

Immunological Memory: Contribution of Memory B Cells Expressing Costimulatory Molecules in the Resting State¹

Amit Bar-Or,^{2*}† Enedina M. L. Oliveira,* David E. Anderson,* Jeff I. Krieger,* Martin Duddy,† Kevin C. O'Connor,* and David A. Hafler*

Traditionally, emphasis has been placed on the roles of Th cells in generating and amplifying both cellular and humoral memory responses. Little is known about the potential contributions of B cell subsets to immunological memory. Resting memory B cells have generally been regarded as poor APC, attributed in part to the relative paucity of costimulatory molecules identified on their surface. We describe a novel subpopulation of human memory B cells that express CD80 in their resting state, are poised to secrete particularly large amounts of class switched Igs, and can efficiently present Ag to and activate T cells. This functionally distinct B cell subset may represent an important mechanism by which quiescent human B cells can initiate and propagate rapid and vigorous immune memory responses. Finally, these studies extend recent observations in the murine system and highlight the phenotypic and functional diversity that exists within the human B cell memory compartment. *The Journal of Immunology*, 2001, 167: 5669–5677.

Immunological memory is a defining feature of adaptive immunity and confers the ability to mount more rapid and more robust responses to subsequent antigenic encounters (1). Although central to our understanding of such diverse processes as normal protective immunity, vaccination immunity, transplantation, and autoimmune disease, the mechanisms underlying the development of both humoral and cellular immune memory responses remain only partially elucidated (2). Early studies demonstrating lower thresholds of activation of memory T cells (3) underscored the role of T cells in the enhanced efficiency of memory responses, and traditional views of the generation of humoral memory to soluble Ags have emphasized the requirement for T cell help. In this context, CD4⁺ memory T cells have been shown to effectively stimulate resting B cells that only subsequently are able to present Ag to the same T cells (4).

A renewed interest in the immunoregulatory roles of B cells has been sparked in recent studies of tolerance and autoimmunity (5, 6) and in studies suggesting that B cells can act as antigenic reservoirs, thereby providing an important potential source for amplifying immune responses in both health and disease (7, 8). Activated B cells as well as autoreactive B cells in models of autoimmunity have been shown to function as effective APCs to naive T cells (9, 10). In contrast, resting B cells are viewed as poor

APCs (11, 12), attributed at least in part to their low levels of expression of costimulatory molecules. The capacity of memory B cells to act as APCs may be particularly important, since, unlike dendritic cells or monocytes, B cells are able to interact with T cells in an Ag-specific manner (13, 14). Nonetheless, very little is known about human B cell subsets and their contributions to immunological memory.

The most definitive marker of memory B cells identified to date is the presence of somatically mutated, high affinity Ag receptors (15). While individual surface markers rarely distinguish perfectly between functionally distinct cell subsets, accumulating evidence has identified surface CD27 as a useful marker of human memory B cells (15–19). In particular, recent single-cell studies of circulating B cells in humans directly confirmed that essentially all circulating CD27⁺ B cells displayed variable Ig gene region somatic mutations, while no mutations were identified in the CD27-B cells (15, 19, 20). We studied the costimulatory profile of circulating CD27⁺ memory B cells in humans, with particular attention to the B7 pathway, which is known to modulate the threshold of activation of both naive and memory T cells (21, 22). It is well established that altering the interactions between the B7.1 (CD80) and B7.2 (CD86) molecules and their T cell counter-receptors, CD28 and CTLA-4 (CD152), can have profound effects on immune responses. Inhibition of B7/CD28 engagement results in enhanced allograft survival, reduced autoantibody production, and amelioration of autoimmunity in both animal models and human disease (23, 24). Unlike professional APCs such as dendritic cells and monocytes that express high constitutive levels of CD86, resting B cells have previously been shown to express low levels of CD86 and no CD80. It has generally been accepted that B cell activation is required to up-regulate both CD80 and CD86 expression, at which time CD86 levels typically rise more rapidly and to a higher extent than CD80 (25–28).

Given the prevailing dogma, we were surprised to identify in normal adult human blood a high frequency of quiescent memory B cells expressing significant levels of CD80, yet negligible levels of CD86. We demonstrate that this novel cell population represents a phenotypically and functionally distinct human memory B cell subset. Although in a resting state, these CD80⁺ memory B cells

*Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; and †Neuroimmunology Unit, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada

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² Address correspondence and reprint requests to Dr. Amit Bar-Or, Neuroimmunology Unit, Montreal Neurological Institute, McGill University, 3801 University Street, Montreal, Quebec, Canada H3A 2B4. E-mail address: abar-or@mni.mcgill.ca

have a lower threshold of activation and can be stimulated to secrete very large amounts of class-switched Igs. Moreover, they are able to efficiently present Ag to and activate T cells. The expression of costimulatory molecules in the resting state and the propensity to mediate vigorous humoral and cellular responses, provide a mechanism by which this novel CD27⁺CD80⁺ memory B cell subset could contribute to the rapid and robust immune responses that constitute the defining features of immunological memory.

Materials and Methods

Phenotyping of whole blood and cell populations

To phenotype cells as closely as possible to the *in vivo* circulating state and to avoid changes in levels of activation markers that may be incurred through *in vitro* processing, we studied whole blood samples directly *in vivo*. Pediatric blood samples (from noninflammatory or infectious cases) as well as postpartum cord blood and adult samples were obtained in accordance with departmental protocols from the Boston Children's Hospital and the Brigham and Women's Hospital (Boston, MA), respectively. Triple-color immunofluorescent staining of fresh samples was performed within 20 min of phlebotomy. Whole blood samples were incubated with predetermined optimal concentrations of the appropriate mAbs or isotype controls (see below) for 30 min at 4°C, followed by lysis of RBC (FACS lysing solution 349202, Becton Dickinson, San Jose, CA). Samples were washed twice in staining buffer (2% FCS in PBS), immediately acquired by flow cytometry using FACSort (Becton Dickinson), and subsequently analyzed by CellQuest FACStation software. For staining of PBMCs or purified T and B cells, an identical approach was used, except for the lysing step. DNA-based cell cycle analysis was performed using the Vybrant kit (Molecular Probes, Eugene, OR) (29). Purified B cell subsets stained for CD27 and B7 were costained with propidium iodide (to exclude dead cells) and Hoechst 33342 (to measure cellular DNA content with UV source). To identify the cell cycle kinetic status of the B cell subsets, we used Ki-67 analysis, which distinguishes quiescent DNA 2n Ki-67-negative (G₀) cells from DNA 2n Ki-67-positive (G₁) cells (30). Purified B cells were first surface stained for expression of CD27 and CD80, then fixed in 4% paraformaldehyde and permeabilized with 0.1% saponin. Samples were incubated with anti-Ki-67 Ab or the appropriate isotype control (see below), then washed twice in permeabilization buffer and once in staining buffer before acquisition. For positive controls in staining experiments, B cells were freshly purified (see below) and incubated with or without activating CD40 ligand (CD40L)-transfected L cells (below) for 40 h before staining and FACS analysis.

The following fluorochrome-labeled mAbs were used for staining CD80 and CD86: PE-L307.4 (mIgG1 anti-CD80/B7.1, Becton Dickinson) and FITC- or PE-2331/FUN1 (mIgG1 anti-CD86/B7.2). Both L307.4 (anti-CD80) and FUN1 (anti-CD86) were of the same isotype (mIgG1), excluding the possibility that nonspecific binding was responsible for the differences in staining patterns. Staining for the B7 molecules on B cells and monocytes in the same whole blood samples provided an additional measure of comparison. The pattern of CD80 and CD86 expression was further confirmed with Cy-L307.4 (mIgG1 anti-CD80/B7.1, PharMingen, San Diego, CA) and FITC-BB1 (mIgM anti-CD80/B7.1), and with PE-IT2.2 (mIgG2b anti-CD86/B7.2), respectively. Other Abs used for staining cells (purchased from PharMingen unless otherwise noted) were: Cy-UCHT1 (mIgG1 anti-CD3), FITC-RPA-T4 (mIgG1 anti-CD4), PE-ICRF44 (mIgG1 anti-CD11b), Cy-B43 (mIgG1, anti-CD19), FITC-2H7 (mIgG2b anti-CD20), FITC-M-A251 (mIgG1 anti-CD25/IL-2R), FITC- or PE-M-T271 (mIgG1 anti-CD27), FITC-5C3 (mIgG1 anti-CD40), Cy-10.1 (mIgG1 anti-CD64/FcγRI), FITC-J4-117 (mIgG1, anti-CD72), PE-G46-2.6 (mIgG1 anti-HLA-A,B,C), FITC-TU39 (mIgG2a anti-HLA-DR,DP,DQ), and FITC-ki-67 (mIgG1 anti-Ki-67, DAKO). The isotype controls used were: FITC-, PE-, or Cy-MOPC-21 (mIgG1); FITC-G155-178 (IgG2a); FITC- or PE-27-35 (IgG2b); and FITC-G155-228 (IgM). FITC-X0927 (mIgG1, DAKO) was used as the control Ab for intracytoplasmic Ki-67 staining.

Cell separation

Peripheral blood leukocytes were obtained by leukapheresis from healthy adult platelet donors, and PBMCs were separated by Ficoll/Hypaque density gradient centrifugation (Pharmacia Biotech, Uppsala, Sweden). B cells were freshly purified using MACS CD19⁺ magnetic microbeads (503-01, Miltenyi Biotec, Auburn, CA), as previously described (31). Purities were consistently >99%. For the isolation of B cell subsets, purified B cells

were sorted on the basis of surface expression of CD27 and CD80. Briefly, B cells at 20 × 10⁶/ml staining buffer were incubated with predetermined optimal concentrations of the appropriate mAbs for 20 min at 4°C, then washed twice in staining buffer. Stained B cells were then immediately sorted (FACSsort, Becton Dickinson) into CD27⁻CD27⁺/CD80⁻, and CD27⁺/CD80⁺ subsets. Highly purified (>97%) CD4⁺ T cells were isolated from fresh PBMCs by a two-step negative selection process using T cell subset enrichment columns (R&D Systems, Minneapolis, MN) followed by magnetic bead depletion of unwanted cells, as previously described (32).

B cell subset activation and Ig secretion

Immediately following separation, B cell subsets were incubated in U-bottom 96-well plates, with irradiated (3000 rad) CD40L-transfected L cells (provided by Y.-J. Liu, DNAX, Palo Alto, CA) and IL-4 (100 U/ml; R&D Systems) and IL-2 (10 U/ml; provided by Teceleukin, National Cancer Institute, Frederick, MD) in complete medium with 10% FCS (modified from Ref. 18). For assays of proliferation kinetics, 2.5 × 10⁴ sorted B cells were incubated with 5.0 × 10³ irradiated L cells in triplicate wells. [³H]Thymidine (1 μCi/well; NEN/DuPont, Boston, MA) was added for 18 h at the indicated times. Plates were then harvested (Harvester 96; Tamtec, Orange, CT), and B cell proliferation was assessed by measuring thymidine incorporation in a beta scintillation counter (Betaplate 1205; Wallac, Gaithersburg, MD). To assess the activation propensity of the different B cell subsets, 2.5 × 10⁴ sorted B cells were incubated with a decreasing titration of irradiated L cells. [³H]Thymidine was added at time zero, and B cell proliferation was assessed at 18 and 48 h.

For Ig measurements, 1.0 × 10⁵ sorted B cells were incubated with 2.0 × 10⁴ irradiated L cells or anti-Igκ and anti-Igλ light chain Abs in triplicate, and day 7 or day 10 supernatants were analyzed by ELISA. Control conditions included triplicate wells with irradiated L cells alone, B cell subsets alone, stained but unsorted purified B cells, and whole PBMCs. When assessing the effects of IL-10 on B cell subsets, triplicate wells were set up as described above, either with or without IL-10 (100 ng/ml; R&D Systems). Sandwich ELISAs were developed for Ig measurements with linear range sensitivities as follows: IgA, 500 pg/ml to ~100 ng/ml; IgM, 2–2000 ng/ml; total IgG, 300 pg/ml to 100 ng/ml; and IgG subtypes: IgG1, 20–5000 ng/ml; IgG2, 1–750 ng/ml; IgG3, 1–500 ng/ml; and IgG4, 1–5000 ng/ml. Briefly, capture Abs were coated on Immulon plates (Dynex, Chantilly, VA) in Tris high salt (THS)³ buffer at 50 μl/well, kept overnight at 4°C, then blocked with 2% BSA (Sigma, St. Louis, MO) in THS (100 μl/well) for 2 h at 37°C. Plates were then washed twice, and supernatant samples and standards (diluted in culture medium) were added at 50 μl/well and kept overnight at 4°C. Plates were washed twice, and biotin-conjugated secondary Abs were added at the indicated concentrations in 50 μl/well THS with Tween 20 (Sigma, St. Louis, MO) and incubated for 45 min. Plates were then washed three times and incubated with 50 μl/well avidin-peroxidase (Sigma) diluted as indicated in THS with Tween for 30 min at room temperature. Finally, plates were washed four times, developed with TMB (Kirkegaard & Perry, Gaithersburg, MD), and stopped with ELISA stop solution (100 μl/well each) before reading at 450 nm.

Assessing APC function

For MLR assays, the capacity of B cell subsets to activate allogeneic CD4⁺ T cells was assessed. B cell subsets or control cells were freshly isolated, treated with mitomycin C (100 μg/ml for 2 h) or irradiated (600 rad), and titrated into wells containing 1.0 × 10⁵ freshly purified allogeneic CD4⁺ T cells in 200 μl complete medium with 10% FCS. Irradiated responders were used as fillers to normalize total cell numbers across wells. Twenty-four-hour [³H]thymidine incorporation was measured on day 5. For Ag-dependent experiments, 1.0 × 10⁵ mitomycin C-treated or irradiated B cell subsets pulsed with glutamate, lysine, alanine, and tyrosine (GLAT; glutaric acetate, 4 μg/ml, provided by TevaMarion Partners Petach-Tikva, Israel), were incubated with 2.0 × 10⁵ autologous CD4⁺ T cells in complete medium with 10% pooled human AB serum (PelFreeze, Brown Deer, WI). Twenty-four-hour [³H]thymidine incorporation was measured at the indicated times. In initial experiments no differences in proliferative responses were found between mitomycin C treatment and irradiation of B cells. Irradiation was chosen as the preferred approach for subsequent experiments to avoid further loss of cells incurred by the additional wash required with mitomycin C treatment. Nonirradiated B cell subsets were also used as APCs to further control for the possibility that the B cell

³ Abbreviations used in this paper: THS, Tris high salt; CD40L, CD40 ligand; GLAT, glutamate, lysine, alanine, and tyrosine.

subsets differed in their radiosensitivity. For CD80 blocking experiments, F(ab')₂ were generated from mIgG1 anti-human CD80 mAb (provided by Mary Collins and Beatriz Carreno, Genetics Institute, Cambridge, MA), using the ImmunoPure F(ab')₂ preparation kit (Pierce, Rockford, IL). Protein recovery was determined by using absorbance at 280 nm, and fragment purity was confirmed by gel electrophoresis. Control F(ab')₂ were similarly prepared from mouse IgG1 (MOPC-21, Sigma). Where indicated, anti-CD80 or control F(ab')₂ were added at 5 μg/ml to triplicate wells at the onset of the cultures.

Results

CD80⁺ expression defines a large subpopulation of circulating human memory B cells that develops gradually into adulthood

We studied the phenotype of circulating human B cells using triple-color immunofluorescent staining of whole blood samples ob-

tained directly ex vivo. Surprisingly, a substantial frequency of normal adult B cells (CD19⁺) expressed significant levels of CD80, but not CD86 (Fig. 1A). In marked contrast, circulating monocytes (CD64⁺) expressed, as expected, high levels of CD86, while only a small fraction (not more than 2%) expressed CD80 (Fig. 1, A and C). The circulating CD19⁺CD80⁺ B cells were predominantly memory (CD27⁺) B cells (Fig. 1B, lower right). Naive (CD27⁻) B cells expressed equally low levels of CD80 and CD86. Thirty-four to 57% (mean, 45%; n = 9) of circulating human adult memory B cells were CD80⁺ (Fig. 1C, far right), representing approximately 12–25% (mean, 17%; n = 9) of the total circulating B cell pool. We predicted that if these CD80⁺ cells developed as a subset of memory B cells (as opposed to merely

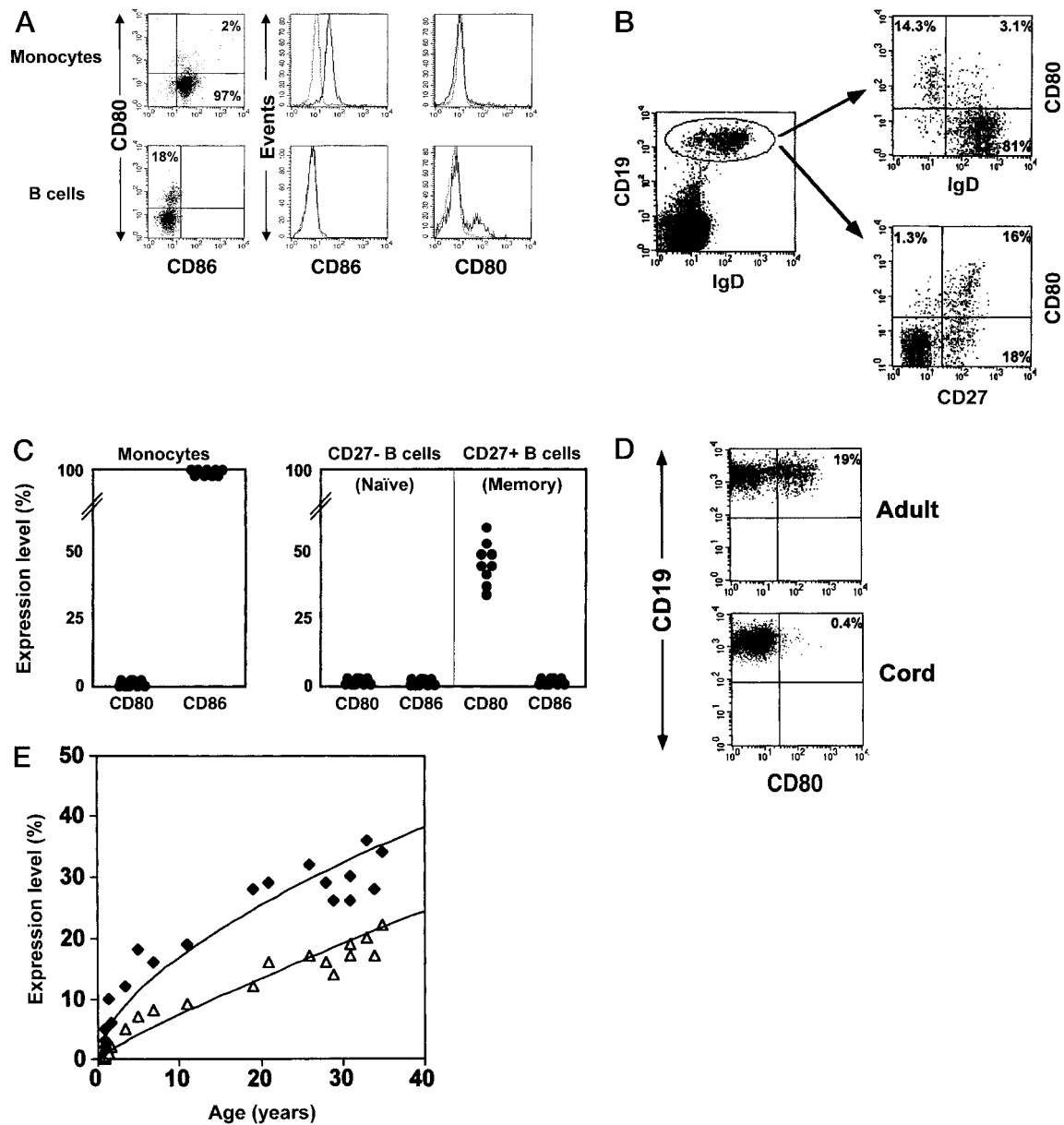


FIGURE 1. The pattern and temporal development of CD80 expression on circulating human memory B cells. *A*, In contrast to circulating monocytes (top row, gated as CD64⁺) that express CD86 and little CD80, ex vivo staining of whole blood reveals a substantial frequency of B cells (bottom row, gated as CD19⁺) expressing CD80 in the face of little or no CD86. Dotted lines show isotype controls. *B*, Gating on (CD19⁺) B cells, the CD80⁺ B cells are largely CD27⁺ (memory) B cells (lower right). *C*, In nine consecutively studied normal adults (●), the CD80⁺CD27⁺ memory B cell subset represents 34–57% of the total circulating memory B cell pool (far right). *D*, Consistent with their development as a memory B cell subset, the CD80⁺ B cells are absent from cord blood. *E*, Depicts percentage of total circulating CD19⁺ B cells that are memory B cells (CD19⁺CD27⁺, ◆) or CD80⁺ memory B cells (CD19⁺CD27⁺CD80⁺, △) over time. Both populations increase gradually into adulthood. Power curve fit.

representing the subset of recently activated memory B cells at any given point in time), then their frequency would increase gradually over time, as has been previously shown for CD27⁺ B cells (17). Indeed, the CD80⁺ B cell subset was essentially absent in cord blood (Fig. 1D), and its frequency in the circulation increased gradually with age, always representing approximately half the circulating memory (CD27⁺) B cell pool (Fig. 1E).

Circulating CD80⁺ memory B cells are in a quiescent state

Since CD80 has been viewed as a marker of B cell activation, it was possible that the CD27⁺CD80⁺ cells simply represented an activated subset of circulating memory cells. However, the observations that the frequency of these cells increases gradually over time (Fig. 1E) and that in the adult they constitute approximately half the circulating memory B cell pool suggested that the expression of CD80 on these memory B cells did not merely reflect recent activation. To directly compare the activation profiles of the memory B cell subsets and naive B cells, freshly purified B cells from normal adults were phenotyped by three-color FACS analysis for a range of activation markers. We found no differences in the activation states of the CD27⁺CD80⁺ and CD27⁺CD80⁻ memory B cells. Both subsets expressed identical levels of CD86, CD25, CD40, and class II (Fig. 2A). DNA-based cell cycle analysis using Hoechst 33342 and propidium iodide revealed equivalent proportions of viable G₀/G₁, S phase, and G₂ cells in both memory B cell subsets (G₀/G₁: 92 ± 4% for CD27⁺CD80⁺, 88 ± 5% for CD27⁺CD80⁻, *p* > 0.3; S phase: 3.3 ± 1.8% for CD27⁺CD80⁺, 5.2 ± 3.6% for CD27⁺CD80⁻, *p* > 0.4; G₂: 5.1 ± 2% for CD27⁺CD80⁺, 6.6 ± 1.8% for CD27⁺CD80⁻, *p* > 0.4; mean ± SD for four independent experiments; significance determined by Student's unpaired *t* test). Furthermore, there were no differences between both memory subsets and the naive CD27⁻ B cells with respect to the intracytoplasmic expression of Ki67 (Fig. 2A), a nuclear proliferation marker used to distinguish between G₀ and G₁ phases of the cell cycle (30). Together our findings are consistent with a prior report that circulating human memory B cells are in a resting, nondividing state (33). We conclude that the circulating CD19⁺CD27⁺CD80⁺ cell subset represents the first description of the expression of the CD80 costimulatory molecule on a population of quiescent memory B cells.

Distinct response properties of the CD27⁺CD80⁺ B cell subset

We next wished to study the capacity of the memory B cell subsets to proliferate and secrete Igs. Since variable expression of costimulatory molecules on the B cell subsets would induce different degrees of T cell help, we chose to stimulate B cells in the absence of T cells. Given the observation that the levels of CD40 were identical on all B cell subsets (Fig. 2A), we used CD40L-transfected L cells (provided by Y.-J. Liu, DNAX) to stimulate highly purified B cell subsets. The kinetics of B cell proliferation were determined by [³H]thymidine incorporation at different times following stimulation. Significantly greater proliferative responses were measured from the CD27⁺CD80⁺ memory B cells compared with either the CD27⁺CD80⁻ memory subset or the CD27⁻ (naive) B cells during the first 2 days following stimulation (Fig. 3A). In contrast, by the fifth day following stimulation, proliferation of the CD27⁺CD80⁺ cells reached a plateau, while CD27⁺CD80⁻ cells were proliferating more rapidly. CD27⁻ (naive) B cells were the slowest to initiate proliferation, but, unlike the memory subsets, followed an exponential growth curve such that by 5 days poststimulation they were incorporating significantly more [³H]thymidine than either of the memory cell subsets. This observation is consistent with prior reports that CD40-mediated stimulation of naive B cells primarily induces proliferation, while sim-

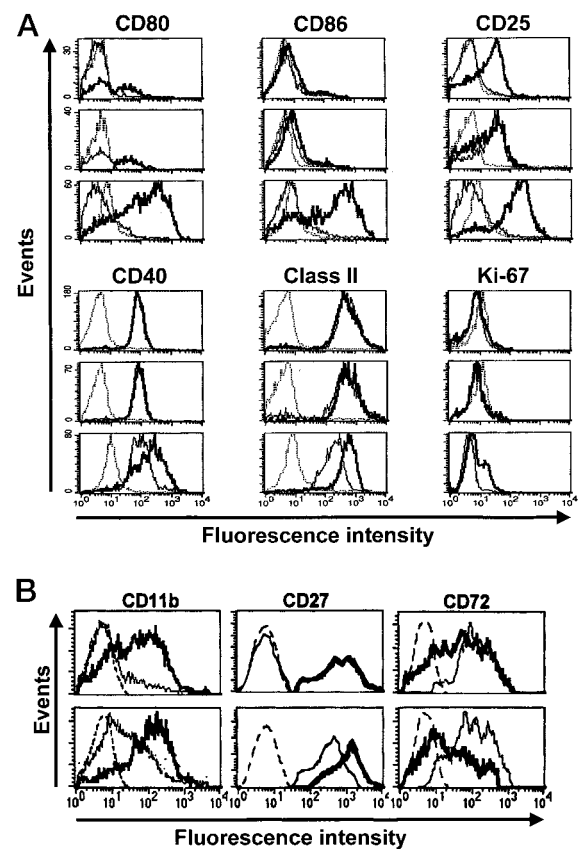


FIGURE 2. Phenotypic characterization of naive and memory B cell subsets by FACS analysis of freshly purified (CD19⁺) B cells. *A*, For each surface marker, three histogram plots are depicted. The *upper histogram* for each marker compares the memory (CD27⁺) B cells (thick line) to naive (CD27⁻) B cells (thin line) and the isotype control (dotted line). The *middle histogram* for each marker compares the CD27⁺CD80⁺ memory B cell subset (thick line) to the CD27⁺CD80⁻ memory B cell subset (thin line) and the isotype control (dotted line). Representative data from one of seven normal adults are shown. Positive controls are provided in the *bottom histogram* for each marker, comparing short term CD40-activated B cells (thick line) with the isotype control (used in the activated condition, dotted line) and unstimulated B cells (thin line). CD80⁺ memory B cells do not have an activated phenotype. The two memory B cell subsets express identical levels of the activation markers CD86, CD25, CD40, and MHC class II. Both memory subsets are essentially negative for the intracytoplasmic activation marker Ki-67, which distinguishes G₀ (Ki-67-negative) from G₁ (Ki-67-positive) cells. DNA-based analysis revealed no cell cycle differences between the memory B cell subsets (see text). Thus, both CD27⁺CD80⁻ and CD27⁺CD80⁺ B cells represent resting, nondividing memory B cells. CD80 expression on the circulating CD27⁺CD80⁺ cells does not reflect a state of enhanced activation. *B*, The CD80⁺ memory B cells have a unique phenotype that predicts their function. For each marker, two histogram plots are depicted. In the *upper histograms*, memory (CD27⁺) B cells (thick line) are compared with naive (CD27⁻) B cells (thin line) and the isotype control (dashed line). The *lower histogram* for each marker compares the CD27⁺CD80⁺ memory B cell subset (thick line) to the CD27⁺CD80⁻ memory B cell subset (thin line) and the isotype control (dashed line). Compared with the CD27⁺CD80⁻ subset, CD27⁺CD80⁺ memory B cells express substantially higher levels of the integrin family adhesion molecule/complement receptor, CD11b and higher levels of CD27, which are important in B cell-T cell interactions. The decreased expression of CD72 on the CD27⁺CD80⁺ subset is consistent with their lowered activation threshold (see text).

ilar stimulation of memory B cells primarily promotes differentiation and Ig secretion, a mechanism thought to prevent B cell repertoire freezing (34).

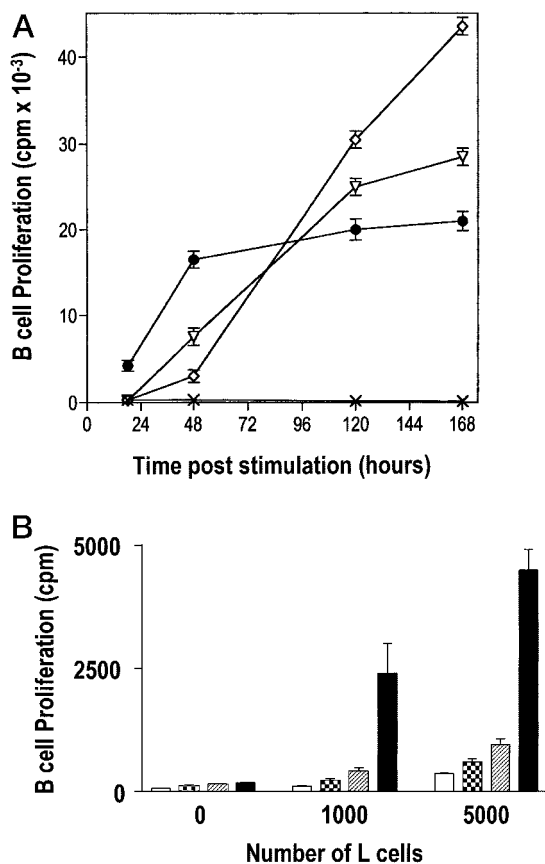


FIGURE 3. B cell subsets have distinct response kinetics and thresholds of activation. *A*, Distinct response kinetics of B cell subsets. Measurement of tritiated thymidine incorporation by B cells at various times following stimulation with CD40L-transfected L cells revealed a rapid response kinetic of the CD27⁺CD80⁺ memory B cells. Each point represents an average of three replicate wells \pm SE (error bars). At the earliest times assessed (18 and 48 h following stimulation) the CD27⁺CD80⁺ memory B cells (●) proliferated significantly more than the CD27⁺CD80⁻ memory B cells (▽; $p < 0.01$) and the CD27⁻ naive B cells (◇; $p < 0.001$). In contrast, CD27⁺CD80⁻ B cell proliferation was not detected until 48 h. Later, however, CD27⁺CD80⁻ B cell proliferation was significantly greater than that measured for CD27⁺CD80⁺ B cells ($p < 0.02$ at 120 and 168 h). CD27⁻ naive B cells followed an exponential proliferation kinetic, with little measured by 48 h, but significantly greater proliferation than both memory subsets at subsequent times ($p < 0.02$ at 120 h; $p < 0.002$ at 168 h). *B*, CD27⁺CD80⁺ memory B cells have a low threshold of activation; a hierarchy of activation is observed even at the earliest time point assessed (18 h) poststimulation. In contrast to the CD27⁻ naive B cells (▩) and CD27⁺CD80⁻ memory B cells (▨), CD27⁺CD80⁺ memory B cells (■) are induced to proliferate with low numbers of stimulators (CD40L-transfected L cells). □, L cells alone. Significance was determined by Student's unpaired, two-tailed t test. Data are representative of four independent experiments. There was no background proliferation in cultures with L or B cells only (marked by X in *A*).

The rapid response kinetic of the CD27⁺CD80⁺ memory B cell subset suggested that these cells have a lower threshold of activation than the CD27⁺CD80⁻ subset. Indeed, a hierarchy of activation thresholds was observed when the CD40L-transfected stimulator cells were used at progressively lower numbers at all time points. Fig. 3*B* demonstrates this at the earliest time point tested (18 h) after stimulation. The CD27⁺CD80⁺ memory cells were readily induced to proliferate with small numbers of stimulators, while measurable induction of proliferation in the CD27⁺CD80⁻ memory cells and in the CD27⁻ naive cells required greater num-

bers of stimulator cells. Consistent with the observation that the CD27⁺CD80⁺ cells can be more readily activated is the demonstration that CD72 is expressed at lower levels on the surface of these memory B cells (Fig. 2*B*). CD72 has been shown to increase the threshold of B cell activation by negatively regulating B cell receptor signaling (35). Lower levels of surface expression of CD72 are therefore associated with a lower threshold of B cell activation.

To study the Ab-producing capacity of the B cell subset, we developed a sensitive sandwich ELISA assay to quantify the levels of secreted IgA, IgM, total IgG, and the IgG subclasses (IgG1–4) in culture supernatants. Following CD40-mediated activation, CD27⁻ cells secreted essentially no Igs, consistent with their naive state and previous reports (16–18). While both memory subsets secreted considerable amounts of IgA, IgG, and IgM, the CD80⁺ subset consistently secreted substantially greater levels of all isotypes measured, which was also true for all IgG subclasses (Fig. 4*A*). These increases in Ab secretion were reproduced with Pokeweed mitogen and with more physiological stimuli such as B cell Ag receptor cross-linking with anti-Ig light chain Abs or stimulation of the freshly isolated B cell subsets with CD3-activated autologous CD4⁺ T cells. In all cases, between 3.5- and 24-fold more Igs were secreted by the CD27⁺CD80⁺ subset compared with the CD27⁺CD80⁻ subset across all isotypes and subclasses (data not shown). In these experiments essentially no Ig secretion was measured when the freshly isolated B cell subsets were cultured in the absence of stimulation (e.g., using mock-transfected L cells or unstimulated CD4⁺ T cells). These observations confirm that while poised to secrete large amounts of Igs, the CD27⁺CD80⁺ B cells circulate as a unique subset of memory B cells and not a population of cells signaled in vivo to differentiate into Ab-producing plasma cells. This is consistent with prior reports that CD27⁺ B cells are not terminally committed to plasma cell differentiation (36).

It has been shown that IL-10, an important human regulatory cytokine, is able to enhance plasma cell differentiation and Ig secretion from memory B cells (37, 38). To examine whether the two memory subsets respond equally to IL-10, we performed an additional series of experiments, with or without the addition of IL-10. Both memory subsets secreted significantly greater amounts of Igs when IL-10 was added, but the CD27⁺CD80⁺ subset displayed a markedly greater sensitivity, such that the enhanced Ig secretion induced by IL-10 was significantly greater in the CD80⁺ memory subset (Fig. 4*B*). Thus, independent of their potential to costimulate T cells (e.g., via CD80/CD28 interactions) and thereby recruit additional T cell help, the CD80⁺ memory B cell subset secreted class-switched Igs much more readily and to significantly higher levels than the CD80⁻ subset, stimulated in the same way.

CD27⁺ CD80⁺ memory B cells are efficient activators of T cells in keeping with their distinct phenotype

We next examined the relative capacity of the B cell subsets to function as APCs. In initial experiments freshly sorted B cell subsets (CD27⁻, CD27⁺CD80⁻, CD27⁺CD80⁺) or whole B cells from the same normal donors were either treated with mitomycin C, or irradiated and then incubated with highly purified allogeneic CD4⁺ T cells. T cell proliferation was assessed at 5 days by [³H]thymidine incorporation. A clear hierarchy of Ag presenting capacity was identified (Fig. 5*A*). As predicted, the CD27⁺CD80⁺ memory B cell subset induced significantly greater proliferation than the CD27⁺CD80⁻ subset, even at the low stimulator:effector ratio of 1:50 (2,000 B cells:100,000 T cells). Naive B cells were relatively poor APCs, consistent with previous reports (11, 12). Since it is known that compared with naive B cells murine memory

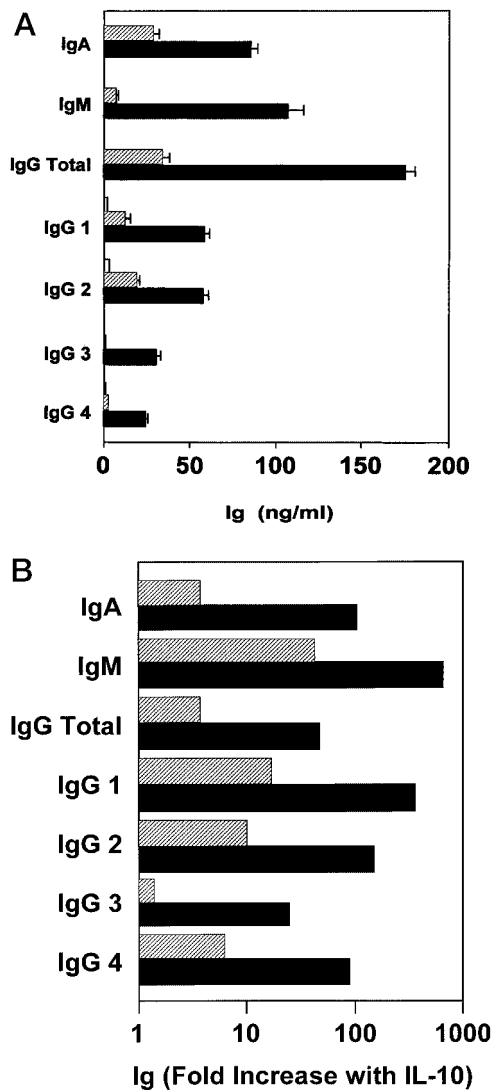


FIGURE 4. Ab-secreting capacity and sensitivity to IL-10. *A*, Ig secretion from purified B cell subsets following stimulation with CD40L-transfected L cells. Day 7 culture supernatants were analyzed for Ig secretion by sandwich ELISA. Bars represent the average Ig concentration measured in supernatants from three parallel cultures \pm SD (error bars). CD27⁺CD80⁺ memory B cells (■) secreted significantly higher amount of Igs of all isotypes and subclasses measured compared with CD27⁺CD80⁻ memory B cells (▨). All differences were significant at $p < 0.001$, by Student's unpaired, two-tailed t test. Naive (CD27⁻) B cells (□) secreted negligible amounts of Ig. The data are representative of four independent experiments. *B*, Enhancement of Ig secretion with IL-10. Purified B cell subsets were stimulated as described above, either with or without the addition of IL-10 (100 ng/ml). Day 7 supernatants were analyzed for Ig content by ELISA as described above. Bars represent the fold increase in Ig secretion induced by the addition of IL-10. While IL-10 augmented Ig secretion from both CD27⁺CD80⁺ (■) and CD27⁺CD80⁻ (▨) memory B cells, the sensitivity of the CD27⁺CD80⁺ subset to IL-10 was significantly greater across all Ig isotypes and subclasses measured than that of the CD27⁺CD80⁻ subset. Data are representative of three independent experiments.

B cells can acquire variable degrees of radioresistance (11), we included nonirradiated B cell subsets as control APCs. The proliferative response to the each B cell subset was not significantly different whether the subset was irradiated or not (Fig. 5A), confirming that proliferation by B cells had a negligible contribution to the measured proliferation and that, importantly, the higher pro-

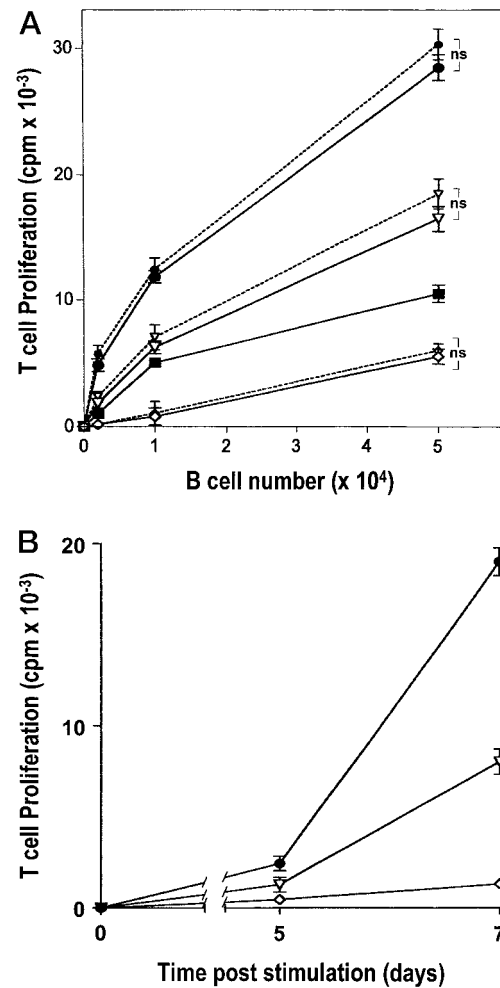


FIGURE 5. Resting CD27⁺CD80⁺ B cells can act as efficient APCs to T cells. *A*, Highly purified CD4⁺ T cell proliferation assessed by tritiated thymidine incorporation 5 days after incubation with various numbers of allogeneic, freshly purified irradiated or nonirradiated B cells. ■, Whole B cells; ◇, CD27⁻ naive B cells; ▽, CD27⁺CD80⁻ memory B cells; ●, CD27⁺CD80⁺ memory B cells. Larger symbols and solid lines show irradiated cells; smaller symbols and dashed lines show nonirradiated cells. Each point represents an average of three replicate wells \pm SD (error bars). Even at the lowest stimulator:responder ratio (2,000:100,000), CD27⁺CD80⁺ memory B cells induced significantly more T cell proliferation compared with the CD27⁺CD80⁻ memory subset ($p < 0.01$), whole B cells ($p < 0.005$), and CD27⁻ naive B cells ($p < 0.001$, by Student's unpaired, two-tailed t test). Data are representative of six independent experiments. Proliferation levels measured for each B cell subset were not significantly different regardless of whether the B cell subset was irradiated (comparing solid and dashed lines for each B cell subset). cpm, counts per minute after backgrounds of either T cells alone or B cell subsets alone (consistently low and between 750 and 2000 cpm) were subtracted. *B*, Kinetics of CD4⁺ T cell proliferation in response to Ag-dependent (GLAT) stimulation by autologous B cell subsets. ◇, CD27⁻ naive B cells; ▽, CD27⁺CD80⁻ memory B cells; ●, CD27⁺CD80⁺ memory B cells. Each point represents an average of three replicate wells \pm SD (error bars). Only the CD27⁺CD80⁺ cells induced significant T cell proliferation at the earliest time assessed ($p < 0.01$). Later, CD27⁺CD80⁺ B cells stimulated significantly more T cell proliferation than CD27⁺CD80⁻ B cells ($p < 0.005$), while the two memory subsets both induced significantly more T cell proliferation than the CD27⁻ subset ($p < 0.001$, by Student's unpaired, two-tailed t test). The data are representative of five independent experiments. cpm, counts per minute after backgrounds (consistently low, and between 400 and 1500 cpm) were subtracted. Thus, CD27⁺CD80⁺ B cells elicited significantly stronger and more rapid Ag-dependent T cell responses than CD27⁺CD80⁻ memory cells or CD27⁻ naive cells.

liferation induced by the CD80⁺ memory cells was due to their enhanced APC capacity, and not to their relative radioresistance.

The addition of blocking anti-CD80 F(ab')₂ (generated from anti-CD80 Ab provided by Mary Collins and Beatriz Carreno, Genetics Institute, Cambridge, MA) diminished the enhanced APC capacity of the CD27⁺CD80⁺ cells by only 50% (data not shown), suggesting that the ability of these memory B cells to more efficiently activate CD4⁺ T cells was not solely due to their expression of CD80. Indeed, compared with the CD80⁻ memory subset, the CD80⁺ memory B cells expressed significantly higher levels of the integrin family adhesion molecule/complement receptor CD11b as well as higher levels of CD27 itself (Fig. 2B). The engagement of CD27 on B cells and of CD70 on T cells is known to play an important role in mediating productive responses of both B cells and T cells (37, 39). Thus, despite their resting state, the CD27⁺CD80⁺ memory B cells are distinguished by several features that promote their ability to interact with and efficiently activate T cells.

Finally, we wished to confirm the enhanced APC capacity of the CD27⁺CD80⁺ B cells in an Ag-dependent system. Because assessment of Ag-dependent interactions between human B cells and T cells is often limited by the low precursor frequencies of responding cells, we chose to use a random copolymer of GLAT, a well-characterized Ag shown to induce high frequency CD4⁺ T cell responses that are mediated through the TCR in an MHC class II-restricted fashion (40, 41). We used freshly sorted B cell subsets to present GLAT to autologous CD4⁺ T cells and defined the kinetics of T cell proliferation. Fig. 5B demonstrates that CD27⁺CD80⁺ B cells elicited significantly stronger and more rapid Ag-dependent T cell responses than CD27⁺CD80⁻ cells.

Discussion

Here we define a novel subset of phenotypically and functionally distinct human memory B cells, underscoring the heterogeneity that exists within the normal circulating memory B cell compartment and extending recent observations from the murine system (42). To our knowledge, these studies provide the first description of the expression of CD80 on the surface of resting B cells. We further demonstrate that despite their quiescent state, these CD19⁺CD27⁺CD80⁺ memory B cells are able to efficiently present Ag to and activate CD4⁺ Th cells, and are poised to deliver rapid and robust memory effector responses.

In a recent report McHeyzer-Williams et al. (42) elegantly described a novel memory B cell subset in the murine system, defined as surface B220⁻. The authors tracked the development of the B220⁻ memory B cells following recall antigenic exposure and identified them as major constituents of the non-Ab-secreting, quiescent memory B cell pool. Upon antigenic rechallenge, these B220⁻ cells displayed a lesser degree of proliferation, but a more robust Ab response compared with the B220⁺ memory subset, reminiscent of the differential responses of our CD80⁺ and CD80⁻ human memory subsets, respectively. While the profile of costimulatory molecule expression on the murine memory subsets was not reported, it is also interesting to note that a subpopulation of the B220⁻ memory cells was shown to express high levels of CD11b, similar to the CD80⁺ memory subset in the current study. In contrast to the circulating human CD27⁺CD80⁺ memory B cell subset, however, the B220⁻ murine memory subset, studied in the bone marrow and spleen, reportedly expressed very low levels of CD19 and did not include IgM-secreting cells. While these murine and human B cell subsets are unlikely to represent equivalent memory B cell subpopulations, the study by McHeyzer-Williams et al. (42) and our current study both provide novel insights into the phenotypic and functional heterogeneity that exists within the

memory B cell compartment. Our findings are underscored by the recent demonstration in the murine system by Harris et al. (43) of distinct effector B cell subsets (Be1 and Be2) that are able to differentially regulate T cell responses.

Several theories have supported an important role for B cells in propagating immune responses, in particular their ability to participate in the activation of naive or primed T cells in an Ag-dependent fashion. Janeway and Mamula (44) proposed a model in which B cells are viewed only as second line APCs after the more professional dendritic cells. More recently, Bretscher (45) extended his original two-signal model of T cell activation to the two-step, two-signal model (8) in which he advocates an important role for memory B cells in the second step of the activation of precursor Th cells. Shared by all prevailing models of precursor T cell activation is the general assumption that any effective B cell contribution requires preactivation of the B cells with the consequent up-regulation of inducible costimulatory molecules (46). Our demonstration of the expression of high levels of costimulatory molecules on a subset of quiescent circulating memory B cells may help to reconcile some of the differences between existing models. These CD27⁺CD80⁺ B cells are able to readily contribute to rapid and productive B cell-T cell interactions and stimulate efficient Ag-dependent CD4⁺ T cell responses without requiring an immediate preactivation step.

Taking advantage of the identical levels of CD40 expression on the B cell subsets, our CD40L-mediated activation assays demonstrate that CD27⁺CD80⁺ memory B cells are capable of secreting very large amounts of Igs compared with the CD80⁻ memory B cells. This is independent of the additional T cell help that the CD27⁺CD80⁺ memory B cells may be able to recruit by virtue of their costimulatory molecule expression. Moreover, these differences are further amplified with the addition of the regulatory cytokine IL-10, which preferentially and powerfully augments Ab secretion from the CD80⁺ subset. The enhanced Ig secretion from the CD80⁺ memory B cells is not restricted to particular isotypes or IgG subclasses, suggesting that the capacity to mount robust Ab effector responses is a broadly defining characteristic of this memory subpopulation.

Although circulating in a resting state, the CD80⁺ memory B cells possess a cell surface phenotype that predicts differential activation requirements and an enhanced capacity to interact with their environment. Decreased levels of surface CD72 expression are consistent with a lower threshold of activation, as is illustrated in our findings of the enhanced responsiveness to suboptimal stimuli and the more rapid proliferation kinetics of the CD27⁺CD80⁺ memory subset compared with the CD27⁺CD80⁻ subset and the naive B cells. The high levels of expression of the integrin family adhesion molecule/type 3 complement receptor, CD11b, coexpressed on CD80⁺ cells, may reflect preferential migration patterns or enhanced complement fixing capacity. CD11b has also been described as a coreceptor for the B cell Ag receptor (47). The increased levels of CD27 enable more efficient interactions with T cells through CD27-CD70 interactions that may further contribute to the ability of these CD80⁺ memory B cells to efficiently stimulate T cells as well as differentiate into powerful Ab-secreting cells.

The mechanisms for the development of the distinct CD27⁺CD80⁺ and CD27⁺CD80⁻ human memory B cell subsets and their respective roles remain speculative. One possibility is that the two subsets develop at the same time during the initial antigenic encounter. It is generally accepted that when a naive B cell encounters Ag during the germinal center reaction, its progeny

will differentiate along either the plasma cell pathway or the memory B cell pathway (48). Little is known, however, about the potential for distinct memory B cell subsets to diverge at that time. One hypothesis is that the CD27⁺CD80⁺ and CD27⁺CD80⁻ B cells develop in such a way to equip the immune system with functionally distinct memory B cells that share the same antigenic specificity, but respond to their target in different contexts, such as unique tissue microenvironments.

Alternatively, the phenotypic and functional differences between the two memory B cell subsets may reflect differences in the number of times that they encountered their unique Ags. In this context, studies of CD80 expression on T cells have shown that CD80 is not expressed on normal naive T cells or following single activation of naive T cells, but can be demonstrated on the surface of normal T cells following the many cycles of antigenic stimulation associated with T cell cloning (49). In the case of B cells, it is well accepted that both CD80 and CD86 expression can be transiently induced upon naive B cell activation (25–27). It is possible, however, that CD80 (unlike CD86) becomes chronically expressed on B cells upon sufficient repeated antigenic exposure and remains expressed even after the memory B cell has returned to a relatively quiescent state. In this way the immune system may ensure that frequently encountered Ags are met with a particularly rapid B cell response that is also capable of efficiently recruiting a cognate T cell response.

In conclusion, we define a novel CD27⁺CD80⁺ B cell subset that develops gradually after birth and comprises a substantial portion of the human adult circulating memory B cell compartment. Although in a resting state, these B cells have a lower threshold of activation and are poised to deliver vigorous memory effector responses. The unique expression of high levels of costimulatory and adhesion molecules on the surface of these resting CD27⁺CD80⁺ memory B cells provides a mechanism by which they may contribute to the efficient Ag-dependent T cell responses observed. As such, this novel memory B cell subset can mediate the rapid and robust immune responses that together constitute the hallmarks of adaptive immune memory. In this context, reports of increased frequencies of CD80⁺ B cells in human autoimmune disease (50, 51) and observations that autoreactive B cells may function as effective APCs (10, 52) raise the possibility that dysregulation of the CD80⁺ memory B cell subset may contribute to autoimmune pathogenesis. Further studies will be required to elucidate the potential roles of these cells in both health and disease, particularly at a time when novel agents are being developed to target the B7 costimulatory pathway in human clinical trials (24).

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References

- Gray, D. 1993. Immunological memory. *Annu. Rev. Immunol.* 11:49.
- Dutton, R. W., S. L. Swain, and L. M. Bradley. 1999. The generation and maintenance of memory T and B cells. *Immunol. Today* 20:291.
- Hafler, D. A., M. Chofflon, D. Benjamin, N. H. Dang, and J. Breitmeyer. 1989. Mechanisms of immune memory: T cell activation and CD3 phosphorylation correlates with Ta₁ (CDw26) expression. *J. Immunol.* 142:2590.
- Noelle, R. J., and E. C. Snow. 1990. Cognate interactions between helper T cells and B cells. *Immunol. Today* 11:361.
- Enyon, E. E., and D. C. Parker. 1993. Parameters of tolerance induction by antigen targeted to B lymphocytes. *J. Immunol.* 151:2958.
- Serreze, D. V., H. D. Chapman, D. S. Varnum, M. S. Hanson, P. C. Reifsnnyder, S. D. Richard, S. A. Fleming, E. H. Leiter, and L. D. Shultz. 1996. B lymphocytes are essential for the initiation of T cell mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.Ig μ null mice. *J. Exp. Med.* 184:2049.
- Rock, K. L., B. Benacerraf, and A. K. Abbas. 1984. Antigen presentation by hapten-specific B lymphocytes. I. Role of surface immunoglobulin receptors. *J. Exp. Med.* 160:1102.
- Bretscher, P. A. 1999. A two-step, two-signal model for the primary activation of precursor helper T cells. *Proc. Natl. Acad. Sci. USA* 96:185.
- Cassell, D. J., and R. H. Schwartz. 1994. A quantitative analysis of antigen-presenting cell function: activated B cells stimulate naive CD4 T cells but are inferior to dendritic cells in providing costimulation. *J. Exp. Med.* 180:1829.
- Mamula, M. S., S. Fatenejad, and J. Craft. 1994. B cells process and present lupus autoantigens that initiate autoimmune T cell responses. *J. Immunol.* 152:1453.
- Krieger, J. I., S. F. Grammer, H. M. Grey, and R. W. Chesnut. 1985. Antigen presentation by splenic B cells: resting B cells are ineffective, whereas activated B cells are effectively accessory cells for T cell responses. *J. Immunol.* 135:2937.
- Chesnut, R. W., and H. M. Grey. 1981. Studies on the capacity of B cells to serve as antigen-presenting-cells. *J. Immunol.* 126:1075.
- Lanzavecchia, A. 1985. Antigen-specific interactions between T and B cells. *Nature* 314:537.
- Garside, P., E. Ingulli, R. R. Merica, J. G. Johnson, R. J. Noelle, and M. K. Jenkins. 1998. Visualization of specific B and T lymphocyte interactions in the lymph node. *Science* 281:96.
- Klein, U., T. Goossens, M. Fischer, H. Kanzler, A. Braeuninger, K. Rajewsky, and R. Kuppers. 1998. Somatic hypermutation in normal and transformed human B cells. *Immunol. Rev.* 162:261.
- Maurer, D., G. F. Fischer, I. Fae, O. Majdic, K. Stuhlmeier, N. Von Jeney, W. Holter, and W. Knapp. 1992. IgM and IgG but not cytokine secretion is restricted to the CD27⁺ B lymphocyte subset. *J. Immunol.* 148:3700.
- Agematsu, K., H. Nagumo, F. C. Yang, T. Nakazawa, K. Fukushima, S. Ito, K. Sugita, T. Mori, T. Kobata, C. Morimoto, et al. 1997. B cell subpopulations separated by CD27 and crucial collaboration of CD27⁺ B cells and helper T cells in immunoglobulin production. *Eur. J. Immunol.* 27:2073.
- Tangye, S. G., Y.-J. Liu, G. Aversa, J. H. Phillips, and J. E. de Vries. 1998. Identification of functional human splenic memory B cells by expression of CD148 and CD27. *J. Exp. Med.* 188:1691.
- Klein, U., K. Rajewsky, and R. Kuppers. 1998. Human immunoglobulin (Ig)M⁺IgD⁺ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. *J. Exp. Med.* 188:1679.
- Agematsu, K., S. Hokibara, H. Nagumo, and A. Komiyama. 2000. CD27: a memory B-cell marker. *Immunol. Today* 21:204.
- McAdam, A. J., A. N. Schweitzer, and A. H. Sharpe. 1998. The role of B7 co-stimulation in activation and differentiation of CD4⁺ and CD8⁺ T cells. *Immunol. Rev.* 165:231.
- Anderson, D. E., A. H. Sharpe, and D. A. Hafler. 1999. The B7-CD28/CTLA-4 costimulatory pathways in autoimmune diseases of the central nervous system. *Curr. Opin. Immunol.* 11:677.
- Lenschow, D. J., Y. Zeng, J. R. Thistlethwaite, A. Montag, W. Brady, M. G. Gibson, P. S. Linsley, and J. A. Bluestone. 1992. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science* 257:789.
- Abrams, J. R., M. G. Leibold, C. A. Guzzo, B. V. Jegasothy, M. T. Goldfarb, B. S. Goffe, A. Menter, N. J. Lowe, G. Krueger, M. J. Brown, et al. 1999. CTLA-4-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *J. Clin. Invest.* 103:1243.
- Azuma, M., D. Ito, H. Yagita, K. Okumura, J. H. Phillips, L. L. Lanier, and C. Somoza. 1993. B70 antigen is a second ligand for CTLA-4 and CD28. *Nature* 366:76.
- Lenschow, D. J., A. I. Sperling, M. P. Cooke, G. Freeman, L. Rhee, D. C. Decker, G. Gray, L. M. Nadler, C. C. Goodnow, and J. A. Bluestone. 1994. Differential up-regulation of the B7-1 and B7-2 co-stimulatory molecules after Ig receptor engagement by antigen. *J. Immunol.* 153:1990.
- Hathcock, K. S., G. Laszlo, C. Pucillo, P. Linsley, and R. H. Hodes. 1994. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. *J. Exp. Med.* 180:631.
- Liu, Y.-J., C. Barthelemy, O. de Bouteiller, C. Arpin, I. Durand, and J. Banchereau. 1995. Memory B cells from human tonsils colonize mucosal epithelium and directly present antigen to T cells by rapid up-regulation of B7-1 and B7-2. *Immunity* 2:239.
- Darzynkiewicz, Z., G. Juan, X. Li, W. Gorczyca, T. Murakami, and F. Traganos. 1997. Cytometry in cell necrobiology: analysis of apoptosis and accidental cell death (necrosis). *Cytometry* 27:1.
- Scholz, T., and J. Gerdes. 2000. The Ki-67 protein: from the known and the unknown. *J. Cell. Physiol.* 182:311.
- Miltenyi, S., W. Muller, W. Weichel, and A. Radbruch. 1990. High gradient magnetic cell separation with MACS. *Cytometry* 11:231.
- Scholz, C., K. P. Patton, D. E. Anderson, G. J. Freeman, and D. A. Hafler. 1998. Expansion of autoreactive T cells in multiple sclerosis is independent of exogenous B7 costimulation. *J. Immunol.* 160:1532.
- Bovia, F., A. C. Nabili-Tehrani, C. Werner-Favre, M. Barnet, V. Kindler, and R. H. Zubler. 1998. Quiescent memory B cells in human peripheral blood co-express bcl-2 and bcl-x_L anti-apoptotic proteins at high levels. *Eur. J. Immunol.* 28:4418.

34. Arpin, C., J. Banchereau, and Y.-J. Liu. 1997. Memory B cells are biased towards terminal differentiation: a strategy that may prevent repertoire freezing. *J. Exp. Med.* 186:931.
35. Tsubuta, T. 1999. Co-receptors on B lymphocytes. *Curr. Opin. Immunol.* 11:249.
36. Agematsu, K., H. Nagumo, Y. Oguchi, T. Nakazawa, K. Fukushima, K. Yasui, S. Ito, T. Kobata, C. Morimoto, and A. Komiyama. 1998. Generation of plasma cells from peripheral blood memory B cells: synergistic effect of IL-10 and CD27/70 interaction. *Blood* 91:173.
37. Nagumo, H., and K. Agematsu. 1998. Synergistic augmentative effect of interleukin-10 and CD27/CD70 interactions on B cell immunoglobulin synthesis. *Immunology* 94:388.
38. Rousset, F., E. Garcia, T. Defrance, C. Peronne, N. Vezzio, D. H. Hsu, R. Kastelein, K. W. Moore, and J. Banchereau. 1992. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA* 89:1890.
39. Lens, S. M. A., K. Tesselaar, M. H. J. van Oers, and R. A. W. van Lier. 1998. Control of lymphocyte function through CD27-CD70 interactions. *Semin. Immunol.* 10:491.
40. Fridkis-Hareli, M., and J. L. Strominger. 1998. Promiscuous binding of synthetic copolymer 1 to purified HLA-DR molecules. *J. Immunol.* 160:4386.
41. Duda, P. W., M. C. Schmied, S. L. Cook, J. I. Krieger, and D. A. Hafler. 2000. Glatiramer acetate (Copaxone) induces degenerate, Th2-polarized immune responses in patients with multiple sclerosis. *J. Clin. Invest.* 105:967.
42. McHeyzer-Williams, L. J., M. Cool, and M. G. McHeizer-Williams. 2000. Antigen-specific B cell memory: expression and replenishment of a novel B220⁻ memory B cell compartment. *J. Exp. Med.* 191:1149.
43. Harris, D. P., L. Haines, P. C. Sayles, D. K. Duso, S. M. Eaton, N. M. Lepak, L. L. Johnson, S. L. Swain, and F. E. Lund. 2000. Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat. Immunol.* 1:475.
44. Mamula, M. J., and C. A. Janeway. 1993. Do B cells drive the diversification of immune responses. *Immunol. Today* 14:151.
45. Bretscher, P. A., and M. Cohn. 1970. A theory of self-nonsel self discrimination. *Science* 169:1042.
46. Constant, S. L. 1999. B lymphocytes as antigen-presenting cells for CD4⁺ T cell priming in vivo. *J. Immunol.* 162:5695.
47. Petty, H. R., and R. F. Todd III. 1996. Integrins as promiscuous signal transduction devices. *Immunol. Today* 17:209.
48. Liu, Y. J., and J. Banchereau. 1997. Regulation of B cell commitment to plasma cells or to memory B cells. *Semin. Immunol.* 9:235.
49. Kipp, B., A. Bar-Or, R. Gausling, E. M. L. Oliveira, S. A. Fruhan, W.H. Stuart, and D. A. Hafler. 2000. A novel population of B7.1⁺ T cells producing intracellular IL-4 is decreased in patients with multiple sclerosis. *Eur. J. Immunol.* 30:2092.
50. Ranheim, E. A., and T. J. Kipps. 1994. Elevated expression of CD80(B7/BB1) and other accessory molecules on synovial fluid mononuclear cell subsets in rheumatoid arthritis. *Arthritis Rheum.* 37:1637.
51. Genc, K., D. L. Dona, and A. T. Reder. 1997. Increased CD80⁺ B cells in active multiple sclerosis and reversal by interferon β -1b therapy. *J. Clin. Invest.* 99:2664.
52. Lin, R.-H., M. J. Mamula, J. A. Harding, and C. A. Janeway. 1991. Induction of autoreactive B cells allows priming of autoreactive T cells. *J. Exp. Med.* 173:1433.