CD40 and B Cell Antigen Receptor Dual Triggering
of Resting B Lymphocytes Turns on a
Partial Germinal Center Phenotype

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Summary

Phenotypic alterations occur when resting human B lymphocytes become germinal center (GC) cells. These include the induction of surface CD38, CD95 (FAS/APO-1), and carboxypeptidase-M (CPM), a recently described GC marker. However, the factors that govern the in vivo induction of these surface molecules on B cells remain unknown. Here, we purified resting (CD38−) human B lymphocytes from tonsils in an attempt to establish culture conditions resulting in the induction of these three GC markers. We show that interferon (IFN) α or IFN-γ, as well as antibodies against the B cell antigen receptor (BCR), could induce CD38 on resting B lymphocytes, a phenomenon further enhanced by CD40 stimulation. Concomitantly, CD95 was upregulated by CD40 ligation and, to a lesser extent, by IFN-γ. By contrast, CPM expression could be upregulated only through BCR triggering. This CPM induction was specifically enhanced by CD19 or CD40 ligation. CD40 + BCR stimulation of resting B cells with CD40 ligand–transfected fibroblastic cells in the presence of cross-linked anti-BCR monoclonal antibodies resulted in the coexpression of CD38, CD95, and CPM. As GC cells, these cells also expressed CD71, CD80 (B7.1), and CD86 (B7.2), but not CD24. However, CD10+ or CD44− B cells could not be detected in these culture conditions, suggesting that yet other signals are required for the induction of these GC markers. Consistent with a GC phenotype, CD40 + BCR–stimulated cells exhibited reduced viability when cultured for 20 h in the absence of stimulus. These results first demonstrate that cotriggering of resting B cells through BCR and CD40 induces both phenotypic and functional GC features. They also show that IFN and CD19 triggering of resting B cells specifically modulate the expression of GC markers.

After antigenic challenge, a large variety of cells sequentially cooperate to develop the humoral response. APC take up the antigen, process it, and present it to specific Th cells in the context of MHC class II molecules. This cognate interaction drives Th cells to express both membrane-associated and soluble factors that control the proliferation and differentiation of B lymphocytes. In secondary lymphoid organs, this Th–B cell interaction is a prerequisite for the formation of germinal centers (GC)1, the histological structures where B lymphocytes proliferate and mature into high-affinity memory cells (for reviews see references 1, 2).

In humans, several phenotypic alterations occur when B lymphocytes enter GC. These include both induction and disappearance of various surface molecules (CD markers) that most likely reflect B cell activation and adaptation to a new environment. Among GC markers, CD38 is a surface enzyme catalyzing the synthesis of cADP-ribose from NAD (3–7). CD38 is believed to participate in the control of GC B cell growth (4) or viability (8) and is coexpressed on GC cells with the carboxypeptidase M (CPM), a zinc-dependent protease known to cleave off basic COOH-terminal amino acids from hormonal peptides (9). In addition to these two ectoenzymes, GC cells express on their surface CD95 (FAS), a TNF receptor–related molecule that also participates in the control of GC B cell survival (10, 11).

Here, we attempted to establish culture conditions resulting in the induction of a GC phenotype on resting tonsillar B cells. This approach allowed us to show that dual triggering of CD40 and B cell antigen receptor (BCR) is required for the coexpression of CD95, CD38, and CPM. It also showed that cells coexpressing these markers are highly susceptible to spontaneous cell death. Finally, it is suggested that IFNs as well as CD19 triggering contribute to the in vivo acquisition of a GC phenotype.

1 Abbreviations used in this paper: BCR, B cell antigen receptor; CPM, carboxypeptidase M; GC, germinal centers.
Materials and Methods

Cytokines. rhIL-1α and rhIL-1β (10^4 U/mg), rhIL-6 (10^5 U/mg), and rhTNF-α (2 × 10^5 U/mg) were purchased from Genzyme Corp. (Boston, MA). They were used at 10, 10, 200, and 2.5 ng/ml, respectively. Purified rhIL-2 (3 × 10^6 U/ml) (Amen Biologicals, Thousand Oaks, CA), rhIL-3 (5 × 10^5 U/mg), rhIL-4 (10^5 U/mg), rhIL-5 (10^5 U/mg), rhIL-10 (10^5 U/mg), rhIL-13 (10^6 U/ml), and rhGM-CSF (2 × 10^6 U/mg) (Schering-Plough Research Institute, Thousand Oaks, CA), rhIL-12 (2–3 × 10^6 U/ml) was kindly provided by Dr. A. O’Garra (DNAX Research Institute, Palo Alto, CA) and was used at 10 ng/ml. TGF-β1 and rhIL-7 were obtained from R&D Systems, Inc. (Minneapolis, MN) and were used at 0.5 and 100 ng/ml, respectively. IFN-γ (10^5 U/mg) was purchased from Amgen Biologicals and was used at 500 U/ml.

Antibodies. The monoclonal and polyclonal antibodies used for phenotypic and functional studies were obtained from the following sources: anti-CD2 and anti-CD3 mAbs used for negative selection of B cells with magnetic beads (Aster Laboratories, La Gaude, France); PE-conjugated anti-CD2 (Leu 5), anti-CD3 (Leu 4), FITC-conjugated anti-CD10 (Calla), anti-CD20 (Leu 16), and anti-CD14 (Leu M3) (Becton Dickinson & Co., Mountain View, CA); FITC-conjugated anti-Bcl-2 oncprotein (Dako, Glostrup, Denmark); anti-α (6E1), anti-λ (C4) anti-CD24 (IOB3), FITC-conjugated anti-CD19 (IOB4), and biotin-conjugated anti-CD24 (IOB3) antibodies (Immunotech, Marseille, France); FITC-conjugated anti-CD44 (A3D8) antibody and biotinylated goat anti-human IgD antibody (Sigma Chemical Co., St. Louis, MO); PE-conjugated anti-CD86 (B70) (PharMingen, San Diego, CA).

The anti-CD40 (mAb 89) (12), anti-CPM (M27) (9), anti-CD21 (FIP6, FIP8, and FIP15), and anti-CD40-L (L2) mAbs were generated and purified in our laboratory. Anti-CD38 (OKT10) antibody was purchased from American Type Culture collection (Rockville, MD) (CRL-8022) and used as ascitic fluid. Anti-CD19 (4G7) and PE-conjugated anti-CD80 (L307) were kindly provided by Drs. R. Levy and L. Lanier, respectively. All other antibodies were obtained from the 4th International Workshop on Leucocyte Typing (Boston, MA, 1993).

Chemicals. FITC-conjugated streptavidin was purchased from Immunotech.

Flow Cytometric Analysis. Antibody binding was analyzed on a FACSScan® flow cytometer (Becton Dickinson & Co.). Gating was set according to forward angle light scatter parameters and propidium iodide staining to exclude subcellular particles, dead cells, and L cells from acquisition data. Data (10^4 events per sample) were subsequently processed using the LYSYS program (Becton Dickinson & Co.). The percentage of positive cells was determined by channel per channel peak subtraction of the control histogram using the Consort 30 program (Becton Dickinson & Co., Immunocytochemistry Systems). For intracellular detection of Bcl-2 protein, cells were permeabilized by 15-min incubation at 4°C in saponine (0.5 mg/ml) before staining with anti-Bcl-2 mAb or an unrelated mAb as a negative control. Cell viability was determined by measurement of the incorporation of the DNA-binding fluorochromes Bisbenzimide HOECHST 33342 (Calbiochem-Novabiochem Corp., San Diego, CA) and propidium iodide, as previously described (13). Cells were incubated for 5 min with 10 μM HOECHST 33342 and 32 μM propidium iodide immediately before analysis with a double laser-equipped FACStar® plus (Becton Dickinson & Co.). This analysis permitted us to delineate three different cell populations: (a) Permeable cell membranes incorporate propidium iodide; (b) apoptotic cells incorporate only HOECHST 33342; (c) viable cells incorporate neither propidium iodide nor HOECHST 33342.

B Cell Cultures. All cultures were performed in RPMI 1640 medium supplemented with 10% heat-inactivated FCS (Flow Laboratories, Irvine, Scotland), 80 μg/ml gentamicin (Genentech; Schering-Plough), in the presence of irradiated (7,000-rad) CD32-transfected (CD32-L cells; American Type Culture Collection, 16.2 CG7) or CD40-L/CD32-double transfected murine Ltk" cells. The CD40-L/CD32-double transfected L cells were produced in our laboratory. Cultures were set up in flat-bottomed 96-well microtiter plates (100 μl final volume) in the presence of 5 × 10^5 transfected L cells/well and 10^5–10^6 purified B cells. Anti-CD or anti-BCR were used at 2 μg/ml or 1:1,000 dilution of ascitic fluid throughout this study.

B Cell Purification. Tonsillar mononuclear cells were separated by standard Ficoll–Hypaque gradient method and were next submitted to E rosetting with SRBC. The following procedure was designed according to Lagresle et al. (14). Nonrosetting cells were labeled with anti-T cell (anti-CD2, -CD3, -CD4, and -CD8 mAbs), anti-monocyte (anti-CD14 mAb), and anti-CD38 (OKT10) mAbs and subsequently incubated twice with magnetic beads coated with anti-mouse IgG antibodies (Dynal, Oslo, Norway). Labeled cells were removed by applying a magnetic field for 15 min. The purity of the B cell subpopulation obtained after this procedure was >98% as estimated by FACSscan® immunofluorescence labelings performed with CD3, CD34, CD19, CD38, and CD44 mAbs. For triple-labeling experiments, cells were labeled by biotinylated goat-anti-IgD antibody + tricolor-streptavidin and indirect mAb + PE-conjugated anti-mouse antibody. After saturation with mouse serum, FITC-conjugated anti-CD38 was added.

Results

CD38+ Tonsillar B Cells Include Both Naive and Memory B Cells. The phenotype of highly purified tonsillar B cells was assessed using triple labeling with (a) anti-CD38 (FITC), (b) anti-IgD (Tricolor), and (c) PE-conjugated anti-CD20, anti-CD45 (FAS), anti-CPM, anti-CD71, anti-CD80 (B7.1), anti-CD86 (B7.2), anti-CD24, anti-CD10, or anti-CD44 mAbs. Results are shown in Fig. 1A. From these labelings, two different tonsillar B cell subsets can be distinguished: (a) CD38low−-cells, which are resting and include both sIgD+ naive and sIgD− memory cells (gate R1) (14–16); and (b) CD38high cells, which correspond to GC B lymphocytes. Consequently, in this report, resting tonsillar B cells were selected by CD38 depletion (14). As expected, the resulting population (Fig. 1B) typically included variable proportions of sIgD− cells. CD38− purified B cells also expressed low levels of CD95, CD80, CD86, and CD71, and no CPM, but high levels of CD24 and CD44 antigens.

IFN-α, IFN-γ, or BCR Ligation Induce CD38 Expression on Resting Tonsillar B Cells. We first examined the in vitro induction of surface CD38 expression on resting (CD38−)
tonsillar B cells. To this end, resting B lymphocytes were cultured with optimal concentrations of cytokines (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IFN-α, IFN-γ, GM-CSF, or TNF-α), anti-BCR or anti-CD40 mAbs in the presence of CD32-L cells. CD38 expression was then determined at day 3 by FACS analysis. Among the cytokines tested, only IFN-α and IFN-γ were found to consistently induce CD38 expression on resting B lymphocytes (Fig. 2 A). This IFN-mediated CD38 induction was observed at as early as 18 h of culture and reached near maximal levels after 24 h (Fig. 2 A). The IFN-α and IFN-γ-mediated induction of CD38 was a dose-dependent process with a half-maximal effect observed at ~5 and ~1 U and an optimal effect at ~1,200 and ~300 U, respectively (Fig. 2 B). This induction was a direct effect of IFNs on B cells because it could be seen in the absence of fibroblastic CD32-L cells (not shown). Triggering of BCR by immobilized anti-κ + λ light chain mAbs was also found to induce a moderate expression of CD38. This anti-BCR-induced CD38 expression displayed slower kinetics than that observed with IFN-α and IFN-γ with an optimal expression around day 5 (Fig. 2 A). At this time point, 22 ± 9% (n = 3) of cells expressed CD38. In contrast, although it is the most efficient mitogenic signal, CD40 triggering by immobilized anti-CD40 mAb (mAb 89) essentially failed to turn on CD38 expression (Fig. 2 A).

IFN- or BCR-induced Expression of CD38 Is Further Enhanced by CD40 Ligation. Because CD40 stimulation sensitizes B cells to anti-BCR triggering (17) and to a large variety of cytokine effects, we tested whether CD40 stimulation would affect anti-BCR- or IFN-mediated induction of CD38. To this end, resting (CD38-) tonsillar B cells were stimulated with either anti-BCR (anti-κ + λ light chains) or anti-CD40 (mAb 89) antibodies cross-linked on CD32-L cells, with or without optimal concentrations of IFN-α or IFN-γ. After 3 d of culture, CD38 expression on activated B cells was determined by FACS analysis. Results are

![Figure 1](image1.png)

Figure 1. (A) Triple labeling of human tonsillar B cells. B lymphocytes were purified from tonsils and labeled as reported in Materials and Methods. Gate R1, resting cells; R2, GC cells. (B) Phenotype of CD38+ tonsillar B lymphocytes. B cells were purified from tonsils and then labeled with anti-CD38 mAb. After adding anti-mouse Ig-coated beads, CD38+ cells were depleted on a magnet. Remaining cells were phenotyped. Each staining profile (solid line) was superimposed onto that of the negative control (dotted line) performed with an isotype-matched unrelated mAb.

![Figure 2](image2.png)

Figure 2. IFNs or BCR stimulation induce CD38 expression on the surface of resting tonsillar B cells. CD38+ resting B lymphocytes were purified from tonsils as reported in Materials and Methods and seeded with CD32+ fibroblasts in the presence of (A) medium (○), IFN-α (2,000 U) (△), IFN-γ (500 U) (□), mAb89 (2 μg/ml) (●), or anti-κ + anti-λ light chain mAbs (2 μg/ml each) (▲), or in the presence of (B) variable concentrations of IFN-α (●) or IFN-γ (○). CD38 expression was measured by FACS. The percentage of positive cells was determined by subtraction of the control peak. Results are representative of three independent experiments.
Figure 3. Double-entry table of in vitro-activated resting B-cell CD38 profiles. CD38- B lymphocytes were cultured in the presence of CD32+ fibroblasts with or without IFN-α, IFN-γ, anti-BCR, anti-CD40, or combinations. On day 3, cells were labeled with FITC-conjugated anti-CD38 mAb. Staining profile of CD38 (solid line) was superimposed onto that of FITC-conjugated isotype-matched unrelated mAb (dashed line). Shaded squares indicate combinations with synergistic or additive effects. These results are representative of four experiments.

Figure 4. Double-entry table of in vitro-activated resting B-cell CD95 profiles. CD38- B lymphocytes were cultured in the presence of CD32+ fibroblasts with or without IFN-α, IFN-γ, anti-BCR, anti-CD40, or combinations. On day 3, cells were labeled with FITC-conjugated anti-CD95 mAb. Staining profile of CD95 (solid line) was superimposed onto that of FITC-conjugated isotype-matched unrelated mAb (dotted line).
CD38- B lymphocytes were cultured in the presence of CD32+ fibroblasts with or without IFN-α, IFN-γ, anti-Ig, anti-CD40, or combinations. On day 3, cells were labeled with biotin-conjugated anti-CPM mAb. Staining profile of CPM (solid line) was superimposed onto that of biotin-conjugated isotype-matched unrelated mAb (dashed line). Shaded squares indicate combinations with synergistic or additive effects.

(Fig. 6 C). However, the minimal anti-BCR concentration needed for day 4 CPM induction was not significantly lowered by CD40 ligand or anti-CD19 mAbs (Fig. 6 C). Finally, because in vivo CPM expression is restricted to CD38+ leukocytes (9), CD40 + BCR- or CD19 + BCR-stimulated resting B cells were labeled with anti-CD38 and anti-CPM mAbs. As illustrated in Fig. 6 D, CPM+ in vitro-activated B cells also expressed the CD38 molecule (Fig. 6 D). Notably, the addition of cytokines (IL-2, IL-3, IL-4, IL-10, or IFN-α) to CD19 + BCR- or CD40 + BCR-activated lymphocytes, CD19 + BCR-activated resting B cells remained CD71low and CD95+. None of these culture conditions, however, could generate CD10+ or CD44+ B lymphocytes from resting tonsillar B cells, suggesting that yet other signals are required to induce a full GC phenotype. Adding IFN-α, IFN-γ, or anti-CD19 to CD40 + BCR–stimulated resting B cells did not significantly change the pattern of expression of these markers (not shown). Similarly, we could not downregulate CD44 expression or induce CD10 by adding GM-CSF (19), supernatant of an anti-CD3–activated T cell clone, anti-CD44 mAbs, or collagen type I, a natural ligand for CD44 (20) (not shown).

As GC B cells undergo spontaneous apoptotic cell death when cultured in medium (21), we asked whether the observed in vitro acquisition of a GC phenotype is associated with reduced viability. Resting B lymphocytes were isolated from tonsils and cultured for 6 d in the presence of CD40 ligand/CD32+ L cells with or without anti-BCR antibodies. B cells were then harvested, washed, and further described in Materials and Methods. Results are representative of three independent experiments. (C) Percentage of CPM+ cells after stimulation with suboptimal concentrations of anti-Ig (×10) (C, D, E, F, G, H). 2 μg/ml anti-CD40-L (IL2) (C, D, E, F, G, H) or 2 μg/ml anti-CD19 (C, D, E, F, G, H) antibodies before labeling with M27 mAb. (D) CD38+ tonsillar B cells were cultured for 4 d in the presence of anti-CD19 + anti-Ig. Cells were then harvested and labeled with FITC-conjugated anti-CD38 mAb and streptavidin-PE + biotinylated anti-CPM mAb.
ther cultured in medium for an additional 20 h in the presence of anti-CD40 ligand antibody to avoid triggering by contaminating L cells. As shown in Fig. 8 A, when harvested, both CD40- and CD40 + BCR–stimulated resting B cells express high and comparable amounts of Bcl-2 protein. However, unlike CD40-activated lymphocytes, CD40 + BCR–triggered cells undergo rapid cell death as measured by trypan blue exclusion (Fig. 8 B). This spontaneous cell death can also be quantified by propidium iodide and HOECHST 33342 double labeling (13). Using this method, after 20 h in medium, only 14% of CD40 + BCR–triggered cells appear viable (Fig. 8 C, right), whereas 68% of cells that received only the CD40 triggering are viable (Fig. 8 C, left). This rapid loss of viability may be due to apoptotic cell death because a sizeable (14%) proportion of CD40 + BCR–activated cells exhibits condensed chromatin and an integral plasma membrane structure.

Discussion

Early histophysiological studies have shown that the GC is a T cell–dependent structure. Indeed, individuals with a complete T cell defect do not form GC (22). The interaction between CD40 on B cells with its ligand on Th cells recently emerged as an essential component of this Th–B cell crosstalk because a defective expression of CD40 or CD40 ligand leads to a GC formation defect (23–26). The implication of CD40–CD40 ligand interaction in the GC reaction is further illustrated by the fact that both isotype switching and the somatic mutation processes—two molecular events that take place in GC—are absent in patients displaying the nonfunctional CD40 ligand gene (27–30, D. Razanajaona, C. Van Kooten, S. Lebeque, J. M. Bridon, S. Ho, S. Smith, R. Callard, J. Banchereau, and F. Briere, manuscript in preparation). As shown in this report, in vitro CD40 stimulation of B cells leads to several phenotypic alterations that occur in the GC. Thus, in agreement with former reports (10, 11, 31, 32), CD40 stimulation induces the coexpression of CD95 (FAS), CD80 (B7.1), CD86 (B7.2), and CD71 surface molecules. In addition, CD40 triggering leads to the disappearance of CD24 antigen which, in vivo, marks B cell entry into the GC (33). When combined with certain cytokines such as IFN-α or

Figure 7. CD40-L + anti-Ig cotriggering induces phenotypic features of GC cells. CD38+ B cells were cultured for 6 d on CD40-L/CD32-transfected fibroblasts with or without anti-κ + anti-λ or on CD32-transfected fibroblasts in the presence of anti-κ + anti-λ and anti-CD19 mAbs. Living cells were labeled with FITC or PE-conjugated antibodies. Staining profile (solid line) was superimposed onto that of a conjugated isotype-matched unrelated mAb (dotted line).

Figure 8. Cells with GC phenotype are highly susceptible to spontaneous cell death. Resting tonsillar B lymphocytes were cultured for 6 d in the presence of CD40-L/CD32-transfected L cells with or without 2 μg/ml of anti-κ + anti-λ antibodies. Cells were then harvested, washed and (A) labeled with Bcl-2 mAb after saponine treatment. Dashed line, CD40-activated cells; solid line: CD40 + Ig-activated cells; dotted line, isotype-matched control. Cells were further cultured in medium. (B) Time course measurement of viability using trypan blue. (C) After 20 h in secondary culture, cells were stained with propidium iodide + HOECHST 33342 as described in Materials and Methods. (Left) CD40-activated cells. (Right) CD40 + Ig-activated cells. The upper quadrant correspond to dead cells, the lower left to viable cells, the lower right to apoptotic cells. Numbers indicate the percentage of events recorded for each of these three subsets.
IFN-γ, CD40 triggering is also able to induce strong CD38 expression. This result is consistent with a previous report showing that immunoreactive IFN-α can be detected in GC tingeобразные body macrophages of human secondary lymphoid tissues (34). A high frequency of spontaneous IFN-γ-producing T cells is also present in human tonsils (35). These IFN-γ-producing T cells are mainly located in the extrafollicular area (36), where CD40-L-activated T cells are also detected (37-39). Accordingly, in vivo CD40 + IFNs stimulation of antigen-specific B lymphocytes could indeed take place in human lymphoid tissues and drive the induction of CD38.

Although it has been clearly demonstrated that antigen is essential for the GC reaction, little is known concerning the site and context of the B cell–antigen encounter. In vitro experiments have shown that antigenic stimulation of B cells is critical for effective Th-B cell collaboration (40). Indeed, BCR triggering by multivalent antigen appears to be necessary for the induction of CD28 ligands (CD80 or CD86) on the B cell surface (41). These CD28 ligands are required for full Th cell proliferation and cytokine production, which, in turn, would control B cell blastogenesis. Interestingly, efficient triggering of BCR is also necessary to sensitize B cells to Th cells expressing low (physiological) levels of CD40 ligand (42). In vivo, this efficient BCR stimulation could be achieved in the presence of accessory cells like follicular dendritic cells (43) retaining the unprocessed antigen on their surface. Here we show that BCR stimulation can contribute to the acquisition of CD38 antigen. In addition, BCR triggering is the only signal tested so far that allows the induction of CPM, a surface membrane enzyme specifically expressed on GC mature B cells (9). Anti-BCR induction of CPM is further enhanced by anti-CD40 or, to a lesser extent, by anti-CD19 cotriggering. The contribution of CD19 or CD40 molecules to the induction of CPM was specific in that antibodies against six other surface molecules failed to display such an activity (see Fig. 6, A and B). Recent experiments in mice have shown that CD19 is necessary for the formation of GC in response to T cell–dependent antigens (44). CD19 is a B cell–restricted type I protein with a large extracytoplasmic portion comprising two Ig-like domains. The CD19 extracellular portion also includes a potential CD77 (globotriaosyl ceramide)-binding site (45). Together, these structural features suggest that a ligand for CD19 may exist. In addition, CD19 is a major component of the signal transduction complex associated with the CD21 (IFN-α receptor) molecule (for reviews see references 46, 47). In our hands, however, IFN-α or anti-CD21 mAbs displayed only a limited effect on CPM induction, suggesting that, if it plays a role in human GC formation, CD19 might act independently of CD21.

Unlike CD19 + BCR stimulation, CD40 + BCR cotriggering drives resting B lymphocytes to exhibit seven GC phenotypic features, including the lack of surface CD24 and the presence of markers like CD80, CD86, CD71, CD95, and CD38. The latter could possibly result from anti-BCR–induced endogenous IFN-α production (48). However, CD40 + BCR stimulation could not generate CD10 + or CD44 + cells, thus suggesting that other stimuli are required for modulating these markers. It should be noted that, in vivo, CD40–CD40 ligand contact is short lasting and tightly regulated. Indeed, within hours after contact, CD40 is released from APC—presumably through proteolytic cleavage (49)—whereas CD40 ligand is internalized in T cells (50). Thus, two step culture experiments using CD40 + BCR–stimulated B cells are in progress to identify the factors responsible for the generation of CD44 + or CD10 + cells. Interestingly, CD40 + BCR–activated B cells undergo spontaneous cell death in medium that is reminiscent of the apoptosis of GC B cells. This in vitro–induced cell death is likely to be the result of continuous triggering of BCR, and is observed on cells exhibiting high levels of Bcl-2 protein. The mechanisms responsible for this cell death induction are unknown and could be dependent upon the expression of other survival/death genes. Because lymphocytes undergoing in vivo somatic hypermutation are highly susceptible to apoptotic cell death (21), we are presently investigating whether CD40 + BCR–stimulated naive B cells undergo somatic mutation in culture. In conclusion, our results imply that the acquisition of a GC phenotype requires efficient cotriggering of BCR and CD40. They also show that IFNs or CD19 triggering could contribute to the induction of GC markers in humans. Finally, consistent with earlier reports on freshly isolated GC cells, in vitro acquisition of a GC phenotype renders resting B lymphocytes highly susceptible to spontaneous cell death.

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