

Multiple players in mouse B cell memory

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Highlights

Multiple memory B-cell subsets are generated by the immune response - IgM⁺ and IgG⁺ memory B cells fulfill different effector functions - Early memory B cells emerge as germinal center-independent subpopulations - Persisting germinal centers modify the outcome of the recall response.

Summary

B cell memory has long been considered the attribute of the sole IgG-positive B cell subset. Since a few years, and due to new B-cell subset identification procedures, increasing heterogeneity has been identified among the memory B cell pool. IgM-positive cells, and germinal center-independent subsets are recent additions to the field. This review describes the diversity of memory B cells, as well as controversial issues on their relative contribution to the recall response. The impact of a protracted germinal center response to the specific mobilization of IgM memory B cells is proposed.

Introduction

Memory within the B-cell lineage is composed of two layers: long lived plasma cells (LLPCs) constantly secreting antibodies (Ab), and memory B cells which, upon re-immunization, generate new memory B cells, short-lived plasma cells and LLPCs. Why have we evolved these two protective barriers? At first, neutralizing Ab secreted by LLPCs, present in serum prior to antigenic re-challenge, appear as the fastest protection against a pathogen, faster than the one provided by innate responses. On the other hand, memory B cells, in case of an insufficiency or absence of neutralizing Ab, will be able to replenish the short lived and long-lived plasma cell pool and therefore take care of the invading pathogen. This step will take more time and will thus only be operative in case of a slow pathogen taking several days to become virulent. Whether re-encounter with a pathogen, either accidentally or through deliberate vaccination, will change the response quantitatively but also qualitatively in terms of affinity or diversity has been in debate in immunology for the last 30 years. Recently this debate has been reanimated by the proposition that, whereas LLPC appear functionally as a rather homogeneous population whose preferential niche is the bone marrow, memory B cells are composed of different sub-populations harboring different effector properties [1]. This new notion of the heterogeneity of memory B cells seems accepted, but the precise phenotypes and functions of each subset according to the different forms of antigens and protocols of immunization used are still a matter of controversy. This review will focus on recent data on memory B cell subset identification in the mouse system. We will also propose that the structure of the antigen, and hence its capacity to generate or not persistent germinal centers, will impact on the differential mobilization of the memory subsets in a recall response.

What role for the structure of the antigen in the initiation of the B cell immune response?

M. Bachmann and his colleagues analyzed how an antigen, either in soluble or particulate form, can trigger a B cell response and documented a key role for natural IgM Ab, i.e IgM pre-existing in the serum prior to any specific immunization [2]. Using their classical bacteriophage Qbeta antigen either as a 28 kDa protein or as a virus-like particle (Qbeta-VLP) of 2.5 MDa, they reported that, when using Qbeta-VLP, immune complexes can be formed by low affinity natural IgM Abs. These immune complexes will be retained by marginal zone B cells and then transported to follicular dendritic cells (FDC) inside B cell follicles. Conversely, the soluble Qbeta antigen required a previous immunization generating specific Qbeta IgM or IgG Ab for the immune complexes to be formed and transported efficiently to FDCs. With both forms of antigen, the binding to marginal zone B cells was non-cognate and

complement dependent. The more potent transport of the particulate form of the antigen led to a stronger germinal center (GC) response.

New players in B-cell memory: IgM memory B cells and persistent germinal centers

Previous work on B-cell memory has largely focused on cells harboring a switched Ig isotype as a surrogate for memory. However, two studies performed in normal, non-Ig transgenic mice, highlighted the importance of the IgM memory subset [3,4].

In one study, memory subsets could be followed up to one year after immunization, through their irreversible labeling at the time of the germinal center reaction. This labeling was achieved by a tamoxifen-inducible Cre under the control of the promoter of activation-induced cytidine deaminase (AID), the key enzyme controlling Ig gene somatic hypermutation (SHM) and isotype switch within GC B cells [3]. Two main observations resulted from this study:

- 1) The presence of a persistent GC reaction up to 8 months after immunization with a particulate antigen (sheep red blood cells, SRBC), easily identified through the intrinsic fluorescent labeling of the responding cells. Such persisting GCs, which had been reported previously on shorter time scales [5], were not observed after immunization with a protein antigen, NP-CGG in alum.
- 2) The different behavior of the IgM and IgG1 subsets upon a boost, performed up to 6 months after the immunization or after transfer of the isolated subsets in pre-immunized hosts. Upon a recall immunization with SRBC, the IgM subset gave rise to a short wave of IgM-secreting anti-SRBC plasma cells, and returned for a large part to GCs in which it underwent more rounds of somatic mutation and switching to IgG1. The IgG1 subset proliferated markedly, and mainly gave rise to anti-SRBC IgG1 plasma cells along with the production of more IgG1 memory B cells. On the contrary, after NP-CGG immunization, boosting at 6 months induced essentially a plasma cell response with no GC reaction. In both cases, the IgM subset harbored a continuum of IgM and IgD surface expression, but distinct functional properties according to the relative expression of the two isotypes were not defined [6].

These results appeared to solve old controversies by establishing that upon a boost at a rather extended time after a vaccination, the host was able to generate antibody-secreting cells but also, depending on the antigen, IgM and IgG centroblasts undergoing new rounds of SHM, the hallmark of an iterative affinity maturation process [7](Fig.1).

The group of M. Jenkins used the protein antigen phycoerythrin (PE) with complete Freund's

adjuvant as an immunizing antigen, allowing the follow-up of responding B cells through a protocol of PE-beads enrichment and enumeration of PE-stained cells [4]. IgM and IgG1 memory B cells were generated after immunization, the former subset dominating the long-term response. Upon a boost with PE in complete adjuvant or after transfer to a host at around one year after the first immunization, the IgG1 subset expanded and gave rise to anti-PE antibody-secreting cells. In contrast, and as opposed to the results mentioned above, the IgM subset only responded and returned to GC when transferred into a naive host, giving rise to IgM and IgG1 GC B cells. The conclusion of the authors was that circulating antibodies against PE prevented the response of the IgM memory B cells, this subset that carried less SHM and harbored less affinity for PE being essentially present in order to respond and generate new variants once circulating anti-PE specific IgG had waned. In a previous study using the same PE immunogen, R. Noelle and colleagues had also reported the generation of plasma cells and the absence of a GC reaction, after an intravenous boost with a low antigen dose [8].

IgM memory B cells had been previously described, in particular in NP-specific transgenic settings [9,10]. In such a model, where transgenic NP-specific cells were challenged after transfer in a naive host, different memory subsets were identified according to the presence of CD73, PD-L2 and CD80 markers: whereas their increased combinatorial expression appeared to parallel the frequency of IgG⁺ B cells, and thus a "hierarchy of maturity", no specific effector function was linked to these different entities [11].

GC-independent memory B cells.

Using the same antigen-based procedure of memory B cell enumeration, Jenkins and colleagues reported that the majority of IgM memory B cells as well as a minor subset of early IgG1 memory B cells were generated early during the response, in a GC-independent mode, and could be distinguished from the GC-dependent IgG1 memory B cells by the absence of the ecto-5'-nucleotidase CD73 surface molecule [12]. In their model, pre-GC B cells, which are already GL7⁺ but also CD38⁺, could either give rise to GC centroblasts (CD38⁻ GL7⁺) or to memory B cells (CD38⁺GL7⁻). In support of their proposition, *Bcl6*^{-/-} mice, which cannot make GCs, still displayed a similar number of CD73⁻ IgM⁺ and IgG1⁺ memory B cells as wild-type animals. In an independent approach, comparing wild type and B-cell restricted *Bcl6*-deficient mice after immunization with NP-CGG, Takemori and colleagues described a similar early IgG1 memory B cell population in both mice, which showed robust secondary response upon adoptive transfer [13] Such a memory subset, with

unmutated Ig genes and low affinity antigen specificity, differs from a classical definition of B-cell memory, as its enhanced responsiveness results from clonal expansion but not from affinity maturation. These two studies also differ as to which extent CD73 may strictly delineate the two GC-dependent and -independent, respectively mutated and unmutated, memory subsets. In the AID-mediated fate labeling experiments previously described, all AID-labeled B cells were CD73⁺, while up to one third of the IgM⁺ memory cells among them carried an unmutated Ig receptor [3].

Localization of memory B cells

T. Kurosaki and his colleagues using adoptive transfer of Ig transgenic B cells harboring a cell cycle marker showed that IgG1⁺ memory B cells were non-dividing and essentially located in clusters near germinal center B cells [14]. These authors also showed that CD4 T cells were present near IgG1⁺ memory B cells, which suggested their possible cognate interaction upon a boost. IgM memory B cells, in contrast, were mainly scattered within follicles. Such challenging observations obviously remain to be conformed in other immunization settings.

Controversial issues on the effector function of IgM memory B cells

Antigenic challenge a few weeks after the initial immunization remobilizes memory B cells for a further round of activation in germinal centers and somatic hypermutation, according to the classical scheme describing affinity maturation as an iterative process upon successive restimulations [7]. As described in the studies cited, this appears to be the hallmark of the IgM memory subset. Nevertheless, IgM memory B cells were shown to respond after a boost in the work of Dogan et al. [3], whereas they did not so in the Jenkins's group report, unless transferred into a naive host [4]. We would like to propose that it is the presence of a pre-existing germinal center reaction that allows such a remobilization to take place. This is the case when the boost is performed early after the first immunization, the germinal center reaction being still ongoing whatever the nature of the immunogen is, a protein or a complex antigen. Later after the initial challenge, the capacity of IgM memory B cells to participate in a recall response would be restricted to immunogens capable of inducing persistent germinal center structures, ready for their rapid recruitment and expansion. Accordingly, there would be competition between the reinitiation of the germinal center reaction and the feedback inhibition mediated by specific IgG generated by rapid plasma cell differentiation (Fig.1). As an alternative explanation, IgM receptors may be unfavorable competitors against circulating

IgGs in the face of protein antigens such as PE, but not in the face of multimeric antigens such as SRBC.

Why would IgM and IgG memory B cells behave differently upon a boost? The difference in the cytoplasmic tail of their Ig receptor that allows the recruitment of specific adaptors (like Grb2) and the triggering of a different signaling cascade could be part of the explanation [15]. A more robust proliferative burst appears also to be specified by the IgG cytoplasmic tail, and organization into membrane microclusters differs between IgM and IgG isotypes [16,17]. Interestingly, the group of T. Kurosaki recently generated a mouse line through nuclear transfer of an IgG1-positive cell, thus allowing the study of IgG1⁺ naive and memory B cells. Unexpectedly, they were able to correlate the heightened differentiation capacity of IgG1 memory B cells into plasma cells not to their signaling capacity, but rather to a specific differentiation program, and more precisely to the expression level of the Bach2 transcription factor [18]. In their study, Bach2 expression appeared high in naive B cells, including the IgG1-expressing ones, whereas it was intermediate in IgM and low in IgG1 memory B cells. Such a low Bach2 expression allows for derepression of Blimp-1 and preferential plasma cell differentiation [19].

The persistence of germinal centers could have multiple contributions to the immune response. First, it could recruit naive B cells that would join an ongoing reaction provided that they encounter appropriate survival and co-stimulatory signals [20]. Second, ongoing diversification might provide the host with Ig variants able to cope with mutants of the original pathogen [21]. In a recent paper M. Diamond and colleagues estimated the protective advantage that could be provided by the diversity generated within the memory B cell pool [21]. After a primary infection of mice with the West Nile virus, antibodies secreted by LLPC could only neutralize the wild-type but not a mutant form of the virus differing by one amino acid in a dominant neutralizing epitope. In contrast, memory B cells generated by this infection differentiated upon challenge into antibody-secreting cells recognizing and neutralizing the wild-type and the mutant virus. It was estimated by the authors that around 10% of the memory B cells produced by infection with the original virus recognized better the mutant form. This report thus supports the notion that LLPC are formed at a rather early stage of the GC reaction while memory B cells may be able to accumulate as long as the GC reaction goes on with specificities somewhat distant from the eliciting antigen.

Lastly, clonal expansions reported in persistent GCs may however represent a risk of neoplastic transformation [3]. Accordingly, recurrent recruitment within germinal centers of

B cells presenting a defective control of apoptosis has been proposed as the mechanism underlying progression towards overt transformation in follicular lymphomas [22](Fig.1).

What role is played by the somatic hypermutation process?

SHM is a major player of the immune response that has been conserved among different species in order to increase the affinity of antibodies raised against invading pathogens. AID is the triggering factor initiating SHM within GCs, and class switch recombination in both GC and extra-follicular sites [23]. The group of T. Honjo, who discovered AID, later showed that AID-deficient mice had hyperplastic GCs in gut-associated lymphoid tissues and in the spleen, along with a large expansion of their anaerobic flora in the small intestine [24]. Since IgA-deficient mice did not show a similar picture, the authors suggested that the absence of SHM could be responsible for this absence of control of the intestinal flora. They have recently described a mutation in the N-terminal portion of AID (G23S) that, upon expression in knock-in mice, reduces drastically SHM while not affecting class switch recombination and V gene repertoire [25]. Class switch recombination was reduced *in vitro* but, due to compensatory amplifications, the serum concentration of all Ig isotypes was normal in these mice. Hyperplastic GCs were observed in spleen and Peyer's patches and more commensal bacteria were found in the gut, although to a lesser degree than in AID-deficient mice. These results, by dissociating SHM from CSR *in vivo*, thus confirmed a specific role for SHM in the control of the gut commensal microbiota.

A specific role of SHM in the control of infections remains to be assessed in this model. In particular, in the control of viral escape variants discussed in the previous chapter [21], SHM may contribute at multiple levels, either in the generation of memory B cells with a more potent repertoire or, during ongoing germinal center reactions, through the provision of altered specificities or newly recruited ones.

Conclusion

Chronic infections such as HIV seem to generate memory B cells bearing antibodies with a large amount of SHM and with sometimes a very distant specificity from the one harbored by their unmutated Ig receptor [26,27]. This suggests that, as long as the response is ongoing in GCs, re-entry of memory and naive B cells is possible as well as the feeding of the memory pool by newly formed GC B cells. It seems too early to draw any conclusion on which vaccination preparation should be used: should one vaccinate with a whole pathogen or with recombinant proteins, such as those expressed at its surface or those, like toxins, secreted in

the outside milieu? The use of a particulate antigen seems to provide several advantages: a more potent triggering of the response and the formation of persistent GCs that may provide additional diversity upon a new challenge and generate more fitted variants. To what extent persistence in germinal centers correlated with prolonged AID expression may, on the other hand, significantly increase oncogenic DNA damages remains obviously to be assessed.

Aknowledgments

INSERM U783 is supported by the Ligue contre le Cancer ("Equipe labellisée"), the Fondation Princesse Grace de Monaco and an ERC Advanced Grant ("Memo-B"). S. L. G. is supported by ERC and Y. H. by the Ligue contre le Cancer.

References and recommended reading

1. Yoshida T, Mei H, Dorner T, Hiepe F, Radbruch A, Fillatreau S, Hoyer BF: **Memory B and memory plasma cells.** *Immunol Rev* 2010, **237**:117-139.
2. Link A, Zabel F, Schnetzler Y, Titz A, Brombacher F, Bachmann MF: **Innate immunity mediates follicular transport of particulate but not soluble protein antigen.** *J Immunol* 2012, **188**:3724-3733.
 - This paper describes the role of non-specific IgM in the enhanced delivery of particulate antigens to the FDC network.
3. Dogan I, Bertocci B, Vilmont V, Delbos F, Megret J, Storck S, Reynaud CA, Weill JC: **Multiple layers of B cell memory with different effector functions.** *Nat Immunol* 2009, **10**:1292-1299.
 - Based on a new mouse reporter line with inducible AID-dependent labeling of memory cells, this paper provides the first description of two IgM and IgG memory layers with distinct effector functions.
4. Pape KA, Taylor JJ, Maul RW, Gearhart PJ, Jenkins MK: **Different B cell populations mediate early and late memory during an endogenous immune response.** *Science* 2011, **331**:1203-1207.
 - Using a new antigen-based enrichment and enumeration procedure, this group describes the different responses of IgM and IgG memory cells, 12-15 months after immunization.
5. Gatto D, Martin SW, Bessa J, Pelliccioli E, Saudan P, Hinton HJ, Bachmann MF: **Regulation of memory antibody levels: the role of persisting antigen versus plasma cell life span.** *J Immunol* 2007, **178**:67-76.
6. Reynaud CA, Descatoire M, Dogan I, Huetz F, Weller S, Weill JC: **IgM memory B cells: a mouse/human paradox.** *Cell Mol Life Sci* 2012, **69**:1625-1634.
7. Berek C, Milstein C: **Mutation drift and repertoire shift in the maturation of the immune response.** *Immunol Rev* 1987, **96**:23-41.
8. Benson MJ, Elgueta R, Schpero W, Molloy M, Zhang W, Usherwood E, Noelle RJ: **Distinction of the memory B cell response to cognate antigen versus bystander inflammatory signals.** *J Exp Med* 2009, **206**:2013-2025.
9. Scholz JL, Crowley JE, Tomayko MM, Steinel N, O'Neill PJ, Quinn WJ, 3rd, Goenka R, Miller JP, Cho YH, Long V, Ward C *et al*: **BLyS inhibition eliminates primary B cells but leaves natural and acquired humoral immunity intact.** *Proc Natl Acad Sci U S A* 2008, **105**:15517-15522.
10. Anderson SM, Tomayko MM, Ahuja A, Haberman AM, Shlomchik MJ: **New markers for murine memory B cells that define mutated and unmutated subsets.** *J Exp Med* 2007, **204**:2103-2114.
11. Tomayko MM, Steinel NC, Anderson SM, Shlomchik MJ: **Cutting edge: Hierarchy of maturity of murine memory B cell subsets.** *J Immunol* 2010, **185**:7146-7150.

12. Taylor JJ, Pape KA, Jenkins MK: **A germinal center-independent pathway generates unswitched memory B cells early in the primary response.** *J Exp Med* 2012, **209**:597-606.
13. Kaji T, Ishige A, Hikida M, Taka J, Hijikata A, Kubo M, Nagashima T, Takahashi Y, Kurosaki T, Okada M, Ohara O *et al*: **Distinct cellular pathways select germline-encoded and somatically mutated antibodies into immunological memory.** *J Exp Med* 2012, **209**:2079-2097.
 - Ref. 12 and 13 describe early, germinal center independent, memory IgM and IgG subsets with unmutated immunoglobulin genes.
14. Aiba Y, Kometani K, Hamadate M, Moriyama S, Sakaue-Sawano A, Tomura M, Luche H, Fehling HJ, Casellas R, Kanagawa O, Miyawaki A *et al*: **Preferential localization of IgG memory B cells adjacent to contracted germinal centers.** *Proc Natl Acad Sci U S A* 2010, **107**:12192-12197.
 - A different localization of IgG and IgM memory B cells is reported in this paper.
15. Engels N, Konig LM, Heemann C, Lutz J, Tsubata T, Griep S, Schrader V, Wienands J: **Recruitment of the cytoplasmic adaptor Grb2 to surface IgG and IgE provides antigen receptor-intrinsic costimulation to class-switched B cells.** *Nat Immunol* 2009, **10**:1018-1025.
16. Martin SW, Goodnow CC: **Burst-enhancing role of the IgG membrane tail as a molecular determinant of memory.** *Nat Immunol* 2002, **3**:182-188.
17. Liu W, Meckel T, Tolar P, Sohn HW, Pierce SK: **Intrinsic properties of immunoglobulin IgG1 isotype-switched B cell receptors promote microclustering and the initiation of signaling.** *Immunity* 2010, **32**:778-789.
18. Kometani K, Nagakagawa, R., Shinnakasu, R., Kaji, T., Rybouchkin, A., Moriyama, S., Furukawa, K., Koseki, H., Takemori, T. and Kurosaki, T.: **Repression of Bach2 contributes to predisposition of IgG1 memory B cells toward plasma cell differentiation.** *Immunity*, *in press*.
 - The comparison of naive and memory IgG1⁺ cells suggest that propensity to plasma cell differentiation is a IgG memory cell-intrinsic program.
19. Muto A, Ochiai K, Kimura Y, Itoh-Nakadai A, Calame KL, Ikebe D, Tashiro S, Igarashi K: **Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch.** *EMBO J* 2010, **29**:4048-4061.
20. Schwickert TA, Alabyev B, Manser T, Nussenzweig MC: **Germinal center reutilization by newly activated B cells.** *J Exp Med* 2009, **206**:2907-2914.
21. Purtha WE, Tedder TF, Johnson S, Bhattacharya D, Diamond MS: **Memory B cells, but not long-lived plasma cells, possess antigen specificities for viral escape mutants.** *J Exp Med* 2011, **208**:2599-2606.
 - This paper describes the generation of memory B cells with specificities for viral variants during a response against yellow fever virus.

22. Roulland S, Faroudi M, Mamessier E, Sungalee S, Salles G, Nadel B: **Early steps of follicular lymphoma pathogenesis.** *Adv Immunol* 2011, **111**:1-46.
23. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T: **Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme.** *Cell* 2000, **102**:553-563.
24. Fagarasan S, Muramatsu M, Suzuki K, Nagaoka H, Hiai H, Honjo T: **Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora.** *Science* 2002, **298**:1424-1427.
25. Wei M, Shinkura R, Doi Y, Maruya M, Fagarasan S, Honjo T: **Mice carrying a knock-in mutation of Aicda resulting in a defect in somatic hypermutation have impaired gut homeostasis and compromised mucosal defense.** *Nat Immunol* 2011, **12**:264-270.
- A knockin mutation of AID that dissociates somatic mutation from isotype switch allows the evaluation of the role of mutated IgAs in the control of the gut flora.
26. Mouquet H, Scheid JF, Zoller MJ, Krogsgaard M, Ott RG, Shukair S, Artyomov MN, Pietzsch J, Connors M, Pereyra F, Walker BD *et al*: **Polyreactivity increases the apparent affinity of anti-HIV antibodies by heterologation.** *Nature* 2010, **467**:591-595.
27. Kwong PD, Mascola JR: **Human antibodies that neutralize HIV-1: identification, structures, and B cell ontogenies.** *Immunity* 2012, **37**:412-425.

Legend to figure**Fig.1. A role for persistent germinal centers in the recall response?**

The scheme represents a proposition for the role of persistent germinal centers in the mobilization of IgM and IgG subsets during a recall response. Persistent germinal centers, elicited by particulate and not by protein antigens, would allow IgM memory B cells to access a pre-existing germinal center reaction, a kinetic process allowing efficient competition against the negative feedback exerted by antigen-specific circulating IgG, whose production massively increases through the preferred plasma cell differentiation of IgG memory B cells. Other possible contributions (ongoing recruitment of naive B cells, ongoing feeding of the memory pool, possible oncogenic risks linked to protracted AID activity) are discussed in the text.

