Studies of In Vitro Activated CD5+ B Cells

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Human B lymphocytes undergo distinct phenotypic changes following activation with antigen and polyclonal mitogens. Increasing interest has focused on the unique subpopulation of B cells that express the CD5 antigen. In this study, we examined the signals that induce the expression of CD5 on normal splenic B cells. Only 12-O-tetradecanoylphorbol-13-acetate (TPA) induced CD5 expression on highly purified splenic B cells, whereas anti-immunoglobulin (anti-Ig), Epstein-Barr virus, anti-CD20, recombinant interleukin-1 (rIL-1), rIL-2, rIL-4, recombinant interferon-γ (rIFN-γ), and B-cell growth factor all failed to induce CD5 expression. The expression of CD5 was detected on the cell surface by 48 hours and decreased by 96 hours.

HUMAN B LYMPHOCYTES can be polyclonally activated in vitro by several pathways. Anti-immunoglobulin,1 Epstein-Barr virus (EBV),2 and interleukin-4 (IL-4)3 all activate B cells via interaction with distinct cell surface receptors. The monoclonal antibody (MoAb) 1F5 directed against the B-cell–restricted antigen CD206 is capable of activating dense tonsillar B cells to leave the G0 phase of the cell cycle.4 Several investigators have also observed that the croton oil–derived tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) is capable of stimulating B cells to proliferate and induce competence to respond to a variety of growth factors.6-12 The mechanism of TPA activation differs from these other stimuli by the fact that TPA does not interact with a cell surface receptor but directly activates protein kinase C.13 Although all these signals activate unstimulated B lymphocytes, the extent to which these stimuli will drive cells to stages of proliferation and differentiation differ. For example, soluble anti-μ does not induce significant proliferation, whereas EBV induces proliferation and Ig secretion.

Depending on the activating signal, B cells undergo distinct phenotypic changes in cell surface antigens. Stimulation with anti-Ig coupled to beads (anti-Ig), EBV, as well as TPA will lead to an increase in major histocompatibility complex (MHC) class II antigen expression and decreases in the expression of surface IgD and CD21.11-18 In addition, a number of cell surface antigens not expressed on unstimulated B cells are induced following activation. For example, the activation antigens B5, transferrin receptor (T9), and BB1 are all induced by anti-Ig, EBV, and TPA.11,18-21 In contrast, IL-4 increases MHC class II antigen expression and induces CD23 antigen.3,22-24 Moreover, CD23 is well expressed following activation with TPA and EBV; however, anti-Ig is a weak stimulus for this activation antigen.3,24 These observations are consistent with the hypothesis that different activating signals stimulate cells by distinct pathways. Increasing evidence suggests that CD5+25-28 B cells represent one or more distinct populations of B lymphocytes. For example, CD5+ B cells have been identified at the edge of the germinal center of secondary follicles in adult tonsil and lymph node30,31 and in fetal spleen and lymph node.32-33 It has been recently reported that TPA activation of unstimulated B cells induces the expression of CD5.31,34 It is presently unclear whether CD5+B cells are a single subset derived from a common progenitor or, alternatively, whether they represent several subsets of B cells.

In the present report, we have attempted to determine which signals induce the expression of CD5 on B cells in vitro. In the studies to be reported later, we demonstrate that of a variety of stimuli and cytokines examined only TPA will induce splenic B cells to express CD5. The differential responses of sorted homogeneous population of CD5+ and CD5– B cells to stimuli of proliferation and differentiation suggest that these populations may be distinct.

MATERIALS AND METHODS

Normal tissues. After securing appropriate Human Protection Committee validation and informed consent, human spleen samples were obtained, and prepared as previously described.18 Mononuclear cells isolated by Ficoll-Hypaque density gradient centrifugation were enriched for B cells by sheep erythrocyte (E) rosetting to deplete T cells. Further enrichment of the E– population for B cells was performed by anti–T cell (anti-CD4, anti-CD8), and anti-monocyte (anti-CD11b, and anti-CD14) MoAb and complement lysis.19 Anti-CD5 MoAb was used for lysis in selected experiments to deplete any CD5+ B cells. Cells were cryopreserved in 10% dimethylsulfoxide and 20% fetal calf serum (FCS) at -196°C in the vapor phase of liquid nitrogen until the time of study.

Fluorescent staining. Indirect immunofluorescence was per-
formulated as previously described. Directly fluorescent isothiocyanate FITC- and phycoerythrin (PE)-conjugated MoAbs were used for dual-laser flow cytometric analysis. Dual fluorescence was undertaken with 1 to 2 x 10^6 cells per sample as previously described. All direct staining correlated with indirect immunofluorescent staining using unlabelled MoAbs anti-CD20, anti-CD5, anti-CD23, and anti-interleukin-2 receptor anti-IL-2R (CD25) developed with goat antiserum IgG/FITC (Tago Diagnostics, Inc., Burlingame, CA). Anti-B1 (CD20) FITC, anti-IL-2R (CD25) FITC, mlgG2a FITC, mlgM FITC, anti-Ti (CD5) PE, and anti-T6 (CD1) PE (used as a negative control) were obtained from Coulter Immunology (Hialeah, FL). Anti-B5 FITC and anti-Blasta-2 (CD23) FITC were prepared as previously described.

* B-cell cultures. Highly purified splenic B cells obtained by anti-T cell and monocyte MoAb and complement lysis of the E fraction of splenic mononuclear cells were cultured at 1 x 10^6 cells/mL in RPMI 1640 supplemented with 10% FCS, 2 mmol/L glutamine, 1 mmol/L sodium pyruvate, 5 μg/mL gentamicin in tissue culture flasks (Corning Glass Works, Corning, NY) with a variety of additions: (a) TPA (Sigma Chemical Co, St Louis) was used at a final concentration of 10 ng/mL. (b) For anti-Ig, affinity-purified rabbit antihuman Ig was coupled to Affigel 702 beads (Bio-Rad Laboratories, Richmond, CA) as previously described. These beads are capable of inducing a five- to tenfold stimulation of titrated thymidine (3H-TdR) uptake above the background of normal splenic B cells at 72 hours of culture. (c) Highly enriched splenic B cells were incubated with EBV (1/4 diluted supernatant from the EBV-producing marmoset cell line B95) for three hours at 37°C, washed three times then cultured for 1, 2, and 6 days at 1 x 10^6 cells/mL. This supernatant is titered in a spleen B-cell 3H-TdR uptake assay and gives between 30,000 and 60,000 cpm for 5 x 10^6 cells at six days of culture. The stimulation index of splenic B-cell proliferation from the EBV supernatant increased with further dilutions. (d) T-cell-conditioned medium (TCM), prepared as previously described, augments the 3H-TdR uptake of anti-Ig bead-activated splenic B cells by two- to fourfold and was used at a 10% final concentration. (e) MoAb 1F5 (anti-CD20) (the gift of Dr Ed Clark) was used at a final concentration of 10 μg/mL. This concentration of 1F5 is comitogenic with TPA for tonsillar B cells. (f) Purified recombinant IL-4 (rIL-4) (gift of Immunex Corp, Seattle) was used at a final concentration of 500 U/mL. This rIL-4 concentration is comitogenic for splenic B cells with anti-Ig beads and augments 3H-TdR uptake two- to threefold. (g) Purified rIL-2 (gift of Biogen Corp, Boston) was used at a final concentration of 50 U/mL. This concentration of rIL-2 is comitogenic with anti-Ig beads for splenic B cells and augments 3H-TdR uptake two- to threefold. (i) Purified recombinant interferon-γ (rIFN-γ) (gift of Biogen) was used at a final concentration of 400 U/mL. Splenic B cells cultured for 24 hours with INF-γ (400 U/mL) and then subsequently stimulated with anti-Ig beads have a two- to threefold augmentation of 3H-TdR uptake as compared with cells precultured in media alone. (j) Partially purified B-cell growth factor (BCGF) (Cellular Products, Buffalo) was used at a final concentration of 5%. This concentration of BCGF is comitogenic with anti-Ig beads for splenic B cells and augments 3H-TdR uptake two- to threefold.

**Cytoluciferometry and cell sorting.** Flow cytometric analysis and cell sorting was performed on an EPICS V cell sorter (Coulter Electronics, Hialeah, FL) as previously described. Single fluorescent samples were scored as the percentage above the highest channel registering background fluorescence when the cells were stained with an irrelevant isotype-identical MoAb. For example, in Fig 1A, CD20 was scored as 90%, but in fact the percentage of B cells was probably significantly greater. The profile showed a single homogeneous peak with no detectable negative cell population, thus indicating that the percentage of B cells was in all likelihood greater than 95%. Dual-fluorescent cells were analyzed after initial calibration of the machine with cells stained with each individual antibody-fluorochrome-labeled cells and with appropriate controls.

**Microcultures.** B cells isolated after cell sorting were cultured in 96-well, round-bottomed microtiter trays (Costar, Cambridge, MA) at 50,000/well. Anti-Ig antibody beads and growth factors including BCGF (5% final concentration) and rIL-2 (50 U/mL final concentration) were added to yield a final culture volume (per well) of 200 μL. Pokeneed antibodies (GIBCO, Grand Island, NY) was used at 1:100 final concentration with autologous splenic T cells at 50,000 cells/well.

**Thymidine uptake assay.** Thymidine uptake was used as an index of mitogenic activity. Microcultures were pulsed with 1 μCi of 3H-thymidine (Amersham Corp, Eastbourne, England) per well and were harvested 15 hours later. Dried filters were counted on a Packard Tri-carb scintillation counter (Downers Grove, IL).

**Assay of IgG production.** A solid-phase radioimmunoassay (RIA) with a sensitivity in the nanogram-per-milliliter range was used as previously described with rabbit antihuman IgG (Dakopatts A/S, Copenhagen). Individual wells were counted (Riagamma, 1274, LKB Instruments, Gaithersburg, MD).

**Labelling of cells with radioisotope.** A modification of the lactoperoxidase iodination technique was used. The iodinated unstimulated and TPA-activated splenic B cells were washed twice with cold phosphate-buffered saline and lysed in 50 mmol/L Tris-HCl containing 150 mmol/L NaCl, 5 mmol/L EDTA, 1 mg/mL bovine serum albumin, 0.1% (wt/vol) NaN3, 1% Nonidet P-40 (NP-40, Sigma, 50 mmol/L iodoacetamide and 1 mmol/L phenylmethylsulfonfylfluoride, pH 7.4) for 30 minutes at 4°C with constant rotation as previously described. Detergent-insoluble material and nuclei were removed by centrifugation at 13,000 rpm for 15 minutes in a microfuge at 4°C. The supernatant was frozen at -70°C until analyzed.

**Immunoprecipitation.** The anti-T1 (CD5) antibody (IgG2a) and W6/32 (IgG2a antibody reactive with HLA-A, -B, and -C antigens) were used as ascites fluid. Cell lysates were preclaried three times for two hours each: twice with 40 μL of a 50% (vol/vol) suspension of protein A-Sepharose CL-4B (Pharmacia, Inc, Piscataway, NJ) per milliliter of lysate, followed by 40 μL of a 50% (vol/vol) suspension of W6/32 coupled to CNBr-Sepharose 4B (Pharmacia). The preclared lysates were incubated with anti-T1 antibody for 30 minutes at 4°C with constant rotation followed by the addition of 20 μL of protein A-Sepharose for three hours at 4°C with constant rotation. Sepharose beads were washed with 1 mL of 100 mmol/L Tris-HCl, pH 8.0, 1% (vol/vol) Triton X-100, 0.2% (wt/vol) sodium deoxycholate, 10 mmol/L EDTA, 10 mmol/L ethylene glycol tetraacetic acid, 10 mmol/L NaCl, 1 mg/mL bovine serum albumin containing 0.5 mol/L NaCl followed by 1 mL of the same buffer containing 0.125 mol/L NaCl, 0.2% (wt/vol) sodium deoxycholate, and 0.05% sodium dodecyl sulfate (SDS). This wash cycle was repeated once. Precipitated proteins were eluted from the Sepharose beads by incubation in 50 μL of 125 mmol/L Tris-HCl, pH 6.8, containing 2% (wt/vol) SDS, 5% (vol/vol) glycerol, and 0.02% (wt/vol) bromphenol blue in a boiling water bath for four minutes. Precipitates were analysed by SDS-polyacrylamide gel electrophoresis (PAGE). Add statistics. The results were analyzed for statistical significance by using the paired Student's t test.
RESULTS

TPA induction of CD5 on splenic B cells. It has been recently reported that the CD5 antigen can be induced on normal peripheral blood and splenic B cells following culture for 48 hours with TPA. We have extended these studies of splenic B cells following stimulation with TPA. Before stimulation, highly purified splenic B cells that were greater than 95% CD20+ contained fewer than 2% T cells and monocytes. To examine the temporal sequence of CD5 expression, cells were then cultured with TPA (10 ng/mL) for 1, 2, 3, and 4 days. The recovery of these cells following culture with TPA was 50% to 70%, likely due to residual adherence of cells to the culture flasks. Viable cells were harvested and examined for the expression of CD20 and CD5 by dual-laser flow cytometric analysis with anti-CD20 FITC and anti-CD5 PE. Before culture with TPA very few cells expressed only CD5 (fewer than 2%) or coexpressed CD20 and CD5 (5% ± 2%). At one day of culture with TPA rare CD20+ CD5+ cells were observed (8% ± 2%). However, by day 2, 41% ± 7% of CD20+ cells coexpressed CD5. By forward-angle light scatter, these CD5+ cells were the same size as the CD5− cells. The expression of CD5 at three days was similar to that observed at two days; however, by four days, the expression of CD5 decreased, with 23% ± 5% of cells coexpressing CD20 and CD5. We then attempted to demonstrate that the CD5 induction observed following TPA stimulation was not simply due to an increase in antigen density of CD5 antigen already present on cells before culture. Highly purified splenic B cells were treated with anti-CD5 followed by rabbit complement to deplete most of the CD5+ B cells from the starting population (less than 1% CD5+) (Fig 1A). These cells were then cultured with TPA (10 ng/mL) for two days, and when examined by dual-laser flow cytometric analysis, 38% of the CD20+ cells coexpressed CD5 (in one representative experiment) (Fig 1B). The absence of contaminating T cells and monocytes coupled with the high percentage of cells expressing CD20 (greater than 95%) supports the notion that the B cells were being induced to express CD5 and that this was not an indirect effect of TPA on T cells or monocytes. The recovery of adequate cell numbers following stimulation further suggests that we were observing antigenic changes and not selecting minor subpopulations of B cells.

We next attempted to determine the optimum concentration of TPA that would induce CD5 expression. Splenic B cells were cultured with 0.1, 1, and 10 ng/mL for two days. At that time, 20% of the cells cultured in TPA at 0.1 ng/mL were CD5+, whereas approximately 40% of cells cultured with 1 and 10 ng/mL expressed CD5.

The cell surface phenotype of these TPA-activated cells was examined further. We have previously observed that following culture with TPA for two days the CD5+ splenic B cells coexpress the activation antigens IL-2R and B5. Cells were dual fluorochrome labeled with either anti-IL-2R FITC and anti-B5 FITC or anti-CD23 FITC and anti-CD5 PE and were examined before and following stimulation. Very few (fewer than 10%) unstimulated splenic B cells expressed these antigens before culture with TPA. However, after two days of culture, approximately 80% of the cells expressed IL-2R and B5, and 50% expressed CD23. We then examined cells for coexpression of these activation antigens and CD5. As seen in Fig 1C, when cells were dual labeled with anti-CD23 and anti-CD5, approximately 25% of the cells expressed only CD23, 20% coexpressed CD23 and CD5, and 20% expressed CD5 alone. Whereas in previous studies we observed that virtually all CD5+ B cells coexpressed B5 and IL-2R, the coexpression of CD23 was significantly more heterogeneous.

To further demonstrate the de novo expression of CD5 by TPA-activated B cells, purified splenic B cells depleted of most of the CD5+ cells by antibody and complement lysis were surface labeled with 125I by the lactoperoxidase method before and following culture for two days with TPA. Labeled cells were then lysed with 1% NP40 lysis buffer, and immunoprecipitation studies were performed with anti-CD5 MoAb and protein A-Sepharose. The immunoprecipitates were analyzed by 10% SDS-PAGE (Fig 2). Autoradiography of the gel showed that a band corresponding to a protein with a molecular weight (mol wt) of 65,000 was precipitated from the TPA-activated B cells but not from the unstimulated B cells. A band with a mol wt of approximately 55,000 was also seen that may represent a difference in glycosylation...
of CD5 protein. This further supports the notion that CD5 is induced after activation of splenic B cells with TPA.  

CD5 expression is specifically induced by TPA. With the demonstration that TPA induces CD5 on splenic B cells we next investigated whether other signals that activate B cells by distinct pathways were capable of inducing T1. The polyclonal B cell mitogen anti-Ig coupled to polyacrylamide beads did not induce CD5 expression when examined at 24 or 48 hours (Table 1) by indirect immunofluorescence. Cells examined at three and four days were similarly CD5− (data not shown). EBV very weakly induced CD5+ cells at two days of culture. EBV-stimulated cells, when examined at six days, however, failed to express CD5. The anti-CD20 antibody 1F5, which will activate dense tonsillar B cells, failed to induce CD5 expression. Three lymphokines that prime or preactivate splenic B cells to have an augmented response to subsequent activation with anti-Ig, including rIL-1β, rIL-4, rIL-1B, and rIL-1B, did not induce CD5 expression. Combinations of rIL-1B, rIL-4, and rIL-1β (including rIL-1 and rIL-4; rIL-1B and rIL-1β; rIL-1β and rIL-1B; rIL-4, and rIL-1β) did not induce CD5 expression. Similarly, rIL-2, partially purified low-mol wt BCGF, and TCM failed to induce CD5. Coculture of anti-Ig with TPA did not augment the intensity of expression or number of cells expressing CD5. Therefore of all the stimuli examined, only TPA induced splenic B cells to express CD5.

Responses of isolated CD20+, CD5+ and CD20+, CD5− cells to stimuli of proliferation and differentiation. With the demonstration that a subset of TPA-activated B cells expressed CD5, we next attempted to isolate the two populations (CD5+ and CD5−) by cell sorting. Highly purified splenic B cells were cultured for two days with TPA, harvested, and stained with anti-CD20 FITC and anti-CD5 PE. The CD20+, CD5− and CD20+, CD5+ cells were isolated as homogeneous populations by cell sorting. These sorted cells were then examined for 3H-TdR uptake in response to either anti-Ig, rIL-2, BCGF, and combinations of anti-Ig and rIL-2 or anti-Ig and BCGF. As seen in Table 2, the CD20+, CD5− cells had a significantly greater proliferative response to the combinations of anti-Ig and rIL-2 and anti-Ig and BCGF 96 hours after cell sorting. We next examined these two populations for the production of IgG. Previous reports have observed that IL-2 but not BCGF is capable of inducing activated B cells to produce IgG. We therefore measured IgG production induced directly by IL-2 as well as with added T cells and PWM as a source of B-cell differentiation factors five days following cell sorting. As seen in Table 3, in both the CD20+, CD5− and CD20+, CD5+ populations, very little IgG production occurred with anti-Ig or rIL-2 alone. However, in the presence of both anti-Ig and rIL-2 or PWM and autologous T cells, both populations secreted IgG. Similar to the observed differences in proliferation between these two populations of cells, the CD20+, CD5− cells produced significantly more IgG than did the CD20+, CD5+ cells. These studies suggest that these two subpopulations of TPA-activated B cells, distinguished by their expression of CD5, are functionally different in response to growth and differentiation signals.

**DISCUSSION**

In the present study, we report the induction and characterization of a subset of in vitro activated cells defined by the coexpression of the CD20 and CD5 antigens. Following activation of highly purified splenic B cells with the phorbol
Cells were CD5 in nanomols per IL-2, anti-Ig plus rIL-2, or PWM and autologous T cells. IgG production populations and cultured TPA (10 ng/mL), the CD5 antigen is induced. Before ester TPA (10 ng/mL), the CD5 antigen is induced. Before stimulation and at 24 hours approximately 7% of the B cells coexpressed CD5. At 48 hours, 40% expressed CD5, and at 96 hours CD5 expression decreases to about 20%. CD5 antigen was induced with as little as 0.1 ng/mL, and maximal induction was seen at both 1.0 and 10 ng/mL. Whereas TPA induced CD5 expression, other B cell stimuli including anti-Ig, EBV, anti-CD20 (1F5), rIL-1, rIL-2, rIL-4, rIFN-γ, and BCGF all failed to induce CD5. Dual-fluorochrome analysis of these T1 + B cells demonstrated coexpression of the B-cell activation antigens CD23, B5, and IL-2R. TPA-activated splenic B cells were then sorted into the CD20+, CD5- and CD20+, CD5+ populations and examined for response to anti-Ig, rIL-2, and BCGF and combinations of anti-Ig and rIL-2 or BCGF. At 96 hours of culture, the CD20+, CD5+ cells demonstrated significantly greater 3H-TdR incorporation and IgG secretion than did the CD20+, CD5+ cells. These phenotypic and functional studies further support the notion that CD5+ B cells are a subset of in vitro activated B cells.

Human B lymphocytes undergo a distinct sequence of cell surface antigenic changes in response to a variety of stimuli. Following in vitro activation with anti-Ig, EBV, or TPA, several antigens expressed on unstimulated B cells are lost, and a large number of activation antigens appear. Since both anti-Ig indirectly and TPA directly activate protein kinase C, the observation that CD5 is expressed only following TPA activation suggests that TPA stimulation of B cells is not solely through the activation of protein kinase C. Further evidence that TPA may activate B cells via alternative pathways is suggested by the observation that TPA in contrast to anti-Ig does not induce increases in intracellular [Ca2+]i. Although it is unclear what additional events are stimulated by TPA, other stimuli examined that preactivate and activate B cells do not induce CD5 antigen. Preliminary studies suggest that stimulation of B cells directly by activated T cells does not induce CD5 antigen.

With the demonstration that CD5 is a B-cell activation antigen following in vitro TPA stimulation, we examined the phenotypic and functional characteristics of this subpopulation of B lymphocytes. The in vitro stimulated CD5+ B cell coexpresses other B-cell activation antigens including B5, IL-2R, and CD23. Previous studies of B cells that express activation antigens (eg, IL-2R and 4F2) demonstrate hyperresponsiveness to growth factors when compared with B cells that lack these activation antigens. These studies are in contrast to the observation that the CD20+, CD5+ subset has a diminished capacity to proliferate and secrete Ig as compared with the CD20+, CD5- subset. These studies are supported by the prior observations that fetal CD5+ splenic B cells do not produce significant amounts of Ig in a PWM-driven system. Although controversial, neoplastic CD5+ B cells are reported to be hyporesponsive to B-cell mitogens and growth factors. This decreased responsiveness may be an inherent property of these cells, or this observation may be only relevant for the growth factors and activation stimuli thus far examined. It will be important to determine whether these CD20+, CD5+ cells demonstrate an enhanced response to other stimuli including EBV and other B-cell growth and differentiation factors.

CD5+ B cells have been observed in normal human adult and fetal lymphoid tissues and in several disease states. Small numbers of CD5+ B cells have been identified in normal lymph node, tonsil, and spleen. In addition, significant numbers of CD5+ B cells have been observed in fetal spleen and lymph node. In disease states, increased numbers of CD5+ B cells have been detected in the peripheral blood of patients with rheumatoid arthritis, and CD5+...
STUDIES OF IN VITRO ACTIVATED CD5+ B CELLS

B cells are reported to be a major subset of B cells in the peripheral blood of patients following allogeneic bone marrow transplantation. CD5 is also expressed on the malignant cells of over 90% of the cases of B-cell chronic lymphocytic leukemia (CLL) and occasional B-cell non-Hodgkin's lymphomas. In addition to the identification of these cells, some of these populations have been more extensively studied. In NZB mice, the Ly1+ subset of B cells spontaneously secrete autoantibodies. Moreover, it has been suggested that the Ly1+ B cell may represent a distinct lineage of murine B lymphocytes. Recent studies have demonstrated that normal CD5+ human B cells, when stimulated with EBV or S aureus Cowan I, produced rheumatoid factor and anti-DNA antibodies. Normal adult CD5+ tonsillar B cells spontaneously proliferate in response to 50-kilodalton (Kd) mol wt BCGF, whereas CD5− B cells required activation with anti-Ig to respond to that growth factor. Although most B-cell CLL cells express activation antigens, the CD5+ cells in fetal spleen are reported to be IL-2R−, and CD5+ cells isolated from normal adult peripheral blood do not express the activation antigens Ba, T9, or IL-2R. The relevance of the TPA-activated CD5+ B cells observed in the present study to these populations of normal and neoplastic B cells is presently unknown. However, it is interesting that the responsiveness of CD5+ tonsillar B cells to 50–Kd BCGF provides in vivo evidence that at least some CD5+ B cells appear to be activated. Further studies comparing these populations will be required to determine the heterogeneity and possible functional diversity of this B cell subset. Moreover, this may provide a model in which to study the function of the CD5 molecule on B lymphocytes.

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