Memory B Cells Are Biased Towards Terminal Differentiation: A Strategy That May Prevent Repertoire Freezing

By Christophe Arpin, Jacques Banchereau, and Yong-Jun Liu

Summary

Isolation of large numbers of surface IgD⁺CD38⁺ naive and surface IgD⁺CD38⁻ memory B cells allowed us to study the intrinsic differences between these two populations. Upon in vitro culture with IL-2 and IL-10, human CD40-activated memory B cells undergo terminal differentiation into plasma cells more readily than do naive B cells, as they give rise to five- to eightfold more plasma cells and three- to fourfold more secreted immunoglobulins. By contrast, native B cells give rise to a larger number of nondifferentiated B blasts. Saturating concentrations of CD40 ligand, which fully inhibit naive B cell differentiation, only partially affect that of memory B cells. The propensity of memory B cells to undergo terminal plasma cell differentiation may explain the extensive extra follicular plasma cell reaction and the limited germinal center reaction observed in vivo after secondary immunizations, which contrast with primary responses in carrier-primed animals. This unique feature of memory B cells may confer two important capacities to the immune system: (a) the rapid generation of a large number of effector cells to efficiently eliminate the pathogens; and (b) the prevention of the overexpansion and chronic accumulation of one particular memory B cell clone that would freeze the available peripheral repertoire.

Materials and Methods

Abbreviations used in this paper: BCR, B cell receptor; CD40L, CD40 ligand; GC, germinal center; PC, plasma cell.
munotech, Marseille, France) and PE-conjugated anti-CD38 (Leu17; Becton Dickinson Monoclonal Center, Mountain View, CA). Antibodies used for cell purification and cell culture were anti-CD4 (QB172, Sigma Chemical Co., St. Louis, MO), anti-CD38 (T16), anti-IgG (6E1), and anti-IgA (C4) purchased from Immunotech, and anti-CD2, -CD3, -CD8 ascites produced in our own laboratory using the OKT hybridomas obtained from American Type Culture Collection (Rockville, MD). Antibodies used for immunoenzymatic stainings are described in the corresponding section. Anti-CD40 ligand (LL48)-blocking mAb and CD40 ligand (CD40L)-transfected murine fibroblasts were produced in our laboratory (31).

Recombinant human IL-2 was purchased from Amgen Biologicals (Thousand Oaks, CA) and recombinant human IL-10 is -transfected murine fibroblasts were produced in our laboratory (31). R recombinant human IL-2 was purchased from Amgen Biologicals (Thousand Oaks, CA) and recombinant human IL-10 is from Schering-Plough Research Institute (Kenilworth, NJ). IL-2 was used at 10 U/ml and IL-10 at 100 ng/ml in cultures. Giemsa-Gurr and Mayer’s hematoxylin staining solutions were purchased from BDH Laboratory Supplies (Poole, England) and Sigma Chemical Co., respectively.

Purification of B Cell Populations. Naive and memory B cells were purified from human tonsils obtained from children undergoing routine tonsillectomy, as previously described (33). In brief, tonsils were finely minced in RPMI 1640 (GIBCO BRL, Paisley, UK). Cell suspension was washed twice and T cells were depleted by sheep RBC rosetting and centrifugation at room temperature on ditrizoate-ficoll (density = 1.077; Eurobio, Les Ulis, France). B cells were then incubated with biotinylated anti-human IgD antibodies. For naive cell purification, two rounds of positive selection were performed with a magnetic activated cell sorter (MACS); M lentiBiotec, Bergisch Gladbach, Germany). For memory cell preparation, two rounds of negative magnetic beads depletion (Streptavadin-coated Dynabeads; Dynal, Oslo, Norway) were performed. Both resulting IgD– and IgD+ populations were further depleted of T cells and CD38+ (i.e., GC) B cells by incubation with anti-CD2, -CD3, -CD4, -CD8, and -CD38 antibodies followed by two rounds of depletion with anti-mouse IgG-coated magnetic beads (Dynal). This procedure lead to 98-99.5% pure naive and 95-99.5% pure memory B cell populations.

Proliferation Assays. For DNA synthesis, 2.5 × 105 B cells were cultured together with 5 × 105. 75 Gy-irradiated, CD40L-transfected fibroblasts in 200 μl I scove medium (GIBCO) complemented with 5% FCS (GIBCO) for 12 d in the presence of IL-2 and IL-10. DNA synthesis was assessed by incubation with 1 μCi of triitated thymidine (A mersham, Les Ulis, France) during the last 8 h of culture. For cellular expansion, 1.5 × 105 B cells were cultured with 5 × 104 CD40L-transfected fibroblasts for 12 d in the presence of IL-2 and IL-10. Cells were harvested and counted in tripia blue (GIBCO) to exclude dead cells.

Two-step Cell Cultures. 1.5-2 × 107 purified naive or memory B cells were cultured for 3 d in 20 ml Iscove medium complemented with 5% FCS in the presence of IL-2, IL-10, and CD40L-transfected fibroblasts (5:1, B cells/fibroblast). Cells were then harvested, washed, and recultured with or without CD40L. In another set of experiments, anti-IgA and IgG antibodies were used to trigger B cell receptor (BCR) at 2 μg/ml final concentration. Secondary cultures consisted of 1.5 × 107 B cells in 1 ml Iscove medium containing IL-2 and IL-10, together with 5 × 104 irradiated murine fibroblasts. Murine fibroblasts were either CD40L-transfected cells or nontransfected cells together with anti-CD40L-blocking antibody at 2 μg/ml to block the signals given by CD40L-transfected cells that could have been harvested from the primary cultures. All secondary cultures were done in triplicate. After 4 d, cultures were harvested, supernatants frozen for antibody titer assays, and cells kept for analysis.

Quantitation of CD40L Molecules on Murine Fibroblasts. The number of CD40L molecules on transfected fibroblasts was estimated using a Qifikit system (Dako, Goldstrup, Denmark) immediately before establishment of cultures. In brief, cells were incubated at saturation with either an anti-CD40L mAb (IgG1 isotype) or a nonrelevant control-matched antibody for 20 min on ice. After two washes, they were incubated with FITC-conjugated sheep anti-mouse immunoglobulins at the same time as different beads suspensions, coated with a known number of mouse Igs. Cells and beads were then analyzed using a FACScan (Becton Dickinson, Sunnyvale, CA). Means of fluorescence intensity were then plotted against the number of mouse Igs on beads and linear regression was calculated (r2 ≥0.998 in all experiments). The number of recognized molecules (CD40L) on stained fibroblasts was calculated using the linear regression and the fluorescence intensity of these cells, after taking account of the fluorescence of the cells stained with the control-matched antibody.

Cell Cultures with Progressive Triggering of CD40. To assess the effect of progressive triggering of CD40 antigen on naive and memory B cells, a second two-step culture was established. Cells were grown in primary cultures as in the previous two-step culture system. After 3 d, cells were recultured under seven different conditions. As the number of CD40L molecules on transfected fibroblasts varies from one experiment to another, a fixed cell ratio, rather than a fixed number of molecules, was chosen to avoid differences in the fibroblast feeder effects. Therefore, 1.5 × 105 B cells were cultured for 4 d in 1 ml Iscove medium containing IL-2 and IL-10, together with 5 × 104 irradiated fibroblasts. One culture condition was established with CD40L-transfected cells whose CD40L molecules number has been determined. These cells are then diluted with nontransfected irradiated fibroblasts for other culture conditions at the ratios of 1/2, 1/4, 1/8, and 1/16. Two other cultures were also set using parental cells, with or without anti-CD40L antibody at 2 μg/ml. All secondary cultures were set in triplicates and designed as the number of CD40L molecules present in the culture per B cell.

Ig Secretion Assays. IgG, IgA, IgM, and IgM concentrations in culture supernatants were measured using ELISA. Total Ig levels are given as the summation of these values.

Cell Sorting. Naive and memory cells were cultured for 3 d in the presence of IL-2, IL-10, and CD40L-transfected fibroblasts. They were then harvested and recultured for an additional 4 d with IL-2, IL-10, and parental fibroblasts. After harvesting, debris and dead cells were depleted from the cultures by centrifugation on ditrizoate-ficoll (Eurobio). Cells were then stained with FITC-conjugated anti-CD20 and PE-conjugated anti-CD38 antibodies. Both CD20-high and CD38-high and CD20-CD38 populations were sorted using a FACS (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Giemsa and Immunoenzymatic Stainings. 7 × 104 sorted cells were cytoco centrifuged on microscope slides. Some slides were stained with Giemsa-Gurr solution, whereas others were kept for immunoenzymatic staining. Human Igs were revealed by anti-human κ and λ light chain antibodies (ABB5 and N10/2 clones, respectively, IgG1 isotypes; Dako), whereas IgM isotype Igs were revealed by anti-human IgM mAb (145-8, IgG1 isotype; Becton Dickinson Monoclonal Center). Enzymatic activity was developed with Fast Red substrate (Dako). All immunoenzymatically colored slides were lightly counterstained with Mayer’s hematoxylin solution.
From naive cells

From memory cells

Figure 1. Naive and memory cells differentiation at the end of secondary cultures, followed by CD20 and CD38 expression. PCs are CD20<sup>-/low</sup>/CD38<sup>high</sup> and non-differentiated B blasts (BLASTS) are CD20<sup>+</sup>/CD38<sup>low</sup> (37). (A) Naive B cells cultured with IL-2 and IL-10 over CD40L-transfected L cells. (B) Naive B cells cultured with IL-2 and IL-10 over parental nontransfected L cells together with an anti-CD40L-blocking antibody at 2 μg/ml. (C) Memory B cells cultured with IL-2 and IL-10 over CD40L-transfected L cells. (D) Memory B cells cultured IL-2 and IL-10 over nontransfected L cells together with an anti-CD40L-blocking antibody at 2 μg/ml.

**Results**

Memory B Cells Undergo Prompt Differentiation into Plasma Cells

Using a two-step culture system, we previously demonstrated that continuous triggering of CD40 antigen on GC cells inhibits their terminal differentiation into plasma cells (PC; 37). To determine the influence of CD40L on the capacity of memory and naïve B cells to generate PCs, similar culture conditions were used. Both populations were cultured for 3 d over CD40L-transfected fibroblasts in the presence of IL-2 and IL-10. Activated B cell blasts were then recultured for 4 d with non-transfected fibroblasts, IL-2, IL-10, and an anti-CD40L-blocking antibody to block the CD40L-transfected fibroblasts carried over from the primary culture. Although naïve B cells yielded 16.4 ± 6.6% CD20<sup>-/low</sup>/CD38<sup>high</sup> plasma cells (mean ± SD, n = 7; Fig. 1 B; Table 1), memory B cells yielded 62.4 ± 11.9% plasma cells (mean ± SD, n = 4; Fig. 1 D; Table 1). Accordingly, naïve B cells yielded three times more non-differentiated CD20<sup>+</sup>/CD38<sup>low</sup> B blasts than did memory cells. Addition of CD40L during the secondary culture (Fig. 1, A and C) considerably inhibited the plasma cell differentiation of B cell blasts, generated from both naïve and memory cells (Table 1).

FACS<sup>®</sup>-sorted CD20<sup>-/low</sup>/CD38<sup>high</sup> cells generated from both naïve and memory B blasts display the morphology of terminally differentiated PCs (Fig. 2 A), as well as an intense Igκ and Igλ light chain staining (Fig. 2 C). In contrast, CD20<sup>+</sup>/CD38<sup>low</sup> populations display the morphology of blasts with a weak surface Ig expression (Fig. 2, B and D). Although 50% of plasma cells generated from naïve B cells contain intracytoplasmic IgM (Fig. 2 E), only 20% of plasma cells generated from memory B cells expressed IgM (Fig. 2 F).

High concentrations of CD40L do not completely block the terminal differentiation of memory B cells. To further understand the propensity of memory B cells to undergo plasma cell differentiation, secondary cultures of naïve and memory blasts were set up in the presence of increasing density of CD40L. For that purpose, absolute numbers of CD40L molecules per fibroblast were estimated using quantitative flow cytometry and CD40L-transfected fibroblasts were gradually diluted with their parental cells (see Material and Methods). As shown in Fig. 3 A, increased CD40 ligation of memory cells results in a decreased production of plasma cells and a concomitant increase of B blasts (Fig. 3 B). In fact, there is a linear correlation between the log (1/CD40L available per memory blast) and the percentage of generated plasma cells ($r^2 = 0.945, 0.966$, and 0.983 from three experiments). Note that CD40L-transfected fibroblasts were indeed carried over from the primary cultures, as the addition of anti-CD40L antibody to the cultures with non-transfected fibroblasts further enhanced the plasma cell generation. As shown in Fig. 3 C, in the absence of CD40L in the secondary culture, memory cells can generate up to eight times more PCs than do naïve cells. Note that very high amounts of CD40L molecules in the secondary cultures do not completely inhibit the generation of PCs from memory cells, since up to $2 \times 10^9$ CD40L molecules/blast led to the generation of $3.6 \times 10^5$ PCs from an initial input of $1.5 \times 10^5$ blasts (Fig. 3 C).

| Table 1. Memory B Cells Promptly Differentiate into PC |
|------------------------------------------------------|
| Secondary cultures | Naive cells | Memory cells |
| CD40L-transfected fibroblasts | 3.7 ± 1.8 [1.1–6.7] | 23.8 ± 7.8 [15–31] |
| Parental fibroblasts + anti-CD40L mAb | 16.4 ± 6.6 [5.4–25] | 62.4 ± 11.9 [50.8–79] |

Mean, standard deviation, and range (brackets) of percentages of CD20<sup>-/low</sup>/CD38<sup>high</sup> cells generated from naïve and memory B cells from seven and four experiments, respectively. Cells were cultured for 3 d in the presence of IL-2, IL-10, and CD40L-transfected fibroblasts before being seeded for 4 d in the secondary cultures with IL-2, IL-10, and fibroblasts. The fibroblasts used in secondary cultures are listed in the table.
Increasing the number of CD40L molecules available in the cultures not only inhibited the plasma cell generation, but also the secretion of Igs (Fig. 4A). Furthermore, in all culture conditions, memory B cells produced more total Igs than naive B cells (Fig. 4A). With regard to secreted isotype, although naive and memory B cells produced comparable levels of IgM (Fig. 4D), memory cells, as expected, produced considerably more IgG and IgA (Fig. 4B and C).

We then questioned whether the poor differentiation capacity of naive B cells, as compared to that of memory B cells, may indeed reflect a reduced activation and proliferation capacity. Thus, purified naive and memory B cells were cultured over CD40L-transfected fibroblasts with IL-2 and IL-10, and proliferation was assessed by measuring thymidine incorporation, as well as viable cell numbers. As shown in Fig. 5, naive B cells proliferate at least as much as memory B cells do.
Memory, but not naive, B cells undergo rapid PC differentiation in cultures even after anti-BCR triggering. Anti-Igs were shown to prevent B cell differentiation (38). Since naive B cells, but not memory B cells, were isolated by positive selection using anti-IgD, we questioned whether the difference in the differentiation capacity between naive and memory B cells could be due to the BCR triggering. Accordingly, in the first 3 d of primary cultures, 2 μg/ml of
anti-Igκ and 2 μg/ml of anti-Igλ antibodies were added into the cultures in the presence of CD40L, IL-2, and IL-10. At the end of the culture, cells were washed and seeded in a 4 d secondary culture with IL-2, IL-10, and different concentrations of CD40L. Fig. 6 shows that in the presence of three different CD40L concentrations (9.6 × 10⁴/cell, 4.8 × 10⁴/cell, no CD40L), 3, 6, and 13% of CD38⁺CD20⁻ plasma cells were generated from the naive B cells. In the same culture conditions, 21, 29, and 43% of CD38⁺CD20⁻ plasma cells were generated from the memory B cells. This experiment indicates that memory B cells, but not naive B cells, preferentially undergo plasma cell differentiation even after BCR triggering.

**Discussion**

This paper describes the striking differentiation ability of memory versus that of naive B cells. This correlates with previous histophysiological observations in vivo showing that secondary antigenic challenge in carrier-primed rats leads to a massive extrafollicular PC reaction and a poor follicular GC reaction in the spleen. In contrast, only small extrafollicular PC reactions, but large GC reactions develop upon primary immunization (Fig. 7; 39, 40). Likewise, in mice infected with reoviruses, adoptively transferred memory B cells give rise to a large extrafollicular PC reaction, but a small GC reaction; in contrast, transferred naive cells generate a large GC reaction (41). Thus, the differences in the capacity of memory versus naive B cells to differentiate is an intrinsic property of the B cells, rather than of the microenvironments. The propensity of memory B cells to undergo rapid differentiation into effector cells may confer two important properties to the immune system. First, it allows the rapid generation of large numbers of effector cells, whose products (antibodies) efficiently eliminate pathogens. This novel feature of memory B cells, together with their low threshold for activation, and their ability to home to the antigen draining sites and to directly present antigen to T cells, may all contribute to the velocity of secondary antibody responses. Second, it prevents the overexpansion and accumulation of a particular memory B cell clone that would otherwise overload the immune system and freeze the available Ig repertoire (26). Since PCs have a relatively short lifespan and do not proliferate in response to further stimulations (42–44), the majority of memory B cells will undergo clonal exhaustion by differentiating into effector cells during secondary immune responses. Interestingly, T memory cells show a similar tendency not to expand and overload the whole immune system, as towards the end of a primary immune response specific T blasts are rapidly eliminated (45–48).

The finding that CD40L inhibits the differentiation of both activated memory and naive B cells, complements the previous observations made with GC B cells (37) or total B cells isolated from blood and tonsils (49, 50). Thus, CD40L represents a differentiation suppressor during not only the
Memory B cells are biased towards terminal plasma cell differentiation in vivo and in vitro. Naive B cells predominantly give rise to germinal center reaction within a rat spleen after primary immunization with DNP-KLH in KLH-primed animals (A, original magnification: 40; B, original magnification: 200). The rats were given BrdU in their drinking water for 48 h before they were killed. Red stains BrdU, blue stains DNP-binding cells, brown stains total B cells. MZ, marginal zone; PALS, periarteriolar lymphoid sheath. Consistent with this in vivo finding, human naive B cells predominantly give rise to proliferating B blasts upon activation in vitro (C). Memory B cells predominantly give rise to plasma cell reaction along the outer edges of the periarteriolar lymphoid sheath and within the red pulp of a rat spleen after 2 d of secondary immunization with DNP-KLH (D and E). The rats have received BrdU in their drinking water for 48 h before killing. DNP-specific plasma blasts are cells with strong blue cytoplasmic staining. Consistent with this in vivo finding, human memory B cells predominantly give rise to plasma cells upon activation in vitro (F). The figures on immunohistology are derived from Y.-J. Liu and I.C.M. MacLennan (40).

Figure 7.
GC, but also the extrafollicular reactions (51). Indeed, CD40L-expressing T cells have been reported by immunohistology, both within the GCs and the extrafollicular T zones (52–54).

Although CD40L inhibits the PC differentiation of naive, GC, and memory B cells, a fraction of the memory cell subset seems to be resistant to this effect. Differential effects of CD40L on mature B cell subsets have already been noticed. For instance, CD40 triggering is an important survival but a minor proliferative signal for GC cells (55–57), whereas it provides a strong and long-term proliferative signal to resting naive and memory B cells (58–61). The molecular mechanisms underlying the propensity of memory B cells to undergo terminal differentiation are still unknown. CD40 triggering on human GCs and resting mature B cells results in the activation of different protein kinases (62, 63). Further comparative studies of CD40 signaling pathways in naive, GC, and memory B cells should now be carried on to explain how mature B cells change their responses to CD40 triggering at different stages of their immunopoiesis.

The authors wish to thank S. Bonnet-Arnaud and M. Vatan for superb editorial assistance; I. Durand for flow cytometry; Dr. J. Chiller for supporting this work; and Drs. F. Brière, I. Fugier-Vivier, and C. Müller for critical reading of the manuscript.

C. Arpin is the recipient of a grant from Fondation Mérieux.

Address correspondence to Dr. Yong-Jun Liu, Schering-Plough, 27 chemin des Peupliers, BP 11, 69571 Dardilly Cedex, France.

Phone: 33-4-72-17-27-00; FAX: 33-4-78-35-47-50; E-mail: lir@schering-plough.fr

R received for publication 30 December 1996 and in revised form 3 July 1997.

References

1. Kroese, F.G.M., W. Timens, and P. Nieuwenhuis. 1990. Germinal center reaction and B lymphocytes morphology and function. In Current Topics in Pathology. Reaction Pattern of the Lymph Node. Springer Verlag, Berlin. 103–148.
2. Liu, Y.J., G.D. Johnson, J. Gordon, and I.C.M. MacLennan. 1993. Tracing B cell development in human germinal centres. Annu. Rev. Immunol. 11:49–77.
3. MacLennan, I.C.M. 1994. Germinal centers. Annu. Rev. Immunol. 12:117–139.
4. Weissman, I.L. 1994. Developmental switches in the immune system. Cell. 76:207–218.
5. Kelsoe, G.J. 1995. In situ studies of the germinal center reaction. Adv. Immunol. 60:267–288.
6. Thorbecke, G.J., A.R. Amin, and V.K. Tsiagbe. 1994. Biology of germinal centers in lymphoid tissue. FASEB J. 8:832–840.
7. Manger, T., L.J. Wysocki, T. Gridley, R.I. Nearn, and M.L. Gefter. 1985. The molecular evolution of the immune response. Immunol. Today. 6:94–101.
8. Berek, C., A. Berger, and M. Apel. 1991. Maturation of the immune response in germinal centers. Cell. 67:1121–1129.
9. Jacob, J., G. Kelsoe, K. Rajewsky, and U. Weis. 1991. Intraclonal generation of antibody mutants in germinal centres. Nat. Rev. 354:389–392.
10. Küssers, R., M. Zhao, M.-L. Hansmann, and K. Rajewsky. 1993. Tracking B cell development in human germinal centres by molecular analysis of single cells picked from histological sections. EMBO (Eur. Mol. Biol. Organ.) J. 12:4955–4967.
11. M. Hezy-Williams, M.G., M.J. McLean, P.A. Lator, and G.J.V. Nossal. 1993. Antigen-driven B cell differentiation in vitro. J. Exp. Med. 178:295–307.
12. Klein, U., R. Küppers, and K. Rajewsky. 1994. Variable region gene analysis of B cell subsets derived from a 4-year-old child: somatically mutated memory B cells accumulate in the peripheral blood already at young age. J. Exp. Med. 180:1383–1393.
13. Pascual, V., Y.J. Liu, A. Magalés, O. de Bouteiller, J. Banchereau, and J.D. Capra. 1994. Analysis of somatic mutations in five B cell subsets of human tonsil. J. Exp. Med. 180:329–339.
14. Rajewsky, K. 1996. Clonal selection and learning in the antibody system. Nature (Lond.). 381:751–758.
15. Toellner, K.M., A. Gulbranson-Judge, D.R. Taylor, D. Man-Yuen, and I.C.M. MacLennan. 1996. Immunoglobulin switch transcript production in vivo related to the site and time of antigen-specific B cell activation. J. Exp. Med. 183:2303–2312.
16. Liu, Y.J., S. Oldfield, and I.C.M. MacLennan. 1988. Memory B cells: antigen specificity and charges in heavy chain class expression. Nature (Lond.). 298:377–379.
17. Liu, Y.J., F. Malisan, O. de Bouteiller, C. Guret, S. Lebecque, J. Banchereau, F.C. Mills, E.E. Max, and H. Martinez-Valdez. 1996. Within germinal centers isotype switching of immunoglobulin genes occurs after onset of somatic mutation. Immunity. 4:241–250.
18. Toellner, K.M., A. Gulbranson-Judge, D.R. Taylor, D. Man-Yuen, and I.C.M. MacLennan. 1996. Immunoglobulin switch transcript production in vivo related to the site and time of antigen-specific B cell activation. J. Exp. Med. 183:2303–2312.
19. Liu, Y.J., S. Oldfield, and I.C.M. MacLennan. 1988. Memory B cells in T cell–dependent antibody responses colonize the splenic marginal zones. Eur. J. Immunol. 18:355–362.
20. MacLennan, I.C.M., and D. Gray. 1986. Antigen-driven selection of virgin and memory B cells. Immunol. Rev. 91:61–85.
21. Rajewsky, K. 1989. Evolutionary and somatic immunological memory. In Progress in Immunology. VII. F. M. richard, editor. Springer Verlag, Berlin. 397–403.
22. Gray, D., and J. Sprent. 1990. Immunological memory. In Current Topics in Pathology. Reaction Patterns of the Lymph Node. Springer Verlag, Berlin. 103–148.
24. Vitetta, E.S., M.T. Berton, C. Burger, M. Kepron, W.T. Lee, and X.M. Yin. 1991. Memory B and T cells. Annu. Rev. Immunol. 9:193–217.
25. Mackay, C.R. 1993. Immunological memory. Adv. Immunol. 53:217–265.
26. Sprent, J. 1994. T and B memory cells. Cél. 76:315–322.
27. Ahmed, R., and D. Gray. 1996. Immunological memory and functional heterogeneity of the IgD+ B cell compartment: identification of two major tonsillar B cell subsets. Int. Immunol. 8:737–744.
28. Lagresle, C., C. Bella, and T. Defrance. 1993. Phenotypic and functional heterogeneity of the IgM– IgD+ B cell compartment: identification of two major tonsillar B cell subsets. Int. Immunol. 5:1259–1268.
29. Liu, Y.J., C. Barthélémy, O. de Bouteiller, C. Arpin, I. Durand, and J. Banchereau. 1996. Fas ligation induces apoptosis of CD40-activated human B lymphocytes. J. Exp. Med. 182:1265–1273.
30. Klein, U., R. Küppers, and K. R. ajewsky. 1997. Evidence for a large compartment of IgM-expressing memory B cells in humans. Blood. 89:1288–1298.
31. Arpin, C., J. Déchanet, C. van Kooten, and Y.J. Liu. 1996. Are Peyer’s patch germinal centre T cell–independent antigens. Nature (Lond.). 379:438–441.
32. Lawton, A.R., R. Asofsky, M.B. Hylton, and D.M. Cooper. 1995. Switch recombination in normal and neoplastic human B lymphocytes. Int. Immunol. 7:1809–1815.
33. van den Ewkeghy, A.M., R. I. Nolle, M. Roy, D.M. Shephard, A. Aruffo, J.A. Ledbetter, W.J.A. Boersma, and E. Claassen. 1993. In vivo CD40–gp39 interactions are essential for thymus-dependent humoral immunity. I. In vivo expression of CD40 ligand; cytokines, and antibody production delineates sites of cognate T–B cell interactions. J. Exp. Med. 178:1555–1565.
34. Cebra, J.J., C.E. Schrader, K.E. Shroff, and P.D. Weinstein. 1991. Are Peyer’s patch germinal centre reactions different from those occurring in other lymphoid tissues? Res. Immunol. 142:222–226.
35. Benner, R., W. Hjmtans, and J.J. Haaijman. 1981. The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. Clin. Exp. Immunol. 46:1–8.
36. Bo, F., J.E. Lortan, I.C.M. M. Alenann, and M. Khan. 1986. Distinct short-lived and long-lived antibody-producing cell populations. Eur. J. Immunol. 16:1297–1301.
37. Ahmed, R., and D. Gray. 1996. Immunological memory and functional heterogeneity of the IgD+ B cell compartment: identification of two major tonsillar B cell subsets. Int. Immunol. 8:737–744.
38. Lawton, A.R., R. Asofsky, M.B. Hylton, and D.M. Cooper. 1995. Switch recombination in normal and neoplastic human B lymphocytes. Int. Immunol. 7:1809–1815.
39. Cebra, J.J., C.E. Schrader, K.E. Shroff, and P.D. Weinstein. 1991. Are Peyer’s patch germinal centre reactions different from those occurring in other lymphoid tissues? Res. Immunol. 142:222–226.
40. Benner, R., W. Hjmtans, and J.J. Haaijman. 1981. The bone marrow: the major source of serum immunoglobulins, but still a neglected site of antibody formation. Clin. Exp. Immunol. 46:1–8.
41. Ho, F., J.E. Lortan, I.C.M. M. Alenann, and M. Khan. 1986. Distinct short-lived and long-lived antibody-producing cell populations. Eur. J. Immunol. 16:1297–1301.
42. Ahmed, R., and D. Gray. 1996. Immunological memory and functional heterogeneity of the IgD+ B cell compartment: identification of two major tonsillar B cell subsets. Int. Immunol. 8:737–744.
43. Lawton, A.R., R. Asofsky, M.B. Hylton, and D.M. Cooper. 1995. Switch recombination in normal and neoplastic human B lymphocytes. Int. Immunol. 7:1809–1815.
Gray, D., K. Siepmann, D. van Essen, J. Poudrier, M. Wykes, S. Jainandunsing, S. Bergthorsdottir, and P. Dullforce. 1996. B–T lymphocyte interactions in the generation and survival of memory cells. Immunol. Rev. 150:45–61.

Clark, E.A., and J.A. Ledbetter. 1986. Activation of human B cells mediated through two distinct cell surface differentiation antigens. Bp35 and Bp50. Proc. Natl. Acad. Sci. USA. 83: 4494–4498.

Gordon, J., M.J. Mills, G.R. Guy, and J.A. Ledbetter. 1987. Synergistic interaction between interleukin 4 and anti-Bp50 (CDw40) revealed in a novel B cell restimulation assay. Eur. J. Immunol. 17:1535–1538.

Spriggs, M.K., R.J. Armitage, L. Strockbine, K.N. Clifford, B.M. Macduff, T.A. Sato, C.R. Maliszewski, and W.C. Fanslow. 1992. Recombinant human CD40 ligand stimulates B cell proliferation and immunoglobulin E secretion. J. Exp. Med. 176:1543–1550.

Banchereau, J., F. Bazan, D. Blanchard, F. Brière, J.P. Galizzi, C. van Kooten, Y.J. Liu, F. Rousset, and S. Saeland. 1994. The CD40 antigen and its Ligand. Annu. Rev. Immunol. 12:881–922.

Uckun, F.M., G.L. Schieven, I. Dibirdik, M. Chandan-Langlie, L. Tuel-Ahlgren, and J.A. Ledbetter. 1991. Stimulation of protein tyrosine phosphorylation, phosphoinositide turnover, and multiple previously unidentified serine/threonine-specific protein kinases by the pan-B-cell receptor CD40/Bp50 at discrete developmental stages of human B-cell ontogeny. J. Biol. Chem. 266:17478–17485.

Knox, K.A., and J. Gordon. 1993. Protein tyrosine phosphorylation is mandatory for CD40-mediated rescue of germinal center B cells from apoptosis. Eur. J. Immunol. 23:2578–2584.