD27^{-}$ B cells are a relatively rare population (8, 29) and T-bet^{+}, $CD11c^{+}$ B cells appear to be expanded in healthy elderly individuals (61). But whether CD21-$CD27^{-}$ T-bet^{+} B cells in healthy adults represent the same population of $CD21^{-}CD27^{-}$ T-bet^{+} aMBCs that are expanded in settings of chronic infections remains unclear. Interestingly, a recent study showed that yellow fever and vaccinia vaccinations stimulated an acute T-bet^{+} B cell response and that the T-$bet^{Hi}CD85j^{Hi}$ population may function as an early responder during acute viral infections (8). Of note, the same study reported that HIV infection maintains an expanded T-bet^{+} B cell population that was primarily comprised of T-$bet^{hi}CD85j^{hi}$ B cells.

aMBCs in autoimmune disease

Several recent studies have described T-bet expression in B cells of individuals with autoimmune diseases. For example, transcriptome analysis of $CD21^{-/Low}$ versus $CD21^{+}$ mature naïve B cells from subjects with rheumatoid arthritis or common variable immunodeficiency found that T-bet expression was upregulated in $CD21^{-/Low}$ B cells (62). Similarly, transcriptome analysis of $CD19^{+}$ B cells isolated from individuals with systemic lupus erythematosus revealed increased T-bet expression as compared to $CD19^{+}$ B cells of healthy controls (63). Importantly, T-bet^{+} $CD19^{+}$ B cells in individuals with autoimmune diseases appear to be able to produce proinflammatory cytokines and autoreactive antibodies (64–66) in contrast to HIV and malaria-associated aMBCs that exhibit markedly reduced cytokine and antibody production capacity (6, 11, 26). Therefore, T-bet^{+} B cells that arise in humans in the context of chronic infections versus autoimmunity may differ phenotypically and functionally, although further studies are needed to determine if this is a consistent pattern.

ABCs in mice

As reviewed in this volume, T-bet^{+}, $CD11c^{+}$ B cells that express unique phenotypic and functional characteristics, termed age-associated B cells (ABCs), appear in mice with age, autoimmunity and viral infections (67–69). T-bet expression in this context was shown to be driven by IFN-γ (70, 71). ABCs are generated through the interplay of IL-4, IL-21, and IFN-γ in concert with Toll-like receptor engagement (72), and have been shown to play a

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role in the pathogenesis of lupus-like autoimmunity (69) and anti-viral immunity (73, 74). Although mouse ABCs are similar to human aMBCs in that they upregulate T-bet and CD11c, and downregulate CD21, unlike aMBCs (26), murine ABCs proliferate in response to TLR agonists, produce IL-10 and IFN-γ and differentiate into ASCs—distinct functional profiles that call into question the relatedness of mouse ABCs and human aMBCs that are associated with chronic infections. Instead, the available evidence suggests that murine ABCs more closely resemble the phenotype and function of T-bet$^+$ B cells in humans with autoimmune diseases described above (63, 64, 66, 75). Although differences in IgG subclasses between mice and humans make direct comparisons difficult, in mice, T-bet is a selective inducer of IFN-γ-mediated class switching to IgG2a (70, 76, 77), which is functionally similar to human IgG1 and IgG3 in terms of FcR binding and complement fixation capacity. Interestingly, a recent study showed that HIV infection drives the expansion and maintenance of T-bet$^+$ B cells that correlate with an overrepresentation of surface-expressed and soluble IgG1 and IgG3 (8). Therefore, there may be a consistent theme in mice and humans: that IFN-γ drives T-bet expression in B cells, which promotes class switching to IgG subclasses that are potent triggers of effector mechanisms.

SUMMARY

As described here, over the last decade it has become increasingly evident that for many chronic human infectious diseases humoral immunity is not readily acquired. Moreover, these chronic infections are accompanied by fundamental changes in the MBC compartment. Here we focused on aMBCs that for the most part have the phenotype: CD10$^-$ CD19$^+$ CD21$^-$ CD27$^-$ T-bet$^+$, CD11c$^+$ and FcRL$^+$. aMBCs also express an array of inhibitory receptors and appear refractory to stimulation through their BCR, TLRs, CD40 and cytokine receptors and consequently cannot be readily induced to proliferate or secrete antibodies or cytokines. What function, if any, aMBCs provide in chronic human diseases is at present only a matter of speculation. It has been postulated that aMBCs either interfere with the acquisition of functional MBCs or at least are an epiphenomenon of the negative impact of chronic infection and inflammation of the development of normal MBC. The expansion of aMBCs appear to be driven, at least in part, by the infecting pathogen *per se*, as shown by the dependence of aMBCs on the presence of the infectious disease-causing pathogen and the accumulation of pathogen-specific B cells in the aMBCs subpopulation. aMBCs highly express T-bet, and the exposure of naïve B cells to Tfh-1 cells correlates with the expansion of T-bet^{hi} B cells. Because the development of long-lived MBCs depends on the function of Tfh cells, there is considerable interest in exploring the possibility that TH1-produced cytokines in chronic infections drive the expansion of Tfh cells with impaired B cell helper function and promote the differentiation of pre-Tfh cells to acquire TH1 features rather than fully functional Tfh cells. The net result of which is GC dysfunction, suboptimal antibody responses and the expansion of aMBCs (Figure 2). It will be of interest in future studies to employ methods such as siRNA gene silencing and ChIP-seq to determine whether T-bet plays a causal role in aMBC differentiation, and if so, how it directly affects B cell programming. Moreover, as described for CD4$^+$ T cell differentiation (78), it is likely that several transcription factors combine to generate aMBCs (79). A high priority should also be placed on ascertaining the 'plasticity' (80) of aMBCs, and whether and how their

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apparent loss of function can be reversed. In this regard, Kardava *et al.* (11) showed that HIV-associated human B cell exhaustion could be attenuated by siRNA downregulation of inhibitory receptors, particularly Fc receptor-like-4 (FCRL4) and sialic acid-binding Ig-like lectin 6. However, emerging data suggests that the array of inhibitory receptors expressed by aMBCs varies by disease; for example, malaria-associated aMBCs upregulate the expression of FCRL3 and FCRL5 rather than FCRL4 (26). An important commonality among the chronic infectious diseases described here, namely, HIV-AIDS, malaria, HCV and TB, is that each has eluded our ability to generate an effective vaccine. The hope is that a detailed understanding of the cellular and molecular mechanisms underlying the expansion and function of aMBCs will contribute to ongoing efforts in vaccine development.

References

1. Silverstein, AM. A History of Immunology. Academic Press; San Diego, CA: 1989.
2. De Silva NS, Klein U. Dynamics of B cells in germinal centres. Nat Rev Immunol. 2015; 15:137–148. [PubMed: 25656706]
3. Kurosaki T, Kometani K, Ise W. Memory B cells. Nat Rev Immunol. 2015; 15:149–159. [PubMed: 25677494]
4. Mesin L, Ersching J, Victora GD. Germinal Center B Cell Dynamics. Immunity. 2016; 45:471–482. [PubMed: 27653600]
5. Bannard O, Cyster JG. Germinal centers: programmed for affinity maturation and antibody diversification. Curr Opin Immunol. 2017; 45:21–30. [PubMed: 28088708]
6. Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA, Roby G, Kottilil S, Arthos J, Proschan MA, Chun TW, Fauci AS. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med. 2008; 205:1797–1805. [PubMed: 18625747]
7. Ferreira CB, Merino-Mansilla A, Llano A, Perez I, Crespo I, Llinas L, Garcia F, Gatell JM, Yuste E, Sanchez-Merino V. Evolution of broadly cross-reactive HIV-1-neutralizing activity: therapy-associated decline, positive association with detectable viremia, and partial restoration of B-cell subpopulations. J Virol. 2013; 87:12227–12236. [PubMed: 24006439]
8. Knox JJ, Buggert M, Kardava L, Seaton KE, Eller MA, Canaday DH, Robb ML, Ostrowski MA, Deeks SG, Slifka MK, Tomaras GD, Moir S, Moody MA, Betts MR. T-bet+ B cells are induced by human viral infections and dominate the HIV gp140 response. JCI Insight. 2017; 2
9. Ehrhardt GR, Hsu JT, Gartland L, Leu CM, Zhang S, Davis RS, Cooper MD. Expression of the immunoregulatory molecule FcRH4 defines a distinctive tissue-based population of memory B cells. J Exp Med. 2005; 202:783–791. [PubMed: 16157685]
10. Wherry EJ, Ha SJ, Kaech SM, Haining WN, Sarkar S, Kalia V, Subramaniam S, Blattman JN, Barber DL, Ahmed R. Molecular signature of CD8+ T cell exhaustion during chronic viral infection. Immunity. 2007; 27:670–684. [PubMed: 17950003]
11. Kardava L, Moir S, Wang W, Ho J, Buckner CM, Posada JG, O'Shea MA, Roby G, Chen J, Sohn HW, Chun TW, Pierce SK, Fauci AS. Attenuation of HIV-associated human B cell exhaustion by siRNA downregulation of inhibitory receptors. J Clin Invest. 2011; 121:2614. [PubMed: 21633172]
12. Kardava L, Moir S, Shah N, Wang W, Wilson R, Buckner CM, Santich BH, Kim LJ, Spurlin EE, Nelson AK, Wheatley AK, Harvey CJ, McDermott AB, Wucherpfennig KW, Chun TW, Tsang JS, Li Y, Fauci AS. Abnormal B cell memory subsets dominate HIV-specific responses in infected individuals. J Clin Invest. 2014; 124:3252–3262. [PubMed: 24892810]
13. Amu S, Lavy-Shahaf G, Cagigi A, Hejdeman B, Nozza S, Lopalco L, Mehr R, Chiodi F. Frequency and phenotype of B cell subpopulations in young and aged HIV-1 infected patients receiving ART. Retrovirology. 2014; 11:76. [PubMed: 25213015]
14. Fogli M, Torti C, Malacarne F, Fiorentini S, Albani M, Izzo I, Giagulli C, Maggi F, Carosi G, Caruso A. Emergence of exhausted B cells in asymptomatic HIV-1-infected patients naive for

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

HAART is related to reduced immune surveillance. Clinical & developmental immunology. 2012; 2012:829584. [PubMed: 22474482]

15. Muema DM, Macharia GN, Hassan AS, Mwaringa SM, Fegan GW, Berkley JA, Nduati EW, Urban BC. Control of Viremia Enables Acquisition of Resting Memory B Cells with Age and Normalization of Activated B Cell Phenotypes in HIV-Infected Children. J Immunol. 2015; 195:1082–1091. [PubMed: 26116511]
16. Pensieroso S, Galli L, Nozza S, Ruffin N, Castagna A, Tambussi G, Hejdeman B, Misciagna D, Riva A, Malnati M, Chiodi F, Scarlatti G. B-cell subset alterations and correlated factors in HIV-1 infection. AIDS. 2013; 27:1209–1217. [PubMed: 23343911]
17. Sciaranghella G, Tong N, Mahan AE, Suscovich TJ, Alter G. Decoupling activation and exhaustion of B cells in spontaneous controllers of HIV infection. AIDS. 2013; 27:175–180. [PubMed: 23135171]
18. Moir S, Buckner CM, Ho J, Wang W, Chen J, Waldner AJ, Posada JG, Kardava L, O'Shea MA, Kottilil S, Chun TW, Proschan MA, Fauci AS. B cells in early and chronic HIV infection: evidence for preservation of immune function associated with early initiation of antiretroviral therapy. Blood. 2010; 116:5571–5579. [PubMed: 20837780]
19. Meffre E, Louie A, Bannock J, Kim LJ, Ho J, Frear CC, Kardava L, Wang W, Buckner CM, Wang Y, Fankuchen OR, Gittens KR, Chun TW, Li Y, Fauci AS, Moir S. Maturational characteristics of HIV-specific antibodies in viremic individuals. JCI Insight. 2016; 1
20. WHO. World malaria report 2014. World Health Organization; Geneva, Switzerland: 2014.
21. Cohen S, Mc GI, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature. 1961; 192:733–737. [PubMed: 13880318]
22. Portugal S, Pierce SK, Crompton PD. Young lives lost as B cells falter: what we are learning about antibody responses in malaria. J Immunol. 2013; 190:3039–3046. [PubMed: 23526829]
23. Weiss GE, Traore B, Kayentao K, Ongoiba A, Doumbo S, Doumtabe D, Kone Y, Dia S, Guindo A, Traore A, Huang CY, Miura K, Mircetic M, Li S, Baughman A, Narum DL, Miller LH, Doumbo OK, Pierce SK, Crompton PD. The Plasmodium falciparum-specific human memory B cell compartment expands gradually with repeated malaria infections. PLoS Pathog. 2010; 6:e1000912. [PubMed: 20502681]
24. Crompton PD, Kayala MA, Traore B, Kayentao K, Ongoiba A, Weiss GE, Molina DM, Burk CR, Waisberg M, Jasinskas A, Tan X, Doumbo S, Doumtabe D, Kone Y, Narum DL, Liang X, Doumbo OK, Miller LH, Doolan DL, Baldi P, Felgner PL, Pierce SK. A prospective analysis of the Ab response to Plasmodium falciparum before and after a malaria season by protein microarray. Proc Natl Acad Sci U S A. 2010; 107:6958–6963. [PubMed: 20351286]
25. Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, Traore B, Kayentao K, Ongoiba A, Doumbo OK, Pierce SK. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. J Immunol. 2009; 183:2176–2182. [PubMed: 19592645]
26. Portugal S, Tipton CM, Sohn H, Kone Y, Wang J, Li S, Skinner J, Virtaneva K, Sturdevant DE, Porcella SF, Doumbo OK, Doumbo S, Kayentao K, Ongoiba A, Traore B, Sanz I, Pierce SK, Crompton PD. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. Elife. 2015; 4
27. Tan J, Pieper K, Piccoli L, Abdi A, Foglierini M, Geiger R, Tully CM, Jarrossay D, Ndungu FM, Wambua J, Bejon P, Fregni CS, Fernandez-Rodriguez B, Barbieri S, Bianchi S, Marsh K, Thathy V, Corti D, Sallusto F, Bull P, Lanzavecchia A. A LAIR1 insertion generates broadly reactive antibodies against malaria variant antigens. Nature. 2016; 529:105–109. [PubMed: 26700814]
28. Sullivan RT, Kim CC, Fontana MF, Feeney ME, Jagannathan P, Boyle MJ, Drakeley CJ, Ssewanyana I, Nankya F, Mayanja-Kizza H, Dorsey G, Greenhouse B. FCRL5 Delineates Functionally Impaired Memory B Cells Associated with Plasmodium falciparum Exposure. PLoS Pathog. 2015; 11:e1004894. [PubMed: 25993340]
29. Li H, Borrego F, Nagata S, Tolnay M. Fc Receptor-like 5 Expression Distinguishes Two Distinct Subsets of Human Circulating Tissue-like Memory B Cells. J Immunol. 2016; 196:4064–4074. [PubMed: 27076679]
30. Zinocker S, Schindler CE, Skinner J, Rogosch T, Waisberg M, Schickel JN, Meffre E, Kayentao K, Ongoiba A, Traore B, Pierce SK. The V gene repertoires of classical and atypical memory B cells

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in malaria-susceptible West African children. J Immunol. 2015; 194:929–939. [PubMed: 25556245]

31. Muellenbeck MF, Ueberheide B, Amulic B, Epp A, Fenyo D, Busse CE, Esen M, Theisen M, Mordmuller B, Wardemann H. Atypical and classical memory B cells produce Plasmodium falciparum neutralizing antibodies. J Exp Med. 2013; 210:389–399. [PubMed: 23319701]
32. Portugal S, Doumtabe D, Traore B, Miller LH, Troye-Blomberg M, Doumbo OK, Dolo A, Pierce SK, Crompton PD. B cell analysis of ethnic groups in Mali with differential susceptibility to malaria. Malar J. 2012; 11:162–168. [PubMed: 22577737]
33. Sullivan RT, Ssewanyana I, Wamala S, Nankya F, Jagannathan P, Tappero JW, Mayanja-Kizza H, Muhindo MK, Arinaitwe E, Kamya M, Dorsey G, Feeney ME, Riley EM, Drakeley CJ, Greenhouse B, Sullivan R. B cell sub-types following acute malaria and associations with clinical immunity. Malar J. 2016; 15:139. [PubMed: 26939776]
34. Lugaajju A, Reddy SB, Wahlgren M, Kironde F, Persson KE. Development of Plasmodium falciparum specific naive, atypical, memory and plasma B cells during infancy and in adults in an endemic area. Malar J. 2017; 16:37. [PubMed: 28109284]
35. Illingworth J, Butler NS, Roetynck S, Mwacharo J, Pierce SK, Bejon P, Crompton PD, Marsh K, Ndungu FM. Chronic exposure to Plasmodium falciparum is associated with phenotypic evidence of B and T cell exhaustion. J Immunol. 2013; 190:1038–1047. [PubMed: 23264654]
36. Ayieko C, Maue AC, Jura WG, Noland GS, Ayodo G, Rochford R, John CC. Changes in B Cell Populations and Merozoite Surface Protein-1-Specific Memory B Cell Responses after Prolonged Absence of Detectable Infection. PLoS One. 2013; 8:e67230. [PubMed: 23826242]
37. Nogaro SI, Hafalla JC, Walther B, Remarque EJ, Tetteh KK, Conway DJ, Riley EM, Walther M. The breadth, but not the magnitude, of circulating memory B cell responses to P. falciparum increases with age/exposure in an area of low transmission. PLoS One. 2011; 6:e25582. [PubMed: 21991321]
38. Ampomah P, Stevenson L, Ofori MF, Barfod L, Hviid L. Kinetics of B cell responses to Plasmodium falciparum erythrocyte membrane protein 1 in Ghanaian women naturally exposed to malaria parasites. J Immunol. 2014; 192:5236–5244. [PubMed: 24760153]
39. Subramaniam KS, Skinner J, Ivan E, Mutimura E, Kim RS, Feintuch CM, Portugal S, Anastos K, Crompton PD, Daily JP. HIV Malaria Co-Infection Is Associated with Atypical Memory B Cell Expansion and a Reduced Antibody Response to a Broad Array of Plasmodium falciparum Antigens in Rwandan Adults. PLoS One. 2015; 10:e0124412. [PubMed: 25928218]
40. Frosch AE, Odumade OA, Taylor JJ, Ireland K, Ayodo G, Ondigo B, Narum DL, Vulule J, John CC. Decrease in Numbers of Naive and Resting B Cells in HIV-Infected Kenyan Adults Leads to a Proportional Increase in Total and Plasmodium falciparum-Specific Atypical Memory B Cells. J Immunol. 2017; 198:4629–4638. [PubMed: 28526680]
41. Scholzen A, Teirlinck AC, Bijker EM, Roestenberg M, Hermsen CC, Hoffman SL, Sauerwein RW. BAFF and BAFF receptor levels correlate with B cell subset activation and redistribution in controlled human malaria infection. J Immunol. 2014; 192:3719–3729. [PubMed: 24646735]
42. Krishnamurty AT, Thouvenel CD, Portugal S, Keitany GJ, Kim KS, Holder A, Crompton PD, Rawlings DJ, Pepper M. Somatically Hypermutated Plasmodium-Specific IgM(+) Memory B Cells Are Rapid, Plastic, Early Responders upon Malaria Rechallenge. Immunity. 2016; 45:402–414. [PubMed: 27473412]
43. Hansen DS, Obeng-Adjei N, Ly A, Ioannidis LJ, Crompton PD. Emerging concepts in T follicular helper cell responses to malaria. Int J Parasitol. 2017; 47:105–110. [PubMed: 27866903]
44. Obeng-Adjei N, Portugal S, Tran TM, Yazew TB, Skinner J, Li S, Jain A, Felgner PL, Doumbo OK, Kayentao K, Ongoiba A, Traore B, Crompton PD. Circulating Th1-Cell-type Tfh Cells that Exhibit Impaired B Cell Help Are Preferentially Activated during Acute Malaria in Children. Cell Rep. 2015; 13:425–439. [PubMed: 26440897]
45. Alter MJ. Epidemiology of hepatitis C virus infection. World J Gastroenterol. 2007; 13:2436–2441. [PubMed: 17552026]
46. Seeff LB. Natural history of chronic hepatitis C. Hepatology. 2002; 36:S35–46. [PubMed: 12407575]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

47. Musset L, Lunel F, Cacoub P, Mannant PR, Silvain C, Lacombe C, Opolon P, Preud'Homme JL. Increased serum immunoglobulin G1 levels in hepatitis C virus infection. Hepatology. 1995; 21:1755–1757. [PubMed: 7768523]
48. Oliviero B, Mantovani S, Ludovisi S, Varchetta S, Mele D, Paolucci S, Baldanti F, Mondelli MU. Skewed B cells in chronic hepatitis C virus infection maintain their ability to respond to virus-induced activation. J Viral Hepat. 2015; 22:391–398. [PubMed: 25258145]
49. Doi H, Tanoue S, Kaplan DE. Peripheral CD27-CD21- B-cells represent an exhausted lymphocyte population in hepatitis C cirrhosis. Clin Immunol. 2014; 150:184–191. [PubMed: 24434272]
50. Chang LY, Li Y, Kaplan DE. Hepatitis C viraemia reversibly maintains subset of antigen-specific T-bet+ tissue-like memory B cells. Journal of Viral Hepatitis. 2017; 24:389–396. [PubMed: 27925349]
51. Sansonno D, Dammacco F. Hepatitis C virus, cryoglobulinaemia, and vasculitis: immune complex relations. Lancet Infect Dis. 2005; 5:227–236. [PubMed: 15792740]
52. Hermine O, Lefrere F, Bronowicki JP, Mariette X, Jondeau K, Eclache-Saudreau V, Delmas B, Valensi F, Cacoub P, Brechot C, Varet B, Troussard X. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med. 2002; 347:89–94. [PubMed: 12110736]
53. Charles ED, Brunetti C, Marukian S, Ritola KD, Talal AH, Marks K, Jacobson IM, Rice CM, Dustin LB. Clonal B cells in patients with hepatitis C virus-associated mixed cryoglobulinemia contain an expanded anergic CD21low B-cell subset. Blood. 2011; 117:5425–5437. [PubMed: 21421840]
54. Kong F, Zhang W, Feng B, Zhang H, Rao H, Wang J, Cong X, Wei L. Abnormal CD4 + T helper (Th) 1 cells and activated memory B cells are associated with type III asymptomatic mixed cryoglobulinemia in HCV infection. Virol J. 2015; 12:100. [PubMed: 26129991]
55. Terrier B, Nagata S, Ise T, Rosenzwajg M, Pastan I, Klatzmann D, Saadoun D, Cacoub P. CD21(-/low) marginal zone B cells highly express Fc receptor-like 5 protein and are killed by anti-Fc receptor-like 5 immunotoxins in hepatitis C virus-associated mixed cryoglobulinemia vasculitis. Arthritis Rheumatol. 2014; 66:433–443. [PubMed: 24504816]
56. Visentini M, Cagliuso M, Conti V, Carbonari M, Cibati M, Siciliano G, Cristofoletti C, Russo G, Casato M, Fiorilli M. Clonal B cells of HCV-associated mixed cryoglobulinemia patients contain exhausted marginal zone-like and CD21 low cells overexpressing Stra13. Eur J Immunol. 2012; 42:1468–1476. [PubMed: 22678901]
57. Achkar JM, Chan J, Casadevall A. B cells and antibodies in the defense against Mycobacterium tuberculosis infection. Immunol Rev. 2015; 264:167–181. [PubMed: 25703559]
58. Joosten SA, van Meijgaarden KE, Del Nonno F, Baiocchini A, Petrone L, Vanini V, Smits HH, Palmieri F, Goletti D, Ottenhoff TH. Patients with Tuberculosis Have a Dysfunctional Circulating B-Cell Compartment, Which Normalizes following Successful Treatment. PLoS Pathog. 2016; 12:e1005687. [PubMed: 27304615]
59. Pupovac A, Good-Jacobson KL. An antigen to remember: regulation of B cell memory in health and disease. Curr Opin Immunol. 2017; 45:89–96. [PubMed: 28319732]
60. Knox JJ, Cosma GL, Betts MR, McLane LM. Characterization of T-bet and eomes in peripheral human immune cells. Frontiers in immunology. 2014; 5:217. [PubMed: 24860576]
61. Frasca D, Diaz A, Romero M, D'Eramo F, Blomberg BB. Aging effects on T-bet expression in human B cell subsets. Cellular immunology. 2017
62. Isnardi I, Ng YS, Menard L, Meyers G, Saadoun D, Srdanovic I, Samuels J, Berman J, Buckner JH, Cunningham-Rundles C, Meffre E. Complement receptor 2/CD21- human naive B cells contain mostly autoreactive unresponsive clones. Blood. 2010; 115:5026–5036. [PubMed: 20231422]
63. Becker AM, Dao KH, Han BK, Kornu R, Lakhanpal S, Mobley AB, Li QZ, Lian Y, Wu T, Reimold AM, Olsen NJ, Karp DR, Chowdhury FZ, Farrar JD, Satterthwaite AB, Mohan C, Lipsky PE, Wakeland EK, Davis LS. SLE peripheral blood B cell, T cell and myeloid cell transcriptomes display unique profiles and each subset contributes to the interferon signature. PLoS One. 2013; 8:e67003. [PubMed: 23826184]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

64. Wang Z, Wang Z, Wang J, Diao Y, Qian X, Zhu N. T-bet-Expressing B Cells Are Positively Associated with Crohn's Disease Activity and Support Th1 Inflammation. DNA and cell biology. 2016; 35:628–635. [PubMed: 27348235]
65. Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoenig M, Driessen G, van der Burg M, van Dongen JJ, Wiech E, Visentini M, Quinti I, Prasse A, Voelxen N, Salzer U, Goldacker S, Fisch P, Eibel H, Schwarz K, Peter HH, Warnatz K. Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. Proc Natl Acad Sci U S A. 2009; 106:13451–13456. [PubMed: 19666505]
66. Claes N, Fraussen J, Vanheusden M, Hellings N, Stinissen P, Van Wijmeersch B, Hupperts R, Somers V. Age-Associated B Cells with Proinflammatory Characteristics Are Expanded in a Proportion of Multiple Sclerosis Patients. J Immunol. 2016; 197:4576–4583. [PubMed: 27837111]
67. Rubtsova K, Rubtsov AV, Cancro MP, Marrack P. Age-Associated B Cells: A T-bet-Dependent Effector with Roles in Protective and Pathogenic Immunity. J Immunol. 2015; 195:1933–1937. [PubMed: 26297793]
68. Naradikian MS, Hao Y, Cancro MP. Age-associated B cells: key mediators of both protective and autoreactive humoral responses. Immunol Rev. 2016; 269:118–129. [PubMed: 26683149]
69. Rubtsova K, Rubtsov AV, Thurman JM, Mennona JM, Kappler JW, Marrack P. B cells expressing the transcription factor T-bet drive lupus-like autoimmunity. J Clin Invest. 2017; 127:1392–1404. [PubMed: 28240602]
70. Peng SL, Szabo SJ, Glimcher LH. T-bet regulates IgG class switching and pathogenic autoantibody production. Proc Natl Acad Sci U S A. 2002; 99:5545–5550. [PubMed: 11960012]
71. Wang NS, McHeyzer-Williams LJ, Okitsu SL, Burris TP, Reiner SL, McHeyzer-Williams MG. Divergent transcriptional programming of class-specific B cell memory by T-bet and RORalpha. Nat Immunol. 2012; 13:604–611. [PubMed: 22561605]
72. Naradikian MS, Myles A, Beiting DP, Roberts KJ, Dawson L, Herati RS, Bengsch B, Linderman SL, Stelekati E, Spolski R, Wherry EJ, Hunter C, Hensley SE, Leonard WJ, Cancro MP. Cutting Edge: IL-4, IL-21, and IFN-gamma Interact To Govern T-bet and CD11c Expression in TLR-Activated B Cells. J Immunol. 2016; 197:1023–1028. [PubMed: 27430719]
73. Barnett BE, Staupe RP, Odorizzi PM, Palko O, Tomov VT, Mahan AE, Gunn B, Chen D, Paley MA, Alter G, Reiner SL, Lauer GM, Teijaro JR, Wherry EJ. Cutting Edge: B Cell-Intrinsic T-bet Expression Is Required To Control Chronic Viral Infection. J Immunol. 2016; 197:1017–1022. [PubMed: 27430722]
74. Rubtsova K, Rubtsov AV, van Dyk LF, Kappler JW, Marrack P. T-box transcription factor T-bet, a key player in a unique type of B-cell activation essential for effective viral clearance. Proc Natl Acad Sci U S A. 2013; 110:E3216–3224. [PubMed: 23922396]
75. Frisullo G, Nociti V, Iorio R, Patanella AK, Marti A, Cammarota G, Mirabella M, Attilio Tonali P, Batocchi AP. Increased expression of T-bet in circulating B cells from a patient with multiple sclerosis and celiac disease. Hum Immunol. 2008; 69:837–839. [PubMed: 18940217]
76. Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. Nat Rev Immunol. 2013; 13:777–789. [PubMed: 24113868]
77. Gerth AJ, Lin L, Peng SL. T-bet regulates T-independent IgG2a class switching. Int Immunol. 2003; 15:937–944. [PubMed: 12882831]
78. Ciofani M, Madar A, Galan C, Sellars M, Mace K, Pauli F, Agarwal A, Huang W, Parkhurst CN, Muratet M, Newberry KM, Meadows S, Greenfield A, Yang Y, Jain P, Kirigin FK, Birchmeier C, Wagner EF, Murphy KM, Myers RM, Bonneau R, Littman DR. A validated regulatory network for Th17 cell specification. Cell. 2012; 151:289–303. [PubMed: 23021777]
79. Ravasi T, Suzuki H, Cannistraci CV, Katayama S, Bajic VB, Tan K, Akalin A, Schmeier S, Kanamori-Katayama M, Bertin N, Carninci P, Daub CO, Forrest AR, Gough J, Grimmond S, Han JH, Hashimoto T, Hide W, Hofmann O, Kamburov A, Kaur M, Kawaji H, Kubosaki A, Lassmann T, van Nimwegen E, MacPherson CR, Ogawa C, Radovanovic A, Schwartz A, Teasdale RD, Tegner J, Lenhard B, Teichmann SA, Arakawa T, Ninomiya N, Murakami K, Tagami M, Fukuda S, Imamura K, Kai C, Ishihara R, Kitazume Y, Kawai J, Hume DA, Ideker T, Hayashizaki Y. An atlas of combinatorial transcriptional regulation in mouse and man. Cell. 2010; 140:744–752. [PubMed: 20211142]

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80. O'Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science. 2010; 327:1098–1102. [PubMed: 20185720]

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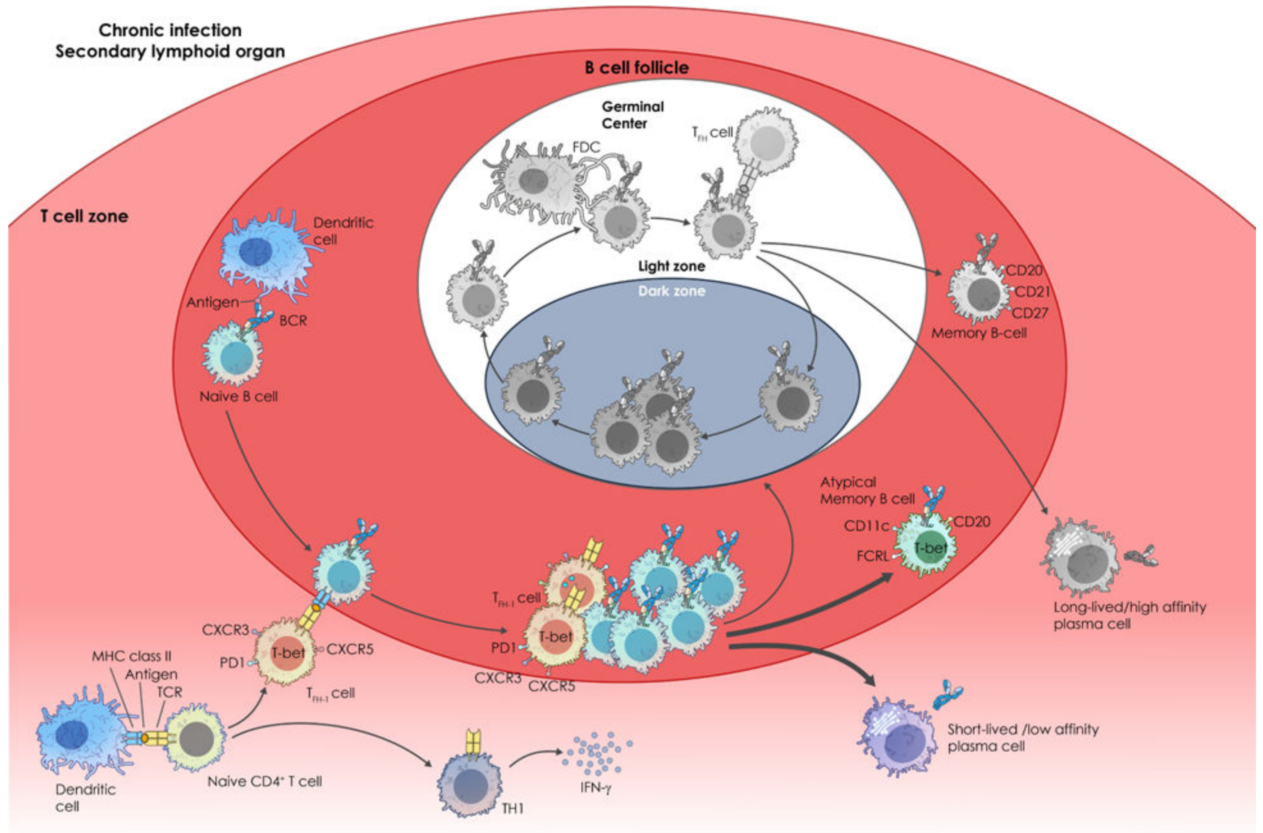

Figure 1. A model for the generation of T cell-dependent B cell memory

As detailed in the text in the follicle naïve B cells gather antigen from DC surfaces and present it to antigen-activated CD4+ T cells resulting in the differentiation of PD1+, CXCR5+ Tfh cells at the T/B border and expansion of B cells that then enter the dark zone of the GC. Following proliferation, class switching and SHM B cells enter the light zone of the GC where high affinity B cells are selected by Tfh cells to ultimately differentiate to long-lived MBCs and plasma cells, based on their ability to gather, process and present antigen. If B cells are unable to adequately process and present antigen at the T-B border, B cells fail to enter GC and rather differentiate into GC-independent MBCs and short-lived plasma cells, according to Kuroski *et al.* (3).

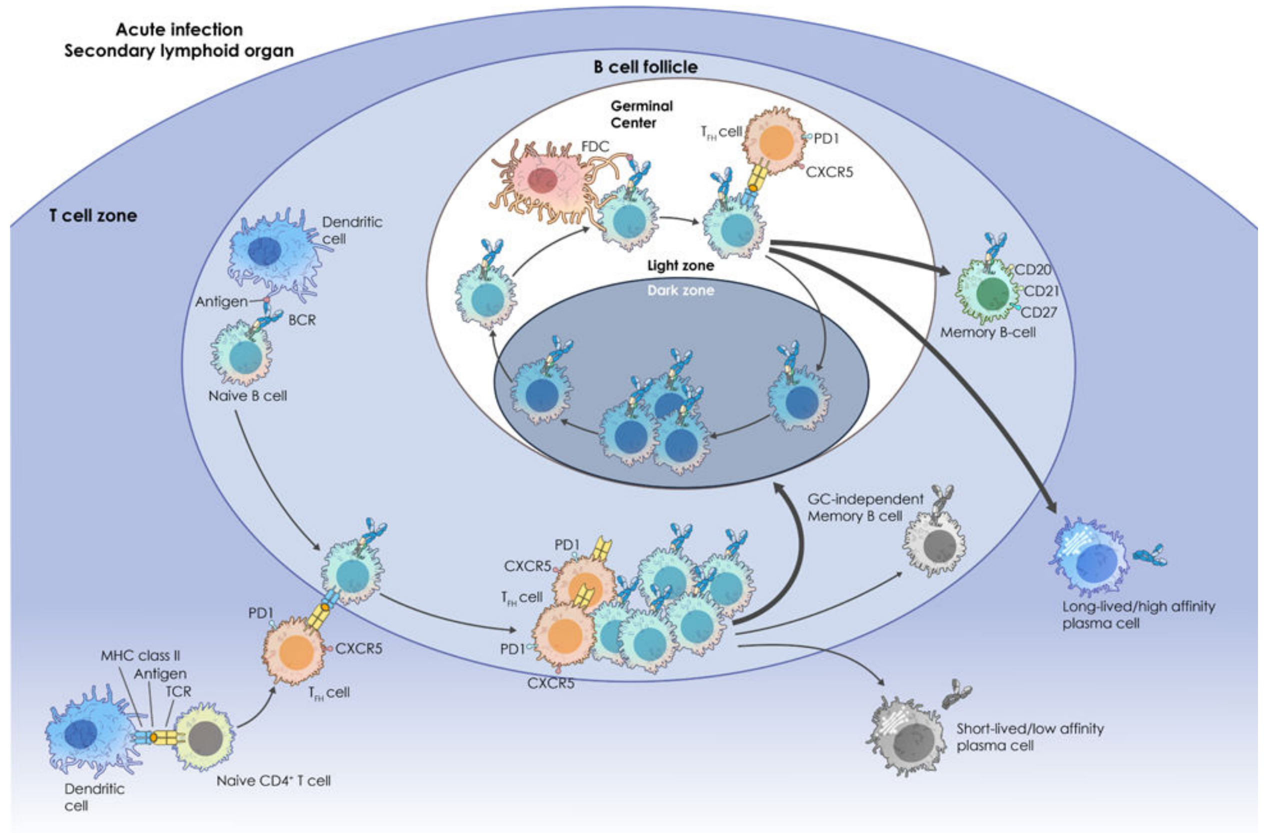

Figure 2. A model for the generation of aMBCs during chronic infection

In the inflammatory environment of chronic infections $CD4^+$ T cells differentiate into IFN-γ expressing TH1 cells and T-bet$^+$ PD1$^+$ CXCR5$^+$ CXCR3$^+$ Tfh-1 cells that drive the differentiation of CD11c$^+$ FcRL$^+$ CD20$^+$ aMBCs and short-lived plasma cells at the expense of GC reactions.

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