

Generation of memory B cells inside and outside germinal centers

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Germinal centers (GCs) are generally considered to be the sole site of memory B-cell generation. However, recent studies demonstrate that memory B cells can also develop in response to a T-cell dependent (TD) antigen before the onset, and independently of, the GC reaction. These two classes of memory cells persist equally over long periods of time and attain functional maturation through distinct but related transcriptional programs. Although the development of both memory B-cell types requires classical T-cell help, the generation of GC-dependent memory B cells requires T_{FH}-cell help, while the generation of GC-independent memory cells does not. These findings led to the conclusion that B-cell memory is generated along two fundamentally distinct cellular differentiation pathways. In this review, we focus on the GC-independent pathway of memory B-cell development, and discuss how the unique features of memory B cells are maintained in the GC-independent pathway.

Keywords: B cells · B cell development · Memory cells

Introduction

Immunological memory is a hallmark of the adaptive immune system, and the existence of humoral immunological memory has been known for decades. However, critical aspects of the cellular and molecular components required for the generation of memory B cells remain incompletely defined. The classical dogma holds that both memory and long-lived antibody-secreting plasma cells (PCs) are derived from germinal centers (GCs) [1]. We have recently provided definitive evidence for a T-cell dependent (TD),

but GC-independent pathway of memory B-cell generation [2], as had been predicted or inferred from earlier work [3–9]. Subsequent investigations support a contribution of GC-independent memory B cells to protective immunity against pathogens [10].

In this review, we focus on this new GC-independent pathway of memory B-cell development. We define memory B cells as “antigen experienced” B cells that persist at a steady level for long periods of time after immunization. The unique features of memory B cells — long lifespan, rapid and robust proliferation in response to antigen, high sensitivity to low doses of antigen, and rapid terminal differentiation into PCs that produce high-affinity antibodies during the secondary response — are retained within the GC independent differentiation pathway.

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B-cell response early after immunization

Following the interaction between antigen-specific B cells and T cells at the border of B- and T-cell zones (termed T-cell dependent (TD) B-cell responses) within the lymphatic organs, a subset of the antigen-engaged B cells initiate a primary antibody response by differentiating into antibody-secreting PCs. Other antigen-engaged B cells upregulate the orphan receptor EBV-induced molecule 2 (EBI-2), which drives their migration into the outer B-cell follicle where they proliferate [11]. Within the B-cell follicle, some B cells undergo class switch recombination and subsequent differentiation into PCs, whereas others are destined to enter the GC reaction.

In parallel, a subset of CD4⁺ T cells differentiates into T follicular helper (T_{FH}) cells, a process that depends on the upregulation of *Bcl6* expression [12–14]. GCs are formed in the spleen as early as day 5 after immunization [15], and can be recognized as clusters of cells expressing *Bcl6* and binding high levels of the plant lectin peanut agglutinin (PNA) [5]. CD38 is expressed on follicular B cells in the mouse but is downregulated on germinal center B cells [16]. In the absence of *Bcl6*, GC formation is completely abolished [17, 18].

Within GCs, B cells undergo massive proliferation accompanied by class switch recombination (CSR) and somatic hypermutation (SHM) of their rearranged Ig variable (V) region genes, a process wherein cells that acquire mutations that increase antibody affinity for the immunizing antigen preferentially survive [19]. This selection process critically depends on sequential antigen presentation processes in the GC microenvironment. Thus, follicular dendritic cells (FDCs) first present antigen to B cells, and these B cells then present antigen in the form of antigenic peptides to antigen-specific T_{FH} cells, which, in turn, deliver survival signals to the cognate B cells [20]. The selected, high-affinity GC B cells then differentiate into either memory B cells or long-lived PCs, concurrent with downregulation of *Bcl6* expression [21]. In accordance with this model, memory B cells and PCs expressing somatically mutated Ig V region genes persist for long periods of time after termination of the GC response [19, 22].

Unmutated memory B cells develop during the early immune response prior to GC formation

Memory B cells are long-lived quiescent B cells that exhibit a phenotype distinct from that of other types of B cells, including the ability to elicit a more rapid and robust response upon antigen re-encounter compared to antigen-inexperienced naïve B cells [23]. Whereas naïve B cells express IgM and IgD on the surface, memory B cells have generally undergone CSR and express antibody of other isotypes. Therefore, mouse memory B cells can be isolated as antigen-binding cells expressing class-switched immunoglobulin in combination with high levels of CD38 and low levels of PNA binding surface molecules [24, 25]. Using this

approach, it became clear that not all IgG memory B cells contain somatic mutations in their Ig V regions [6, 25, 26]. In addition, blockade of inducible costimulator (ICOS) early in the immune response caused a significant reduction in the frequency of somatically mutated memory and GC B cells but had no effect on the total number of memory B cells [5]. Additionally, under these conditions, the memory B cells generated were largely devoid of somatic mutations. These findings led us to speculate that these unmutated memory cells emerged early from the GC reaction [27] or, alternatively, developed independently of GCs. This latter hypothesis was supported by evidence that unmutated memory B cells can be generated in irradiated mice reconstituted with *Bcl6*-deficient bone marrow [3]. However, since *Bcl6* germline deletion results in an inflammatory disease due to overexpression of Th2 cytokines [17, 18] that may induce aberrant properties in B cells prior to immunization [28], it remained uncertain whether a GC-independent pathway contributed significantly to memory cell generation under physiological conditions.

Jenkins and colleagues recently reported the generation of antigen-specific B cells with a CD38⁺/GL-7⁻ memory phenotype in a GC-independent manner at an early stage of the immune response to immunization with PE plus CFA (complete Freund's adjuvant) [9, 29]. These presumed GC-independent memory B cells could be distinguished from GC-dependent IgG1 memory B cells by the absence of the CD73 surface molecule, whose expression was enriched in mutated memory B cells [2]. However, the functional properties of these cells have not been studied.

Taking advantage of a novel mouse strain in which *Bcl6* is selectively depleted from B-lineage cells, Kaji et al. recently characterized two distinct types of memory B cells that are generated in response to a TD antigen in a GC-dependent and -independent manner [2]. We found that, whereas ablation of *Bcl6* in B cells essentially precluded the formation of GC B cells, it did not affect IgG1 memory B-cell formation, as determined by the antigen binding activity of these cells and their expression of various surface and genetic markers. Not surprisingly, the *Bcl6*-deficient memory B cells that had formed independently of GCs did not carry somatic mutations and thus did not undergo affinity maturation. However, they were quiescent, long-lived cells, capable of producing greater amounts of antibodies in the recall response compared to naïve B cells. These findings were corroborated in a different model that did not rely on genetic ablation of *Bcl6* [5]. Furthermore, analysis of sequential expression of memory B-cell markers on wild type donor B cells in adoptively transferred recipient mice after antigen stimulation revealed that antigen-activated IgG1⁺ B cells could differentiate toward memory B cells as early as day 3 after immunization through initial proliferative expansion. Together, these results demonstrate that antigen engaged B cells develop into IgG memory cells prior to GC formation.

Several studies identified memory B cells expressing IgM during the TD immune response in normal mice [2, 9, 29, 30]. However, IgM memory B cells do not contribute much to the overall secondary antibody response, at least in the case of soluble protein antigens. Most IgM memory B cells develop in the GC-independent pathway and their recall response shows little

evidence of affinity maturation [10, 29]. Whereas PE-specific IgM⁺ memory cells did not undergo CSR upon antigen rechallenge [29], IgM⁺ memory cells specific for sheep red blood cells underwent CSR in GCs after rechallenge and gave rise to IgG antibody-secreting cells [30]. This discrepancy may reflect the different nature of the antigens used in the two studies.

GC-dependent and -independent memory B-cell development exhibit distinct requirements of T-cell help

During the early immune response, CD4⁺ T cells primed by dendritic cells (DCs) are polarized into either effector T helper (Th) cells, which support and regulate the efficacy of humoral immunity. Effector Th cells consist of several subsets, such as Th1, Th2, Th17, and regulatory T (Treg) cells or T_{FH} cells. T_{FH} cells arise by a distinct developmental pathway from other effector T cells, depending on expression of transcription factor Bcl6 and interaction with antigen-specific B cells [31]. The migration of antigen-activated CD4⁺ T cells to B-cell areas of lymphoid tissues is important for mounting TD antibody responses. ICOS triggered by ICOS ligand (ICOSL)-expressing follicular bystander B cells, but not by DCs, increases the motility of T cells at the T–B border, resulting in an efficient T-cell recruitment from the T–B border into the follicular parenchyma [32]. The T_{FH} cell program is associated with the upregulation of CXCR5 and the inhibitory receptor PD-1, and the downregulation of the C-C chemokine receptor CCR7 [33–37]. Surface CXCR5 expression is not required for the positioning of T cells at the T-B border. However, in combination with CCR7 downregulation, CXCR5 expression enables the T_{FH} cells to migrate into B-cell follicles in a CXCL13-dependent manner. This process assists the antigen-specific B cells to mount a GC response and to promote the selection of B cells expressing high-affinity antibodies in the GC environment [2, 35, 36].

Neither IgG1⁺ memory B cells nor GC B cells are generated in CD40-deficient mice after immunization with a TD antigen, NP-chicken gamma globulin (CGG), or in wild type mice immunized with a T-cell independent (TI) antigen, NP-Ficolin [2]. These results indicate that the development of both GC-dependent and -independent IgG1⁺ memory B cells requires classical T-cell help. B cells also receive innate nonclassical help from natural killer T (NKT) cells [38], although both GC-dependent and -independent memory B cells develop normally in mice lacking NKT cells [2].

However, GC-dependent and -independent memory B cells are distinct with respect to their dependence on T_{FH} help for their generation and maintenance. We showed in a recent study that the loss of T_{FH} cells caused by T-cell specific deletion of *Bcl6* resulted in complete absence of GCs for at least 40 days [2]. However, total numbers of memory B cells were reduced only about twofold in the absence of T_{FH} cells. This reduction resulted predominantly from the loss of mutated high-affinity memory B cells, consistent with the notion that the generation of these cells significantly relies on T_{FH} cells. Significantly, unmutated memory B cells still developed upon conditional deletion of *Bcl6* in both either B and or T cells,

demonstrating the existence of a TD memory B-cell developmental pathway independent of GCs and T_{FH} cells.

Whether naïve B cells are intrinsically programmed for recruitment into either the GC-independent or GC-dependent pathway, or can enter either pathway depending on signals received upon activation, remains to be explored. Clearly, both pathways require TD antigenic stimulation. However, the processes following initial B-cell activation are dynamic and involve sequential cellular interactions of different duration [39], which would provide ample opportunities for activated B cells to branch out into alternate differentiation pathways. As discussed above, the polarization of antigen-specific CD4⁺ T cells into effector Th-cell populations is completed within 3 days during the DC priming period [12]. Based on our study, antigen-binding IgG1⁺ B cells with a memory B-cell gene expression signature appear at around day 3 after immunization [2]. Therefore, it is conceivable that within 3 days after immunization a heterogeneous population of antigen-engaged T cells interacts with antigen-engaged B cells via CD40-CD40L at the border of the T- and B-cell zones, and that different kinds of T-B cell interactions determine the differentiation of B cells toward either (unmutated) memory or GC derived B cells.

Both types of memory B cells consistently upregulate the orphan receptor EBI-2 (T. Kaji and T. Takemori, unpublished), allowing them to migrate into the outer B cell follicle [11]. However, it remains uncertain whether GC-independent memory B cells develop at the border of T- and B-cell zones or in the follicle. Although T-cell CXCR5 is needed for optimal GC responses, CXCR5-deficient T cells are able to access follicles and induce GCs, albeit smaller in size compared with wild-type T cells [36, 40]. Likewise, a small number of GC B cells were generated in the spleen of mice in the absence of T_{FH} cells at day 7 after immunization [2], raising the possibility that non-T_{FH} cells may also access follicles and help B cells to respond at an early stage of the immune response. T_{FH} cells secrete IL-21 [41]. IL-21 signaling profoundly affects GC function by promoting the proliferation of GC B cells and their differentiation into memory B cells. Accordingly, in mice deficient for IL-21, memory B cells exhibit lower levels of somatic mutations in rearranged Ig V region genes compared with memory B cells from wild-type controls [8].

Cell surface markers on GC-independent and -dependent memory B cells

There is no specific cell surface marker known for memory B cells, although PD-L1, PD-L2, CD35, CD80, and ecto-5'-nucleotidase CD73 have been reported to be expressed on memory B cells in the spleen in contrast to naïve B cells [26] or naïve and GC B cells [42]. Along these lines, we have confirmed that the levels of PD-L2 and CD80 expression are significantly increased in both GC-independent and -dependent memory B cells compared with those in naïve and GC B cells [2] (Fig. 1). However, as previously reported [9], CD73 is expressed on GC B cells and a subset of memory B cells in wild type mice as the immune response progresses. On GC-independent memory B cells, CD73 is expressed

| | Naive | IgM memory | GC-independent IgG memory | GC-dependent IgG memory | IgG GC B cells |
|---|----------------------|-----------------------------|--|--------------------------|--------------------------|
| BCR isotype: IgD/IgM or IgG | IgD+ / IgM+ | IgD± / IgM+ | IgD- / IgG+ | IgD- / IgG+ | IgD- / IgG+ |
| Surface Phenotype: CD38 PD-L2 CD80 | + - - | + ? ± | + ++ + | + ++ + | dull + dull |
| Localization | Follicle | Follicle | Follicle | Follicle | Follicle |
| T cell help | | CD4 T | CD4 T (nonT _{FH}) | CD4 T _{FH} | CD4 T _{FH} |
| SHM | - | ± | - | + | + |
| Affinity (1 st) | Low | Low | Low | High | Low-High |
| Survival | Short | Long | Long | Long | Short |
| Molecular requirement: Bcl-6 CD40 ICOS IL-21 | No No No No | No Yes ? ? | No Yes ? ^{a)} No ^{b)} | Yes Yes Yes Yes | Yes Yes Yes Yes |
| 2 nd Response: Proliferation GC response/SHM | No No | ^{c)} + + / ± | + + / + | + + / + | No No |

Figure 1. Comparative properties of murine memory, GC, and naïve B cells. The properties listed are for the comparison between GC-dependent and -independent IgG memory B cells and are not intended to be an exhaustive list. ^{a)}ICOS may be required before day 5 [5]. ^{b)}Based on the observation by Zotos et al. [8]. ^{c)}IgM memory response is inhibited in immune hosts by negative regulatory factors [29].

at a low level. In our study, approximately 80% of CD73⁺ memory B cells in wild-type mice carried somatically mutated Ig V region gene segments [2]. Thus, CD73 expression may preferentially mark somatically mutated memory cells.

Although we observed costimulatory MHC class II, CD40, and CD80 molecules to be almost equally expressed on both day 7 and day 40 GC-independent and -dependent memory B cells, the cell surface expression level of PD-L2 increased from day 7 to day 40 after immunization on both types of cells [2]. Thus, GC-independent and -dependent memory cells express several common surface markers at comparable levels, except for CD73.

GC-independent memory B cells are long-lived, entering the memory compartment before B-cell progeny

The memory B-cell population consists of clones that have proliferated in response to an antigen and then remain in a resting state for a long period of time [23]. Their survival is independent of T-cell help and of continuous contact with cognate antigen [43, 44]. It has been suggested that memory B cells localize in

spleen and other secondary lymphoid organs [26], and also circulate in blood [6]. Similar to GC-dependent memory cells, GC-independent IgG1 memory B cells are detectable in the spleen from early on [2]. They are positive for B-cell markers and CD38, negative for CD138 (a marker for plasma cells; [45]) and exhibit low PNA-binding activity. Histology revealed the average number of IgG1⁺ memory B cells in splenic follicles to be comparable between wild-type and mutant mice with conditional deletion of *Bcl6* in B cells, consistent with the results of the flow cytometric analysis. These results suggest that in the spleen both unmutated and mutated memory B cells mostly localize to B-cell follicles.

In wild-type mice, large numbers of GC B cells accumulate mutations in their Ig V_H genes by day 7 after immunization [2]. In parallel with GC development, studies of the frequency of mutated V_H genes among IgG1 memory B cells showed an increase from ≤5% at day 7 to 50–60% at day 40 after immunization. There was no significant increase in the frequency of mutated cells in the memory B-cell population from day 40 to day 100 after immunization [2]. This observation supports the notion that memory B cells that develop independently of GCs within the first week of the response are maintained for a long period, and then are joined by mutated GC B-cell progeny as the immune response

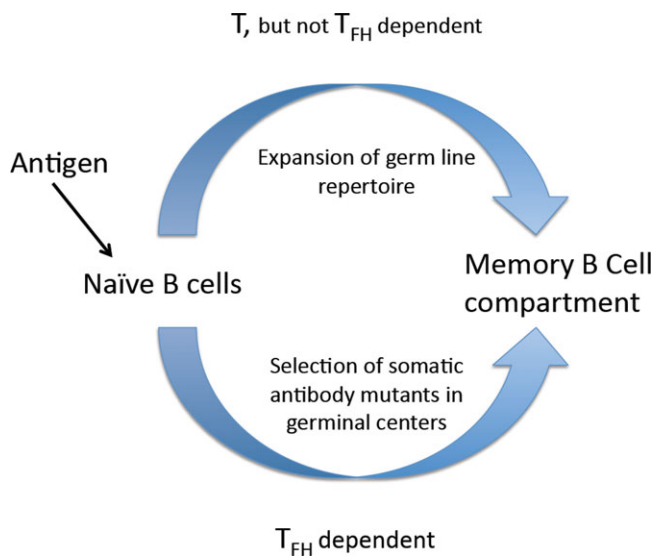


Figure 2. The immune system has evolved two distinct memory B-cell developmental pathways in association with Bcl6 expression in B-cell and T-cell compartment. One of these pathways represents the classical generation of memory B cells through antibody affinity maturation in GC reaction as a consequence of Bcl6 expression in antigen-engaged B cells. This pathway requires the help from CXCR5^{high} Bcl6^{high} T_{FH} cells. Loss of T_{FH} cells by T-cell specific deletion of Bcl6 eradicates this GC-dependent pathway and abolishes the generation of mutated memory B cells during T-cell dependent response. In contrast, antigen-engaged B cells can be driven into the GC-independent memory pathway with the help of T cells other than T_{FH} cells. This pathway does not require Bcl6 expression in B cells and T cells and produces unmutated memory B cells with a germ-line repertoire. Loss of T_{FH} cells by T-cell specific deletion of Bcl6 does not interfere this pathway.

progresses. Despite the recruitment of substantial numbers of GC B-cell progeny into the memory compartment over time, the absolute numbers of memory cells were similar between normal wild type mice and mice in which the GC response was ablated by *Bcl6* deletion. These results indicate that the splenic environment has a limited capacity to sustain memory B cells. We speculate that GC-dependent and -independent memory B cells compete for hypothetical “niches” in the spleen for survival. It seems unlikely that competition for antigen is the determining factor in this process, since memory B cells persist over a long period in the apparent absence of immunizing antigen, under competitive conditions [44]. The postulated niches for memory B cell maintenance in peripheral lymphoid organs may thus serve some trophic function.

Concluding remarks

For over two decades, GCs have been considered to be the sole site for memory B-cell generation. Recent studies challenge this dogma and demonstrate that memory B cells can also develop before the onset and independently of the GC reaction (Fig. 2). How important are such low-affinity memory B cells and their immune response for protective immunity? We have recently shown that IgG1 memory B cells proliferate in response to antigen re-exposure

and accumulate somatic mutations in their rearranged V_H genes [10], regardless of whether they express unmutated or mutated V_H genes. In this process, unmutated memory B cells generate large numbers of progeny expressing somatically mutated antibodies with high affinity for the antigen to which they responded. Extrapolating these findings to immune defense, memory B cells can efficiently adapt their antibody specificity to an invading cross-reacting pathogen or a pathogen variant selected in the course of an infection. In this context the preservation of germ-line encoded antibody specificities in the memory B-cell population provides the system with a unique flexibility that would be lost if only somatic antibody mutants persisted that are selected for high-affinity binding to the original pathogen.

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References

- Zotos, D. and Tarlinton, D. M., Determining germinal centre B cell fate. *Trends Immunol.* 2012. 33: 281–288.
- Kaji, T., Ishige, A., Hikida, M., Taka, J., Hijikata, A., Kubo, M., Nagashima, T. et al., Distinct cellular pathways select germline-encoded and somatically mutated antibodies into immunological memory. *J. Exp. Med.* 2012. 204: 2079–2097.
- Toyama, H., Okada, S., Hatano, M., Takahashi, Y., Takeda, N., Ichii, H., Takemori, T. et al., Memory B cells without somatic hypermutation are generated from Bcl6-deficient B cells. *Immunity* 2002. 17: 329–339.
- Weller, S., Faili, A., Garcia, C., Braun, M. C., Le Deist, F. F., de Saint Basile, G. G., Hermine, O. et al., CD40-CD40L independent Ig gene hypermutation suggests a second B cell diversification pathway in humans. *Proc. Natl. Acad. Sci. U.S.A.* 2001. 98: 1166–1170.
- Inamine, A., Takahashi, Y., Baba, N., Miyake, K., Tokuhisa, T., Takemori, T. and Abe, R., Two waves of memory B-cell generation in the primary immune response. *Int. Immunol.* 2005. 17: 581–589.
- Blink, E. J., Light, A., Kallies, A., Nutt, S. L., Hodgkin, P. D. and Tarlinton, D. M., Early appearance of germinal center-derived memory B cells and plasma cells in blood after primary immunization. *J. Exp. Med.* 2005. 201: 545–554.
- Chan, T. D., Gatto, D., Wood, K., Camidge, T., Basten, A. and Brink, R., Antigen affinity controls rapid T-dependent antibody production by driving the expansion rather than the differentiation or extrafollicular migration of early plasmablasts. *J. Immunol.* 2009. 183: 3139–3149.
- Zotos, D., Coquet, J. M., Zhang, Y., Light, A., D’Costa, K., Kallies, A., Corcoran, L. M. et al., IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. *J. Exp. Med.* 2010. 207: 365–378.

- 9 Taylor, J. J., Pape, K. A. and Jenkins, M. K., A germinal center-independent pathway generates unswitched memory B cells early in the primary response. *J. Exp. Med.* 2012. 209: 597–606.
- 10 Kaji, T., Furukawa, K., Ishige, A., Toyokura, I., Nomura, M., Okada, M., Takahashi, Y. et al., Both mutated and unmutated memory B cells accumulate mutations in the course of the secondary response and develop a new antibody repertoire optimally adapted to the secondary stimulus. *Int. Immunol.* 2013. 12: 683–695.
- 11 Pereira, J. P., Kelly, L. M. and Cyster, J. G., Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses. *Int. Immunol.* 2010. 22: 413–419.
- 12 Choi, Y. S., Kageyama, R., Eto, D., Escobar, T. C., Johnston, R. J., Monticelli, L., Lao, C. et al., ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. *Immunity* 2011. 34: 932–946.
- 13 Kerfoot, S. M., Yaari, G., Patel, J. R., Johnson, K. L., Gonzalez, D. G., Kleinstein, S. H. and Haberman, A. M., Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. *Immunity* 2011. 34: 947–960.
- 14 Kitano, M., Moriyama, S., Ando, Y., Hikida, M., Mori, Y., Kurosaki, T. and Okada, T., Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. *Immunity* 2011. 34: 961–972.
- 15 Camacho, S. A., Kosco-Vilbois, M. H. and Berek, C., The dynamic structure of the germinal center. *Immunol. Today* 1998. 19: 511–514.
- 16 Oliver, A. M., Martin, F. and Kearney, J. F., Mouse CD38 is downregulated on germinal center B cells and mature plasma cells. *J. Immunol.* 1997. 158: 1108–1115.
- 17 Ye, B. H., Cattoretti, G., Shen, Q., Zhang, J., Hawe, N., de Waard, R., Leung, C. et al., The BCL6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. *Nat. Genet.* 1997. 16: 161–170.
- 18 Dent, A. L., Shaffer, A. L., Yu, X., Allman, D. and Staudt, L. M., Control of inflammation, cytokine expression, and germinal center formation by BCL6. *Science* 1997. 276: 589–592.
- 19 Rajewsky, K., Clonal selection and learning in the antibody system. *Nature* 1996. 381: 751–758.
- 20 Victora, G. D., Schwickert, T. A., Fooksman, D. R., Kamphorst, A. O., Meyer-Hermann, M., Dustin, M. L. and Nussenzweig, M. C., Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell* 2010. 143: 592–605.
- 21 Calame, K., Transcription factors that regulate memory in humoral responses. *Immunol. Rev.* 2006. 211: 269–279.
- 22 Tarlinton, D. M., B-cell memory: are subsets necessary? *Nat. Rev. Immunol.* 2006. 6: 785–790.
- 23 Tangye, S. G. and Tarlinton, D. M., Memory B cells: effectors of long-lived immune responses. *Eur. J. Immunol.* 2009. 39: 2065–2075.
- 24 Ridderstad, A. and Tarlinton, D. M., Kinetics of establishing the memory B cell population as revealed by CD38 expression. *J. Immunol.* 1998. 160: 4688–4695.
- 25 Takahashi, Y., Ohta, H. and Takemori, T., Fas is required for clonal selection in germinal centers and the subsequent establishment of the memory B cell repertoire. *Immunity* 2001. 14: 181–192.
- 26 Anderson, S. M., Tomayko, M. M., Ahuja, A., Haberman, A. M. and Shlomchik, M. J., New markers for murine memory B cells that define mutated and unmutated subsets. *J. Exp. Med.* 2007. 204: 2103–2114.
- 27 Good-Jacobson, K. L. and Shlomchik, M. J., Plasticity and heterogeneity in the generation of memory B cells and long-lived plasma cells: the influence of germinal center interactions and dynamics. *J. Immunol.* 2010. 185: 3117–3125.
- 28 Shaffer, A. L., Yu, X., He, Y., Boldrick, J., Chan, E. P. and Staudt, L. M., BCL6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. *Immunity* 2000. 13: 199–212.
- 29 Pape, K. A., Taylor, J. J., Maul, R. W., Gearhart, P. J. and Jenkins, M. K., Different B cell populations mediate early and late memory during an endogenous immune response. *Science* 2011. 331: 1203–1207.
- 30 Dogan, I., Bertocci, B., Vilmont, V., Delbos, F., Mègret, J., Storck, S., Reynaud, C. A. and Weill, J. C., Multiple layers of B cell memory with different effector functions. *Nat. Immunol.* 2009. 10: 1292–1299.
- 31 Johnston, R. J., Poholek, A. C., DiToro, D., Yusuf, I., Eto, D., Barnett, B., Dent, A. L. et al., Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 2009. 325: 1006–1010.
- 32 Xu, H., Li, X., Liu, D., Li, J., Zhang, X., Chen, X., Hou, S. et al., Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility. *Nature* 2013. 496: 523–527.
- 33 Nurieva, R. I., Chung, Y., Hwang, D., Yang, X. O., Kang, H. S., Ma, L., Wang, Y. H. et al., Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. *Immunity* 2008. 29: 138–149.
- 34 Breitfeld, D., Ohl, L., Kremmer, E., Ellwart, J., Sallusto, F., Lipp, M. and Förster, R., Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J. Exp. Med.* 2000. 192: 1545–1552.
- 35 Schaerli, P., Willmann, K., Lang, A. B., Lipp, M., Loetscher, P. and Moser, B., CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J. Exp. Med.* 2000. 192: 1553–1562.
- 36 Haynes, N. M., Allen, C. D., Lesley, R., Ansel, K. M., Killeen, N. and Cyster, J. G., Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1-high germinal center-associated subpopulation. *J. Immunol.* 2007. 179: 5099–5108.
- 37 Yu, D., Rao, S., Tsai, L. M., Lee, S. K., He, Y., Sutcliffe, E. L., Srivastava, M. et al., The transcriptional repressor Bcl6 directs T follicular helper cell lineage commitment. *Immunity* 2009. 31: 457–468.
- 38 Vinuesa, C. G. and Chang, P. P., Innate B cell helpers reveal novel types of antibody responses. *Nat. Immunol.* 2013. 14: 119–126.
- 39 Okada, T., Miller, M. J., Parker, I., Krummel, M. F., Neighbors, M., Hartley, S. B., O'Garra, A. et al., Antigen-engaged B cells undergo chemotaxis toward the T zone and form motile conjugates with helper T cells. *PLoS Biol.* 2005. 3: e150.
- 40 Arnold, C. N., Campbell, D. J., Lipp, M. and Butcher, E. C., The germinal center response is impaired in the absence of T cell-expressed CXCR5. *Eur. J. Immunol.* 2007. 37: 100–109.
- 41 Bryant, V. L., Ma, C. S., Avery, D. T., Li, Y., Good, K. L., Corcoran, L. M., de Waal Malefyt, R. and Tangye, S. G., Cytokine-mediated regulation of human B cell differentiation into Ig-secreting cells: predominant role of IL-21 produced by CXCR5+ T follicular helper cells. *J. Immunol.* 2007. 179: 8180–8190.
- 42 Good-Jacobson, K. L., Szumilas, C. G., Chen, L., Sharpe, A. H., Tomayko, M. M. and Shlomchik, M. J., PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nat. Immunol.* 2010. 11: 535–542.
- 43 Vieira, P. and Rajewsky, K., Persistence of memory B cells in mice deprived of T cell help. *Int. Immunol.* 1990. 2: 487–494.
- 44 Maruyama, M., Lam, K. P. and Rajewsky, K., Memory B-cell persistence is independent of persisting immunizing antigen. *Nature* 2000. 407: 636–642.

45 Angelin-Duclos, C., Cattoretti, G., Lin, K. I. and Calame, K., Commitment of B lymphocytes to a plasma cell fate is associated with Blimp-1 expression in vivo. *J. Immunol.* 2000. **165**: 5462–5471.

Abbreviations: CSR: class switch recombination · PC: plasma cell · PNA: peanut agglutinin · TD: T cell dependent

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