B cell memory: building two walls of protection against pathogens

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Abstract | Surviving a single infection often results in lifelong immunity to the infecting pathogen. Such protection is mediated, in large part, by two main B cell memory ‘walls’ — namely, long-lived plasma cells and memory B cells. The cellular and molecular processes that drive the production of long-lived plasma cells and memory B cells are subjects of intensive research and have important implications for global health. Indeed, although nearly all vaccines in use today depend on their ability to induce B cell memory, we have not yet succeeded in developing vaccines for some of the world’s most deadly diseases, including AIDS and malaria. Here, we describe the two-phase process by which antigen drives the generation of long-lived plasma cells and memory B cells and highlight the challenges for successful vaccine development in each phase.

An appreciation of the protective power conferred by immunological memory preceded our understanding of the cellular and molecular basis of this memory by centuries. During a plague in Athens in 430 B.C.E., the citizens understood that having the good fortune to recover from the disease, they could care for the newly stricken because no one was ‘attacked twice, at least not fatally’ 1. The simple principle that survival of an infection or exposure to a less virulent or attenuated form of a pathogen leads to lifelong immunity formed the foundation of Edward Jenner’s development of a vaccine for smallpox, an infectious disease that had the power to decimate entire populations in Europe in the mid-1700s2. Even fairly recent vaccines, such as those that elicit protective immunity to polio, measles and rubella, were developed for the most part without comprehensive knowledge of the mechanisms underlying immunity. Unfortunately, not all attempts to develop vaccines have been successful; worse, some vaccines were actually shown to be harmful to humans, as was the tragic case for respiratory syncytial virus3. In addition, to date, we have not succeeded in producing effective vaccines for some of the world’s most lethal diseases, including AIDS and malaria4,5. The hope is that more detailed knowledge of the cellular and molecular mechanisms that underlie the generation of protective, long-lived antibody responses and B cell memory will allow the development of safe, effective vaccines for pathogens for which we currently have none. Moreover, such knowledge may have broader benefits; for example, for the development of therapies for both systemic autoimmune disease and B cell tumours that may be, in part, unintended consequences of the drive to generate B cell memory6,7.

We now understand that immunological memory for many infectious diseases is acquired after a single infection and is dependent on the acquisition of two main ‘walls’ of memory; namely, long-lived plasma cells that produce protective antibodies and memory B cells that are able to respond on reinfection to pathogens and their variants. This process is detailed in several excellent recent reviews8–11. The antigen-driven generation of long-lived plasma cells and memory B cells from the naïve B cell repertoire in the primary response to antigen occurs predominantly in secondary lymphoid organs (SLOs) in B cell follicles and in germinal centres (GCs) in two consecutive phases12–14. In phase 1, antigenic stimulation through B cell antigen receptors (BCRs) induces naïve B cells to differentiate into short-lived plasma cells and GC B cells in the B cell follicles. In phase 2, antigens drive GC B cells to differentiate into long-lived plasma cells and memory B cells in GCs. In subsequent recall responses to antigens, memory B cells respond by differentiating into long-lived plasma cells or by re-entering the GC reaction. In this Review, we describe these two main phases of memory B cell development, highlighting some of the key variables that predict success at each step. We also briefly describe how chronic infectious diseases, including AIDS and malaria, may derail the acquisition of protective B cell memory. In this context, we speculate on the utility of leapfrogging over the development of vaccines for such chronic infectious diseases and providing protection through prophylactic, broadly neutralizing antibodies.
**Phase 1 of B cell memory**

*Antigen-driven differentiation of naive B cells.* Naive B cells first encounter antigen in the B cell follicles of SLOs (Fig. 1). Antigen binding to BCRs results in downstream BCR signalling and in the internalization, processing and presentation of the BCR-bound antigen on MHC class II molecules. Antigen-activated naive B cells increase their metabolic activity and express chemoattractant receptors (CC-chemokine receptor 7 (CCR7) and EBI2) that direct them to the border of the T cell zone, where they interact with antigenspecific T helper cells (T<sub>H</sub> cells) that have been primed.

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**Fig. 1 | Two phases of the acquisition of B cell memory.** In phase 1 of the primary response (left), naive B cells exit the circulation, enter B cell follicles in the secondary lymphoid organ and survey the environment for antigen. Antigens encountered on follicular dendritic cells (FDCs) activate B cells through the B cell receptor (BCR), and the antigens are processed and presented to T cells at the T cell–B cell border, driving naive B cells to proliferate and differentiate into three main cell types: germinal centre (GC)-independent memory B cells, GC B cells or short-lived plasma cells. In phase 2 of the primary response (right), newly differentiated GC B cells form GCs and undergo proliferation and somatic hypermutation in the dark zone before exiting to the light zone, where the GC B cells encounter antigen on FDCs, present the antigen to T follicular helper cells (T<sub>H</sub> cells) and undergo three main fates: namely, differentiation into memory B cells, differentiation into long-lived plasma cells or re-entry into the GC dark zone. In the secondary response (bottom right), memory B cells respond to antigen and differentiate into long-lived plasma cells or GC B cells that undergo GC reactions. TCR, T cell receptor.
The different antigen-driven T cell dependent fates have a profound impact on the outcome of an infection, resulting in either short-lived plasma cells that provide immediate short-lived antibody responses capable of controlling the initial infection or GC B cells that contribute to long-lived B cell memory by differentiating into long-lived plasma cells and GC-dependent memory B cells, albeit after a delay of several days to complete the GC reaction. A fundamental question for which we do not yet have a complete answer is what parameters govern this fate decision?

Precursory frequency, antigen affinity and avidity. A recent study provided important insights into the processes involved in antigen-driven naive B cell differentiation. The authors examined the factors that influence the outcome of vaccination in mice containing naive B cells expressing human germline VRC01-class BCRs that recognize the broadly neutralizing epitope of HIV gp120 (REF.\(^\text{25}\)). Taking advantage of the remarkable array of tools available to determine the frequency and antigen affinity of VRC01-class B cells and detailed knowledge of the VRC01-class B cell antigen, this study provided evidence that the precursor frequency of naive B cells expressing germine VRC01-class BCR (~1 in 10\(^8\), antigen affinity (less than 1 μM) and avidity (an antigenic epitope valency greater than 60) were interdependently limiting for successful GC completion. High-affinity multimeric antigens were shown to be capable of driving relatively rare VRC01-class naive B cells to differentiate into GC B cells that underwent extensive SHM and differentiated into memory B cells following a single immunization.

Recent studies of the transcriptional regulation of GC B cell differentiation versus plasma cell differentiation provided additional evidence for a role for antigen affinity in fate decisions. The transcription factor interferon regulatory factor 4 (IRF4), which regulates plasma cell differentiation, was shown to also initiate the generation of GC B cells, depending on the level of expression of IRF4, determined by the strength of BCR signalling\(^\text{26}\). Transient low levels of IRF4 favoured GC cell fates in contrast to sustained and higher levels of IRF4, which promoted the generation of plasma cells. These findings suggest that low-affinity antigen–BCR interactions that result in weak induction of IRF4 will initiate GC B cell differentiation, whereas high-affinity antigen–BCR interactions that induce high levels of IRF4 will promote plasma cell differentiation. T\(_{FH}\) cell help provided both through CD40L-induced CD40 signalling and through IL-21 appear to play roles in directing naive B cells towards GC differentiation versus plasma cell differentiation\(^\text{27}\). However, chronic CD40 signalling antagonized the differentiation of GC B cells and promotes plasma cell differentiation\(^\text{28,29}\). Recently, evidence was provided that B cell fate decisions are made in a multi-step process in which CD40 signalling is required only early to achieve increases in BCL-6 expression and that prolonged CD40 signalling drives precursors away from GC B cell fates\(^\text{30}\).

These observations raise an important question: how stringent is the influence of affinity of the BCR on the fate of naive B cells? Recent studies comparing the affinity...
thresholds for BCR-induced activation of human tonsil naive B cells versus GC B cells provided evidence that naive B cells have an intrinsic affinity threshold that is 1/100 of that of GC B cells. The estimated affinity of the BCRs expressed by naive B cells for the antigen in this study was ~0.5 µM, similar to the suggested minimal affinity threshold for naive VRC01-class B cell selection in the study described earlier (~1 µM). Given that these affinities are in the lowest range of affinities of functional antibodies it appears that there may be very little affinity-dependent exclusion of antigen-specific naive B cells in the first phase of antigen-driven differentiation. Even relatively low-affinity antigen-specific naive B cells would enter GCs and have an opportunity to differentiate into high-affinity long-lived plasma cells and memory B cells. Thus, very few antigen-specific B cells may be ‘wasted’ in phase 1 as a high affinity for antigen is not essential.

**Molecular mechanisms of fate instruction.** Several recent studies addressed the molecular mechanisms that instruct B cells in plasma cell and GC fates in vivo, emphasizing the importance of both metabolic changes and T<sub>FH</sub> cell interactions. In one study, mice that contained B cells deficient in protein kinase Cβ (PKCβ), a key regulator of both cell polarization and mechanistic target of rapamycin (mTOR) complex-dependent metabolic reprogramming, failed to develop plasma cells or GCs following immunization with a T cell-dependent antigen. Following this, BCR signalling in naive B cells was shown to trigger a shift from canonical to non-canonical autophagy, and enhancement of the non-canonical pathway changed mitochondrial homeostasis and influenced the GC and plasma cell fate decisions. In another study, mice containing B cells that were diminished in their ability to form long-term conjugates with T cells were severely impaired in their ability to form GCs and to produce affinity mature antibodies following immunization. In this study, the diminished ability to form B cell–T cell conjugates was due to a deficiency in intersectin 2 (ITSN2), a guanine exchange factor for CDC42, a GTPase that is essential for the remodelling of the actin cytoskeleton in response to BCR signalling.

**Impact of antigen structure.** The nature and structure of antigens also impact the outcome of immunization. Of particular interest are complexes of weakly immunogenic recombinant subunit antigens coupled to bacterial membrane complexes, such as the outer membrane complex of Neisseria meningitidis, or to virus-like particles, which are non-replicating shells composed of viral structural proteins that when overexpressed assemble into dense, multiprotein arrays. These antigens are proving to be exceptionally immunogenic and capable of routinely inducing high-tited persistent antibody responses, although the mechanisms underlying their potency are not well understood. Indeed, the highly successful human papillomavirus (HPV) vaccine, which comprises virus-like particles assembled from the major L1 proteins from four HPV types, provides near-complete protection from sexually transmitted HPV infections in naive individuals and may even be effective when administered in a single dose. The HPV vaccine was shown to induce memory B cells in vaccinated naive individuals expressing potently neutralizing antibodies but ones that have not undergone extensive SHM.

Another important feature of antigens is the form they take on in vivo. Antigens arrive in the SLO through afferent lymphatics via subcapsular sinuses. LYVE1-expressing lymphoid endothelial cells and a network of surrounding CD169-expressing macrophages create a tight barrier that prevents movement of large antigens and antigen complexes into B cell follicles. However, small antigens such as hen egg white lysozyme (HEL; 14.4 kDa) have been shown to penetrate B cell follicles within minutes after injection and pass into pores of the subcapsular sinuses or lymphatic conduits. Larger antigens (more than 70 kDa), viruses and virus-like particles are captured by subcapsular sinus macrophages in the form of immune complexes or complement-coupled complexes. These are rapidly shuttled to non-cognate B cells that transport the antigens into follicles, where the complexes are deposited onto follicular DCs (FDCs) that provide a continuous supply of antigen during the B cell response. FDCs also provide a 3D network on which B cells move within the follicle and secrete CXC-chemokine ligand 13 (CXCL13), which recruits and retains CXC-chemokine receptor 5 (CXCR5)-expressing B cells in GCs. The fact that in SLOs, B cells are exposed to both soluble and FDC-associated antigens raises an important question: is the outcome of a B cell’s exposure to soluble antigens versus membrane-associated antigens similar? It is well established that membrane-associated antigens are highly effective at triggering B cell activation. In addition, several studies in vitro have provided evidence that the requirements for B cell responses to soluble antigens versus membrane-associated antigens differ in a variety of parameters, including the requirement for co-receptors, responses to monovalent antigens, the regulation of cytoskeleton and the kinetics and regulation of BCR clustering. However, at present we have little information concerning the fate of B cells responding to soluble antigens versus membrane-associated antigens either in vitro or in vivo. It would seem that vaccine design would benefit from such information.

**Effect of pathogen products in the microenvironment.** The impact of the microenvironment within the B cell follicle on the fate of naive B cells is just beginning to be explored. One important environmental factor is the presence of the pathogen or pathogen products and the signals of imminent danger these send to the host through innate immune receptors. A recent study showed that pathogen-associated molecular patterns (PAMPs), in particular the CpG ligand for Toll-like receptor 9 (TLR9), had the unexpected effect on naive B cells of blocking antigen processing and presentation at a point after antigen internalization but before the delivery of the antigen to processing compartments. Thus, CpG-stimulated B cells were less able to acquire T cell help. Parallel studies provided evidence that activated B cells that were unable to acquire T cell help shortly after BCR signalling underwent apoptosis due...
to induced mitochondrial dysfunction acting as a ‘metabolic clock’ \(^{17}\). These studies also showed that TLR9 stimulation rescued the antigen-activated B cells from apoptosis and drove their proliferation and differentiation into low-affinity short-lived plasma cells. Taken together these observations provide a mechanism by which naive B cells activated in the presence of pathogen products are fated to rapidly differentiate into short-lived plasma cells rather than committing to participate in time-consuming GC reactions \(\text{Fig. 2}\).

The discovery of an antagonistic effect of CpG on B cell responses to a T cell dependent antigen was surprising as responses to immunization with T cell-dependent antigen are well known to require adjuvants, many of which contain PAMPs as immunostimulatory components. Conventional DCs respond to PAMPs in peripheral tissues, where they encounter antigen, by increasing their antigen-presenting cell function before travelling to SLOs to activate T cells, a clear benefit of adjuvants. Indeed, the yellow fever vaccine YF-17D, one of the most successful empiric vaccines ever developed, activates DCs through multiple TLRs \(^{51,52}\). However, the study just described suggests that if PAMPs accumulate in B cell follicles, B cells will respond to the signal of impending danger by differentiating immediately into relatively low-affinity short-lived plasma cells. That study showed that immunization of mice and humans with protein antigens with CpG as an adjuvant resulted in high levels of antibody that failed to affinity mature, consistent with GC-independent plasma cell generation \(^{50}\). These observations suggest that PAMPs or CpG in particular may not be ideal adjuvants for pathogen-specific responses that require high levels of SHM, such as broadly neutralizing HIV-specific antibody responses.

Are there better adjuvant formulations to drive naive B cells to differentiate into GC B cells that would accumulate SHM in GCs? A recent comparison of the impact of eight different commonly used adjuvants on the antibody response to the HIV envelope protein in a non-human primate model showed that formulation of the envelope protein with adjuvants increased the levels of HIV-specific antibodies but did not increase the frequency of SHMs essential for the development of broadly neutralizing antibodies \(^{53}\). Thus, future evaluations of the efficacy of adjuvants may require an assessment of not only antibody titres and the duration of the response but

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**Fig. 2 | The impact of pathogens on naive B cell fates.** Naive B cells express both B cell receptors (BCRs) for antigen and Toll-like receptors (TLRs; depicted as the intracellular TLRs) that respond to pathogen-associated molecular patterns (PAMPs). In the presence of antigen alone (left), B cells internalize, process and present antigen on MHC class II molecules. Engagement with antigen-specific preactivated T cells results in B cell proliferation and differentiation into germinal centre (GC) B cells. In the presence of antigen and PAMPs (right), signalling via TLRs blocks the processing of antigens internalized by BCRs and stimulates B cells to proliferate and differentiate into short-lived plasma cells. TCR, T cell receptor.
Fig. 3 | Antigen affinity discrimination by germinal centre B cells. On antigen engagement (step 1) germinal centre (GC) B cells form unique actin- and ezrin-rich pod-like structures that concentrate B cell receptors (BCRs) (step 2) GC B cells exert force on BCR–antigen complexes through myosin-dependent processes (step 3), leading to the deformation of the antigen–associated membrane (steps 4, 5) and ultimately to the capture of the antigen and associated membrane fragments (step 6) and their trafficking away from the synapse for internalization (step 7). High-affinity BCRs are better able than low-affinity BCRs to resist the pulling force exerted by the GC B cell on the BCR–antigen complex and, consequently, high-affinity BCRs capture, process and present larger amounts of antigen to T follicular helper cells.

Affinity thresholds and GC B cell selection. To understand affinity selection in GCs, the mechanisms by which B cells sense their affinity for antigens is of central importance. As mentioned earlier, both soluble antigens and membrane-associated antigens may be present in GCs. At present, the mechanisms by which B cells sense their affinity for soluble antigens are only poorly understood. In contrast, the ability of B cells to engage antigen, signal in response to it and internalize it for processing and presentation were shown to be directly dependent on the B cells’ affinity for the antigen(s). B cells capture antigen from membranes through the exertion of pulling forces on the BCR–antigen complex. Higher-affinity interactions are better able to resist the pulling forces and are more successful at internalizing antigen for presentation to T<sup>h</sup> cells. Thus, B cell affinity is indirectly determined by the amount of antigen a B cell is able to capture from an FDC and present to a T<sup>h</sup> cell in the GC (Fig. 3).

If affinity selection is paramount in the GC reaction, are GC B cells particularly well suited to discriminate antigen affinity and to be selected by high-affinity antigens? As mentioned earlier, recent studies provided evidence that the intrinsic affinity threshold of GC B cells for both BCR signalling and antigen internalization is at least 100-fold higher than that of naive B cells. Recent studies also provided evidence that GC B cells have ‘rewired’ CD40 and BCR signalling such that CD40 signals are transduced through nuclear factor-κB and BCR signals are transduced through forkhead box protein O1 (FOXO1), and both of these signalling pathways are required for induction of MYC, which is critical for GC B cell survival. In contrast, naive B cells can signal through either BCR or CD40 alone to activate MYC. The net result of this rewiring is that, compared with naive B cells, GC B cells show a more stringent requirement for antigen and T cells for them to become activated.

In addition, GC and naive B cells differ in their expression of cell surface receptors that may serve to further increase the affinity threshold of GC B cells. For example, compared with naive human B cells, human GC B cells express very little VLA4 (also known as α4 integrin), which is an integrin that stabilizes the initial interactions with antigen-bearing DCs by binding to VCAM1 and thereby lowers the affinity threshold for B cell activation. In addition, compared with naive B cells, GC B cells were shown in mice to recognize antigen through specialized immune synapse architecture. Subsequent studies provided a detailed picture of the GC B cell architecture, showing that BCRs expressed by GC B cells are concentrated in unique, highly dynamic actin- and ezrin-containing pod-like structures through which the BCRs exert pulling forces and test affinity. Remarkably, the affinity of the antigen engaged by the GC B cell dictates the behaviour of the pod-like structures. Low-affinity antigens induce a dynamic searching behaviour with engagement and release of the antigen, whereas high-affinity antigens induce stable long-lived engagement of the GC B cell with the antigen-containing membranes. A more detailed understanding of the mechanisms underlying the initiation of BCR signalling in these GC B cell pod-like structures may provide insight into the design of vaccines to maximize selection of high-affinity GC B cells.
**Further differentiation of GC B cells.** A central question for which we are just beginning to get answers concerns the control of the differentiation of GC B cells into long-lived plasma cells versus memory B cells in GCs. At present, the data support the concept of a fundamental dichotomy between the GC processes that drive plasma cell differentiation versus memory B cell differentiation. Studies using 5-bromodeoxyuridine pulse labelling provided evidence that GC responses undergo a temporal switch as they mature, first producing memory B cells with limited SHM and then generating long-lived plasma cells that contain more highly mutated variable (V) genes\(^6\). The observed paucity of mutations in the memory B cell compartment suggests that B cells have lower levels of affinity-based selection and have more broadly cross-reactive BCRs as compared with more highly mutated BCRs of long-lived plasma cells. Recent studies directly demonstrated that affinity selection is not equally applied to precursors of memory B cells and long-lived plasma cells, resulting in highly selected, high-affinity long-lived plasma cells and broadly reactive lower-affinity memory B cells. Analyses of single nitrophenyl-specific plasma cells and memory B cells provided evidence that the initial output of GCs in vitro immunization was stringently selected, high-affinity plasma cells and IgG memory B cells that persisted in GC-like structures for several months and IgM and IgG memory B cells that accumulated outside the follicle. On antigen challenge, the IgM memory B cells entered GCs in contrast to IgG memory B cells, which differentiated into plasma cells. In a transgenic mouse model, IgG1 memory B cells were shown to be predisposed to differentiate into plasma cells and were the major source of IgG1 antigen-specific antibody in the secondary response\(^6\).

The fate of antigen-specific B cells that expressed IgM (IgM\(^\text{+}\)) or isotype-switched immunoglobulin (swIg\(^\text{+}\)) in C57BL/6 mice was traced following immunization in a separate study and it was found that both IgM\(^\text{+}\) and swIg\(^\text{+}\) memory B cells were generated\(^6\). However, in subsequent challenges, although IgM\(^\text{+}\) memory B cells were more numerous and longer-lived than swIg\(^\text{+}\) memory B cells, swIg\(^\text{+}\) memory B cells dominated the challenge response, producing plasma cells and new memory B cells but no GC B cells\(^6\). As antigen-specific serum immunoglobulin levels dropped, the numbers of swIg\(^\text{+}\) memory B cells declined but IgM\(^\text{+}\) memory B cells, which contained few SHMs but had the ability to produce GC B cells, persisted and became the reservoirs of durable memory. However, a subsequent study by the same group of authors suggested an alternative mechanism for the instability of swIg\(^\text{+}\) memory B cells and the generation of large numbers of IgM memory B cells in C57BL/6 mice was dominated by an unusual single heavy chain variable domain (VH) that conferred high-affinity binding to antigen\(^6\).

Another study provided evidence for functionally distinct memory B cell subsets on the basis of their expression of CD80 and PD-L2, independently of isotype\(^7\). On challenge, CD80\(^{\text{+}}\)PD-L2\(^{\text{+}}\) memory B cells that were of relatively high affinity differentiated into plasma cells but not GC B cells, and conversely CD80\(^{\text{+}}\)PD-L2\(^{\text{+}}\) memory B cells that were of lower affinity produced few plasma cells but robustly differentiated into GC B cells. In a separate study, CD80\(^{\text{+}}\)PD-L2\(^{\text{+}}\) memory B cells were shown to be generated from high-affinity B cells during the primary immune response by mechanisms that depended on T\(_{\text{H}1}\) cell-inducing strong CD40 signalling in contrast to the development CD80\(^{\text{+}}\)PD-L2\(^{\text{+}}\) memory B cells, which did not require strong CD40 signalling\(^7\).

Taken together, these studies provide strong evidence that the ability of memory B cells to differentiate into plasma cells versus GC B cells is compartmentalized into perhaps several subpopulations. To exploit this compartmentalization of memory B cell functions in vaccine design it will be necessary to further elucidate the mechanisms that drive the differentiation of these memory B cell subsets.
Roles of long-lived plasma cells and memory B cells in protective immunity. The current model of B cell memory suggest that the highly-selected, high-affinity antibodies produced by long-lived plasma cells form the first line of defence against homologous challenge and that memory B cells provide a second layer of defence against challenge by variant pathogens that escape the long-lived plasma cell-mediated defence (FIG. 4). How strong is the evidence that memory B cells function in this way and is the ability of memory B cells to provide this function dependent on accumulating additional SHMs?

Studies using a mouse model of West Nile virus infection with wild-type and variant viruses that differed in only one amino acid in a dominant neutralizing epitope demonstrated that the antibodies produced by long-lived plasma cells generated in response to the wild-type virus only poorly neutralized the variant virus75. However, memory B cell-derived plasma cells produced antibodies that recognized both the wild-type virus and the variant virus equivalently, or remarkably, recognized the variant virus better than the wild-type virus and did so without accumulating additional SHMs. Consistent with these results, studies that traced influenza virus haemagglutinin (HA)-specific B cells in mice immunized first with the Narita virus strain followed by challenge with the homologous virus or the heterologous PR8 virus strain demonstrated that pre-existing antibodies secreted by long-lived plasma cells protected against homologous challenge, whereas protection from heterologous challenge required memory B cell activation. These memory B cells were primarily directed towards the relatively invariant HA stem76. Studies of the antibody response in humans to vaccination with the pandemic 2009 H1N1 influenza vaccine showed that individuals who had low levels of pre-existing antibodies to the vaccine generated broadly reactive antibodies to the HA stem, whereas high pre-existing levels of antibody to the vaccine correlated with strain-specific, HA-head responses, suggesting that antibodies to the HA head blocked the generation of broadly protective stalk-specific antibodies77. Thus, an individual’s immune history with influenza virus affects the ability to produce broadly protective B cell responses on challenge.

Of interest was the finding in mice that administration of the mTOR inhibitor, rapamycin, during immunization with influenza virus subtype H3N2 reduced the formation of GCs and inhibited class switching by B cells but resulted in a unique repertoire of antibodies that protected against lethal infection with hetero-subtypic H5N1 virus78. Studies of the antibody response of mice immunized with Dengue virus envelope proteins and challenged with the same or variant virus proteins showed that the variant proteins stimulate predominantly IgM+ memory B cells with the most diverse and least mutated V genes79. Taken together, these studies provide strong evidence for the role of highly diverse memory B cell populations in providing broad protection against variant virus infections. The challenge for the future will be to determine how to differentially evoke these memory B cell populations.

Effect of chronic infections on memory B cells

Nearly all of the studies described thus far in this Review investigated the generation and function of B cell memory in models of acute infections or vaccinations followed by challenge. The findings from these studies are most relevant to antibody-mediated development of vaccines to induce protective immunity in naive individuals. However, there is an urgent need for vaccines for individuals who have chronic infections, including AIDS, malaria and hepatitis virus infections. The development of such vaccines may be highly challenging as current evidence indicates that such chronic infections have a profound impact on the memory B cell compartment, the repercussions of which we are just beginning to understand80. For example, one common feature of chronic infections is the large expansion of a novel population of ‘T-bet’ memory B cells termed ‘atypical memory B cells’. We do not yet understand the function of these memory B cells in humans, and it is not entirely clear whether mouse models of ‘T-bet’ memory B cells that arise with age and in certain infections are relevant to human atypical memory B cells and can be used as
models to study atypical memory B cells. Thus, we may be quite a distance from designing vaccines for chronic infections.

**Prophylactic antibodies**

Although vaccination to induce B cell memory is the most effective and low-cost method of preventing infectious diseases, the development of effective vaccines for many of the world’s most deadly pathogens, including those that cause AIDS and malaria, have proven to be extremely challenging and thus far have met with little success. The failure to develop vaccines for these two diseases as well as several others have led to efforts to leapfrog over vaccine development entirely and to directly provide highly effective broadly neutralizing antibodies as prophylactics (reviewed in REFS 14,15). This is not a new idea as passively transferred antibodies have been used for more than a century as therapeutics for infectious diseases such as diphtheria and tetanus and subsequently for several viral diseases, including infections with respiratory syncytial virus, hepatitis B virus and hepatitis C virus. What is new, however, is the successful generation of rare, highly potent and broadly cross-reactive human monoclonal antibodies for several viruses, including HIV, and for the parasite that causes malaria. In addition, antibody engineering can increase the potency, cross-reactivity and half-life of such antibodies. Key to the discovery of these rare human antibodies was the careful selection of donors with desirable serum antibody profiles and the development of high-throughput human B cell isolation technologies. At present, passive antibody prophylaxis appears to be a promising alternative to vaccination for HIV infection. In addition, recent successes in vector-mediated antibody transfers in mice and macaques in which a single injection provided continuous and sustained delivery of antibody may provide an alternative form of antibody prophylaxis16,17. A number of antiviral and antimalaria monoclonal antibodies are in clinical development, and the results of these studies over the next several years will tell whether the current promise of this approach is fulfilled.

**Summary**

We have come a long way towards providing the plague survivors of 430 B.C.E. Athens with an explanation of their good fortune to never be ‘attacked twice’. We now have a good understanding of the cellular basis of B cell memory, namely long-lived plasma cells and memory B cells, and of how these cells develop from naïve B cells in two consecutive antigen-driven processes. We also have a general concept of how the two B cell memory walls are built and the protection against invading pathogens they afford. What we know far less about is how to effectively design vaccines and adjutants to reproducibly generate B cell memory. At present, it is not clear if general guidelines will emerge from current research or if vaccine design will continue to be basically an empirical process. Given the recent rapid pace of progress in understanding B cell memory, we are optimistic that it will not be long before future advances provide new prescriptions for the development of badly needed vaccines to protect against humankind’s worst enemies.

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