Memory B Cells of Mice and Humans

Florian Weisel and Mark Shlomchik

Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261; email: fweisel@pitt.edu, mshlomch@pitt.edu

Annu. Rev. Immunol. 2017. 35:255–84

First published online as a Review in Advance on January 30, 2017

The *Annual Review of Immunology* is online at immunol.annualreviews.org

https://doi.org/10.1146/annurev-immunol-041015-055531

Keywords

immune memory, vaccine, memory B cell, germinal center, plasma cell, B cell subsets

Abstract

We comprehensively review memory B cells (MBCs), covering the definition of MBCs and their identities and subsets, how MBCs are generated, where they are localized, how they are maintained, and how they are reactivated. Whereas naive B cells adopt multiple fates upon stimulation, MBCs are more restricted in their responses. Evolving work reveals that the MBC compartment in mice and humans consists of distinct subpopulations with differing effector functions. We discuss the various approaches to define subsets and subset-specific roles. A major theme is the need to both deliver faster effector function upon reexposure and readapt to antigenically variant pathogens while avoiding burnout, which would be the result if all MBCs generated only terminal effector function. We discuss cell-intrinsic differences in gene expression and signaling that underlie differences in function between MBCs and naive B cells and among MBC subsets and how this leads to memory responses.

INTRODUCTION

Immunological memory is a hallmark of the vertebrate immune system. Although nonclonal forms of innate immune memory have recently been elucidated (1), the mainstay of immune memory is adaptive and antigen-specific, among both T and B cell lineages. The roles and mechanisms of memory in immune protection are variable and depend on the specific pathogen or antigen.

Memory emerges from a range of changes that evolve over time in the whole body of the host after and even during an infection or artificial challenge. Among T cells, multiple types of long-lived memory cells are generated, including those that circulate centrally, those that reside within tissues, and those that are poised more for effector function than for proliferation (2, 3). In addition, polarization toward secretion of certain cytokine programs and other effector functions is overlaid on this framework, particularly for CD4 T cells. Moreover, the frequency of antigen-specific cells of all these types is increased in the immune animal. The situation in the B cell compartment is to some degree parallel, yet in certain areas it is much less understood.

As for T cells, long-lived memory B cells (MBCs) are generated during and following challenge. It is clear that many of those B cells recirculate and in both mice and humans are found in blood and in secondary lymphoid tissues (SLTs). There is also some evidence for tissue-resident MBCs, though this is much less well defined. Like T cells, antigen-experienced MBCs can secrete cytokines and in some cases can be polarized (4–6). Both human and murine MBCs show both phenotypic and functional heterogeneity in other dimensions, as well, as discussed below. These cell-intrinsic changes, along with the diversification of the MBC compartment, are the primary subject of this review.

A major difference between T and B cells is that the B cell compartment generates antibodies, some of which remain for long periods, being secreted by long-lived plasma cells that mainly reside in the bone marrow of immune animals (7, 8). These antibodies can act at long range to prevent reinfection and can even protect external spaces at barrier sites. Importantly, it can further affect secondary responses by opsonizing antigen and redirecting its cellular processing via Fc receptors and complement receptors, as well as by changing its valency or receptor cross-linking ability. This effect influences subsequent T and B cell responses.

Unique to the B cell compartment, and affecting both long-lived MBCs and plasma cells, is the process of somatic hypermutation (SHM) and its effect on attendant affinity maturation. This impinges on both the quality and potency of subsequent circulating antibody, as well as on the sensitivity of the B cell receptors (BCRs) expressed on MBCs. In addition, SHM leads to diversification of the response and also provides the substrate for affinity-based selection. As such, SHM and selection endow the B cell response an anticipatory element in the context of pathogens that themselves undergo rapid mutation, such as in the case of retroviruses or influenza.

All of these features working together lead to an immune host. Because of their interdependency and also because the response depends on the type, route, and dose of infection or challenge, it is difficult to predict how individual components will behave and will contribute to overall host protection. It is in this sense that memory emerges from a series of changes in multiple aspects of the immune system. Research aims to dissect the individual changes and the roles that these changes play in context.

In this review we first discuss the identification of MBC subsets in both mice and humans. We next cover the generation of MBCs by various stimuli, at various sites, and at different time points during the response. We also discuss data on the residence and maintenance of MBCs. We then go on to discuss functional differences inherent in MBCs versus naive B cells (NBCs) and in different types of MBCs. Finally, we consider how MBCs can be reactivated to generate protective

function. Though MBCs can be made in a T-independent response (9, 10), this review focuses on T-dependent memory.

WHAT ARE MEMORY B CELLS?

Notions of the MBC

It is important to begin by defining the concept of a MBC. In the past, this concept may have been skewed by preconceived notions about the origins, characteristics, and functional capabilities that a MBC should have. For example in mice and for a long time in humans, MBCs were assumed to have a switched (i.e., non-IgM) isotype (11), a notion that persists (12). Similarly, MBCs were expected to carry V region mutations and to be of higher affinity than their NBC counterparts (11). Since memory responses per se (that emerge from a multiplicity of changes as described above) are faster, it has also been assumed that MBCs themselves must respond faster. All of the above are distinctive properties overall of the memory response but not necessarily of every MBC.

An Agnostic Definition of a MBC

A more agnostic definition of a MBC is possible and desirable: a cell that has encountered and responded to antigen and that has returned to a quiescent state and remains present in the animal at a given later time point (13). This cell should remain responsive to a second challenge with antigen. (This rules out nondividing plasma cells from consideration as MBCs. Hence, we would reject as confusing the term memory plasma cell; plasma cell alone should be used.) By this definition, MBCs can be found and studied at any interval after immunization, their longevity being defined by the chosen time point after initial stimulation. Of note, at the population level, MBCs are expected to remain at higher precursor frequency than their naive counterparts, which operationally seems to be usually or always the case.

How MBCs Have Been Generated and Defined in Mice

Though this definition of MBCs is very attractive, it is much more easily implemented in mice than in humans (though it can be applied in humans under certain conditions, as discussed below). Using this definition, our lab has performed cell transfer experiments and dye-dilution studies in mice and found that after several weeks the only residual cells were those that divided in response to antigen (14, 15). We have also used BrdU labeling to mark cells undergoing division after immunization in either intact mice or transfer recipients (13, 16, 17). We then identified the antigen-specific B cells using labeled antigen or other surrogate markers. The cell transfer system we have used in most of our studies is illustrated in **Figure 1**; controls show how bona fide memory cells are easily identifiable using labeled antigen, appearing only in recipients that received both cells and immunization more than eight weeks prior.

Other groups have identified antigen-specific B cells in intact animals as well. In one set of studies, tamoxifen-inducible Cre action under the control of the activation-induced cytidine deaminase (AID) promoter, which is turned on after most types of B cell activation including but not limited to germinal centers (GCs), was used to mark cells that had responded to antigen in intact animals (18). Some type of labeling approach can be useful in distinguishing true MBCs from residual NBCs that exist in intact animals, albeit at lower precursor frequencies than their MBC counterparts.

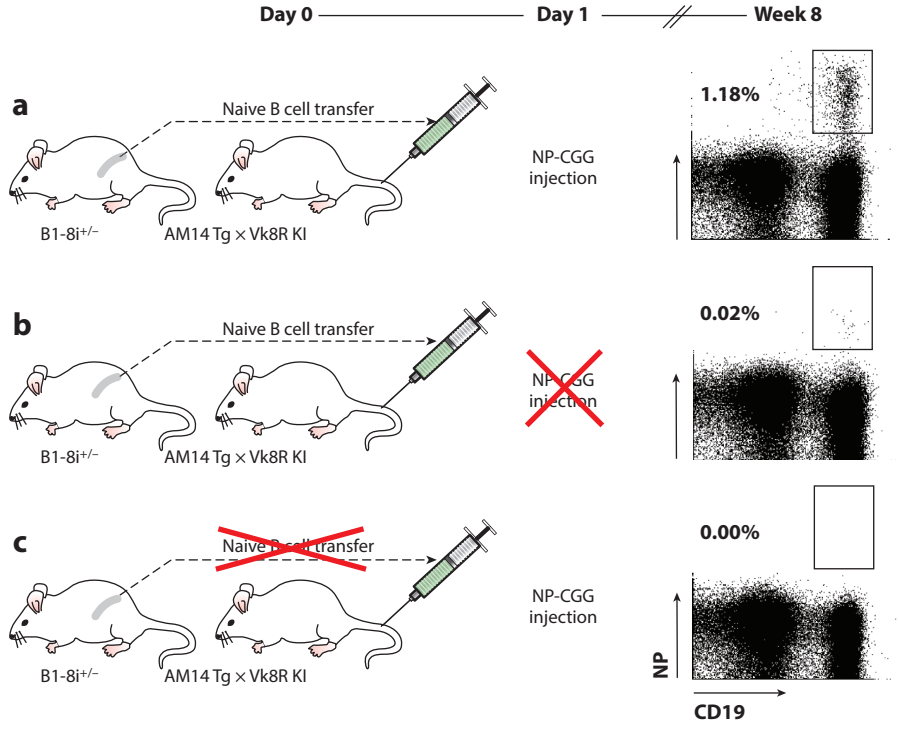

Figure 1

Adoptive transfer system to generate MBCs. B1-8i$^{+/-}$ genetically targeted BALB/cJ mice carry a germ line–encoded, unmutated Vh186.2 site–directed transgene that, when paired with λ1 light chains, encodes a BCR with moderate affinity for the hapten nitrophenyl (NP). To generate MBCs, an equivalent of 2×10^5 naive, NP-reactive B cells from B1-8i$^{+/-}$ genetically targeted BALB/cJ mice were adoptively transferred into AM14 transgenic × Vk8R (AM14 Tg Vk8R KI) BALB/cJ mice. FACS plots are gated on live singlets. Numbers adjacent to outlined areas indicate percentages of live cells in the gated population. (*a*) Immunization with NP-CGG resulted in robust splenic NP-reactive MBC generation as determined by flow cytometric analysis eight weeks later. (*b*) No expansion of adoptively transferred naive B1-8i$^{+/-}$ B cells was observed in the absence of NP-CGG immunization, indicating that MBC generation is antigen dependent. (*c*) NP-CGG immunization of AM14 Tg Vk8R KI mice in the absence of adoptive B1-8i$^{+/-}$ B cell transfer did not result in NP-specific MBC formation because these mice harbor an irrelevant monoclonal B cell population but otherwise display normal lymphoid architecture.

In some cases in humans there is a known exposure to antigen, such as a vaccination or a diagnosis of infection such that the evolution of a specific immune response can be tracked. This in turn requires a way to identify antigen-specific B cells, which usually is accomplished via a labeled antigen "bait." This approach can be used in both mice and humans. For example this has been done with tetanus toxoid, influenza, haptens such as nitrophenyl (NP), virus-like particles, and fluorescent proteins (19–27). An alternative approach used by many, mainly in humans but also in mice, has been to enumerate MBCs in limiting dilution cultures with polyclonal activators (with or without nominal antigen; 28) that generate antibody-secreting cells and then assay supernatants by ELISA for antigen-binding activity (29–32). One important caveat to this approach is that it may detect only the higher-affinity B cells and could exclude a substantial proportion of lower-affinity

responsive clones. Affinity thresholds for detection in general are difficult to quantify, and it has become clear that many responding B cells have extremely low affinities that would be undetectable in such assays. This has likely led to a substantial underestimation of antigen-specific B cell frequencies among both MBCs (which, though affinity-selected in part, include many B cells that express unmutated V regions and hence could be low affinity) and especially NBCs (33–35). Nonetheless, these approaches have been revealing in tracking MBCs at known time points after antigen exposure and in some cases at steady state after more distant or less defined immune encounters.

A complementary approach has been used to identify presumptively antigen-specific responding B cells during an acute immune response, again either to vaccination or infection. This response can elicit a sharp increase in plasmablasts in peripheral blood, which in turn are identified by surface marker expression in flow cytometry (36, 37). Because of the markedly increased frequencies, even without a specific antigen probe, the peripheral blood will be highly enriched for cells that in fact do recognize or were stimulated by the antigen(s) in question. This has been established in some cases by sorting and cloning BCRs from single plasmablasts, followed by expression as secreted IgG (36, 37). The binding of this reconstructed IgG can show that the originating cell was antigen specific.

MBC Markers in Humans

A most useful alternative approach to the identification of antigen-specific cells in the context of known antigen exposures is to use the expression of unique memory-specific surface markers that can be identified by flow cytometry. CD27 was identified as one marker of MBCs and indeed shows many properties that correlate with MBC identity, such as isotype switch and presence of V region mutations (38, 39). Further, extensive in vitro functional analysis has shown that the $CD27^+$ pool of cells en masse behaves differently than the $CD27^-$ counterparts (40–45). CD27 has also been used to separate human B cells for subsequent gene expression analysis, showing interesting and consistent differences between the $CD27^+$ and $CD27^-$ cells (42).

Nonetheless, it is difficult to prove that MBCs belong exclusively to the $CD27^+$ pool and that, conversely, all $CD27^+$ cells are true MBCs. In fact, over the last decade it has become apparent that there are indeed minor, but unique and bona fide, subsets of $CD27^-$ MBCs, as discussed below (46–48). Further, it is likely that CD27 in fact defines a postactivation B cell, perhaps one that has been in a GC, but does not necessarily mark long-lived cells. This concern is based in part on data from model systems in mice, where the kinetics of MBCs can be accurately tracked after immunization. In the mouse, we found that populations of B cells with memory phenotypes after activation decreased rather dramatically between 4 and 12 weeks after immunization, before reaching plateaus in cell numbers that were presumably populated with long-lived MBCs (16). Similar kinetics have been observed for CD8 T cells (49). Hence, expression of a memory marker does not distinguish cells fated to carry true long-term memory in the host. If one considers that humans are much "dirtier" than mice (50, 51), then postactivation B cells may be generated at a substantially higher rate in humans. This would lead to, at any given time, a higher percentage of $CD27^+$ cells being "young" postactivation cells rather than long-lived MBCs. Indeed, deuterium labeling experiments in humans produced the seemingly paradoxical result that $CD27^+$ cells had a faster labeling (turnover) rate, even though they were thought to comprise long-lived MBCs (52), a finding confirmed using additional markers and Ki67 staining (53). This result, however, would be compatible with the compartment containing a relatively high fraction of cells that recently experienced antigen stimulation.

MEMORY B CELL SUBSETS

Subsets of Murine MBCs

In mice it has been possible to identify antigen-specific and antigen-experienced cells at known time points after immunization and to assess their surface marker expression as a first step in identifying heterogeneity among MBCs. Initially our lab used BrdU labeling in intact immunoglobulin (Ig)-transgenic mice that carried a transgene encoding a heavy chain (B1-8) that, together with a λ1 light chain, encoded an anti-NP BCR (16). Mice given BrdU at the peak of the GC response retained an elevated frequency of λ1-expressing B cells that retained BrdU, an indicator that they had divided in response to antigen during the GC response, thus marking them as MBCs. By trial and error, we then defined surface markers that identified some, but not all MBCs, including CD80 and CD21 (interestingly, both were also differentially expressed among $CD27^+$ B cells in humans; see below) (54). We then used these markers to sort the cells for V region sequencing, identifying both mutated and unmutated MBCs and their subsets. We provisionally identified a number of other markers at this time. Seeking a more comprehensive approach, we next developed a transfer system in which essentially all antigen-specific cells, at eight or more weeks after immunization, were resting MBCs, as the host recipients lacked antigen-specific B cells and could not respond because of the expression of a transgene-encoded, irrelevant BCR on host B cells. This set the stage for sorting highly purified MBCs and their naive counterparts, and the subsequent analysis of their transcriptome with Affymetrix microarrays (15). We identified a host of additional differentially expressed genes; among them we selected the surface markers PD-L2 and CD73 as our focus for further study. These, along with CD80, defined at least five subsets of MBCs as revealed with multicolor flow cytometry and, combined with Ig isotype (as the transfer system could utilize BCR knock-in or site-directed transgenes that could undergo normal isotype switch), defined a total of at least ten populations (14).

We then concentrated further on the three major populations of NP-specific MBCs defined by expression of the B7 family members CD80 and PD-L2: double negatives (DN), single positives (SP) for PD-L2, and double positives (DP). Based on several lines of evidence, these populations appeared to be stable and not interchanging, leading to the notion that they were MBC subsets, as opposed to transient differentiation states. There was a hierarchy of SHM content, with DN MBCs mostly carrying no mutations, DP carrying the most, and SP an intermediate number. Similarly, most DN were IgM, and the DP were about 50% IgG, with SP again in between. Since both V region mutation and isotype switch are indelible marks in the DNA that are introduced during the proliferative phase of the response, the fact that the frequencies of these events differ between individual populations of nonproliferating MBCs (which are not undergoing further SHM and switching) indicates that the populations are not undergoing substantial mixing during the resting phase (14). This also suggests that the populations were formed at different times during the response, particularly given that SHM accumulates as a function of time and cell division. Indeed, we recently showed that DN, SP, and DP MBCs are formed in three overlapping periods (55).

Identification of Murine MBC Subset Markers in a Variety of Immune Settings

The markers for murine MBCs that we identified were initially characterized in the antihapten response to protein antigen given in alum. Subsequently, other groups have found similar MBC subsets in a variety of conditions and locations. Takahashi and colleagues found lung-resident MBCs after influenza infection that variably expressed CD80, PD-L2, and/or CD73, though

because of very low cell numbers they did not resolve subsets with multicolor staining (56). Bemark et al. (57) found that mucosal immunization led to the development of CD80$^+$ IgM, IgG, and IgA MBCs in the gut. Lindner et al. (58) used CD80 and CD73 as MBC markers to retrieve spontaneously formed IgM and IgA MBCs from murine Peyer's patches (PPs), showing that both IgM and IgA MBCs carried V region mutations and that IgA MBCs shared clonotypes with gut IgA plasma cells in the same animals. Recently, Pepper and colleagues reported that malaria infection promotes the development of IgM and IgG MBC subsets that express CD80 and CD73 (59). They also found that among IgM MBCs, those that expressed lower levels of IgD tended to lack CD80 expression and carried V regions that mainly lacked mutations, in agreement with data from the anti-NP response, showing that CD80$^-$ MBCs had substantially fewer SHMs (14, 17). Together these data suggest that while the presence or absence of CD73, CD80, or PD-L2 expression correlates with memory status on some or even most MBCs, specific aspects of immunization and/or detection control differences in the extent and pattern of expression of these markers. This bears substantial further examination to understand the functional meaning and ontogeny of these various subsets of MBCs and the surface markers that they express.

Distinct IgM and IgG Subsets of MBCs

Initially, and not surprisingly, several labs focused great attention on isotype differences among MBCs, with the theory in mind that isotype would not just mark cells of differential fate and function but would actually control, via differential function of the IgM versus IgG BCR, functional outcome. This concept and line of research were applied to both mouse and human studies [with the caveat that in humans bulk CD27$^+$ cells among spleen or peripheral blood lymphocytes (PBLs) were taken as MBCs and then divided by isotype].

Dogan et al. (18) used a novel approach to mark B cells that had been activated by antigen and hence expressed the activation marker AID, which is required for both SHM and isotype switch. They constructed a mouse that expressed tamoxifen-inducible Cre from the *aicda* locus. AID is prominently expressed in GC B cells but is also expressed in non-GC B cells, which needs to be kept in mind for interpretation. By administering tamoxifen to mice that carried this gene along with a rosa26-flox-STOP-YFP allele irreversibly modified to express YFP upon Cre action, they could permanently mark cells that were transcribing the *aicda* locus at any chosen time during the response. Using this clever approach they were able to unequivocally mark both IgM and IgG MBCs and determine their characteristics without using cell transfers or Ig transgenic–type systems and without specific identification of antigen-specific cells, which can be problematic in immune mice (60–62). The identification of IgM MBCs in mice confirmed work of others who measured functional secondary IgM responses and/or identified antigen-experienced IgM cells engaging in secondary responses (63, 64).

Pape and colleagues used a bead-based method to enrich antigen-specific MBCs, thereby identifying populations of cells that remained in immune mice at expanded frequencies (22, 23). Like Dogan et al. (18) they divided MBCs by isotype only. They concluded that IgM MBCs are not functional in the presence of higher-affinity IgG MBCs but that IgG MBCs have a much shorter half-life, implying that once these cells decayed, the IgM MBCs might regain functional relevance. This finding remains controversial. Multiple groups, including our own, have not found a shorter half-life for IgG MBCs (55, 65). Classic studies by Schittek & Rajewsky (25), who used phycoerythrin as an immunogen and BrdU labeling to identify MBCs, also did not find a fall-off in MBC numbers up to 200 days after immunization, and they focused on IgM/IgD-negative (i.e., IgG) cells. However, Gitlin et al. (66) recently reported a somewhat similar finding to that of Pape et al. using a very elegant system to separate isotype switch from AID expression. They concluded

that the expression of AID per se, rather than IgG isotype expression, leads to a shorter MBC half-life.

These differences in half-life were quite striking, with an up to tenfold loss from peak levels in one year. It is hard to reconcile these differences among the studies. Those that did not detect a shorter half-life for IgG MBCs used direct enumeration of cells by flow cytometry. The two that showed a shorter half-life for IgG MBCs used a bead-based technique to preenrich B cells before enumerating them by flow cytometry. It is hard to argue that an artifact would have led to more IgG MBCs being detected than were actually present, suggesting that the finding of IgG MBC stability is valid. However, depending on detection methods it is possible that some IgG MBCs could have been missed: It could be that the bead-capture approach did not detect all IgG MBCs as time went on; it is extremely difficult to assess the efficiency of such bead capture, and neither study documented this. With time, there is accumulation of abundant and high-affinity serum antibodies, which can coat cells (cytophilic antibodies); speculatively, this may have led to an under-recovery of IgG MBCs from bead-based preisolation procedures. On the other hand, this could have affected the studies of NP responses, thus leading to artifactual overcounting. However, the transfer studies used by Weisel et al. (55) generate minimal serum antibodies and would not have been so affected, making this explanation less likely. Another possible explanation for the Gitlin finding in particular is the slow rejection of YFP^+ cells, likely due to lack of complete tolerance to the foreign protein. While it could be argued that the differences between YFP^+ cells that expressed AID and those that did not would control for this, it is notable that YFP^+ cells from either type of mouse decay with approximately the same slope from day 120 to nearly 600 days. These decayed by four to seven times over this span, whereas YFP^- cells from either mouse did not decay detectably. If YFP expression per se were irrelevant and only AID were relevant, then YFP^+ and YFP^- cells from mice that never expressed AID should have had the same decay curves, but they did not. As there are many variables that have yet to be explored and controlled for, the half-life of IgG MBCs remains an open question.

The notion that AID expression could result in reduced half-life is nonetheless intriguing and worthy of further investigation. Gitlin et al. (66) further showed that very-long-lived IgG MBCs had less polyreactivity than did MBCs isolated earlier (a population that includes many cells destined to die, based on other experiments in their study). It also appeared that late MBCs may have had improved binding to antigen, though this was not specifically quantitated. In any case, these studies raise the notion that the MBC compartment is curated, perhaps by both negative and positive selection, after it is initially formed. Another implication is that DN MBCs, which have few mutations, might be particularly long-lived.

Human MBC Subsets

As noted, CD27 was identified some time ago as a marker of MBCs, or, as we now understand, a marker of postactivation B cells, including MBCs. Numerous flow cytometry and functional studies have uncovered several levels of additional complexity and heterogeneity among human MBCs or MBC-like cells. First, among $CD27^+$ classical cells, surface markers such as CD80 (67) and IL-18 (68) were found to be expressed on some but not all cells. Nonetheless, the functional significance of surface markers that may divide human $CD27^+$ cells into subpopulations has been studied little. Second, multiple groups noted that among $CD27^-$ cells there were IgG^+ and even IgA^+ B cells that lacked CD27 expression, implying that there do exist additional populations of antigen-experienced cells that are not captured by the CD27 marker (46–48). These were initially dubbed atypical MBCs, an appropriate term that we will use here. Notably, these cells express a variety of inhibitory cell receptors that are homologous to Fc receptors and are called

Fc-receptor-like, or FCRL, molecules. These include FCRL2, 3, 4, and 5 and CD300a (69–73). These cells were also noted to be $CD21^-$ (69).

Though $CD27^-/CD21^-$ atypical MBCs were found in subepithelial locations in lymphoid tissues such as tonsil (46) as well as in peripheral blood of healthy individuals, several groups noted their relative expansion in blood of patients with a variety of conditions. Sanz and colleagues identified them in patients with systemic lupus erythematosus (48) and rheumatoid arthritis (74). Patients with common variable immunodeficiency also have elevated frequencies of $CD27^-$ MBCs (75). Several chronic or recurrent infections—such as malaria, HIV, and hepatitis C—are all associated with an increase in the frequencies of various types of atypical MBCs in PBLs (70, 76, 77). A study of pregnant women who underwent primary cytomegalovirus (CMV) infection also observed expansion of both activated ($CD27^+CD20^+CD21^{low}$) and atypical ($CD27^-CD20^+CD21^{low}$) MBCs (78).

These atypical MBCs, notable for their expression of inhibitory receptors (73), were found by several groups to be less functional in a variety of assays and hence were dubbed anergic and exhausted (73, 74, 79). Their functional significance in disease and whether they play effective roles in containing infection or autoimmunity, or the reverse, are far less clear. There is some evidence that these subsets [or related ones (80)] are enriched in self-reactivity, or pathogen reactivity in the case of infection (76, 77).

Relationship of Human and Mouse MBC Subsets

There is at present little information about the homology between mouse and human MBC subsets, because the murine subsets were identified recently and are relatively unexplored and perhaps in part because human tissue (including spleen) B cells have been studied much less, with most information coming from PBLs. Clearly, both of these issues require intensive investigation, which will likely bear much fruit in terms of our understanding of immune memory.

Nonetheless, there are some data regarding marker overlap, focusing on those that define heterogeneity. CD80 is expressed on a substantial fraction of MBCs, but there are notable $CD80^-$ populations (14, 67). Whether the function of these (see below) matches between humans and mice is yet to be determined. PD-L2, another marker in mice, is evidently not expressed among human $CD27^+$ B cells among PBL (our unpublished data). Similarly, a screen did not pick up CD73, which is expressed on subsets of murine MBCs with as yet undefined functional correlation, on human $CD27^+$ B cells (our unpublished data).

It is well known that homologous populations of hematolymphoid cells in human and mice often share some but not all markers. For this reason functional similarities and—most decisively—gene expression similarities should be used in lieu of markers to align the two systems. A substantial amount of work will be required to reach this stage but if accomplished could help in the translation of mouse findings to human studies.

DIFFERENTIAL FUNCTIONS OF MEMORY B CELL SUBSETS

Murine Functional Studies

The in vivo and in vitro functions of various types of MBCs have been investigated in the mouse. Seminal studies by Dogan et al. (18) and Pape et al. (22) delineated differences between separated IgM and IgG MBCs. They found that IgG MBCs were prone to make rapid antibody-forming cell (AFC) responses and not reenter GCs, whereas IgM MBCs could generate second rounds of GCs but were not enhanced in AFC generation. These data also addressed a long-standing

controversy generated by two papers with conflicting results about whether MBCs can undergo a second round of SHM, by showing that some MBCs can and others cannot. They also harmonized with observations that IgG and IgM could transmit unique signals owing to differences in their cytoplasmic tails (81–85).

Our group investigated MBC subsets defined by PD-L2 and CD80 (14, 86). To account for the findings concerning IgM and IgG MBCs, we controlled for this by sorting and comparing subsets of B cells that all shared the same isotype. As DN MBCs are predominantly IgM, we compared DN, SP, and DP MBCs of this isotype and found that the DN, and to a lesser extent the SP, MBCs could reenter the GC reaction, but there was no detectable GC seeding by DP IgM MBCs. Conversely, the DP MBCs were much more potent in generating rapid, IgG AFC responses, with the SP again being intermediate. We also compared IgG-expressing subsets, though in this case we could not study DN, as there were too few of these cells expressing IgG (<5%). Again, the subset markers and not the isotype were decisive, with the DP making only AFCs, whereas the SP could give rise to both AFC and GC reactions. Indeed, cell for cell, the IgM and IgG SP were equally efficient at making GC B cells, again showing that isotype was not a determining factor.

These seemingly conflicting data could be reconciled by realizing that IgM MBCs are enriched for DN, so that an IgM population should yield detectable GCs based on its B7 family–based subset composition, whereas an IgG MBC population will be enriched for DP and hence will make a more notable AFC reaction. The two models are compared side-by-side in **Figure 2**, which helps to illustrate how they are in some ways compatible, but the subset model provides more precision.

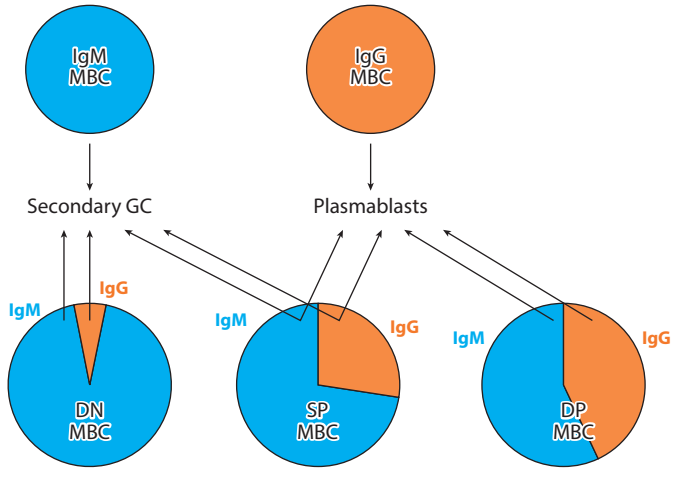

Figure 2

Comparison of the isotype and subset models to explain heterogeneity of MBC function upon reimmunization. (*Top*) Isotype model proposed by Dogan et al. (18), in which IgM isotype MBCs (*blue*) all differentiate into germinal center (GC) B cells, whereas IgG MBCs (*orange*) spawn plasmablasts as indicated by arrows. (*Bottom*) Subset model proposed by Zuccarino-Catania et al. (86), in which the expression of the B7-family members' surface molecules CD80 and PD-L2 better predict the function of MBC subsets upon restimulation. These molecules are heterogeneously expressed by MBCs; those MBCs lacking both (double negative, DN) predominantly generate new GC B cells, whereas those expressing both markers (double positive, DP), mainly generate plasmablasts and those expressing only PD-L2 (single positive, SP) can do both, as indicated by arrows. The isotype composition of each subset is indicated by the pie charts, showing how the two independent models would generally correlate with each other.

What would be missed in the IgM/IgG subset schema is that IgM DP are not capable of making that GC reaction. Interestingly, Kometani et al. (87) produced mice that expressed IgG1 on NBCs and found that their responses correlated not with isotype, but rather with stimulation history, in agreement with our findings. Nonetheless, work showing that IgG has distinct functional signaling is also not inconsistent with any of our results. On top of other layers of differentiation, IgG could still deliver distinctive signals and outcomes. Others and we are now searching for the mechanisms that underlie different functional capabilities of MBC subsets defined by CD80 and PD-L2.

We found that Zbtb32, a member of the large transcriptional repressor family that also includes Bcl6, was highly overexpressed in DP MBCs (86). Recently the Bhattacharya lab in collaboration with ours demonstrated that Zbtb32 expression in MBCs actually restrains the secondary AFC response, such that in its absence many more antibodies are produced upon secondary challenge (88). This is an initial clue into how differential gene expression between MBCs and NBCs, and possibly between MBC subsets, can in turn control different functional outcomes.

Human Functional Studies

Studies in humans have largely used CD27 to separate memory (i.e., in our view, inclusive of both memory and, more recently, antigen-experienced cells) from NBCs and then compared their responses to various stimuli in vitro. In some cases, IgM and IgG expression were used to further subdivide the $CD27^+$ population. Several studies noted intrinsic differences in proliferation, particularly the propensity of MBCs to enter cell cycle earlier than NBCs (40, 42, 89). This was related to higher expression of quiescence factors, including members of the Kruppel-like factor (Klf) family of transcription factors (42, 89). Gene expression profiling and flow cytometry have suggested some salient differences between IgM and IgG human $CD27^+$ cells (90). In addition, Davey & Pierce (91) demonstrated key signaling differences between IgG BCRs of $CD27^+$ cells versus IgM BCRs of $CD27^-$ NBCs. Limited studies in that report also suggested that both CD27 and BCR identity contributed to the tempo and strength of very early BCR signaling as read out by phospho-Syk and BCR accumulation in B cell immune synapses at planar lipid bilayers.

Implications of Subsets for Design of the Memory Response

In the last decade and especially in the last several years, a great deal of heterogeneity has been appreciated in both mouse and human MBC compartments. Naive B cells in general, and follicular-type B cells in particular, seem to have multiple potentials upon initial stimulation—notably, they can both make short-lived AFCs and seed GC reactions. Memory responses are known for delivering stronger and faster effector functions; so presumably, MBCs differentiate more quickly into AFCs. However, MBC subsets that lack expression of some of the typical MBC markers (i.e., DN MBCs), are mainly IgM, and carry relatively few V region mutations appear to be dedicated not to making immediate effector function but rather to reseeding GCs, which they do almost as efficiently as NBCs. On the other hand, more "memory-like" MBC subsets, all of which express markers like CD80 and some of which are IgG, seem committed to making immediate effector function. Such IgM MBCs have been found in antihapten, antiprotein, antibacterial, and antimalaria responses (14, 22, 59, 63) and can rapidly make both IgM and IgG AFCs.

This division of labor in the MBC compartment could permit both rapid and effective responses upon rechallenge while avoiding burnout, whereas if all MBCs were to differentiate to a terminal effector stage immediately, strong secondary responses would deplete or even extinguish MBC pools in an antigen-specific fashion. In contrast, the follicular B cells of the naive compartment

can give rise both to early AFC reactions (albeit not as quickly as MBCs do) and to GCs; in other words, NBCs have a broader potential than either MBC compartment, but taken together, by division of labor, the MBC compartment can deliver both a faster and a stronger response and still regenerate a new crop of MBCs.

GENERATION OF MEMORY B CELLS

Germinal Centers and Other Sources of MBCs

Historically it was thought that MBCs derived specifically and solely (or at least primarily) from GCs, in keeping with the view that MBCs were isotype switched and harbored V region mutations. Indeed, evidence shows that many MBCs do emanate from GC reactions. However, commensurate with the emerging recognition that the normal MBC repertoire includes cells that both are IgM and contain few mutations, data from multiple sources indicate that MBCs do also arise from extra-GC sources. Indeed, even IgG1 MBCs can have few or even no mutations, as first demonstrated by Takahashi et al. (92).

Inamine et al. (93) observed antigen-specific expanded populations of B cells with a memory phenotype as early as 10 days after immunization and found that ICOS-blocking antibodies had little effect on even IgG1 MBCs for as long as 70 days into the response; however, the GC responses were markedly attenuated though not eliminated (a limitation that reduced the conclusiveness of these studies at the time). Mice lacking Bcl6 specifically in B cells in a mixed bone marrow chimera setting completely lacked GCs but could generate unmated IgM and IgG1 MBCs, again dissociating GC formation from MBC formation (94); these data were confirmed and extended using conditional Bcl6 deletion (95). Taylor et al. carried out similar chimera experiments that further confirmed that B cells lacking this molecule could make MBCs (23). However, these studies could have revealed or exaggerated alternative pathways that emerged when normal interactions or gene expression was blocked. Taylor et al. (23) used CD73 as a marker of GC-derived MBCs to reach the conclusion; however, it was later shown that CD73 was expressed by MBCs that formed prior to the GC reaction as well as during the reaction (55). Hence, though the conclusions of Taylor et al. agree with prior work, they do not provide definitive proof, because the marker they relied on was not validated.

Weisel et al. (55) performed a comprehensive BrdU-labeling time course experiment to label cells in the proliferative precursor phase of MBC and plasma cell development over the first 42 days of the immune response and then assayed MBCs and bone marrow long-lived plasma cells 8 weeks after immunization. Cells that remained labeled after the pulse would have been dividing when BrdU was given but then quickly stopped dividing and differentiated to long-lived nonproliferating products (i.e., splenic MBCs and bone marrow plasma cells), whereas cells that continued to proliferate and thus did not differentiate would have diluted out the BrdU and appeared nonlabeled. They showed that approximately 25% of IgM MBCs that are detectable at 8 weeks were formed during the first 2 days of the immune response, prior to any detectable GC reaction. Around 3–5% of IgG1 MBCs were also formed at this time point, and overall, IgG1 MBCs were formed with more delayed kinetics compared to IgM MBCs.

Remarkably, nearly all of the MBC formation was complete before the peak of the GC response. They also found that DN MBCs were on average formed earlier than SP, which in turn preceded DP MBCs, and that most of the MBCs that were produced at later time points were of the DP subset. In contrast, long-lived bone marrow plasma cells were formed at much later time points, with relatively little overlap between MBC formation. The overall results of these studies are depicted in **Figure 3**.

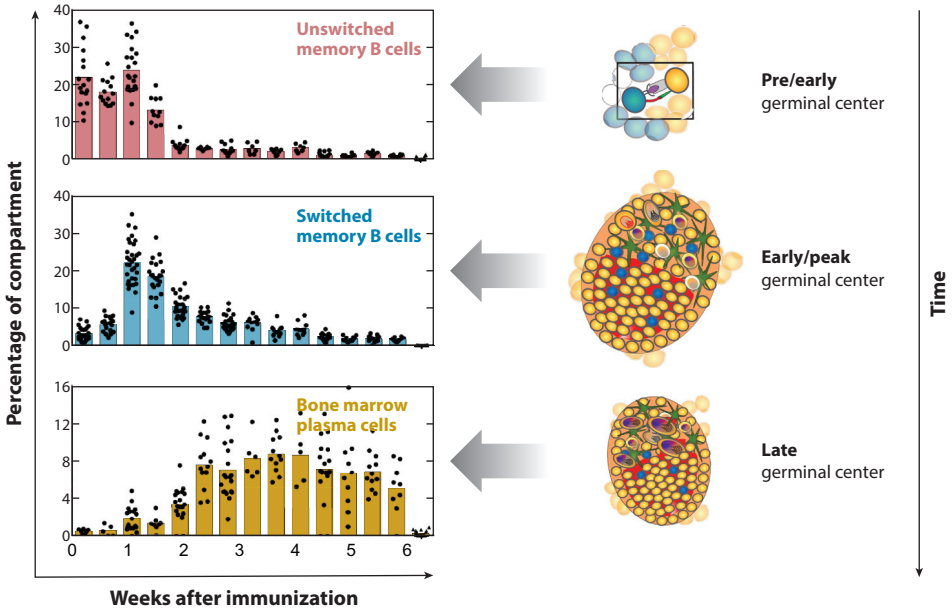

Figure 3

Formation of long-lived humoral memory compartments is separated in time. BrdU pulse-chase in vivo labeling experiments of adoptive transfer recipients (as described in **Figure 1**) during the process of memory generation revealed that unswitched MBCs are generated in two main waves very early during the immune response—even before the onset of germinal centers (*pink*). The *y*-axis on the left represents the percentage of the total compartment that was formed during each time window indicated on the *x*-axis. MBCs of switched isotype were found to be stably formed later than their unswitched counterparts with peak MBC generation at about one week after immunization (*blue*). Bone marrow–resident, antigen-specific, long-lived plasma cells of IgG1 isotype were found to originate much later during the immune response (*yellow*). Modified from Reference 55 with permission.

Hence, as originally put forth by Inamine et al. (93), MBCs are generated in two waves, with one occurring prior to GC formation. Interestingly, histologic detection of EdU-labeled cells at key time points showed that the very early MBCs are likely being formed in T cell zones and to a lesser extent B cell follicles that do not contain GCs (55). At later time points there is strongly detectable EdU uptake in the GCs, as expected, indicating that these would be generative sites; but, notably, there is continued proliferation at the same sites as early proliferation occurred. This suggests that the extrafollicular "wave" may actually continue on, though it becomes overshadowed by the larger GC-derived source of MBC formation.

Questions Remaining on Extra-GC Versus GC-Derived MBC Origins and Functions

The last several years have brought major progress in understanding the origins of MBCs and how different ontogenies generate different types of MBCs. These data have mostly come from the murine system, where the required types of experiments are more readily conducted, although analysis of V region mutations, Ig isotype, and CD27 expression as well as origins in CD40L-deficient patients has led some to postulate GC-independent pathways in humans as well (96, 97). These advances highlight the formation of MBCs outside of GCs at early time points, and

possibly in an ongoing fashion. Some salient questions remain. The longevity of early-appearing (DN) IgM MBCs is not entirely clear, though several groups including our own have found that IgM MBCs can have very long half-lives. This suggests an important adaptive purpose for such unmutated and relatively lower-affinity B cells. It is also unclear whether such MBCs have unique trafficking patterns and/or residence sites within the immune organism. Given that some MBCs are formed very early and presumably before antigen and pathogens are eliminated, it is interesting to consider that they could potentially rejoin the immune reaction at a later time during the same response cycle. These cells will be mostly DN and hence capable of seeding GC reactions. One could imagine that if an infecting pathogen were to escape control and antigen levels were to increase, then newly formed early MBCs could be recruited into new GC reactions or even to join nascent ones, as it is likely that their affinity will be similar to B cells in early GCs (98). Further, if these MBCs begin to recirculate, they might join or initiate GC reactions in distant sites in the event of disseminated infection.

Cellular Precursors for MBCs

There are several types of NBCs, and an important question is whether they all have the potential to generate MBCs, or at least all types of MBCs. It is widely accepted that follicular B cells are precursors to GC B cells, which in turn are clearly precursors for MBCs. Song & Cerny (99) reported that, whereas follicular B cells do indeed seed GCs and generate memory as expected, marginal zone (MZ) B cells can seed both AFC and GC reactions, and they suggested that this may reflect heterogeneity among MZ B cells. MBCs derived from MZ B cells mounted a much higher IgM secondary response upon rechallenge after transfer, suggesting that MZ B cells may more efficiently make early, DN, IgM and potentially GC-independent MBCs. This remains to be tested.

Mice have prominent populations of so-called B1 B cells, which in turn are often divided into B1a and B1b subsets based on expression of CD5. These cells are important for protection from, and do respond to, various types of pathogen, though in differential fashion (reviewed in Reference 100). While a detailed discussion is beyond the scope of this review, data from several systems do suggest that B1b B cells can generate antigen-specific, antigen-driven memory to pathogen-derived epitopes. These examples include responses to bacterial dextrans among peritoneal B1b B cells (101) or *Borrelia hermsii* after infection (102); in the latter case, only B1b phenotype B cells were seen to stably expand.

LOCATION OF MEMORY B CELLS

Secondary Lymphoid Tissue Versus Organ Localization of MBCs

It has long been known that once MBCs are formed they can be found in all SLTs. Classical and recent experiments show that memory responses can be transferred by passaging cells from either spleen or lymph node to naive recipients. More recently, surface markers that identify MBCs in both mice and humans have been used to enumerate MBCs in multiple tissues. At steady state, $CD27^+$ cells can be found in the gut, tonsillar epithelium (103), and PPs of humans (104), as well as in the healthy gingiva (105). MBCs expressing CD80 and/or CD73 of either IgM or IgA isotype can be found in PPs of mice (58). Oral immunization of mice with proteins and cholera toxin adjuvant generates functionally detectable MBCs eight months after immunization in spleen, mesenteric lymph nodes, PPs, and intestinal lamina propria (106). We have also found MBCs in lamina propria of mice that had been immunized intraperitoneally with nitrophenyl

chicken gamma globulin (NP-CGG) in alum (unpublished observations). Recently Bemark and colleagues (107) found that oral immunization with NP conjugated to cholera toxin generates MBCs that populate spleen, PPs, lamina propria, and mesenteric lymph nodes. Most of these express CD73 and PD-L2, and many but not all express CD80. Notably, many of these MBCs elicited by oral immunization are IgA^+, whereas intraperitoneal immunization did not generate this isotype. Aging mice accumulate a population of $CD80^+$ B cells that contain V region mutations, and the frequency of such mutations also increases with time (57). Similarly, murine MBCs that express CD80, PD-L2, CD73 or some combination of these can be found in lung after influenza infection. This confirms pioneering work by Sangster and colleagues, who reached a similar conclusion using functional assays for MBCs and showed that MBCs can disseminate even after a focused infection such as influenza in the lung (32). These flu-specific MBCs in lung may reside in inducible bronchus-associated lymphoid tissue (iBALT) and/or be scattered in the interstitial spaces (108).

It was recently shown that commensal colonization can lead to long-term IgA production in mouse small intestine (58, 109). However, it is less clear whether this is due to generation of long-lived MBCs (110, 111). Indeed, it could be that in many cases, intestinal colonization that does not result in frank mucosal border breach and more central immune responses (i.e., in draining lymph nodes) generates relatively short-lived AFC responses instead (112). It is not clear that attenuated *Salmonella* given orally, for example, generates true long-lived memory as it does not readily generate a GC response (113), although GC formation is a desirable and sought-after outcome. Reports of protection could readily be attributed to persistent antibody responses when no cell transfer and/or rechallenge occurred relatively quickly after initial infection (114).

The specific properties of tissue-resident MBCs are thus incompletely understood. As noted, in the mouse they do express at least some of the markers found among splenic MBCs, but whether these patterns of expression differ, whether there are additional subsets or markers, and whether there are unique functions largely remain to be determined. The same can be said of human MBCs in tissues. This is an area for further investigation that may help us to understand why and how MBCs function to protect mucosal surfaces.

Microanatomic Localization of MBCs Within Secondary Lymphoid Tissues

Where do MBCs reside in SLTs? Based on studies in rats two weeks after immunization it was originally thought that MBCs colonized mainly the splenic MZ (115). Subsequent studies in mice carried out at later time points identified MBCs in both splenic follicles and MZs, at frequencies approximately proportional to the sizes of the zones (17). MBCs are also heterogeneous for expression of CD35, a marker of MZ B cells. However, preliminary experiments suggested that at least some of the follicular MBCs were expressing high levels of CD35 (17). In humans, most of the MZ B cells of human spleen are $CD27^+$, although $CD27^+$ cells are also seen scattered in follicles. Whereas MZ B cells at least in rodents are thought to be relatively sessile, in humans MBCs are known to recirculate, given that they are abundant in blood, PPs, tonsils, and gut lamina propria. The relationship of the $CD27^+$ B cells in MZs and follicles of humans and true "marginal zone" B cells that are antigenically naive is not clear. This parallel is further clouded by the significant functional differences between rodent and primate splenic architecture, and specifically the MZ (116).

The mechanisms and signals that localize MBCs within SLTs are poorly characterized. However, MBCs do express CXCR5, a primary receptor for follicular localization, and so it stands to reason that MBCs should at the least circulate in follicles. They also express EBI2, which is needed for outer follicular localization, at levels similar to or possibly even higher than naive

follicular B cells (117). Further research of the locations of MBCs and how they are maintained is needed, especially in light of newly uncovered heterogeneity. In parallel, greater understanding is needed of how MBCs are located in specific niches within tissues, such as lamina propria and lung parenchyma.

MAINTENANCE OF MEMORY B CELLS

MBCs live a long time, almost by definition. What are the signals and intrinsic changes that keep them alive? Is there heterogeneity of MBC lifespan and maintenance requirements? A major advance was the discovery that the TNF family cytokine BAFF/BLyS is not essential to the survival of MBCs, unlike the situation with NBCs, which require BAFF-R signals mediated by BAFF (118, 119). This was first found in mice and was later confirmed when anti-BAFF or TACI-Ig treatments were tested in humans in connection with developing treatments for autoimmune diseases. In addition, MBCs require Syk for their continued survival (120); this may relate to a consistent need for Syk-mediated tonic BCR signaling (121). In this regard they may even differ from NBCs, which were originally thought to require Syk for survival based on constitutive signaling (122, 123), but which may also survive at least in part via BAFF-R signals (which presumably are not important for MBCs) if Syk is deleted once cells have matured (124).

Since MBCs and NBCs differ in their requirements for BAFF-R signaling, this leaves open the question of what if any cytokine or contact-dependent signals in fact are required to keep MBCs alive. MBCs could have switched to a different cytokine system, much like how CD8 memory T cells rely on IL-7 and IL-15 for survival. However, we are unaware of any reports that MBCs depend on IL-7, IL-15, or any other cytokine. One report claimed that nerve growth factor is an autocrine survival factor for MBCs (125); however, we have found no recent confirmatory reports. MBCs also upregulate bone morphogenetic protein receptor 1a, as well as a stem cell–like program; hence, they could rely on cytokines important for regulating stemness, such as bone morphogenetic proteins or leukemia inhibitory factor (15, 126, 127).

T cells are not required for MBC survival: T cell depletion had no effect on recall responses in intact mice (128). Although it was previously (129), and more recently (130), suggested that antigen (131), most likely trapped on follicular dendritic cells in the form of immune complexes, is a reservoir that maintains MBCs by providing intermittent or low-level exposure to antigen, two pivotal studies suggest that this mechanism is in fact dispensable for MBC maintenance. Rajewsky and colleagues devised a clever system in which mouse BCRs could be switched to irrelevant ones in already established MBCs (132). This would render any retained antigen irrelevant. The half-life of such MBCs was not reduced. In a separate approach, Hannum et al. (133) generated a mouse that had B cells that could not secrete any Ig and hence were unable to deposit antigen on follicular dendritic cells in the form of immune complexes. Again, MBCs in these mice had a normal and very long half-life. These issues are reviewed more comprehensively elsewhere (134).

Apart from external signals for MBC survival, which notably have not yet been identified, differential expression of either prosurvival or proapoptotic genes could explain the longer life span of MBCs. Based on higher numbers of MBCs found in Puma-deficient mice after immunization, Clybouw et al. (135) suggested that Puma regulates MBC survival. However, Puma was absent throughout B cell differentiation in these experiments, and Puma could plausibly control the survival and/or differentiation of MBC precursors either exclusively or in addition. More precise experiments would be required to gather better support for this idea. Mcl-1, a prosurvival member of the Bcl-2 family of genes, was required for GC survival, and substantially fewer MBCs were formed in its absence; but again it was unclear if Mcl-1 was required for persistence of MBCs

once they would have formed normally (136). In contrast, ABT-737—an inhibitor of three other members of the Bcl-2 family of proteins that does not affect Mcl-1—did have an effect on the established MBC compartment (137).

MEMORY B CELL FUNCTION UPON REEXPOSURE

Recall Responses

The main function of immunological memory is to protect the individual from recurrent infection. This can be achieved at multiple levels: standing antibody titers to opsonize or neutralize invading pathogens, accelerated recall responses to form immediate effector cells for antibody or cytokine production (138), and initiation of second rounds of GC reactions (86). Given that—across many immunizing or infectious antigens in mice (e.g., NP-CGG, PE, NP-KLH, LCMV, HSV, influenza, VSV, TT, DT)—50 to 3,000 MBCs are generated for every million splenic B cells, the finding that adoptive transfer of 25 virus-specific IgG MBCs can generate a strong antibody titer in recipients upon T cell–independent antigen recall (27) highlights the immense immunological power and redundancy of humoral immunity (11, 24, 139–145). Estimating an antibody synthesis rate of 5×10^7 molecules per hour (146) and a serum half-life of about a week (147), it could be calculated that indeed reactivation of 10–100 MBCs could result in the generation and maintenance of biologically relevant serum antibody titers.

MBCs in both mice and humans respond faster in a number of respects. They are known to enter the cell cycle faster, to differentiate into AFCs faster, and possibly to reseed GCs faster (42, 89, 148). Undoubtedly, much of this is encoded in cell-intrinsic differences in gene expression, likely due to as yet uncharacterized epigenetic alterations (149), as discussed above. However, reactivation differences may also be seen at the level of signaling from the BCR and other initial activation receptors, such as CD40 and innate immune receptors. Part of this could be due to the cell-intrinsic differences in gene expression just mentioned; nonetheless, it would be of great interest to understand how signaling networks are remodeled in MBCs versus NBCs. As already noted, signal reprogramming is a subject of ongoing study (12, 83).

Differences in MBC Signaling

One area of recent progress is the differences in signaling between IgM and IgG BCRs (150). An emerging body of work has shown that all IgG BCRs allow for inducible phosphorylation on a tyrosine residue in a motif termed the immunoglobulin tail tyrosine (ITT) motif. This allows for amplification and costimulation of the standard Igα/ Igβ ITAM-based signaling system (summarized in 85). The adaptor protein growth factor–bound protein 2 (Grb2) binds to the phosphorylated ITT of IgG and IgE BCRs (151) and recruits Bruton's tyrosine kinase (Btk), which is the activator of PLCγ2 (152). This ITT-based costimulation lowers the activation threshold of IgG BCRs and therefore might explain why IgG MBCs (which are in general also of higher affinity) are preferentially activated compared to their IgM counterparts, as B cell–specific Grb2-deficient mice mount strongly reduced memory IgG responses (153). Inducible loss of PLCγ2 in the established MBC compartment leads to its decrease over time, which points to the fact that BCR signaling is important for IgG MBCs (154).

Another mechanism for discriminating IgM and IgG signaling may stem from the inhibitory coreceptor CD22. It was originally reported to downmodulate signaling through IgM and IgD BCRs, whereas the cytoplasmic tail of IgG was thought to prevent CD22-mediated signal inhibition (155). However, more recent data have challenged this notion (156). IgD is expressed at

least at some level on a fraction of—perhaps most—IgM MBCs in both mice and humans (157). The coexpression of this receptor might also dampen the reactivation of these MBCs (158, 159). Hence, multiple mechanisms might mediate the relatively more brisk and qualitatively distinct signaling inherent in IgG MBCs.

Effects of Preexisting Antibodies

In many circumstances reactivation of MBCs happens under different serologic conditions compared to initial antigen encounter of NBCs, since antigen-specific antibody titers (serological memory) are present during recall responses. Therefore, antigen-IgG immune complexes add another layer in directing and enhancing antigen presentation during secondary immune responses, compared to initial antigen encounter. Another potential difference between primary responses by NBCs and secondary responses by MBCs is antigenic load. During primary responses low antigen-specific serum antibody titers allow for unchecked pathogen multiplication, thus increasing antigenic load, whereas high antibody titers of generally higher affinity can capture, opsonize, and neutralize recurring antigens during recall responses. Together these two effects can both redirect antigen and reduce its availability during a MBC response. The net effect could be to restrict MBC activation to only a subset of the highest-affinity MBCs, which may have led to an overestimation of the repertoire affinity of the MBC compartment (160). This is because in most cases the MBC compartment has been interrogated by reimmunization in intact animals. This has led to the suggestion that IgM MBCs (which tend to be of lower affinity) per se do not participate in secondary responses as long as antibody titers are present (22). However, these B cells may be just as functional as IgG MBCs (86) save for their affinity, which would render them inferior if, and only if, antigen dose were low and relevant preexisting antibody were present.

While it would at first blush seem to be the norm that MBC responses occur only in the presence of preexisting serum antibody titers, there are exceptions to consider. The exceptions would include two scenarios: if long-term AFCs were not made while MBCs were made or if the pathogen mutated such that the highly selected and focused long-lived plasma cell (LLPC) repertoire and hence serum antibodies were rendered irrelevant. In each case, the result upon rechallenge or the next season of infection would be MBC exposure in the absence of relevant serum antibodies. Interestingly, though in experimental systems this is rarely modeled, in reality it could often be the case. Multiple pathways are known that when mutated or inhibited lead to only partial or interrupted GC development. In these cases, the result is that LLPC production and hence serum antibodies are abrogated while MBC development is preserved. These pathways notably include signals mediated by complement receptor, PD-1 or IL-21R (161–163). If pathogens can block or if immunization conditions minimize such signals, then MBCs will form without long-lived antibody titers and hence reimmunization will expose MBCs to antigen in the absence of such antibody, even in an intact host. In fact, multiple pathogens are known to elicit strong B cell responses in the absence of much, if any, GC response (113, 164). Yet, in the case of *Ehrlichia* infection, there is still T cell–dependent but GC-independent MBC formation, and these IgM MBCs will be recalled to generate secondary IgG responses (165). As detailed in this review, it is abundantly clear that a wave of IgM MBCs is indeed formed independent of and prior to GC reactions (55, 93, 94). In another equally relevant scenario, pathogens evolve over time to generate escape mutants that are no longer recognized by the dominant antibodies produced in a prior infection. The most notorious example is influenza. Here, studies have shown that whereas an antigenically drifted strain in a subsequent year has avoided relevant serum antibodies and can infect, the host will mount a response drawn largely from last year's MBC compartment,

which undergoes a new round of SHM to adapt to the antigenically drifted influenza HA (36). Thus, situations in which MBCs are exposed in the absence of serum antibodies may be relatively common in nature, and not just an artifact of B cell transfer experiments that have been used to interrogate MBC potential in the absence of preexisting serum antibodies. The latter (e.g., 14, 86) have been critiqued as unphysiological (22) but in fact are informative with respect to not only native MBC biology but also physiological situations that arise constantly in natural infection scenarios.

REQUIREMENTS FOR REACTIVATION

Upon reexposure to antigen, the immune system has to accomplish two seemingly disparate functions that need to be carefully balanced: immediate effector function to ensure maximum protection via differentiation into AFCs and avoiding burnout to ensure subsequent protection upon further reexposure to antigen. At another level, secondary immune responses also need to be able to adapt to drifted antigens and therefore optimize existing immunity by adapting to changes within the original immunogen. How this is accomplished in detail is not clear at the moment, but it would be reasonable to assume that particular immune-effector functions are segregated among different subsets of MBCs. Quantity and quality of MBC formation as well as recall responses depend critically on the immunizing as well as challenging antigens, as IgG1 MBCs generated after immunization with the T cell–dependent antigen NP-CGG cannot be reactivated with the T cell–independent type II antigen NP-Ficoll (166). In contrast, murine IgG MBCs generated in response to immunization with human CMV–like particles could readily be reactivated after adoptive transfer in T cell– and B cell–deficient Rag1$^{-/-}$ mice when challenged with virus-like particles again (167). However, challenge with monomeric, soluble viral protein did not result in T cell–independent reactivation of MBCs (167), whereas particularization and multimerization of the same viral protein on microbeads were sufficient to induce T cell–independent IgG MBC responses, indicating that the valency of the antigen plays a pivotal role in MBC reactivation (27).

Location of reactivation may also be a factor, though studies show a fair amount of flexibility in where MBCs can be reactivated. Weisel et al. (27) observed T cell–independent MBC reactivation in TNF/LT$\alpha^{-/-}$ mice that had been reconstituted with normal bone marrow. Such mice lack most lymphoid structures but have normally functioning hematopoietic systems. This indicates that reactivation can occur independently of lymph nodes and PPs, since these tissues are not generated de novo after bone marrow reconstitution (27). In this study MBCs were hardly detectable in bone marrow, peritoneal cavity, and peripheral blood. In line with these findings, at least three other studies failed to detect MBC activation in the bone marrow (168–170), whereas vesicular stomatitis virus–specific MBCs were reported to be activated in the bone marrow (171). MBCs could be reactivated in the absence of follicular dendritic cells and in the absence of clodronate-sensitive cell populations like dendritic cells and macrophages (172). Interestingly, reactivation of virus-specific MBCs was not restricted to the splenic MZ, given that FTY720-mediated relocation of MZ-resident B cells into the follicle did not alter the memory response (172), whereas anti-CXCL13-mediated impairment of MBC homing to lymphoid follicles (173) resulted in significantly impaired MBC responses (27). Taken together, these data highlight that MBC recall responses can occur in settings of immunosuppression, without T cells, and at least in some settings in the absence of SLT. Moreover, even in a situation where helper T cells are potentially available, they may be limiting or not in the proper location at least initially. In this vein, it is particularly interesting that there may be both early T cell–independent and later T cell–dependent memory responses to infectious challenge (174).

MEMORY B CELLS AND PROTECTION FROM PATHOGENS

How do MBCs afford protection from pathogens in various contexts? Context does matter. In fact the possibilities of how and when and where MBCs could contribute to both resistance to reinfection and clearance or suppression of ongoing infection could be quite broad. These possibilities cannot all be covered here; indeed, this is an area that requires substantially more research in the context of specific pathogen infections. Here we briefly touch on three pathogens—CMV, influenza, and HIV.

CMV

CMV infection is a major problem after immunosuppression (175). Therefore, MBCs may be called on to respond to infection in the absence of optimal T cell help. To investigate this, the protective capacity of adoptive MBC transfer in a murine CMV (mCMV) infectious model was analyzed (175). Adoptive transfer of mCMV-specific MBCs into T cell– and B cell–deficient mice resulted in the generation of strong mCMV-specific serum titers and significant reduction of viral titers following active mCMV infection. Furthermore, this approach provided long-term protection from lethality. Adoptive transfer of mCMV-specific MBCs into immunocompromised mice after infection also resulted in protection of the animals, indicating therapeutic potential of adoptive MBC transfer (176). Together these data showed that, at least for certain viral infections, the protective and functional effects of MBCs can occur independently of T cells.

Influenza

Despite vaccination programs, influenza still causes approximately 20,000 deaths annually in the United States, with elderly people being affected most (177). Antibodies alone can prevent influenza infection (summarized in 178). Moreover, naturally arising anti-influenza antibodies are long-lasting: Survivors of the 1918 influenza pandemic had IgG MBCs specific for this strain 90 years later (179). During primary infection IgA^+ B cells are a major component of the response. During the course of infection inducible tertiary lymphoid aggregates may form (iBALT), that contain follicles and T cell areas. iBALT can be the site of GC reactions (180), which persist longer in iBALT than in SLT (181). In murine models there are persistent GCs in the lung with higher rates of SHM and more cross-reactive B cells that are able to neutralize escape variants (182). In rhesus monkeys MBCs distribute mainly to mediastinal lymph nodes (183). The immune response in vaccinated or exposed adults is characterized by activation of preexisting MBCs (36, 184), which are the predominant precursors to the influenza-specific plasmablast response (185). Furthermore, previous exposures to certain influenza strains shape subsequent responses, suggesting that they are drawn from MBCs against cross-reactive strains (186).

These observations also have implications for influenza vaccination. Seasonal influenza vaccines have to be reformulated every year to allow for the generation of neutralizing antibodies to antigenic drift virus variants, since the majority of generated antibodies are strain-specific. Therefore the field has endeavored to design vaccines that elicit broadly neutralizing antibodies [e.g., directed against the conserved stalk domain instead of the highly variable head domain to enable targeting a wide spectrum of influenza strains (summarized in 187)]. However, since MBCs also offer protection to cross-reactive strains, the nature of the MBC compartment should probably be considered further. In particular, based on animal models, infection in the lung generates local responses that may be of higher or different protective quality, in a way that would not be replicated by killed vaccines administered intramuscularly.

HIV

Vaccine approaches are being developed to effect both cure and prevention of HIV infection. Owing to the immune evasion mechanisms and the complexity of the infection, particularly its attendant severe immunological dysfunction (188), there are as yet no effective strategies for either goal. B cells have been underestimated for many years in combating HIV. Viral diversification is a major hurdle to finding effective vaccines, and as with influenza the emphasis has been on strategies to find broadly neutralizing antibodies. These generally only arise in a subset of infected patients and typically only after years of infection (189). The antibodies themselves have very large numbers of V region mutations (190, 191), which points to a prolonged affinity maturation process. The pathways for generating such antibodies naturally and via vaccination are far from clear, despite a great deal of work and theory development (192, 193). Whether these cells spend extended periods of time within GC reactions or whether MBCs are generated that are constantly reactivated is not clear. Mutational load increases during the course of the GC reaction (55), while late-appearing murine MBCs are mostly of $CD80^+$ and $PD\text{-}L2^+$ phenotype (55) and therefore prone to generate plasmablasts rather than reenter GC reactions upon antigen reencounter (86). For these reasons, it seems possible that antibodies with high V region mutational load would emanate from constantly reactivated MBCs that are more of a DN or memory stem cell type. If so, then immunization strategies should be developed to forestall switching to IgG and/or promote generation of DN-type MBCs from early GC reactions (86). As mentioned above, HIV is one of several chronic infections associated with atypical $CD27^-$ MBCs, which are relatively less responsive to stimuli. These have been termed exhausted, but in fact their function in controlling disease remains uncertain. Whether these would be precursors for eventual generation of broadly neutralizing antibodies is an interesting question.

Thus, with respect to immunization, more emphasis might be placed on understanding the MBC compartments as well as the AFC compartments, because these can diverge considerably in repertoire, as has been noted in both people and mice. While there may be hypermutated V regions among AFCs, it is less clear what the antigen-specific MBC compartment looks like and whether these two compartments are truly connected. Hence, further study is required to understand how MBCs could control ongoing HIV infection and how they contribute to generating broadly neutralizing or other antibodies either naturally or upon vaccination.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Netea MG, Latz E, Mills KHG, O'Neill LAJ. 2015. Innate immune memory: a paradigm shift in understanding host defense. *Nat. Immunol.* 16:675–79
2. Yamane H, Paul WE. 2012. Memory $CD4^+$ T cells: fate determination, positive feedback and plasticity. *Cell. Mol. Life Sci.* 69:1577–83
3. Restifo NP, Gattinoni L. 2013. Lineage relationship of effector and memory T cells. *Curr. Opin. Immunol.* 25:556–63
4. Harris DP, Haynes L, Sayles PC, Durso DK, Eaton SM, et al. 2000. Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat. Immunol.* 1:475–82
5. Lund FE, Garvy BA, Randall TD, Harris DP. 2005. Regulatory roles for cytokine-producing B cells in infection and autoimmune disease. *Curr. Dir. Autoimmun.* 8:25–54

6. Duddy M, Niino M, Adatia F, Hebert S, Freedman M, et al. 2007. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J. Immunol.* 178:6092–99
7. Slifka MK, Antia R, Whitmire JK, Ahmed R. 1998. Humoral immunity due to long-lived plasma cells. *Immunity* 8:363–72
8. Hammarlund E, Lewis MW, Hansen SG, Strelow LI, Nelson JA, et al. 2003. Duration of antiviral immunity after smallpox vaccination. *Nat. Med.* 9:1131–37
9. Obukhanych TV, Nussenzweig MC. 2006. T-independent type II immune responses generate memory B cells. *J. Exp. Med.* 203:305–10
10. Taillardet M, Haffar G, Mondière P, Asensio M-J, Gheit H, et al. 2009. The thymus-independent immunity conferred by a pneumococcal polysaccharide is mediated by long-lived plasma cells. *Blood* 114:4432–40
11. McHeyzer-Williams MG, Nossal GJV, Lalor PA. 1991. Molecular characterization of single memory B cells. *Nature* 350:502–5
12. Pierce SK, Liu W. 2013. Encoding immunological memory in the initiation of B-cell receptor signaling. *Cold Spring Harb. Symp. Quant. Biol.* 78:231–37
13. Anderson SM, Tomayko MM, Shlomchik MJ. 2006. Intrinsic properties of human and murine memory B cells. *Immunol. Rev.* 211:280–94
14. Tomayko MM, Steinel NC, Anderson SM, Shlomchik MJ. 2010. Cutting edge: hierarchy of maturity of murine memory B cell subsets. *J. Immunol.* 185:7146–50
15. Tomayko MM, Anderson SM, Brayton CE, Sadanand S, Steinel NC, et al. 2008. Systematic comparison of gene expression between murine memory and naive B cells demonstrates that memory B cells have unique signaling capabilities. *J. Immunol.* 181:27–38
16. Anderson SM, Hannum LG, Shlomchik MJ. 2006. Cutting edge: memory B cell survival and function in the absence of secreted antibody and immune complexes on follicular dendritic cells. *J. Immunol.* 176:4515–19
17. Anderson SM, Tomayko MM, Ahuja A, Haberman AM, Shlomchik MJ. 2007. New markers for murine memory B cells that define mutated and unmutated subsets. *J. Exp. Med.* 204:2103–14
18. Dogan I, Bertocci B, Vilmont V, Delbos F, Megret J, et al. 2009. Multiple layers of B cell memory with different effector functions. *Nat. Immunol.* 10:1292–99
19. Hougs L, Juul L, Ditzel HJ, Heilmann C, Svejgaard A, Barington T. 1999. The first dose of a *Haemophilus influenzae* type B conjugate vaccine reactivates memory B cells: evidence for extensive clonal selection, intraclonal affinity maturation, and multiple isotype switches to IgA2. *J. Immunol.* 162:224–37
20. Frolich D, Giesecke C, Mei HE, Reiter K, Daridon C, et al. 2010. Secondary immunization generates clonally related antigen-specific plasma cells and memory B cells. *J. Immunol.* 185:3103–10
21. Giesecke C, Frolich D, Reiter K, Mei HE, Wirries I, et al. 2014. Tissue distribution and dependence of responsiveness of human antigen-specific memory B cells. *J. Immunol.* 192:3091–100
22. Pape KA, Taylor JJ, Maul RW, Gearhart PJ, Jenkins MK. 2011. Different B cell populations mediate early and late memory during an endogenous immune response. *Science* 331:1203–7
23. Taylor JJ, Pape KA, Jenkins MK. 2012. A germinal center-independent pathway generates unswitched memory B cells early in the primary response. *J. Exp. Med.* 209:597–606
24. Hayakawa K, Ishii R, Yamasaki K, Kishimoto T, Hardy RR. 1987. Isolation of high-affinity memory B cells: phycoerythrin as a probe for antigen-binding cells. *PNAS* 84:1379–83
25. Schittek B, Rajewsky K. 1990. Maintenance of B-cell memory by long-lived cells generated from proliferating precursors. *Nature* 346:749–51
26. Schittek B, Rajewsky K, Forster I. 1991. Dividing cells in bone marrow and spleen incorporate bromodeoxyuridine with high efficiency. *Eur. J. Immunol.* 21:235–38
27. Weisel FJ, Appelt UK, Schneider AM, Horlitz JU, van Rooijen N, et al. 2010. Unique requirements for reactivation of virus-specific memory B lymphocytes. *J. Immunol.* 185:4011–21
28. Silvy A, Lagresle C, Bella C, Defrance T. 1996. The differentiation of human memory B cells into specific antibody-secreting cells is CD40 independent. *Eur. J. Immunol.* 26:517–24
29. Crotty S, Felgner P, Davies H, Glidewell J, Villarreal L, Ahmed R. 2003. Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J. Immunol.* 171:4969–73

30. Bernasconi NL, Traggiai E, Lanzavecchia A. 2002. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* 298:2199–202
31. Clarke ET, Williams NA, Findlow J, Borrow R, Heyderman RS, Finn A. 2013. Polysaccharide-specific memory B cells generated by conjugate vaccines in humans conform to the $CD27^{+}IgG^{+}$ isotype-switched memory B cell phenotype and require contact-dependent signals from bystander T cells activated by bacterial proteins to differentiate into plasma cells. *J. Immunol.* 191:6071–83
32. Joo HM, He Y, Sangster MY. 2008. Broad dispersion and lung localization of virus-specific memory B cells induced by influenza pneumonia. *PNAS* 105:3485–90
33. Dal Porto JM, Haberman AM, Shlomchik MJ, Kelsoe G. 1998. Antigen drives very low affinity B cells to become plasmacytes and enter germinal centers. *J. Immunol.* 161:5373–81
34. Dal Porto JM, Haberman AM, Kelsoe G, Shlomchik MJ. 2002. Very low affinity B cells form germinal centers, become memory B cells, and participate in secondary immune responses when higher affinity competition is reduced. *J. Exp. Med.* 195:1215–21
35. Di Niro R, Lee SJ, Vander Heiden JA, Elsner RA, Trivedi N, et al. 2015. *Salmonella* infection drives promiscuous B cell activation followed by extrafollicular affinity maturation. *Immunity* 43:120–31
36. Wrammert J, Smith K, Miller J, Langley WA, Kokko K, et al. 2008. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature* 453:667–71
37. Wrammert J, Onlamoon N, Akondy RS, Perng GC, Polsrila K, et al. 2012. Rapid and massive virus-specific plasmablast responses during acute dengue virus infection in humans. *J. Virol.* 86:2911–18
38. Klein U, Kuppers R, Rajewsky K. 1997. Evidence for a large compartment of IgM-expressing memory B cells in humans. *Blood* 89:1288–98
39. Tangye SG, Liu YJ, Aversa G, Phillips JH, de Vries JE. 1998. Identification of functional human splenic memory B cells by expression of CD148 and CD27. *J. Exp. Med.* 188:1691–703
40. Tangye SG, Avery DT, Deenick EK, Hodgkin PD. 2003. Intrinsic differences in the proliferation of naive and memory human B cells as a mechanism for enhanced secondary immune responses. *J. Immunol.* 170:686–94
41. Good KL, Bryant VL, Tangye SG. 2006. Kinetics of human B cell behavior and amplification of proliferative responses following stimulation with IL-21. *J. Immunol.* 177:5236–47
42. Good KL, Avery DT, Tangye SG. 2009. Resting human memory B cells are intrinsically programmed for enhanced survival and responsiveness to diverse stimuli compared to naive B cells. *J. Immunol.* 182:890–901
43. Tangye SG, Tarlinton DM. 2009. Memory B cells: effectors of long-lived immune responses. *Eur. J. Immunol.* 39:2065–75
44. Bernasconi NL, Onai N, Lanzavecchia A. 2003. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. *Blood* 101:4500–4
45. Arpin C, Banchereau J, Liu Y-J. 1997. Memory B cells are biased towards terminal differentiation: a strategy that may prevent repertoire freezing. *J. Exp. Med.* 186:931–40
46. Ehrhardt GRA, Hsu JT, Gartland L, Leu C-M, Zhang S, et al. 2005. Expression of the immunoregulatory molecule FcRH4 defines a distinctive tissue-based population of memory B cells. *J. Exp. Med.* 202:783–91
47. Fecteau JF, Cote G, Neron S. 2006. A new memory $CD27^{-}IgG^{+}$ B cell population in peripheral blood expressing V_H genes with low frequency of somatic mutation. *J. Immunol.* 177:3728–36
48. Wei C, Anolik J, Cappione A, Zheng B, Pugh-Bernard A, et al. 2007. A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J. Immunol.* 178:6624–33
49. Kaech SM, Wherry EJ, Ahmed R. 2002. Effector and memory T-cell differentiation: implications for vaccine development. *Nat. Rev. Immunol.* 2:251–62
50. Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, et al. 2016. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* 532:512–16
51. Reese TA, Bi K, Kambal A, Filali-Mouhim A, Beura LK, et al. 2016. Sequential infection with common pathogens promotes human-like immune gene expression and altered vaccine response. *Cell Host Microbe* 19:713–19

52. Macallan DC, Wallace DL, Zhang Y, Ghattas H, Asquith B, et al. 2005. B-cell kinetics in humans: rapid turnover of peripheral blood memory cells. *Blood* 105:3633–40
53. Wirths S, Lanzavecchia A. 2005. ABCB1 transporter discriminates human resting naive B cells from cycling transitional and memory B cells. *Eur. J. Immunol.* 35:3433–41
54. Anderson S, Tomayko M, Ahuja A, Haberman A, Shlomchik M. 2007. New markers for murine memory B cells that define mutated and unmutated subsets. *J. Exp. Med.* 204:2103–14
55. Weisel FJ, Zuccarino-Catania GV, Chikina M, Shlomchik MJ. 2016. A temporal switch in the germinal center determines differential output of memory B and plasma cells. *Immunity* 44:116–30
56. Onodera T, Takahashi Y, Yokoi Y, Ato M, Kodama Y, et al. 2012. Memory B cells in the lung participate in protective humoral immune responses to pulmonary influenza virus reinfection. *PNAS* 109:2485–90
57. Bemark M, Bergqvist P, Stensson A, Holmberg A, Mattsson J, Lycke NY. 2011. A unique role of the cholera toxin A1-DD adjuvant for long-term plasma and memory B cell development. *J. Immunol.* 186:1399–410
58. Lindner C, Thomsen I, Wahl B, Ugur M, Sethi MK, et al. 2015. Diversification of memory B cells drives the continuous adaptation of secretory antibodies to gut microbiota. *Nat. Immunol.* 16:880–88
59. Krishnamurty AT, Thouvenel CD, Portugal S, Keitany GJ, Kim KS, et al. 2016. Somatically hypermutated *Plasmodium*-specific IgM^+ memory B cells are rapid, plastic, early responders upon malaria rechallenge. *Immunity* 45:402–14
60. Driver DJ, McHeyzer-Williams LJ, Cool M, Stetson DB, McHeyzer-Williams MG. 2001. Development and maintenance of a $B220^-$ memory B cell compartment. *J. Immunol.* 167:1393–405
61. Bell J, Gray D. 2003. Antigen-capturing cells can masquerade as memory B cells. *J. Exp. Med.* 197:1233–44
62. Wolniak KL, Noelle RJ, Waldschmidt TJ. 2006. Characterization of (4-hydroxy-3-nitrophenyl)acetyl (NP)-specific germinal center B cells and antigen-binding $B220^-$ cells after primary NP challenge in mice. *J. Immunol.* 177:2072–79
63. Dell CL, Lu Y, Claflin JL. 1989. Molecular analysis of clonal stability and longevity in B cell memory. *J. Immunol.* 143:3364–70
64. Vajdy M, Lycke N. 1995. Mucosal memory B cells retain the ability to produce IgM antibodies 2 years after oral immunization. *Immunology* 86:336–42
65. Jones DD, Wilmore JR, Allman D. 2015. Cellular dynamics of memory B cell populations: IgM^+ and IgG^+ memory B cells persist indefinitely as quiescent cells. *J. Immunol.* 195:4753–59
66. Gitlin AD, von Boehmer L, Gazumyan A, Shulman Z, Oliveira TY, Nussenzweig MC. 2016. Independent roles of switching and hypermutation in the development and persistence of B lymphocyte memory. *Immunity* 44:769–81
67. Bar-Or A, Oliveira EM, Anderson DE, Krieger JI, Duddy M, et al. 2001. Immunological memory: contribution of memory B cells expressing costimulatory molecules in the resting state. *J. Immunol.* 167:5669–77
68. Airoldi I, Raffaghello L, Cocco C, Guglielmino R, Roncella S, et al. 2004. Heterogeneous expression of interleukin-18 and its receptor in B-cell lymphoproliferative disorders deriving from naive, germinal center, and memory B lymphocytes. *Clin. Cancer Res.* 10:144–54
69. Ehrhardt GR, Hijikata A, Kitamura H, Ohara O, Wang JY, Cooper MD. 2008. Discriminating gene expression profiles of memory B cell subpopulations. *J. Exp. Med.* 205:1807–17
70. Weiss GE, Crompton PD, Li S, Walsh LA, Moir S, et al. 2009. Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J. Immunol.* 183:2176–82
71. Llinàs L, Lázaro A, de Salort J, Matesanz-Isabel J, Sintes J, Engel P. 2011. Expression profiles of novel cell surface molecules on B-cell subsets and plasma cells as analyzed by flow cytometry. *Immunol. Lett.* 134:113–21
72. Li H, Borrego F, Nagata S, Tolnay M. 2016. Fc receptor-like 5 expression distinguishes two distinct subsets of human circulating tissue-like memory B cells. *J. Immunol.* 196:4064–74
73. Sullivan RT, Kim CC, Fontana MF, Feeney ME, Jagannathan P, et al. 2015. FCRL5 delineates functionally impaired memory B cells associated with *Plasmodium falciparum* exposure. *PLOS Patbog.* 11:e1004894
74. Isnardi I, Ng Y-S, Menard L, Meyers G, Saadoun D, et al. 2010. Complement receptor 2/$CD21^-$ human naive B cells contain mostly autoreactive unresponsive clones. *Blood* 115:5026–36

75. Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoenig M, et al. 2009. Circulating CD21^{low} B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. *PNAS* 106:13451–56
76. Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, et al. 2008. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J. Exp. Med.* 205:1797–805
77. Charles ED, Brunetti C, Marukian S, Ritola KD, Talal AH, et al. 2011. Clonal B cells in patients with hepatitis C virus–associated mixed cryoglobulinemia contain an expanded anergic CD21^{low} B-cell subset. *Blood* 117:5425–37
78. Dauby N, Kummert C, Lecomte S, Liesnard C, Delforge ML, et al. 2014. Primary human cytomegalovirus infection induces the expansion of virus-specific activated and atypical memory B cells. *J. Infect. Dis.* 210:1275–85
79. Portugal S, Tipton CM, Sohn H, Kone Y, Wang J, et al. 2015. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. *eLife* 4:e07218
80. Nicholas MW, Dooley MA, Hogan SL, Anolik J, Looney J, et al. 2008. A novel subset of memory B cells is enriched in autoreactivity and correlates with adverse outcomes in SLE. *Clin. Immunol.* 126:189–201
81. Wakabayashi C, Adachi T, Wienands J, Tsubata T. 2002. A distinct signaling pathway used by the IgG-containing B cell antigen receptor. *Science* 298:2392–95
82. Martin SW, Goodnow CC. 2002. Burst-enhancing role of the IgG membrane tail as a molecular determinant of memory. *Nat. Immunol.* 3:182–88
83. Xu Y, Xu L, Zhao M, Xu C, Fan Y, et al. 2014. No receptor stands alone: IgG B-cell receptor intrinsic and extrinsic mechanisms contribute to antibody memory. *Cell Res.* 24:651–64
84. Lutz J, Dittmann K, Bosl MR, Winkler TH, Wienands J, Engels N. 2016. Reactivation of IgG-switched memory B cells by BCR-intrinsic signal amplification promotes IgG antibody production. *Nat. Commun.* 6:8575
85. Wienands J, Engels N. 2016. The memory function of the B cell antigen receptor. *Curr. Top. Microbiol. Immunol.* 393:107–21
86. Zuccarino-Catania GV, Sadanand S, Weisel FJ, Tomayko MM, Meng H, et al. 2014. CD80 and PD-L2 define functionally distinct memory B cell subsets that are independent of antibody isotype. *Nat. Immunol.* 15:631–37
87. Kometani K, Nakagawa R, Shinnakasu R, Kaji T, Rybouchkin A, et al. 2013. Repression of the transcription factor Bach2 contributes to predisposition of IgG1 memory B cells toward plasma cell differentiation. *Immunity* 39:136–47
88. Jash A, Wang Y, Weisel FJ, Scharer CD, Boss JM, et al. 2016. ZBTB32 restricts the duration of memory B cell recall responses. *J. Immunol.* 197:1159–68
89. Good KL, Tangye SG. 2007. Decreased expression of Krüppel-like factors in memory B cells induces the rapid response typical of secondary antibody responses. *PNAS* 104:13420–25
90. Seifert M, Przekopowitz M, Taudien S, Lollies A, Ronge V, et al. 2015. Functional capacities of human IgM memory B cells in early inflammatory responses and secondary germinal center reactions. *PNAS* 112:E546–55
91. Davey AM, Pierce SK. 2012. Intrinsic differences in the initiation of B cell receptor signaling favor responses of human IgG$^+$ memory B cells over IgM$^+$ naive B cells. *J. Immunol.* 188:3332–41
92. Takahashi Y, Ohta H, Takemori T. 2001. Fas is required for clonal selection in germinal centers and the subsequent establishment of the memory B cell repertoire. *Immunity* 14:181–92
93. Inamine A, Takahashi Y, Baba N, Miyake K, Tokuhisa T, et al. 2005. Two waves of memory B-cell generation in the primary immune response. *Int. Immunol.* 17:581–89
94. Toyama H, Okada S, Hatano M, Takahashi Y, Takeda N, et al. 2002. Memory B cells without somatic hypermutation are generated from Bcl6-deficient B cells. *Immunity* 17:329–39
95. Kaji T, Ishige A, Hikida M, Taka J, Hijikata A, et al. 2012. Distinct cellular pathways select germline-encoded and somatically mutated antibodies into immunological memory. *J. Exp. Med.* 209:2079–97

96. Berkowska MA, Driessen GJA, Bikos V, Grosserichter-Wagener C, Stamatopoulos K, et al. 2011. Human memory B cells originate from three distinct germinal center–dependent and –independent maturation pathways. *Blood* 118:2150–58
97. Tangye SG, Good KL. 2007. Human IgM$^+$CD27$^+$ B cells: memory B cells or "memory" B cells? *J. Immunol.* 179:13–19
98. Schwickert TA, Victora GD, Fooksman DR, Kamphorst AO, Mugnier MR, et al. 2011. A dynamic T cell–limited checkpoint regulates affinity-dependent B cell entry into the germinal center. *J. Exp. Med.* 208:1243–52
99. Song H, Cerny J. 2003. Functional heterogeneity of marginal zone B cells revealed by their ability to generate both early antibody-forming cells and germinal centers with hypermutation and memory in response to a T-dependent antigen. *J. Exp. Med.* 198:1923–35
100. Baumgarth N. 2013. Innate-like B cells and their rules of engagement. *Adv. Exp. Med. Biol.* 785:57–66
101. Foote JB, Kearney JF. 2009. Generation of B cell memory to the bacterial polysaccharide α-1,3 dextran. *J. Immunol.* 183:6359–68
102. Alugupalli KR, Leong JM, Woodland RT, Muramatsu M, Honjo T, Gerstein RM. 2004. B1b lymphocytes confer T cell-independent long-lasting immunity. *Immunity* 21:379–90
103. Liu Y-J, Barthelemy C, de Bouteiller O, Arpin C, Durand I, Banchereau J. 1995. Memory B cells from human tonsils colonize mucosal epithelium and directly present antigen to T cells by rapid up-regulation of B7-1 and B7-2. *Immunity* 2:239–48
104. Dunn-Walters DK, Isaacson PG, Spencer J. 1996. Sequence analysis of rearranged IgVH genes from microdissected human Peyer's patch marginal zone B cells. *Immunology* 88:618–24
105. Mahanonda R, Champaiboon C, Subbalekha K, Sa-Ard-Iam N, Rattanathammatada W, et al. 2016. Human memory B cells in healthy gingiva, gingivitis, and periodontitis. *J. Immunol.* 197:715–25
106. Vajdy M, Lycke N. 1993. Stimulation of antigen-specific T- and B-cell memory in local as well as systemic lymphoid tissues following oral immunization with cholera toxin adjuvant. *Immunology* 80:197–203
107. Bemark M, Hazanov H, Stromberg A, Komban R, Holmqvist J, et al. 2016. Limited clonal relatedness between gut IgA plasma cells and memory B cells after oral immunization. *Nat. Commun.* 7:12698
108. Moyron-Quiroz JE, Rangel-Moreno J, Hartson L, Kusser K, Tighe MP, et al. 2006. Persistence and responsiveness of immunologic memory in the absence of secondary lymphoid organs. *Immunity* 25:643–54
109. Bunker JJ, Flynn TM, Koval JC, Shaw DG, Meisel M, et al. 2015. Innate and adaptive humoral responses coat distinct commensal bacteria with immunoglobulin A. *Immunity* 43:541–53
110. Yuvaraj S, Dijkstra G, Burgerhof JG, Dammers PM, Stoel M, et al. 2009. Evidence for local expansion of IgA plasma cell precursors in human ileum. *J. Immunol.* 183:4871–78
111. Hapfelmeier S, Lawson MAE, Slack E, Kirundi JK, Stoel M, et al. 2010. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science* 328:1705–9
112. Martinoli C, Chiavelli A, Rescigno M. 2007. Entry route of *Salmonella typhimurium* directs the type of induced immune response. *Immunity* 27:975–84
113. Cunningham AF, Gaspal F, Serre K, Mohr E, Henderson IR, et al. 2007. *Salmonella* induces a switched antibody response without germinal centers that impedes the extracellular spread of infection. *J. Immunol.* 178:6200–7
114. Mittrucker HW, Raupach B, Kohler A, Kaufmann SH. 2000. Cutting edge: role of B lymphocytes in protective immunity against *Salmonella typhimurium* infection. *J. Immunol.* 164:1648–52
115. Liu YJ, Oldfield S, MacLennan IC. 1988. Memory B cells in T cell-dependent antibody responses colonize the splenic marginal zones. *Eur. J. Immunol.* 18:355–62
116. Steiniger B, Timphus E-M, Jacob R, Barth PJ. 2005. CD27$^+$ B cells in human lymphatic organs: re-evaluating the splenic marginal zone. *Immunology* 116:429–42
117. Takemori T, Kaji T, Takahashi Y, Shimoda M, Rajewsky K. 2014. Generation of memory B cells inside and outside germinal centers. *Eur. J. Immunol.* 44:1258–64
118. Scholz JL, Crowley JE, Tomayko MM, Steinel N, O'Neill PJ, et al. 2008. BLyS inhibition eliminates primary B cells but leaves natural and acquired humoral immunity intact. *PNAS* 105:15517–22
119. Benson MJ, Dillon SR, Castigli E, Geha RS, Xu S, et al. 2008. Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. *J. Immunol.* 180:3655–59

120. Ackermann JA, Nys J, Schweighoffer E, McCleary S, Smithers N, Tybulewicz VLJ. 2015. Syk tyrosine kinase is critical for B cell antibody responses and memory B cell survival. *J. Immunol.* 194:4650–56
121. Kraus M, Alimzhanov MB, Rajewsky N, Rajewsky K. 2004. Survival of resting mature B lymphocytes depends on BCR signaling via the Igα/β heterodimer. *Cell* 117:787–800
122. Turner M, Gulbranson-Judge A, Quinn ME, Walters AE, MacLennan IC, Tybulewicz VL. 1997. Syk tyrosine kinase is required for the positive selection of immature B cells into the recirculating B cell pool. *J. Exp. Med.* 186:2013–21
123. Cornall RJ, Cheng AM, Pawson T, Goodnow CC. 2000. Role of Syk in B-cell development and antigen-receptor signaling. *PNAS* 97:1713–18
124. Hobeika E, Levit-Zerdoun E, Anastasopoulou V, Pohlmeyer R, Altmeier S, et al. 2015. CD19 and BAFF-R can signal to promote B-cell survival in the absence of Syk. *EMBO J.* 34:925–39
125. Torcia M, Bracci-Laudiero L, Lucibello M, Nencioni L, Labardi D, et al. 1996. Nerve growth factor is an autocrine survival factor for memory B lymphocytes. *Cell* 85:345–56
126. Bhattacharya D, Cheah MT, Franco CB, Hosen N, Pin CL, et al. 2007. Transcriptional profiling of antigen-dependent murine B cell differentiation and memory formation. *J. Immunol.* 179:6808–19
127. Luckey CJ, Bhattacharya D, Goldrath AW, Weissman IL, Benoist C, Mathis D. 2006. Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. *PNAS* 103:3304–9
128. Vieira P, Rajewsky K. 1990. Persistence of memory B cells in mice deprived of T cell help. *Int. Immunol.* 2:487–94
129. Tew JG, Phipps RP, Madnel TE. 1980. The follicular dendritic cell: long-term antigen retention during immunity. *Immunol. Rev.* 53:29–59
130. Kosco-Vilbois MH. 2003. Opinion: Are follicular dendritic cells really good for nothing? *Nat. Rev. Immunol.* 3:764–69
131. Gray D, Skarvall H. 1988. B-cell memory is short-lived in the absence of antigen. *Nature* 336:70–73
132. Maruyama M, Lam KP, Rajewsky K. 2000. Memory B-cell persistence is independent of persisting immunizing antigen. *Nature* 407:636–42
133. Hannum LG, Haberman AM, Anderson SM, Shlomchik MJ. 2000. Germinal center initiation, variable gene region hypermutation, and mutant B cell selection without detectable immune complexes on follicular dendritic cells. *J. Exp. Med.* 192:931–42
134. Haberman AM, Shlomchik MJ. 2003. Reassessing the function of immune-complex retention by follicular dendritic cells. *Nat. Rev. Immunol.* 3:757–64
135. Clybouw C, Fischer S, Auffredou MT, Hugues P, Alexia C, et al. 2011. Regulation of memory B-cell survival by the BH3-only protein Puma. *Blood* 118:4120–28
136. Vikstrom I, Carotta S, Luthje K, Peperzak V, Jost PJ, et al. 2010. Mcl-1 is essential for germinal center formation and B cell memory. *Science* 330:1095–99
137. Carrington EM, Vikstrom IB, Light A, Sutherland RM, Londrigan SL, et al. 2010. BH3 mimetics antagonizing restricted prosurvival Bcl-2 proteins represent another class of selective immune modulatory drugs. *PNAS* 107:10967–71
138. Shen P, Fillatreau S. 2015. Antibody-independent functions of B cells: a focus on cytokines. *Nat. Rev. Immunol.* 15:441–51
139. Amanna I, Slifka M. 2006. Quantitation of rare memory B cell populations by two independent and complementary approaches. *J. Immunol. Methods* 317:175–85
140. Bachmann M, Kundig T, Kalberer C, Hengartner H, Zinkernagel R. 1994. How many specific B cells are needed to protect against a virus? *J. Immunol.* 152:4235–41
141. Lalor PA, Nossal GJ, Sanderson RD, McHeyzer-Williams MG. 1992. Functional and molecular characterization of single, (4-hydroxy-3-nitrophenyl)acetyl (NP)-specific, IgG_1^+ B cells from antibody-secreting and memory B cell pathways in the C57BL/6 immune response to NP. *Eur. J. Immunol.* 22:3001–11
142. Li X, Vanitha D, Joo H, He Y, Rouse B, Sangster M. 2006. A strategy for selective, $CD4^+$ T cell-independent activation of virus-specific memory B cells for limiting dilution analysis. *J. Immunol. Methods* 313:110–18

143. Slifka M, Ahmed R. 1996. Limiting dilution analysis of virus-specific memory B cells by an ELISPOT assay. *J. Immunol. Methods* 199:37–46
144. Vanitha D, Joo H, Rouse B, Sangster M. 2007. Quantitative analysis of herpes simplex virus type 1-specific memory B cells generated by different routes of infection. *Virology* 360:136–42
145. Weiss U, Rajewsky K. 1990. The repertoire of somatic antibody mutants accumulating in the memory compartment after primary immunization is restricted through affinity maturation and mirrors that expressed in the secondary response. *J. Exp. Med.* 172:1681–89
146. Helmreich E, Kern M, Eisen H. 1961. The secretion of antibody by isolated lymph node cells. *J. Biol. Chem.* 236:464–73
147. Vieira P, Rajewsky K. 1988. The half-lives of serum immunoglobulins in adult mice. *Eur. J. Immunol.* 18:313–16
148. Tarlinton D, Good-Jacobson K. 2013. Diversity among memory B cells: origin, consequences, and utility. *Science* 341:1205–11
149. Good-Jacobson KL. 2014. Regulation of germinal center, B-cell memory, and plasma cell formation by histone modifiers. *Front. Immunol.* 5:596
150. Wienands J, Engels N. 2016. Control of memory B cell responses by extrinsic and intrinsic mechanisms. *Immunol. Lett.* 178:27–30
151. Engels N, Konig LM, Heemann C, Lutz J, Tsubata T, et al. 2009. Recruitment of the cytoplasmic adaptor Grb2 to surface IgG and IgE provides antigen receptor–intrinsic costimulation to class-switched B cells. *Nat. Immunol.* 10:1018–25
152. Engels N, Konig LM, Schulze W, Radtke D, Vanshylla K, et al. 2014. The immunoglobulin tail tyrosine motif upgrades memory-type BCRs by incorporating a Grb2-Btk signalling module. *Nat. Commun.* 5:5456
153. Ackermann JA, Radtke D, Maurberger A, Winkler TH, Nitschke L. 2011. Grb2 regulates B-cell maturation, B-cell memory responses and inhibits B-cell Ca^{2+} signalling. *EMBO J.* 30:1621–33
154. Hikida M, Casola S, Takahashi N, Kaji T, Takemori T, et al. 2009. PLC-γ2 is essential for formation and maintenance of memory B cells. *J. Exp. Med.* 206:681–89
155. Wakabayashi C, Adachi T, Wienands J, Tsubata T. 2002. A distinct signaling pathway used by the IgG-containing B cell antigen receptor. *Science* 298:2392–95
156. Waisman A, Kraus M, Seagal J, Ghosh S, Melamed D, et al. 2007. IgG1 B cell receptor signaling is inhibited by CD22 and promotes the development of B cells whose survival is less dependent on Igα/β. *J. Exp. Med.* 204:747–58
157. Shi Y, Agematsu K, Ochs HD, Sugane K. 2003. Functional analysis of human memory B-cell subpopulations: $IgD^{+}CD27^{+}$ B cells are crucial in secondary immune response by producing high affinity IgM. *Clin. Immunol.* 108:128–37
158. Hobeika E, Maity PC, Jumaa H. 2016. Control of B cell responsiveness by isotype and structural elements of the antigen receptor. *Trends Immunol.* 37:310–20
159. Übelhart R, Hug E, Bach MP, Wossning T, Dühren-von Minden M, et al. 2015. Responsiveness of B cells is regulated by the hinge region of IgD. *Nat. Immunol.* 16:534–43
160. Cumano A, Rajewsky K. 1986. Clonal recruitment and somatic mutation in the generation of immunological memory to the hapten NP. *EMBO J.* 5:2459–68
161. Chen Z, Koralov SB, Gendelman M, Carroll MC, Kelsoe G. 2000. Humoral immune responses in $Cr2^{-/-}$ mice: enhanced affinity maturation but impaired antibody persistence. *J. Immunol.* 164:4522–32
162. Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, et al. 2010. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J. Exp. Med.* 207:365–78
163. Good-Jacobson KL, Szumilas CG, Chen L, Sharpe AH, Tomayko MM, Shlomchik MJ. 2010. PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nat. Immunol.* 11:535–42
164. Racine R, Jones DD, Chatterjee M, McLaughlin M, MacNamara KC, Winslow GM. 2010. Impaired germinal center responses and suppression of local IgG production during intracellular bacterial infection. *J. Immunol.* 184:5085–93
165. Yates JL, Racine R, McBride KM, Winslow GM. 2013. T cell–dependent IgM memory B cells generated during bacterial infection are required for IgG responses to antigen challenge. *J. Immunol.* 191:1240–49

166. Haniuda K, Nojima T, Ohyama K, Kitamura D. 2011. Tolerance induction of IgG^+ memory B cells by T cell-independent type II antigens. *J. Immunol.* 186:5620–28
167. Hebeis BJ, Klenovsek K, Rohwer P, Ritter U, Schneider A, et al. 2004. Activation of virus-specific memory B cells in the absence of T cell help. *J. Exp. Med.* 199:593–602
168. Koch G, Osmond DG, Julius MH, Benner R. 1981. The mechanism of thymus-dependent antibody formation in bone marrow. *J. Immunol.* 126:1447–51
169. Shepherd D, Noelle R. 1991. The lack of memory B cells in immune bone marrow. *Transplantation* 52:97–100
170. Slifka M, Antia R, Whitmire J, Ahmed R. 1998. Humoral immunity due to long-lived plasma cells. *Immunity* 8:363–72
171. Ochsenbein A, Pinschewer D, Sierro S, Horvath E, Hengartner H, Zinkernagel R. 2000. Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. *PNAS* 97:13263–68
172. Cinamon G, Matloubian M, Lesneski M, Xu Y, Low C, et al. 2004. Sphingosine 1-phosphate receptor 1 promotes B cell localization in the splenic marginal zone. *Nat. Immunol.* 5:713–20
173. Forster R, Mattis A, Kremmer E, Wolf E, Brem G, Lipp M. 1996. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* 87:1037–47
174. Onodera T, Hosono A, Odagiri T, Tashiro M, Kaminogawa S, et al. 2016. Whole-virion influenza vaccine recalls an early burst of high-affinity memory B cell response through TLR signaling. *J. Immunol.* 196:4172–84
175. Boeckh M, Nichols W, Papanicolaou G, Rubin R, Wingard J, Zaia J. 2003. Cytomegalovirus in hematopoietic stem cell transplant recipients: current status, known challenges, and future strategies. *Biol. Blood Marrow Transplant.* 9:543–58
176. Klenovsek K, Weisel F, Schneider A, Appelt U, Jonjic S, et al. 2007. Protection from CMV infection in immunodeficient hosts by adoptive transfer of memory B cells. *Blood* 110:3472–79
177. Weinberger B, Herndler-Brandstetter D, Schwanninger A, Weiskopf D, Grubeck-Loebenstein B. 2008. Biology of immune responses to vaccines in elderly persons. *Clin. Infect. Dis.* 46:1078–84
178. Chiu C, Ellebedy AH, Wrammert J, Ahmed R. 2015. B cell responses to influenza infection and vaccination. *Curr. Top. Microbiol. Immunol.* 386:381–98
179. Yu X, Tsibane T, McGraw PA, House FS, Keefer CJ, et al. 2008. Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* 455:532–36
180. Moyron-Quiroz JE, Rangel-Moreno J, Kusser K, Hartson L, Sprague F, et al. 2004. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nat. Med.* 10:927–34
181. Baumgarth N, Choi YS, Rothaeusler K, Yang Y, Herzenberg LA. 2008. B cell lineage contributions to antiviral host responses. *Curr. Top. Microbiol. Immunol.* 319:41–61
182. Adachi Y, Onodera T, Yamada Y, Daio R, Tsuiji M, et al. 2015. Distinct germinal center selection at local sites shapes memory B cell response to viral escape. *J. Exp. Med.* 212:1709–23
183. Pichyangkul S, Yongvanitchit K, Limsalakpetch A, Kum-Arb U, Im-Erbsin R, et al. 2015. Tissue distribution of memory T and B cells in rhesus monkeys following influenza A infection. *J. Immunol.* 195:4378–86
184. Li G-M, Chiu C, Wrammert J, McCausland M, Andrews SF, et al. 2012. Pandemic H1N1 influenza vaccine induces a recall response in humans that favors broadly cross-reactive memory B cells. *PNAS* 109:9047–52
185. Andrews SF, Huang Y, Kaur K, Popova LI, Ho IY, et al. 2015. Immune history profoundly affects broadly protective B cell responses to influenza. *Sci. Transl. Med.* 7:316ra192
186. Li Y, Myers JL, Bostick DL, Sullivan CB, Madara J, et al. 2013. Immune history shapes specificity of pandemic H1N1 influenza antibody responses. *J. Exp. Med.* 210:1493–500
187. Neu KE, Henry Dunand CJ, Wilson PC. 2016. Heads, stalks and everything else: How can antibodies eradicate influenza as a human disease? *Curr. Opin. Immunol.* 42:48–55
188. Moir S, Fauci AS. 2009. B cells in HIV infection and disease. *Nat. Rev. Immunol.* 9:235–45
189. Kwong PD, Mascola JR. 2012. Human antibodies that neutralize HIV-1: identification, structures, and B cell ontogenies. *Immunity* 37:412–25

190. Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, et al. 2011. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. *Science* 333:1593–602
191. Lynch RM, Tran L, Louder MK, Schmidt SD, Cohen M, et al. 2012. The development of CD4 binding site antibodies during HIV-1 infection. *J. Virol.* 86:7588–95
192. Klein F, Mouquet H, Dosenovic P, Scheid JF, Scharf L, Nussenzweig MC. 2013. Antibodies in HIV-1 vaccine development and therapy. *Science* 341:1199–204
193. Mouquet H, Nussenzweig MC. 2013. HIV: roadmaps to a vaccine. *Nature* 496:441–42