Multiple routes to B-cell memory

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Abstract

B-cell memory describes the populations of cells that provide long-term humoral immunity: long-lived antibody-secreting plasma cells that reside mainly in the bone marrow and memory B cells. Interestingly, the memory B-cell population is heterogeneous, although the importance of this heterogeneity has been unclear. Recent studies have investigated the formation and function of memory in different settings. In particular, T-independent memory-like cells and T-dependent (TD) IgM memory B cells qualitatively differ from canonical TD class-switched memory B cells; however, these studies suggest that IgM memory cells preserve the memory population over long periods of time. These subsets are evocative of the evolution of the humoral immune response, with memory-like cells appearing before acquisition of germinal centers, suggesting that there are multiple pathways to producing B-cell memory.

Keywords: antibody, evolution, IgM memory, plasma cells, T-independent

Introduction

The immune system has one fundamental function—to recognize foreign invaders and reject them from the body, while ensuring that such defense mechanisms do not destroy the host. This function remains the center around which the immune system, at the cellular, biochemical, genetic and epigenetic levels, has evolved. The immune system has also, at various points in evolution, acquired the ability for heightened responses to pathogens if the host had been infected prior—this is termed immune memory. As such, patients with immunodeficiencies that affect the humoral system often have an inability to form and/or maintain memory, resulting in increased susceptibility to infections (1, 2). Similarly, the ability to induce B-cell (and T-cell) memory is the foundation for protective immunity generated by vaccines. Yet, despite the clinical relevance of B-cell memory, the mechanisms underlying formation and function of memory B cells remain elusive.

Various questions have been posed about B-cell memory. What signals and genetic networks are required for its formation? How do memory B cells respond more rapidly and robustly during a secondary response? What maintains the population for long periods of time and, on the flipside, what prevents exhaustion of the population? Although many molecules have been identified to be important for the formation, maintenance and regulation of germinal center (GC) responses (reviewed in ref. 3), thus impacting on the quality and quantity of B-cell memory, these fundamental questions remain unsolved.

In the past decade, studies on B-cell memory in non-disease states have had three foci: gene expression studies, utilization of gene-deficient animals to study the roles of genes expressed during humoral responses and the formation of memory subsets. In particular, recent studies have provided a more in-depth characterization of T-independent (TI) formation of B-cell memory (4–6) and the function of murine IgM memory B cells (7, 8). The identification of multiple routes to memory B-cell formation has led to the idea that memory can be formed in ‘layers’, reminiscent of how evolution of the immune system has been described (9). In this review, we examine recent data demonstrating that memory B cells are formed in response to either TI or T-dependent (TD) stimuli.

Memory B-cell formation in TD responses

During a primary TD humoral response, antigen-stimulated B cells interact with activated T cells and other accessory cells, resulting in the formation of foci of short-lived plasma cells (PCs), GC-independent ‘early’ memory cells or a GC reaction, which can last a number of weeks before subsiding (reviewed in ref. 9). Within the GC, B cells undergo rounds of division and affinity maturation, in which antibody affinity increases due to somatic hypermutation (SHM). Class switch recombination (CSR) from IgM to IgG, IgA or IgE can occur, although these processes can also occur outside a GC. Eventually, high-affinity mutants are selected and differentiate into memory B cells or long-lived PCs (LLPCs). During a secondary response, memory B cells differentiate rapidly into high-affinity plasmablasts, thus clearing the infection more quickly than the primary response.
Memory B-cell subsets

The memory B-cell population in higher vertebrates is heterogeneous (3). Subsets in both humans and mice can be clearly identified by different Ig isotypes (e.g. IgM or class-switched isotypes) (7, 8, 10–12), and IgM memory B cells and IgG memory B cells have been shown to be intrinsically different (13). However, there are also less distinct differences, such as the levels of expression of different surface proteins (e.g. co-stimulatory factors) (14, 15) that may reflect different experiences of each cell during the primary response (3). In these latter studies, memory B cells can be categorized into different subgroups based on cell surface markers, and this could be correlated to precursor frequency and mutation rate (but not to class switch) (15).

Other groups had previously identified heterogeneity within the memory B-cell population (14–19) in both humans and mice. For example, Cooper et al. have identified a tissue-specific population of memory B cells in humans, identified by the expression of FCRL4 (17, 18).

Finally, atypical memory B-cell populations found in HIV and malaria patients have high expression of inhibitory receptors, the presence of which is correlated to defective function of the humoral system in these patients (20, 21). Taken together, these studies suggest that the memory population may be subdivided based on origin and function.

Interestingly, many gene-deficient mice and immunodeficient patients that lack productive GCs have cells that resemble memory cells, but the population appears to have an incomplete phenotype, such as few mutations, inadequate affinity maturation or no class-switched isotypes (reviewed in ref. 1, 3). These studies have revealed many genes that are important for different phases of the GC response—i.e. formation, magnitude or resolution—affecting output of the GC and thus the quality and quantity of the memory population but not whether the memory population exists.

As a result, the definition of a memory B cell has become contentious. Some researchers have made a priori assumptions based on observations of the cells that have been found to persist in mice and man; i.e. the precursor cell of a canonical memory B cell is a GC cell, and therefore, its Ig must be class switched and mutated. Thus, the main sources of contention are whether IgM and/or low-affinity early memory B cells, which can be found in the absence of a GC or paucity of GCs; (1, 3, 22–24), can be defined as memory cells. Similarly, TI B cells have not traditionally been considered as canonical memory B cells. We will focus this review on the recent in vivo research into what might be regarded as non-canonical memory formation.

TI memory

TI antigens can be TI type I (TI-I; for soluble antigens) or TI-II (where antigen is cell-bound). Recently, various groups have described LLPCs and memory-like cells formed during TI responses in mice. First, LLPCs can be generated to TI-II antigens, which secrete protective antibody from both the secondary lymphoid organs (25) and the bone marrow (4, 5). These LLPCs can either be IgM-secreting or be IgG-secreting; however, in at least one study, TI LLPCs secreted lower amounts of antibody compared with LLPCs generated during a TD response (4).

Unlike ‘conventional’ B cells (B2 cells), B1 cells were thought to self-renew in the periphery, to be TI, to express IgM but not to form memory cells; B1-a and B1-b subsets have been described but it is not clear exactly how mouse B1-a/B1-b subsets relate to the human equivalents. Alugupalli et al. (26) demonstrated that mouse B1-b cells give rise to an IgM-expressing B-cell population that persists, controls Borrelia hermsii infection and can confer protection on transfer, suggesting that these are in fact memory cells. Obukhanych and Nussenzweig used (4-hydroxy-3-nitrophenyl)-acetyl (NP)-Ficoll, a TI-II antigen, to generate cells that persisted for many months post-immunization (6). When these cells were adoptively transferred into naive hosts, they rapidly divided, although it is unclear whether they generated a burst of plasmablasts. Interestingly, both these groups confirmed older studies demonstrating that serum Ig, specifically class-switched Ig, inhibits the proliferation of TI memory cells (27, 28).

It remains unclear whether the B1-b memory population is continually proliferating or whether, once generated, these cells have an intrinsic survival advantage. There were some differences between these studies. For example, in response to NP-Ficoll, the memory population contains both IgG+ and IgG− cells (6), whereas in the study by Alugupalli et al., the B1-b memory population was only IgM+ (26). Furthermore, it is unclear if the precursor cells that respond to NP-Ficoll are of B1 or B2 origin or both, or even whether aborted GCs were generated (29).

In sum, both these studies (6, 26) demonstrate that immunity formed during a TI response can be conferred when transferred into naive hosts and that these ‘recall’ responses are inhibited by serum Ig. However, it appears that this is a quantitative rather than qualitative attribute; thus, it is as yet unexplored whether these cells have an intrinsic advantage—as happens with TD memory in vivo and IgM and IgG memory in humans (1, 13, 30, 31)—to respond more rapidly and robustly compared with naive B cells. Thus, it is clear that at least TI-II antigens can form B-cell memory, although it is different to TD memory in regards to its longevity, secretion and phenotype.

TD IgM memory B cells

Memory B cells that retain IgM on their surface have been shown to exist in both mice (32) and man (1, 10–12). The identity of these cells in humans is contentious, with some groups identifying them as memory based on their functional and molecular similarity to IgG memory and others suggesting that they are ‘circulating’ marginal zone (MZ) B cells (33) (splenic MZ B cells are generally mature, IgM-expressing and non-recirculating cells).

Two recent studies have investigated the origin, function and persistence of murine IgM memory cells during a TD response (7, 8). First, Weill et al. utilized a mouse encoding a reporter for activation-induced cytidine deaminase expression to track cells that have been activated during an immunization with sheep red blood cells (7) (a classical TD antigen). Taking this a step further, Jenkins et al. immunized...
mice with PE (a fluorescent TD antigen) and thus were able to track antigen-specific cells throughout the response (8).

Taken together, both groups (7, 8) confirm that IgM memory can be formed, and there are qualitative differences between IgM and IgG1 memory. The two main findings were, first, that IgM memory persists longer than IgG1 memory and, second, that class-switched memory B cells differentiate rapidly into plasmablasts during a secondary response, whereas IgM memory B cells had a slower turnover rate and were less likely to participate in a secondary response in the presence of serum Ig (8). These IgM memory B cells were less mutated than IgG memory B cells, paralleling the IgM*CD27+ memory B cells found in healthy people. Weill et al. proposed the concept of ‘layers’ of memory (7), describing the existence of IgM memory and IgG memory, as well as their proliferating precursors within chronic GCs.

Thus, it appears that IgM memory B cells can be generated in both TI and TD responses and in the presence of different adjuvants. Although TI and TD IgM memory cells may qualitatively differ and/or have different precursors, they have overarching characteristics regardless of the response. The population can persist for months in vivo in mice, they divide and confer protection when transferred into naive recipients and are inhibited by serum Ig. Hence, it was concluded that IgM memory B cells are important for persistence of the population, whereas IgG memory B cells are the frontline responders. It is still unclear whether these murine populations are equivalent to IgM*CD27+ memory B cells found in humans, although Obukhanych and Nussenzweig suggest that the inhibition by IgG is an important regulatory mechanism in humans, highlighted by the fact that immunodeficient patients that do not generate IgG display hyper-IgM syndrome (6).

Can subsets inform memory biology?

It could be proposed that the presence of memory subsets answers some long-standing questions about memory B-cell biology. Arguably, the three most important questions regarding memory biology are (Q1) how is the population maintained over long periods of time, sometimes for the life of the host; (Q2) what are the mechanisms underlying rapid and robust secondary responses and (Q3) how are memory B cells formed? We will discuss these recent papers within the context of the first two questions.

The data presented in papers by both Weill and Jenkins (7, 8) suggest that IgM memory B cells may maintain the memory population (Q1). Both papers demonstrate that IgM memory B cells persist for longer periods of time than IgG memory B cells—in fact, the same number of IgM memory cells is maintained after an initial decrease during the resolution of the primary response (8), whereas IgG memory B cells diminish in number over time. It is important to note that all these studies are in mice, and therefore, it is difficult to directly compare how long these cells may be maintained in humans. Despite this, the data in these papers suggest that it may be IgM memory B cells that maintain the memory population.

On the other hand, these data do not explain how a qualitatively better response is generated, beyond the known advantages of canonical IgG memory B cells (Q2; 34) as murine IgM memory B cells do not have enhanced response kinetics over naive B cells in vivo (8). It is important to note that, in contrast, human IgM memory B cells have an intrinsic advantage over IgM* naive B cells with respect to proliferation, co-stimulation, time taken to first division and survival (13, 30, 31); therefore, it is likely that there are modifications in addition to expression of IgG rather than IgM that account for enhanced secondary responses. In mice, however, IgM memory B cells appear to have only two main advantages over IgM* naive B cells: an increase in number over naive B cells and a higher-affinity B-cell receptor. Would this be enough to induce rapid and robust secondary responses after the eventual loss of IgG memory B cells?

On the flipside, the presence of IgM memory B cells may be why the memory B-cell population is not exhausted during a secondary response—although they are poor responders in the presence of serum Ig, in the absence of it, they can generate secondary GCs and replenish the IgG memory compartment.

Although these studies do not reveal the molecular mechanisms underlying the formation of memory B cells, they raise an important question: are there multiple independent pathways to becoming B-cell memory (Fig. 1)? Or are the different ‘types’ of memory (TI, IgM memory and early memory) in GC-deficient conditions intrinsically related? As Weill et al. suggest, have we formed layers of memory over time?

Evolution of memory B cells

Protection, defined as the ability to produce a heightened response to a previously seen infection, has been identified in lower vertebrates, such as bony fish and amphibians [tables summarizing data for secondary responses in vertebrates can be found in (35–37)]. For example, repeated immunizations of trout have demonstrated that successive responses induce heightened antibody levels compared with the primary response. It has even been shown that invertebrates, such as the bumblebee, are able to clear secondary infections more rapidly and with specificity (38). Therefore, it is likely that a memory-like cells exists in lower vertebrates and several invertebrates.

The protective antibody responses observed in lower vertebrates do not show an increase in affinity compared with the primary response. Unlike higher vertebrates that can regulate body temperature (homeotherms), poikilothersms do not have the ability to form GCs (36). Yet, lower vertebrates have transcription factors that have homology to those that regulate B-cell differentiation in higher vertebrates, such as Bcl-6 and Blimp-1 (39–42). Although the series of evolutionary events that occurred in order to form GCs are unclear, it appears to at least correlate with enhanced T-cell responses (and thereby T-cell help) (43, 44) that occurred upon the increase and regulation of body temperature (36) and the existence of secondary lymphoid organs with complex organization and the presence of follicular dendritic cells (reviewed in ref. 37). Evidence of affinity maturation and selection appears with GCs in higher vertebrates (36, 37) explaining why secondary antibody responses in lower vertebrates are not of higher affinity than the primary response.
Humans with immunodeficient diseases that affect GC development, as well as lower vertebrates, similarly do not form fully functional humoral responses (as discussed in detail in ref. 45). Various GC-deficient or GC-defective mice, such as those that lack Bcl-6, ICOS or IL-21 (22–24), are still able to produce early memory-like cells, as can B1-b cells in mice. Interestingly, B cells in lower vertebrates can undergo SHM in the absence of a GC. The amphibian Xenopus, for example, contains B cells with somatic mutations (46); however, because there is a lack of evidence for affinity maturation in secondary responses in this species, it appears that these somatic mutants are not expanded and selected into the pool of persisting cells.

Similarly, patients with mutations in Cd40L (47) or Sh2d1a (48) that have a paucity of GCs still have B cells that undergo SHM, generating mutated IgM+ cells with a memory phenotype. B cells in these patients can class-switch in the absence of a GC, as can those in lower vertebrates such as Xenopus (which switch to IgY) (46). Therefore, the cellular machinery for SHM and CSR, normally associated with affinity maturation and selection, appears to exist in B cells in both lower vertebrates and patients that lack effective GC responses. Taken together, investigation of primitive immunity demonstrates that B cells in lower vertebrates are able to somatically mutate, differentiate into antibody-producing cells and produce heightened protective responses, indicating that a memory-like population exists in these animals.

Are memory B cells an unintended consequence of infection in lower vertebrates? Memory B cells may have been an intermediate state between naive B cells and PCs, i.e. activated B cells that have undergone at least one round of division but do not yet produce antibodies. Instead, they were able to escape death signals and revert back to a ‘naive-like’ state. If so, at some point during evolution, the cells within this population acquired the ability to persist. Evidence for an early induction of a memory state post-activation has been observed in T cells: CD4+ T cells that have been rested for 2 days post-activation were observed to have almost identical transcriptional profiles to those that had been rested for 2 months (49).

Differentiation into PCs induces a large change in transcriptional networks. In contrast, naive and memory B cells have very similar transcriptional profiles. While no ‘master regulator’ of memory B-cell differentiation has been found,
the differences in transcription consist of a number of changes in cell cycle regulators, as well as ligands and receptors that allow interaction with T cells (13, 30, 50, 51). Similarly, upon activation, B cells integrate signals delivered via surface receptors, leading to a genetic re-programming that allows cell cycle and differentiation. Taken together, we hypothesize that an activated B cell has the ability to differentiate into a memory B cell after only a few rounds of division, and without the large changes in transcriptional networks seen in GCs and PCs. GC responses were an addition to the lineage relationship of naive and memory B cells that further honed memory B-cell qualities. Once GCs were part of the microenvironment, these cells were then able to go into a GC ‘state’ and undergo affinity maturation and selection. Memory B-cell evolution can therefore be split into two stages, separated into the ability to produce a cell that can persist to provide protection and the acquisition of qualities that further enhance the efficiency of secondary responses.

Evolution of mechanisms that regulate the humoral response was necessary for stable GC function and have in turn shaped the characteristics of the memory B-cell population. Within the GC, regulatory mechanisms provided by Fas–FasL, PD-1–PD-L and limiting the amount of T-cell help are vital in shaping the quality and quantity of the memory population (22, 52–55). This is evident in mice lacking molecules important for a fully functional GC—rarely does the ability to form a memory cell become completely disrupted; however, the quality and quantity of the memory population are different to that present in a control animal (reviewed in ref. 3). The characteristics attributed to memory that are attained within the GC, such as selection of high-affinity mutants, are layered over other the characteristics required for persistence. Heterogeneity within the immune system may therefore reflect the importance of layering of attributes of the memory population, both during the immune response and over time.

Moving forward

There are of course questions that remain unanswered. Are human IgM memory B cells counterparts of B1 or B2 memory in mice? Why is there a difference in longevity and responses of IgM and IgG memory B cells? Is this difference governed by intrinsic differences, and if so, are they at the genetic or epigenetic level? In particular, research into histone modifications that shutdown cell cycle to allow differentiation, or alternatively to allow quick transcription of genes during a secondary response, may reveal the answers to the questions that still consume the memory field.

Conclusions

Adaptation is required for survival—this is the main tenet of evolution. Thus, it would be advantageous for a naive B cell to be structured in such a way that it is able to differentiate into PCs, GCs or memory B cells, with extrinsic factors and epigenetic modifications influencing the fate of the activated B cell.

When the humoral adaptive system is deconstructed into its components—i.e. the ability to respond to and clear foreign antigen, the ability to produce antibody, the ability for a population of antigen-specific cells to persist and the ability of these cells to respond to and clear the immunizing antigen more rapidly during secondary responses—it becomes clear that during evolution, the qualities and subsets of memory B cells routinely studied in mice and humans have been layered over the more primitive structure that originally existed. Genetic and epigenetic changes that allowed persistence of the memory population, and thus induced protection, were beneficial when selective pressures were applied. Therefore, evolution has resulted in the continual selection of traits that enhance the main function of the immune system—to protect the host.

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