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Review Article

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Increased B Cell Understanding Puts Improved Vaccine Platforms Just Over the Horizon

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AID, activation-induced deaminase; AP, activated precursor; ASC, Ab secreting cell; BCL, B-cell lymphoma; BCR, B cell receptor; BRM, lung-specific memory B cell; COVID-19, coronavirus disease 2019; CSR, class switch recombination; DZ, dark zone;

ABSTRACT

In the face of an endlessly expanding repertoire of Ags, vaccines are constantly being tested, each more effective than the last. As viruses and other pathogens evolve to become more infectious, the need for efficient and effective vaccines grows daily, which is especially obvious in an era that is still attempting to remove itself from the clutches of the severe acute respiratory syndrome coronavirus 2, the cause of coronavirus pandemic. To continue evolving alongside these pathogens, it is proving increasingly essential to consider one of the main effector cells of the immune system. As one of the chief orchestrators of the humoral immune response, the B cell and other lymphocytes are essential to not only achieving immunity, but also maintaining it, which is the vital objective of every vaccine.

Keywords: Vaccine; Immunology; B cell; Rational vaccine design

AN INTRODUCTION TO THE B CELL

The B cell originates in the bone marrow and migrates to other lymphoid organs, such as the spleen, to complete its development before migrating to the lymph nodes to become activated by Ag. The B cell population can be subdivided into naive and Ag-experienced B cells, with the former consisting of the fetal-derived B-1, the follicular (Fo) B cells (FoB), and the marginal zone subsets. B cells which have been exposed to Ag differentiate along one of several fates and become either plasma cells (PCs) or memory B cells (MBCs). The former population of short-lived plasma cells (SLPCs) and long-lived plasma cells (LLPCs) can be distinguished by Ag affinity. While SLPCs are chiefly responsible for rapidly secreting broadly neutralizing and low-affinity Abs during the first stages of infection, known as the humoral immune response, the LLPC population constitutively secretes protective and high affinity Abs as part of the adaptive immune response, which is also part of the immune memory response. MBCs orchestrate the other part of this response by secreting Abs of varying affinity when faced with a secondary infection, amplifying both breadth and depth of the protective immunity afforded following primary infection or immunization. Together, LLPCs and MBCs can maintain immunity against a particular Ag for months, years, or, in some cases up to the lifespan of the host (1).

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EF, extrafollicular; eMBC, early memory B cell; FDC, follicular dendritic cell; Fo, follicular; FoB, follicular B cells; GC, germinal center; GCBC, germinal center B cell; HIV, human immunodeficiency virus; LLPC, long-lived plasma cell; LZ, light zone; MBC, memory B cell; PAMPs, pathogen-associated molecular patterns; PB, plasmablast; PC, plasma cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SHM, somatic hypermutation; SLPC, short-lived plasma cell; TFH, follicular helper T cells.

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Upon exposure to Ag, a B cell undergoes activation and proliferation, followed by either mutation towards increased Ab affinity for Ag and proliferation into a MBC or LLPC, or immediate differentiation into a low-affinity Ab-secreting SLPC. The former process chiefly occurs in a specialized lymphoid compartment called the germinal center (GC), while the GC-independent response, known as the extrafollicular (EF) response, includes a major population of SLPCs and "unswitched" MBCs and LLPCs derived independently from GC reactions. Both GC-dependent and -independent MBCs and PCs are contributors to long-term immunity. Though LLPCs are generally found to result only from GC reactions, it has also been shown that sustained Ab production can be formed without a GC reaction, though the mechanism by which this phenomenon arises is still unknown (1).

To increase the affinity of B cells for Ag, they undergo several rounds of selection, during which the structure of their B cell receptor (BCR) undergoes random mutations to increase affinity for Ag, and consequent clones are selected based on the level of this affinity. Somatic hypermutation (SHM) and affinity maturation, respectively, are drivers of B cell affinity, while additional processes such as class switch recombination (CSR) occur concurrently and alter the isotype of their BCR from the natively expressed IgM and IgD to IgA, IgE, or IgG. While IgG is most often implicated in the adaptive immune response, IgA and IgE also play their own protective roles within specialized immunological niches. IgA, which is mainly found in the small intestine and other mucosal tissues, can also provide protection in mucosal tissues, and therefore are relevant in the context of mucosal immunity (2). On the other hand, IgE cells formed in the GC are transient; in fact, most IgE cells are PCs. This isotype is primarily implicated in allergic responses, and will not be discussed further (3); unless specified, Abs discussed in this review will generally refer to the IgG subtype.

To understand how to prolong the efficacy of patients' immunity, this review is primarily concerned with detailing the fates and functions of B cells, as well as the external and internal cues which guide them toward conferring lasting immunity. Methods to elucidate the characteristics of these responses have been reviewed succinctly elsewhere and will not be discussed here (4).

LIMITATIONS OF RECENT VACCINES

There has never been a more important time to improve the understanding of the B cell response, as not only has the severe need for more advanced immunology research been demonstrated by the recent and ongoing coronavirus disease 2019 (COVID-19) pandemic, but there is now improved technology to make use of this information for potentially life-saving purposes. It was unheard of for vaccine development to proceed at the rate it did prior to the COVID-19 pandemic. The resulting safe, effective, and first-of-its-kind prophylactic mRNA vaccine which protected against the initial strain of highly transmissible severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus was a significant step forward, but shortcomings of the vaccines are becoming apparent. Recipients have not been able to maintain long-lasting immunity, partially because the SARS-CoV-2 virus developed several mutations which limited the effectiveness of the original vaccine. Though mRNA vaccines have been demonstrated to induce the production of Ag-specific MBCs following immunization, there is no evidence that the mRNA-based SARS-CoV-2 vaccines were able to induce the effective formation of LLPCs, which in addition to MBCs form the foundation of immunological memory that protects the body from secondary infections (5).

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Studies point out that the inability of the vaccine to generate long-term immunity could be partially a result of most people already having coronavirus-experienced memory cells, given that many commonplace seasonal diseases, such as the common cold, are caused by coronaviruses (6). These pre-existing Abs do not allow for the immune response generated by vaccination to proceed long enough to induce effective LLPC formation. These cells not only arise later in the immune response than MBCs but are also generally of higher affinity, a result of increased time in the GC compartment undergoing affinity maturation and positive selection towards higher-affinity clones (5).

Patients who have actually contracted COVID-19 have been demonstrated to produce LLPCs in addition to MBCs, as well as an expanded Ab response, affording patients that survive the infection a higher degree of immunity than those who are infection-naive but are fully vaccinated (7). Studies concur that this increased immunity is likely due to severely ill patients being able to generate a robust EF B cellular response following an increase in proinflammatory cytokines and neutralizing Ab titers. Recent data suggests previously COVID-19-infected patients that are consequently vaccinated may have enhanced immunity to new variants of the virus compared to naive individuals who have been vaccinated, which could be due to the ongoing clonal evolution of B cells as a result of actual infection (8). Because most data indicate that protection provided by the vaccine against SARS-CoV-2 wanes dramatically 4-6 months following vaccination (5) and induction of immune memory after priming with the mRNA vaccine produces fewer plasmablasts (PBs) and atypical MBCs (9), optimization of this and future vaccines hinges on determining how to increase the efficacy of the induction of LLPCs during an immune response, potentially in the presence of cross-reactive memory cells.

As with the SARS-CoV-2 vaccine, immunity generated by mRNA vaccines has been found to be limited for several reasons; first, among the many coronavirus family members, humoral immunity against one subtype provides little or no protection against others, including zoonotic strains. Like other virus subtypes, coronaviruses exhibit wide variability, and consequently generated Abs may exhibit “preference” to target non-conserved regions of viral proteins, which accumulate mutations readily, as has been seen in infection with influenza A viruses. Virus families such as coronaviruses, which are highly mutating, bring additional complications due to the ability to rapidly evade the immune system by undergoing mutations (10). Significant mutations allow viruses like coronaviruses to evade even cross-reactive Abs; such Abs produced by Pfizer and Moderna’s mRNA vaccines were consequently effective against the SARS-CoV-2 Delta variant’s 9 mutations but faltered against the Omicron variant’s significantly increased 34 mutations, necessitating a booster shot for effective protection (11). Additionally, studies in mice have shown that some individuals may be genetically predisposed to generate non-canonical MBC response to certain Ags, generating lower-affinity Abs by default due to higher binding affinities in the non-mutated BCR. This changes the way these Abs are directed to differentiate into MBCs by the immune system, though they are still a naturally occurring feature of the B cell repertoire (9,12).

Immunizations are generally intended to trigger the immune response to generate not only Abs but also long-lived effector cells such as MBCs and LLPCs, allowing for multiple tiers of protection. While LLPCs secrete Abs constitutively, MBCs must be recalled to re-differentiate into effector cells during secondary Ag encounter. Recall responses of MBCs in secondary reactions strongly depend on immunization conditions, including the delay between priming and boosting, the level of circulating Abs, the nature of the Ag, the persistence of GC reactions, the site of immunization, the location of the response, and the type of adjuvant used (13).

Because MBCs are more broadly reactive than LLPCs, they can be crucial in cases of reinfection with mutants of the same virus. This is useful in cases where cross reactive Abs can elicit an immune response to conserved viral regions. Vaccination strategies taking advantage of this rely on effectively finding broadly conserved surface domains among pathogens of a similar lineage (14). Some suggest that vaccination could be used to skew the MBC population towards the GC fate with the goal of increasing SHM. However, given studies showing that MBCs recalled to the GC during secondary immune responses are quite rare (14,15) in addition to the fact that MBCs are useful *because*, and not despite, the fact that they are broadly reactive (15), this method may not be beneficial. Thus, understanding the immune response generated toward a particular virus is key to developing a way to induce the same immune response without necessitating contraction of the disease.

CANONICALLY DERIVED EFFECTOR B CELLS ARISE FROM BONE MARROW PRECURSORS

"Conventional" B cells produced in the bone marrow are also known as B-2 or FoB and are the most numerous subset in lymph nodes and spleen (16,17). During an infection, B cell differentiation proceeds through an initial primary or EF phase during which B cells undergo proliferation and differentiation following Ag stimulation to become short-lived Ab secreting cells (ASCs). This "initial phase" immune response is the critical source of early protective Abs, which are secreted sooner after the onset of infection but have minimal Ab affinity (16, 17). Prior to infection, FoB specific for the Ag are rare (16).

By the second phase, activated B cells not yet differentiated into PBs reenter the follicle and, after receiving follicular helper T cells (T_{FH}, referred to herein interchangeably as T cells), form the GC. The GC is the site of the most extensive SHM, though not exclusively (17). GCs form around follicular dendritic cell (FDC) networks within secondary lymphoid organs 4-7 days following challenge with foreign T cell-dependent Ags. GC B cell responses are sustained via signals presented on the surface of FDCs and T_{FH} which bind processed Ag presented on MHC class II molecules on the surface of activated B cells (18). While GC responses are considered the major orchestrators of B cell-mediated immune responses, their formation is not the only way to clear an infection; many infections are able to be cleared before GCs even form (16). Despite the main role of the GC in producing effector cells of higher affinity, it is now known that not only do many effector cells arise concurrently with GC maturation, but many MBCs arise before GC formation is even detected, are still long-lived, and have a significantly increased affinity on average than naive cells. These long-lived IgM MBCs form independently of the GC pathway (2).

Ag SENSING BY THE BCR: THE INITIATION OF THE B-CELL MEDIATED IMMUNE RESPONSE

B cells' ability to enter the GC and differentiate into an effector cell is mediated by their activation by Ag via the BCR, though a basal or tonic BCR signal is also necessary for continued cell survival and maturation (19). In the first day following immunization, activated B cells which have bound their cognate Ag migrate to the interfollicular zone in the lymph node, guided by increased expression of GPCR receptor EBI2 (20), where they have higher chances of encountering cognate Ag. Following a successful meeting, these cells

consequently proliferate; these initial pathways are still not fully understood. The binding of Ag results in several main outcomes, beginning with reorganization and internalization of the BCR signalosome and endosome-mediated loading of Ag-derived peptides onto MHC class II molecules. Resulting transcriptional changes increase B cell survival and facilitate entry into the cell cycle for clonal expansion, and upregulation of MHC II, costimulatory molecules, and chemokines support B-T cell interactions (16). Some of the cells' progeny will migrate back to the follicles to populate GCs or give rise to EF PBs within the next few days. While a single naive B cell can give rise to all 3 lineages, cell death limits the contribution of many cell clones to only 1 or 2 subsets. The choice between these three lineages is mainly regulated by BCR affinity and the amount of available T-cell help (16,21).

Evidence supports the idea that membrane-bound Ags are responsible for eliciting the strongest B cell immune response (22-24). Despite canonical BCR signaling pathways being decreased in GCBCs relative to those in naive B cells (25), GCBCs can transmit BCR-mediated signals via synaptic interactions between GCBCs and FDCs, which encourage an improved response to membrane-bound Ags in an affinity-dependent manner (26). This and other studies, alluded to in recent reviews (27-29), have similarly concluded that that the immune system preferentially interacts with membrane-bound Ag over soluble molecules.

FoB which have bound Ag in this manner are guided to follicles in the lymph node and positioned at the borders of T cell zones, giving them a higher chance of meeting a T cell with its cognate Ag and receiving "help" to become an effector cell and proceed to further differentiation (16). The initial meeting between B and T cells at the Fo border region triggers a variety of different cascades (30) that influence B cell differentiation. T cells canonically mediate further B cell differentiation through contact of B cell-expressed CD40 and T cell-expressed CD40L interactions which upregulate B cell survival and proliferation (23,31), including the expression of the activation-induced deaminase (AID) essential for gene rearrangements responsible for class switching. In addition to CD40L, the cytokine IL-21 and IL-4, especially in concert with IL-21, can contribute to the establishment and growth of the GC by promoting expression of the B-cell lymphoma (BCL)-6 transcription factor, which functions primarily as a transcriptional repressor and helps regulate the expression of a wide range of genes controlling various critical cellular processes involved in cell life cycle and differentiation (23,31,32).

BCR CO-RECEPTORS AND TLRs MAY BE KEY MEDIATORS OF IMMUNE RESPONSES

While this review primarily is focused on BCR-induced B cell activation and consequent effector functions, B cell surface receptors such as the TLRs, in addition to BCR co-receptors, also provide important signals to mediate both vaccine- and infection-induced immune responses. These include the co-stimulatory molecules CD22 and CD38 and the TLRs, which have been shown to have a clear impact on the immune response in various ways. BCR-associated TLRs can have different effects on the immune response than other immune cell-associated TLRs (32); adjuvant stimulation of co-receptors referred to here and elsewhere in this paper refer specifically to their impact on B cells. One such example is TLR9. Studies have demonstrated that stimulation of B cell TLR9 led to B cell differentiation into PCs by decreasing the expression of key PC differentiation repression factors such as *Bcl6*. Signaling via TLR9 also appeared to deter the B cell from delivering Ag into late endosomes following

Ag internalization, which also reduced their ability from getting T cell help (33). This is only one example of how B cell co-receptors play an important role in B cell fate; information about other TLRs and co-receptors is described in more detail in several publications (33-36) and will not be further explained here.

THE GC REACTION

GCs are sites of affinity maturation, SHM, and consequently, clonal selection (**Fig. 1**). The GC reaction is essential to increase the affinity of B cells for Ag; since according to recent studies, there is apparently little affinity-dependent exclusion of Ag-specific naive B cells in the first phase of Ag-dependent differentiation. GCs have 2 distinct zones, known as the light zone (LZ) and the dark zone (DZ) that also includes a recently characterized "gray zone" (37). The light zone, which encompasses the major populations of Ag-presenting cells (FDCs and CD4 T_{FH}), allows for selection of high-affinity clones for further proliferation. FDCs are able to retain Ag in the form of immune complexes after an infection is cleared, often for long periods of time, allowing the trajectory of the GC reaction to continuously evolve towards higher affinity immune cells (16,26). Activation of high affinity GC B cells in the LZ is therefore considered to mediate positive selection (18,26). LZ B cells that bind Ag internalize BCR-Ag complexes and subsequently present Ag on specialized MHC class II molecules to T cells, from which they receive help. Cells presenting Ag induce the expression of MYC, a critical regulator for GC maintenance and proliferation; cells expressing high levels of MYC are thus more "licensed" to be in the GC (38,39). GCBCs in the LZ restart the cell cycle and travel to the DZ for further proliferation, after which GC reactions eventually result in high-affinity Ab secreting PCs and MBCs (26). It remains unclear where or how PC differentiation is initiated within the GC. In addition to this, IgM^+ GCBCs, which have not undergone CSR, are consistently depleted from GCs, via a selection mechanism determined by the constant region of the BCR rather than its variable region-defined affinity leading to their depletion in the GC despite undergoing normal SHM and increasing their affinity (40). Not only has CSR has been shown to not occur predominantly (or indeed even significantly) in the GC (41), it is also a permissive process allowing for the entrance of IgM^+ cells into the GC as well as the formation of IgM^+ MBCs (41).

Canonical high-affinity B cells in the GC have improved ability to contact displayed Ag, which consequently improves their odds of positive selection and leads to an increase in stimulatory signals received from both T_{FH} and supporting cells in the GC. This increases the cell's life span in addition to inducing proliferation (40). Within the DZ, they may also differentiate into LLPCs that take up residence in the bone marrow, or MBCs, which have no effector function until recalled during secondary infection (32). Positive selection in the GC enables the immune system to select for higher affinity clones and produce B cells which can effectively target Ag. T cell-mediated signals are potentially the largest driving force behind positive selection, and are likely delivered preferentially to GCBCs which have most efficiently captured peptides, a function of BCR affinity (23). Therefore, boosting T_{FH} help could drive GCBCs to differentiate into PCs at a higher rate and with broader receptor affinity. Signals necessary to initiate PC differentiation appear to be delivered directly upon BCR engagement of intact Ag (probably from the surface of FDCs). Thus, either Ag engagement acts as the master regulator of both positive selection and PC differentiation within the GC, or the 2 key processes rely on distinct selective mechanisms (18). Concurrently, other studies show that providing strong T cell help to the total GCBC population during GC responses greatly

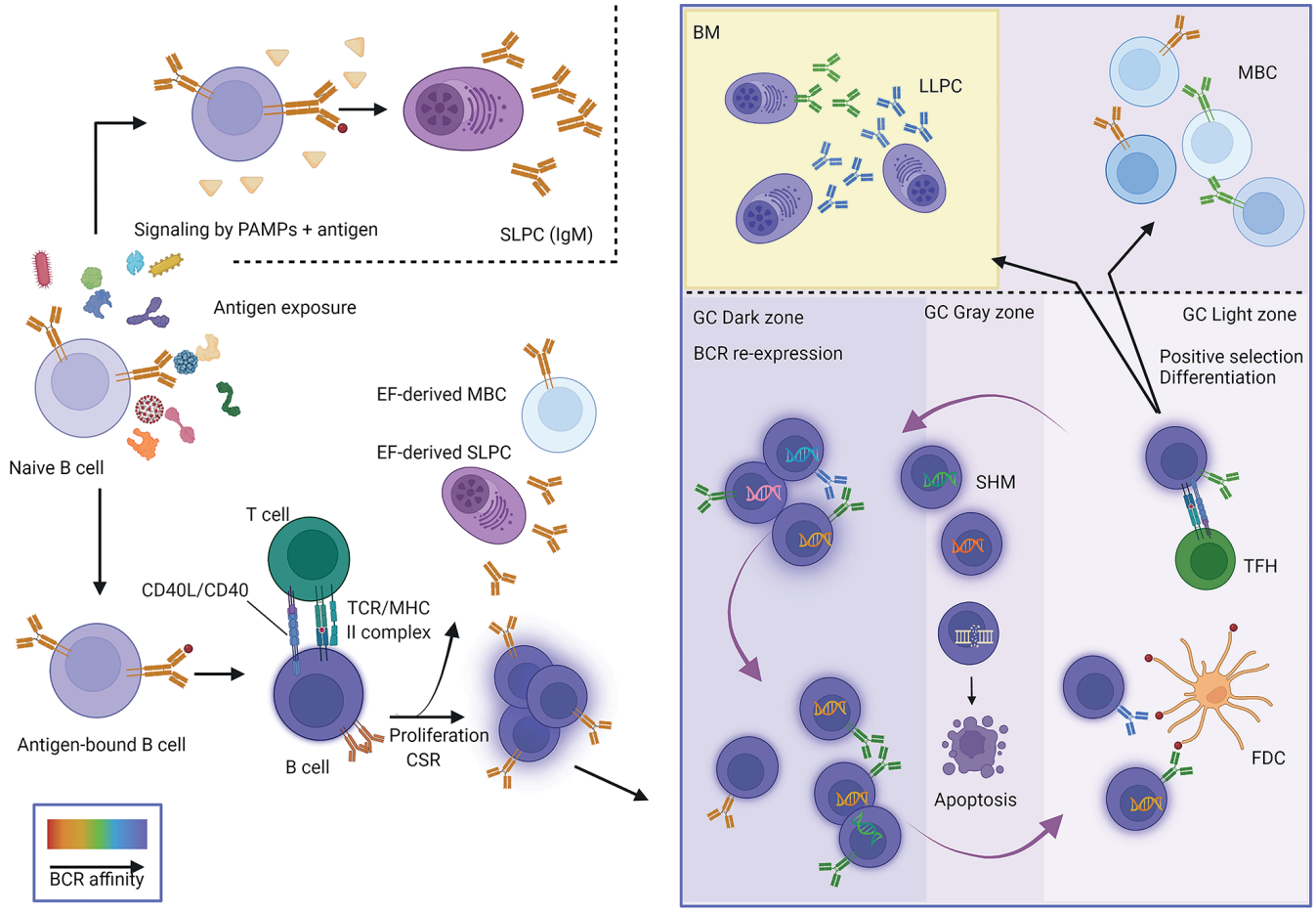

Figure 1. GC reaction and fate.
Upon being exposed to Ag, B cells may differentiate immediately into effector cells due to their ability to sense PAMPs associated with certain microbes via a combination of BCR stimulation and TLR stimulation (not shown). B cells with only BCR stimulation internalize the Ag-BCR complex and present Ag to activating T cells, may undergo class-switch recombination, and then migrate to the lymph node, where they form specialized structures known as GCs. Within the GC, B cells undergo SHM of the BCR variable region and undergo positive selection to increase affinity against the Ag. Cells leaving the GC differentiate into the effector cells that actively fight pathogens via secretion of neutralizing Abs and maintain immunological memory.

increases the rate of positive selection and proliferation due to positive feedback loops induced by increased Ag presentation capabilities of high-affinity GCBCs (26). Cell extrinsic factors, such as the dominance of one clonal subtype over another, can also determine which cells are selected, regardless of relative population frequencies (23).

Additionally, MYC induction is likely linked to successful positive selection despite only being expressed by a small minority of GCBCs (23). This transcription factor is transiently induced in positively selected cells that are therefore fated for GC expansion and PC formation, in correlation with strong T-cell help (23,42). CD40 expression is positively correlated with MYC expression in GCBCs, probably due in part to the fact that CD40 expression on B cells is shown to be enhanced in vitro by survival factor BAFF secreted by FDCs. This may therefore confer upon GCBCs that have collected Ag from FDCs another advantage to obtaining T cell help via preferential association with T cells expressing higher levels of CD40L and other stimulatory cytokines favorable to positive selection (26,42).

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The balance of cell differentiation pathways varies depending on the nature of the infection or immunization. Examination of early B cell response to T-dependent Ags in mice showed that early after immunization, a homogenous population of activated precursor (AP) cells gave rise to a transient wave of PBs, followed a day later by the emergence of germinal center B cells (GCBCs) (21). At this early stage, most precursors exited the cell cycle and gave rise to non-GC-derived early memory B cells (eMBCs) which retained an AP-like transcriptional profile (21). Fate mapping indicated that a significant portion of the MBC pool consisted of non-GC derived MBCs, and quiescent cells with an MBC phenotype also dominated the early response to immunization in primates. It was consequently concluded that limited Ag availability contributes to the early cell cycle exit of APs and that provision of additional Ag rescues this withdrawal but also simultaneously enhances PB lineage differentiation to account for the perceived inability to contain the threat due to excess presence of Ag (21). The microenvironment present at the time of B cell activation can also impact a differentiation trajectory. For example, B cells that come into contact with pathogen-associated molecular patterns (PAMPs) can obtain signals that regulate Ag processing, Ag presentation, and even their ability to receive T cell help, which affects their tendency to differentiate into PCs (32).

MBC POPULATIONS ARISE BEFORE AND DURING THE GC RESPONSE

MBCs are able to survive long-term and rapidly differentiate into Ab-secreting cells upon Ag reencounter. They can also re-enter the GC during recall responses to undergo further SHM and tend to emerge from the early phases of the GC response, typically displaying reduced levels of SHM and affinity maturation compared to PCs. During a viral infection, this reduced mutational load allows MBCs to be more flexible in the face of various viral strains relative to PCs, which are usually more specific for a particular strain or subtype. As a byproduct of this high cross-reactivity, however, the MBC population in both mice and humans has an elevated fraction of broadly reactive clones relative to the more specific PC pool (31). While primarily found in various lymphoid organs, such as the spleen, which has the largest population of B cells, MBCs also populate major secondary lymphoid organs and the blood, and peripheral tissues such as the lung, gingiva, and the gut (43). Though they can persist for extended periods of time, potentially the lifetime of the organism, MBCs have no effector function without re-stimulation in the face of a secondary infection. As a population, they are generally of higher affinity than the precursors they came from, and seem to require minimal help from T_{FH} (26). They are frequently considered to be a "quiescent" population derived from cells which were not positively selected prior to differentiation (43). However, this would imply that MBC precursors, which do exhibit specificity for BCR affinity, would have to be selected via an alternative method while still receiving pro-survival signals essential to survival during the normal process of positive selection. In fact, high affinity clones are still able to be selected for MBC differentiation within a pool of GCBCs with varied affinity. Additionally, MBCs have been seen to remain constant in number and frequency after a primary challenge, despite generating downstream effector cells (26,43).

Like LLPCs and SLPCs, MBCs can arise by both T cell dependent or independent mechanisms (2,17,31,32,43,44). Three days post-immunization, GC-independent derived MBCs (sometimes eMBCs) can be detected making a substantial contribution to the memory compartment during the early phase of the immune response. This initial majority may later be overcome by nascent populations of GC-dependent MBCs, assuming the GC reaction

proceeds (44). The decision to enter the non-GC derived MBC pool therefore occurs early, before CSR, widespread clonal expansion, or SHM, since these cells still express both IgM and IgD, are not found in large expanded clones, and carry few somatic mutations (44). Activated B cell differentiation into an MBC prior to GC entry is influenced largely by Ag binding affinity. In the GC, MBC precursors have low Ag binding affinity relative to cells remaining in the GC while higher affinity cells develop into plasma and GC cells. MBC differentiation has been found to be encouraged by FDC-mediated sequestration of IL-4 (45). B cells continue to differentiate into non-GC derived MBCs during the immune response despite the ongoing proliferation of cells within the GC, in concurrence with the activated B cell compartment persisting throughout the immune response; therefore, higher affinity activated B cells can continue to join GCs (44). GC MBCs are closely related to GCBCs while GC-independent MBCs cells most resemble cells of Fo origin. These subsets have documented functional differences; for example, non-GC experienced MBCs preferentially participate in secondary GCs upon secondary immunization, while those which are class switched (and therefore may have increased mutations) do not (12,44,46). During secondary immunization, GC-derived MBCs undergo preferential differentiation into ASCs (44). This suggests that it is more favorable to have a population of GC-experienced MBCs able to secrete Abs right away, while using lower affinity MBCs to augment the affinity-matured immune response. As a whole, non-GC-derived MBCs are higher in affinity than naive B cells, but generally lower in affinity than GC-derived MBCs, which makes them closer to the affinity required to reenter secondary GCs during reinfection at a more frequent rate than their GC-derived counterparts (44). Therefore, the expression of high-affinity BCRs and increased signals associated with T-cell help favor immediate differentiation into PB over GCBC and GC-independent MBC fates (21).

Like canonical B cells, MBCs tend to express known B cell surface markers such as CD19 and CD20 in human cells. Additionally, they can be distinguished from GCBCs by their lack of CD38; human GCB cells are $CD38^{+}$. Unlike PCs, MBCs lack CD138 and have a small cellular size, making identification from the general population of B cells relatively unproblematic (43). There are various subsets of MBCs currently characterized, all of which exhibit particular cell surface markers and fate tendencies in the face of secondary reinfection or recall. Directing the MBC population towards higher specificity or higher cross-reactivity according to the Ag could depend on the further characterization of these MBC populations. Since many of these subsets have been widely explored in various studies and reviews (2,3,13,46-50), despite potential relevance in understanding the reactions of different subsets of B cells to immunization, they will not be described in the interest of space. These subsets could be important targets of vaccination to increase the robustness of the reacting B cell response.

LONG LIVED PCs PROVIDE HIGHLY SPECIFIC PROTECTION

While MBCs must be reactivated to have effector functions, some PCs - more specifically, LLPCs - can secrete large amounts of Abs over extended periods of time, though the precise way in which LLPCs and other shorter-lived ASCs are committed to this lineage is still unknown (17,51). While precise mechanisms for the long-term persistence of LLPCs are also not yet fully understood, it is widely held to be promoted by a wide variety of factors which include cell types in the physiological niche where LLPCs are located, and humoral and genetic factors.

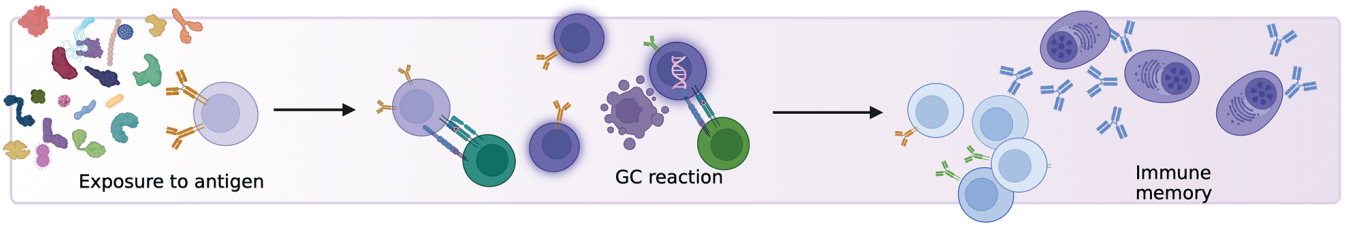
Here it serves to note that while SLPCs and LLPCs are collectively referred to as PCs, and, generally speaking, the former is a result of EF reactions and the latter is a product of the GC, PC lifespans vary significantly, and both long- and short-lived clones can be present in both populations. Regardless, here we will refer to LLPCs and SLPCs as separate populations based on their lifespan and function as either transient or long-term ASCs (51).

PB and PC fates are facilitated by increased T_{FH} help, with enhanced NF-κB signaling caused by CD40-CD40L signaling. PB or PC precursors in the light zones of GCs expressing MYC, high levels of CD69, and low Bcl6 also express relatively high levels of IRF4, a critical transcription factor for PC differentiation that is induced by NF-κB signaling. According to previous findings, these precursors generally consist of high affinity GCBCs and are detectable soon after positive selection (26). Selectivity of PC differentiation for high-affinity GC cells indicates an underlying mechanism closely related to Ag affinity (18).

Stable contact between T_{FH} and GCBCs as a consequence of greater Ag complex presentation induces Ca^{2+} dependent expression of IL-21 and IL-4; stronger T cell help-inducing signals promote PB/PC fate differentiation by triggering the production of supporting cytokines (26). This output of PB and PCs is also influenced by Ag valency, which reduces the Ag affinity threshold in EF PC responses, so multivalent Ag presentation may also play a role in this differentiation program (26). After B cells undergo clonal selection, activated B cells may become PCs secreting mostly IgM of low affinity, while others enter the GC to undergo further differentiation. Resulting cells are either MBCs or switched PCs. Increasing evidence suggests that the choice between rapid PB differentiation vs GC entry could be mediated by the degree of PI3K-Akt signaling and thus by FOXO transcription factor activity (52), which in turn impacts the level of upregulation of the gene AICDA, essential for class switching (52). Other metabolic processes have been found to be linked to LLPC differentiation and maintenance as well, reviewed in several studies (53,54), such as the observation that decreasing mTOR activity modulates the Ab response and improves cross-protective immunity in cases of lethal influenza infections (**Fig. 2**) (55).

While SLPCs can be switched and unswitched, LLPCs are usually the latter, having formed in the GC at advanced stages of the immune response. Sometimes known as “memory plasma cells” to distinguish them from their transient counterparts, they constitutively secrete Abs for prolonged periods, often extending to the lifespan of the organism (51). Despite significant confusion surrounding their origins, it has recently been proposed that cells that have differentiated into LLPCs began as migratory PBs that became integrated into a specific bone-marrow localized “niche” (51), after which they are provided with various signals in order to keep them alive, such as B cell survival factors BAFF and APRIL, and activation of the CD28 receptor and Bcl-2 family member and anti-apoptotic protein Mcl-1. The niche is populated by both stromal and hematopoietic cells which participate in the maintenance of LLPCs by secreting various key factors.

The persistence of LLPCs remains relatively nebulous despite extensive characterization, especially by recent reviews (51) describing factors associated with LLPC survival. In one study of the smallpox vaccine in humans, vaccine-specific $CD19^+$ PCs were detected in bone marrow, despite mature LLPCs normally downregulating CD19 following their maturation (56). Because most patients were vaccinated decades prior to the study, these $CD19^+$ LLPCs could not have recently migrated from GCs. This observation, along with the fact that $CD19^-$ LLPCs are also present, suggests that the generation of new vaccinia-specific LLPCs



VACCINES CAN TARGET...	...SINCE	ON THE OTHER HAND,
1. Antigen valency	High antigen valency allows for increased recruitment of low-affinity clones into the GC, in addition to high affinity clones.	Low antigen valency can stimulate a full GC response only when the cognate cells are high affinity.
2. Antigen availability	Appropriate antigen concentration ensures maximal recruitment of B cells into GC reaction.	Excessive antigen availability can increase immediate differentiation into plasma cells due to percieved inability to contain the pathogen, leading to increased generation of low-affinity antibodies.
3. B cell TLR stimulation	Stimulation of TLR7 or TLR7 and TLR4 simultaneously with BCR leads to increased MBC formation and secondary response.	PAMP (such as CpG) stimulation of B cell TLR9 receptors decreases GC recruitment by reducing T cell help, and encourages immediate SLPC differentiation.
4. Time of antigen delivery	Prolonged antigen delivery time increases B and T cell response and vaccine efficacy overall while augmenting broadly neutralizing antibody titres.	Bulk antigen delivery reduces the length of the GC response, resulting in overall lower memory immune cell affinity.
5. Metabolic regulation	Decreased mTORC activity increases cross-protective immunity.	Increased Blimp1 stimulation positively regulates mTOR through upregulation of amino acid import, leading to plasma cell fate differentiation programs.

Figure 2. Potential areas for vaccine-mediated interventions.
Vaccination allows for controlled activation of the immune response. Understanding how certain stages or processes can be influenced by vaccines is the foundation to inducing safe immune responses which induce effective protective memory. While this is not a comprehensive list, it serves to illustrate the broad areas within B cell biology that have the possibility of being targeted effectively by man-made immunizations.
mTORC, mammalian target of rapamycin complex.

is ongoing even decades after vaccination (56). Though this information is speculative and based on limited evidence, studies like this suggest that LLPCs can not only long-lived but may, in some cases, be able to maintain their population via generation of new vaccine-specific cells. Factors that may impact the ability of LLPCs to exhibit this type of long-term persistence most likely are dependent on the type of microenvironment in the bone marrow, the method of vaccination, age at immunization, and changes in bone marrow due to aging (57). As new insights narrow in on how various Ags can impact LLPC formation and persistence in the bone marrow, effective ways to maintain these populations through vaccines will be more clearly understood.

MBCs RECALLED TO SECONDARY GCs MUST COMPETE WITH NAIVE B CELLS FOR GC ENTRY

In addition to the tendency to undergo rapid differentiation into PCs upon secondary Ag encounter, which appears to vary across several MBC subpopulations (32), some MBCs can reenter GC reactions upon re-immunization. Those which preferentially do so have been

defined as a subset with no mature memory markers, identifying them as "unswitched." Though previous research seemed to conclude that MBCs were not able to re-enter GCs upon recall, recent observations indicate that despite secondary GCBCs consisting mostly of GC-inexperienced clones of seemingly naive origin, there is a small population of mature MBCs in GCs during secondary infection restricted to only a few high-affinity clones (14,15) (**Fig. 3**). Therefore, the originally GC-derived MBC compartment has the greatest impact in the EF response generated by a secondary infection event, as these memory cells immediately differentiate into PCs upon challenge. Since selection for the MBC fate may require a lower affinity BCR than LLPCs (indicated by lower mutational rates) due to their earlier exit from the GC, MBCs have an increased ability to respond to cross-reactive Ags compared to LLPCs. Additionally, GC responses only become particularly useful during a primary response if the infection is not cleared quickly, since if it is, EF responses make up the bulk of the effective response while the GC is still forming. In the case that a pathogen cannot be eradicated in a short time, GC-derived effector cells take over the immune response (16). When recalled during a secondary immune response, the isotype of MBCs may directly impact their manner of differentiation. Though not always, switched MBC populations have been shown to be unstable

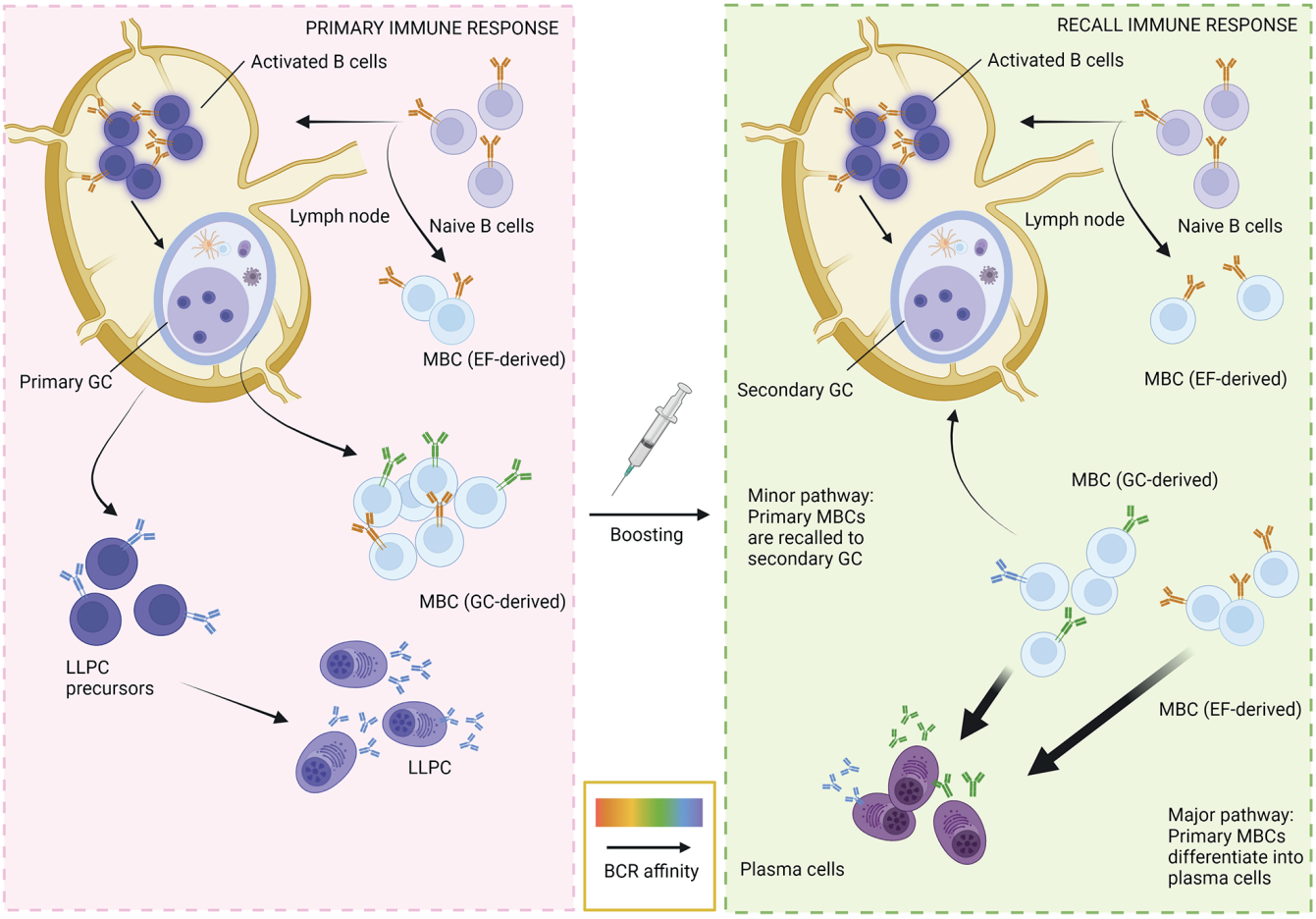


Figure 3. Secondary GC formation after boosting.
The first encounter of the immune system with Ag triggers the GC response and results in the formation of SLPCs and immunological memory cells, the former of which mostly participate in targeting Ag during ongoing infection and the latter which are responsible for long-term immunity. Long-lived bone marrow associated PCs constitutively secrete Abs against the primary Ag. During repeat immune encounters, such as boosting, primary MBCs differentiate into PCs of higher affinity for the Ag than naive cells. Generally, only a few high affinity MBC clones can compete with the enormous starting naive repertoire and participate in secondary GCs.

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and decline over time (58,59). On the other hand, IgM memory cells, which can be long-lived, retain a higher capacity to enter GCs and undergo class switching and affinity maturation, which probably allows greater flexibility in the response to secondary infections (46). This may be particularly useful for designing vaccines able to provide protection despite high viral mutation rates, such as the SARS-CoV-2 virus. This IgM memory can be maintained even in the presence of chronic Ag, pointing to a potential function of these MBCs as maintaining long-term protective Abs rather than rapid PC differentiation (46).

MUCOSA-ASSOCIATED B CELLS ARE FRONTLINE GATEKEEPERS AGAINST EXOGENOUS Ags

The mucosal immune system encompasses sites where both Ag-specific B and T cell responses are induced and where adaptive immune effector responses are orchestrated. The interplay between these two sites is mediated by varying expression of B and T cell-associated chemokine receptors and integrins. Despite compartmentalization of many of these responses, extensive communication between different mucosae makes it possible to vaccinate at a single mucosal site but induce immune responses at another. The mucosal system's constant exposure to Ags necessitates strongly immunoregulatory mechanisms to prevent chronic inflammation, but this results in many adjuvants commonly used in intramuscular vaccinations being ineffective or often completely useless. This is partially due to the mucosal tissues' inherent tolerance of the intestinal microbiome, which is not only extremely diverse but shares many of the same surface characteristics and markers as pathogenic strains of bacteria (60). The population of ASCs present in mucosal tissues varies extensively, as during infection or other inflammatory responses, additional ASC populations are recruited which are able to engage with the Ag and cooperate with effector processes (60) Similarly, MBCs are found in both lymphoid and nonlymphoid tissues, which suggests that some of them may be tissue-resident cells. Typically, MBCs generated in the course of a normal immune response are also observed traveling to mucosal surfaces (61). Further, though their existence was previously disputed, it has now been confirmed that tissue-specific infections can generate MBC subpopulations distinct from their canonical MBC counterparts, contributing to early PB responses following challenge infection (61). Lung-specific MBC (BRM) formation was observed without formation of tertiary lymphoid organs in similar studies done in mice (62). These mice also demonstrated LLPC formation upon recovery from infection. Class-switched BRM cells have also been found in human lungs, and are now considered a common component of the lung adaptive immunity response (62).

The effectiveness of mediating the immune response via the mucosal surfaces has also been demonstrated in other studies (63) relating to SARS-CoV-2. Since viruses and infections often enter the body via the mucosae, inducing the formation of mucosal-specific memory cell types able to protect the "frontlines" during an infection could potentially clear viral infections more efficiently and more quickly, preventing more serious onset of symptoms.

INTEGRATION OF B CELL KNOWLEDGE ALLOWS FOR IMPROVED VACCINE DESIGNS

The main goal of this review is to illustrate key B cell pathways for differentiation and activation to understand how to maximize the effectiveness of the resulting immune

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response among all subsets of B cells. Though there is still a long way to go towards improving vaccine efficacy, there are many studies which have shown precisely how effective vaccination-induced immunity can be. Some vaccines which have been in use for decades are still providing protection against their respective pathogenic counterparts. Studies in primates immunized with tetanus vaccines showed that even after removal of potential B cell reservoirs from solid tissues (namely the spleen and draining lymph nodes), as well as detectable tetanus-specific MBCs, tetanus-specific Ab titers were maintained above the protective threshold limit for the lifespan of the host. These resulting Ab titers were maintained long-term, which suggested that the bone marrow could potentially be a major site of systemic vaccine-induced Ab production in humans as well as in primates (57).

Various factors could contribute to this prolonged maintenance; it has been shown that the pathogens themselves can skew the resulting response. While some pathogens are able to suppress the secondary GC response to evade eradication following an initial wave of the EF response (16), some, like SARS-CoV-2 infection, are associated with leukocyte infiltration (mostly macrophages and neutrophils) and an increase in type I and type II interferon, other proinflammatory cytokines, and chemokines, exacerbating the immune response to a dangerous degree and causing an autoimmune response (63,64) Up until now, various methods for developing vaccines to circumvent these and myriad other problems have been proposed, including adjuvants or delivery constructs which emulsify viral agents or further stabilize the immunogen (10,32,60).

Because stable Ab repertoires can arise from both T cell-independent and -dependent processes, and because the formation of LLPCs and MBCs is not solely attributable to a GC response and, indeed, appears to arise via independent mechanisms, several studies imply that a vaccine's effectiveness in forming MBCs or LLPCs appears to have less to do with how much they stimulate immune cells and more on how other factors, such as the adjuvant, affect the formation of cell populations with increased longevity (2). Aside from alum, which is currently the most widely used adjuvant (65), there are very few adjuvants currently approved for use in humans. However, adjuvants able to stimulate B cells via their pattern recognition receptors have recently shown great promise in preliminary studies (66). Induction of TLR homo- or hetero-dimerization, stimulation of cytokine production, or simultaneous activation of the C-type lectin-like receptors and Nod-like receptors are among the several other methods which can induce a broad spectrum of immune responses and fate programs (32,66), making them valuable targets for vaccination (**Fig. 2**). This does not always involve the direct stimulation of the receptor by the adjuvant itself but may trigger immune responses via induction of damage-associated molecular patterns by inducing cell damage which activates dendritic cells and other APCs.

The dependency of B cell activation on Ag valency (**Fig. 2**) (22,24,27,67), has already been widely considered as a key factor in designing vaccines, but Ag amount and availability are also important factors. Interplay between these is also important, as has been seen in studies (67) showing that immunization with a higher valency Ag is better in all cases, though GC response is improved to the greatest degree when immunizing with a low-affinity Ag. This study also revealed that immune reactions dominated by high affinity B cells tend to result in high rates of PC differentiation. Ag multimerization increases the likelihood of lower-affinity B cells differentiating into PCs by increasing induction of IRF4 (67). Because of this, a possible result of altering Ag valency could be expansion of the PC library into a more broadly reactive population.

As previously mentioned, limited Ag availability (**Fig. 2**) can contribute to variation in the rate of differentiation of PCs and MBCs (21). Even Ag that is not highly immunogenic has been shown to recruit B cells into subsequent immune responses, provided there is available T cell help, and they have taken up adequate Ag during initial activation (48). B cells transiently acquiring Ag can still generate several subsets of MBCs, inciting long-term participation of B cells in GC responses and the differentiation of several MBC subsets into PCs during a secondary infection. Studies demonstrate that increased or recurrent exposure to Ag was not shown to significantly increase the populations of eMBC numbers (26,48), indicating that increasing the Ag amount may not offer significant advantages to an immune response. Conversely, slowed or delayed delivery of Ag after immunization has been shown in an human immunodeficiency virus (HIV) model to improve the GC response and result in increased activation of B and T cells (**Fig. 2**), improving the resulting memory immune response (68).

The creation of vaccines which effectively induce protective immunity depends on understanding the capacities of MBCs and LLPCs, the 2 major subsets of immunological memory. Since LLPCs provide more specific constitutive protection and MBCs are capable of broadly cross-reactive Ab generation (32,44), it may seem reasonable to consider vaccines targeted towards bolstering one or both pathways, depending on the Ag. While the major tactical advantage of MBCs in cases of rapidly mutating pathogens is their cross-reactivity (and therefore relatively lower affinity), consistently increasing the diversity of the immune repertoire is difficult due to differences in patients' starting immune repertoire. Therefore, targeting highly conserved regions of otherwise rapidly mutating proteins appears to be an excellent way to ensure that a vaccine's protection can last a long time. Recent observations in H1N1 influenza patients revealed that memory cells targeted towards relatively conserved regions of a previous influenza virus produced the most effective Abs against a newly encountered virus hemagglutinin rather than the variant head region (32). These Abs, generated by MBCs rather than resident LLPCS, were in some cases more effective against the new virus than the original. Therefore, the patient's ability to respond effectively to the vaccinating strain of influenza hinges on the patient's previous repertoire of immune cells against that strain. More specifically, those with increased Ab titers against the vaccinating strain have increased Ab titers against immunodominant epitopes, preventing them from effectively activating rare MBCs against conserved stalk regions to induce broadly protective immunity. (32,69). The creation of "universally" targeting vaccines should build on this research to find a way to bypass varying immune backgrounds in patients and instead reliably activate MBCs against conserved viral regions.

Though current searches for conserved viral regions that can be targeted by universal vaccines are ongoing and complicated, especially due to constant evolution by pathogens to evade the immune system using myriad strategies (70), the information presented in this review emphasizes 2 points: first, that vaccines are and will continue to be an effective means of preventing the spread of communicable diseases, and second, that the most important tool at our disposal is our own immune system, which can be harnessed by these vaccines.

Though recent progress indicates significant advances, there are still many unanswered questions. Aside from unknown fate programs, functional subsets of MBCs, or the mysterious self-maintenance of LLPCs, many aspects of even the B cell response remain uncertain. For example, what causes the variability in GC diversity among different individuals? To what extent do sequential immunizations with variant viruses facilitate the activation of MBCs against conserved regions? Does past immunity affect how newly formed

GCs prioritize diversity vs. affinity? Are mRNA vaccines a viable solution to future pandemics, despite the limited longevity of Ag specific Ab responses? These questions, among the many others, effectively paint the frontline of research that must be understood to improve the capacity of vaccine technologies now and in the future.

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