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Activation of human B cells mediated through two distinct cell surface differentiation antigens, Bp35 and Bp50

(B lymphocytes/growth/monoclonal antibodies/cell cycle)

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ABSTRACT **Two human B-cell differentiation antigens, Bp35 and Bp50, apparently play distinct roles as signal receptors in B-cell activation. Monoclonal antibodies (mAbs) to either Bp35 or Bp50 deliver positive signals to B cells that stimulate their transition through the cell cycle. mAb to Bp35, like anti-immunoglobulin antibodies, functions principally to activate resting B cells to become competent to enter the G_1 phase of the cell cycle. In contrast, mAb to Bp50, a 50-kDa polypeptide expressed on all B cells, functions to stimulate activated B cells to traverse the cell cycle. mAb to Bp35, like anti-immunoglobulin antibodies, activates tonsillar B cells and induces low levels of B-cell proliferation. In contrast, anti-Bp50 mAb alone neither activates B cells nor induces B cells to proliferate but, together with anti-Bp35 or anti-immunoglobulin, augments B-cell proliferation. In this respect the action of anti-Bp50 antibody resembles the activity of B-cell growth factor(s) (BCGF). As little as 0.05 μg of anti-Bp50 per ml is needed to augment proliferation and, like BCGF, anti-Bp50 is effective even when added 12–24 hr after B cells are activated with anti-immunoglobulin or anti-Bp35. Without additional exogenous signals, anti-Bp35 and anti-Bp50 together induce strong proliferation of purified resting B cells. These results suggest that the Bp35 and Bp50 surface molecules function in the regulatory control of B-cell activation and progression through the cell cycle.**

The activation of resting B cells from G_0 to G_1 phase of the cell cycle and the subsequent induction of activated B cells to proliferate are distinct steps requiring distinct regulatory mechanisms (1–6). Some agents, including murine B-cell-stimulating factor 1 (BSF-1) (7) or low doses of anti-immunoglobulin (5, 6, 8, 9), are "activation" or "competence" factors. That is, they induce B cells to enlarge, synthesize more RNA, and enter G_1, but alone they do not induce DNA synthesis in B cells. Other "growth" factors, such as human B-cell growth factor (BCGF) and interleukin 2 (IL-2) cause activated B cells to traverse the cell cycle and enter S phase but do not trigger resting B cells (2, 9–11).

These activation and growth signals presumably regulate B cells by interacting with specific B-cell surface structures. In addition to the antigen-specific signal through surface immunoglobulin (sIg), several other candidate B-cell surface polypeptides have been identified that may in some way function in the activation or growth of B cells. For instance, Subbarro and Mosier (12) found that a monoclonal antibody (mAb) to the murine B-cell antigen Lyb2 activates B cells, and evidence has been presented that Lyb2 may be the receptor for BSF-1 (13). Similarly, appropriate mAb (1F5) to a 35-kDa polypeptide, Bp35, activates human B cells from G_0 into G_1 (refs. 14 and 15; E.A.C. and G. Shu, unpublished results). Aggregated complement component C3d or antibodies to the 140-kDa C3d receptor, Bp140, cause proliferation of B cells that are T-cell dependent (16–18). BCGFs have been identified in both mouse and human (1–3). However, the receptors for these factors have not been isolated. Wang *et al.* (19) made a rabbit polyclonal antiserum that identified a 54-kDa glycosylated polypeptide (gp54) on human B cells and showed that the antiserum to gp54 induced tonsillar B cells to divide. Jung and Fu (20) isolated a mAb (AB-1) to a 55-kDa antigen restricted to activated B cells and found that it blocks BCGF-dependent proliferation. However, whether or not either anti-gp54 or AB-1 recognize the BCGF receptor is not known.

In this report, we describe a 50-kDa B-cell surface marker, Bp50, that apparently functions in B-cell proliferation but not in early B-cell activation. A mAb to Bp50, like BCGF, augments B-cell proliferation. Unlike anti-Bp35 mAb, which can induce resting B cells in G_0 to enter G_1, anti-Bp50 mAb does not activate resting B cells. Anti-Bp35 and anti-Bp50 mAbs together, without any additional exogenous signals, induce strong activation and proliferation of purified B cells.

METHODS

Cell Preparations. Mononuclear cells were isolated from normal or leukemic heparan-treated peripheral blood by Ficoll/Hypaque gradients (Pharmacia). Mononuclear cells were obtained from tonsillar tissues as described (14). T cells were depleted by *S*-(2-aminoethyl)isothiouronium bromide (AET)-treated sheep erythrocyte rosetting and Ficoll-Hypaque gradient separation. In some experiments, blood B cells were enriched by isolating nylon wool-adherent cells. Monocytes were removed by incubation on plastic petri dishes one or two times at 37°C for 45 min unless otherwise stated. Buoyant or dense tonsillar B-cell fractions were isolated by Percoll step gradients as described (14). Dense tonsillar B-cell preparations consistently had 95% sIg^+, $Bp35^+$ cells. Blood B-cell-enriched preparations had 60–85% sIg^+ cells.

Monoclonal Antibodies. The G28-5 antibody to Bp50 was generated by immunizing BALB/c mice with human erythrocyte-rosette-negative tonsillar lymphocytes and fusing immune spleen cells with the NS-1 myeloma (21, 22). Hybrid cell cultures secreting antibody reactive with tonsillar B cells and not with T cells were identified by the use of indirect immunofluorescence and analysis with a FACS IV (Becton Dickinson) fluorescence-activated cell sorter; cultures with antibody giving histogram patterns similar to known mAb to pan-B-cell markers (e.g., Bp35, see ref. 14) were cloned and selected for further study. The G28-5 clone produced an IgG1

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Abbreviations: BCGF, B-cell growth factor; IL-1 and IL-2, interleukins 1 and 2; mAb, monoclonal antibody.

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mAb that reacted only with normal or malignant B cells or B-cell lines. Other mAbs used in this study have been described in detail (14, 23–25). These include 1F5 (IgG2a) anti-Bp35 (14), HB10a (IgG2a) anti-HLA-DR, 2C3 (IgG1) anti-μ chain (23, 24), G19-4 (IgG1) anti-CD3 (25), FC-2 (IgG2a) anti-Fc receptor CD16 (25), and 9.6 (IgG2a) anti-CD2 (erythrocyte receptor) provided by P. Martin (Fred Hutchinson Cancer Center). The IgG1 mAbs were purified by precipitation with 45% saturated ammonium sulfate and DEAE-Sephacryl column chromatography, and the IgG2a mAbs were purified by the use of protein A-Sepharose columns. The 2C3 mAb to μ chains was conjugated to cyanogen bromide-activated Sepharose 4B (Pharmacia).

Fluorescein and Phycoerythrin Conjugations. Purified mAbs were either directly conjugated with fluorescein (green fluorescence), using fluorescein isothiocyanate (Molecular Probes, Plano, TX) as described by Goding (26), or conjugated to R-phycoerythrin (red fluorescence) by using *N*-succinimidyl 3-(2-pyridyldithio)propionate (Pharmacia) as detailed elsewhere (27). Lymphoid cells were incubated in round-bottom microwell plates for 30 min with an appropriate dilution of green and/or red mAb, washed twice, and then analyzed on a FACS IV cell sorter.

Two-Color Immunofluorescence. Two-color studies were done with a FACS IV fluorescence-activated cell sorter (Becton Dickinson) by using a 560-nm dichroic mirror to split the beam and a 580-nm long-pass filter and a 540-nm short-pass filter (Ditric Optics, Hudson, MA) in front of the red and green photomultiplier tubes, respectively. In addition, a two-color compensator (T. Nozaki, Stanford University) was used to correct for minor spillover of green and red signals. For each two-color stain, data from 40,000 cells were collected and stored on floppy disks. Data are presented as cell number (vertical) vs. log green fluorescence vs. log red fluorescence on a 64 × 64 dot grid. Approximately 4.5 dots represents a doubling of fluorescence. Our flow-cytometry system for two-color immunofluorescence with fluorescein and phycoerythrin is described in more detail elsewhere (25, 27).

Cell Culture. Blood or tonsillar lymphoid cells were cultured at 5–10 × 10^5 per ml in quadruplicate in 96-well plates; each well contained 200 μl of RPMI 1640 medium supplemented with 15% fetal bovine serum, antibiotics, glutamine, and pyruvate. After 2 or 3 days, cells were incubated with [*methyl*-^{3}H]thymidine (New England Nuclear, 6.7 Ci/mmol; 0.5 μCi per well; 1 Ci = 37 GBq) for 18 hr. Cells were then harvested onto glass-fiber filters with a cell harvester (PHD, Cambridge Technology), and radioactivity was measured in a scintillation counter. In some experiments, antibodies or factors were added at various times after the start of cultures; proliferation (i.e., thymidine incorporation) in these experiments was measured on day 3.

Costimulatory Factors. Partially purified BCGF was purchased from Cytokine Technology (Buffalo, NY) and contained no detectable IL-1, IL-2, or interferon activity. IL-1 purified to homogeneity (28) was the generous gift of S. Dower of Immunex. Recombinant IL-2 was kindly provided by Cetus Corporation.

Detection of Cell Activation. Changes in cell volume induced by mAb and/or factors were measured using a cell sorter and forward-angle light-scattering. Cell cycle changes in cellular RNA and DNA levels were measured by staining activated cells with acridine orange and measuring relative cellular RNA (red) and DNA (green) content with a cell sorter, by the method of Darzynkiewicz *et al.* (29). Changes in relative levels of cell surface antigens were monitored by use of mAb directly conjugated with fluorescein and then quantitated by direct immunofluorescence levels with an Epics V (Coulter) cell sorter.

Biochemical Characterization of Bp50. Immunoprecipitation of Bp50 from ^{125}I-surface-labeled tonsillar cells was performed as described (30). Isolated antigens were electrophoresed in $NaDodSO_4$/10% polyacrylamide slab gels without reduction. Labeled proteins were visualized by autoradiography at −70°C using Cronex Lightning Plus intensifying screens (DuPont).

RESULTS

Identification of a B-cell-Specific 50-kDa Cell Surface Marker, Bp50. A mAb to Bp50 was raised by immunizing BALB/c mice with human tonsillar lymphocytes and fusing immune spleen cells with the NS-1 myeloma. One clone, G28-5, produced an IgG1 mAb that did not contain the NS-1 light chain (data not shown). Upon immunofluorescence analysis, G28-5 was found to react only with normal or malignant B cells or B-cell lines. A comprehensive screening of normal tissues by established methods (14, 24, 25) revealed that the G28-5 antibody reacts with erythrocyte-rosette-negative (E_r^-) cells from blood or tonsils but not with nylon-wool-nonadherent T cells, with phytohemagglutinin (PHA)-induced T-cell blasts, or with blood granulocytes, monocytes, erythrocytes, or platelets. It reacted strongly with all seven B-lymphoblastoid cell lines tested and with three Burkitt lymphoma lines (Raji, Daudi, and Namalwa), but not with four T-cell lines (CEM, HSB-2, Jurkat, and HPB-ALL). All (3/3) chronic lymphocytic leukemias tested and 90% (9/10) of B lymphomas tested expressed the Bp50 marker, whereas only 28% (2/7) of non-T, non-B, common acute lymphoblastic leukemia antigen-positive ($CALLA^+$) acute lymphocytic leukemias expressed Bp50.

The restricted distribution of Bp50 on normal tissues was further confirmed by quantitative two-color analyses (25). Using an R-phycoerythrin-conjugated antibody (red) to the pan-B-cell antigen Bp35 (B1, CD20) and fluorescein-conjugated anti-Bp50 antibody (green), we found that Bp50 was expressed only on $Bp35^+$ B cells (Fig. 1) in blood or tonsils. Blood B cells consistently expressed somewhat lower levels of Bp50 than tonsillar B cells; this is similar to HLA-DR expression, which is also lower on blood B cells (24), and to gp54 expression (19). Bp50 was expressed at similar levels on

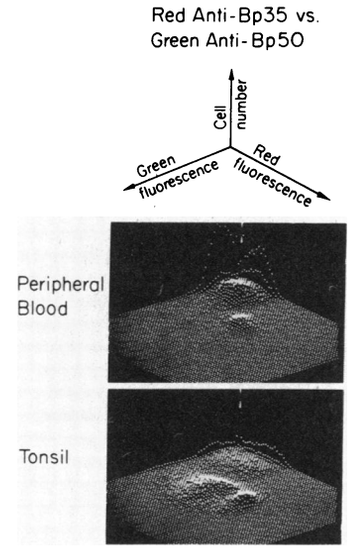

FIG. 1. Expression of Bp50 is restricted to $Bp35^+$ B cells. Two-color flow cytometric analysis of 50,000 cells was done as described (14). Data are plotted as cell number vs. log green fluorescence and log red fluorescence, where 4–5 dots represent approximately a doubling of fluorescence. The data are presented to show autofluorescent negative cells. Phycoerythrin-conjugated (red) anti-Bp35 (mAb 1F5) vs. fluorescein-conjugated (green) anti-Bp50 (mAb G28-5) staining shows that all $Bp50^+$ cells are also $Bp35^+$.

tonsillar B-cell subpopulations separated on Percoll gradients into buoyant and dense fractions (data not shown). Using our phycoerythrin-conjugated mAbs to the T-cell marker CD3 (T3) and the natural killer (NK) cell-associated marker CD16 (Fc receptor) (22), we found that Bp50 is not expressed on T cells or NK cells (J.A.L., G. Shu, M. Gallagher, and E.A.C., unpublished data). Using two-color immunofluorescence, we also found that $CD3^+$ phytohemagglutinin-induced blasts that expressed high levels of IL-2 receptors did not express Bp50.

The G28-5 antibody reacted with a single polypeptide on tonsillar lymphocytes that migrated at ≈50 kDa under nonreducing conditions (Fig. 2*A*). This molecule is larger than previously reported B-cell markers in the same molecular mass range such as Bp39 or Bp45 (31–35) (Fig. 2*B*). The exposure time for this gel was selected so that the molecular weights of the other B-cell markers could be readily compared with Bp50. The Bp39 marker, unlike Bp50, is expressed on granulocytes, and Bp45, unlike Bp50, is restricted to B-cell blasts. Antibodies to Bp39 (41-H16) and Bp45 (MNM6, Blast-1, and Blast-2), made available through an international workshop (33), did not block the binding of fluoresceinated anti-Bp50 antibodies to B cells (data not shown). Thus, based on tissue distribution, biochemical analysis, and blocking studies, the G28-5 antibody recognizes a 50-kDa structure distinct from other known B-cell antigens.

Augmentation of B-Cell Proliferation with Anti-Bp50 Antibody. B cells can be activated with low doses of μ-chain-specific antibodies (5, 6, 8, 9). The B-cell-specific marker Bp35 (CD20), a 35-kDa polypeptide, may also function in early B-cell activation: the 1F5 mAb to Bp35, like low doses of anti-μ antibody, activates B cells to increase in cell volume and RNA content and to become responsive to BCGF (14, 15). Therefore, it was of interest to compare the effect of anti-Bp50 mAb on the proliferation of untreated B cells and B cells activated with either anti-Bp35 or anti-μ antibodies (Table 1). Either anti-Bp35 in solution or anti-μ-Sepharose alone, under appropriate conditions, stimulated some B-cell proliferation; in contrast, anti-Bp50 antibodies alone did not stimulate proliferation. However, anti-Bp50 mAb augmented proliferation considerably when present along with anti-μ beads or with anti-Bp35. In this respect anti-Bp50 resembled BCGF (Table 1). Thus, it was important to determine whether anti-Bp50 and BCGF together could induce B-cell proliferation. As shown in Table 1, anti-Bp50 and BCGF together induced no proliferation but did augment proliferation of either anti-μ- or anti-Bp35-activated cells somewhat more than either stimulant alone. When used with anti-Bp50 without other signals, BCGF over a 3-log concentration range had no effect on proliferation of dense B cells even when anti-Bp50 was used at doses ranging from 0.1 to 10 μg/ml (data not shown).

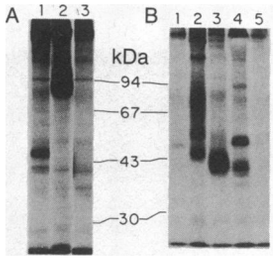

FIG. 2. Biochemical comparison of Bp50 polypeptide with other B-cell surface antigens. Immunoprecipitates from ^{125}I-surface-labeled tonsillar cells were electrophoresed in $NaDodSO_4$/10% polyacrylamide slab gels without reduction. Proteins were visualized by autoradiography with intensifying screens. (*A*) Lanes: 1, anti-Bp50 (G28-5); 2, anti-Bp95 (G28-8); 3, Sepharose-conjugated goat anti-mouse immunoglobulin only. Exposure time was 4 days. (*B*) Lanes: 1, anti-Bp50 (G28-5); 2, anti-Bp45 (Blast-2); 3, anti-Bp39 (G28-1); 4, anti-Bp39 (41-H16); 5, Sepharose-conjugated goat anti-mouse immunoglobulin only. An exposure time of 2 days was selected so that the bands in lanes 2–4 were not overexposed and could be clearly distinguished relative to Bp50. Experiment was repeated twice with similar results.

Table 1. Anti-Bp50 augments anti-Ig- or anti-Bp35-induced B-cell proliferation

Costimulant	[^{3}H]Thymidine incorporation, cpm (mean ± SEM, n = 4)		
	Medium	Anti-μ beads	Anti-Bp35
None	1212 ± 547	10,219 ± 462	5,539 ± 308
Anti-Bp50	719 ± 718	38,792 ± 1,329	25,465 ± 616
BCGF	456 ± 217	14,217 ± 445	9,443 ± 343
Anti-Bp50 + BCGF	1456 ± 126	54,393 ± 2,537	46,488 ± 3,387

Proliferation of dense tonsillar erythrocyte-rosette-negative B cells (>95% $sIgM^+$ cells) was measured on day 3 as described in *Methods*. Briefly, 2×10^5 cells per 200 μl per well were cultured in quadruplicate wells for 48 hr in medium without antibody or with either 2C3 monoclonal antibody to IgM μ heavy chains coupled to Sepharose ("anti-μ beads," 50 μg/ml) or free 1F5 anti-Bp35 antibody (5 μg/ml). BCGF (5% final concentration, Cytokine Technology, Buffalo, NY; has no detectable IL-1 or IL-2 activity) and/or anti-Bp50 (1:1000 dilution of ascites) were added as costimulants. After 48 hr, [^{3}H]thymidine was added, and incorporation was measured 18 hr later.

Anti-Bp50 mAb Augments Proliferation Only After B Cells Are Activated by Anti-Bp35 or Anti-μ. The results in Table 1 suggest that anti-Bp50 mAb could not induce proliferation by itself. As shown in Fig. 3, doses of anti-Bp50 ranging from 0.05 to 2.0 μg/ml had no effect on [^{3}H]thymidine incorporation. However, in the presence of optimal levels of anti-Bp35 mAb, as little as 0.1–0.5 μg of anti-Bp50 per ml augmented proliferation substantially (Fig. 3). Incorporation as high as 50,000–70,000 cpm was detected at the optimal time of proliferation when highly purified B cells were cultured only with anti-Bp35 plus anti-Bp50 (data not shown). A consistent observation was that higher concentrations of anti-Bp50 (2–5 μg/ml) were less effective than concentrations in the 100–200 ng range.

These results suggested that anti-Bp50 may function only after B cells are activated by other signals. Data shown in Fig. 4 suggest that this is indeed the case. If B cells were first activated with anti-Bp35, anti-Bp50 added as late as 24–48 hr

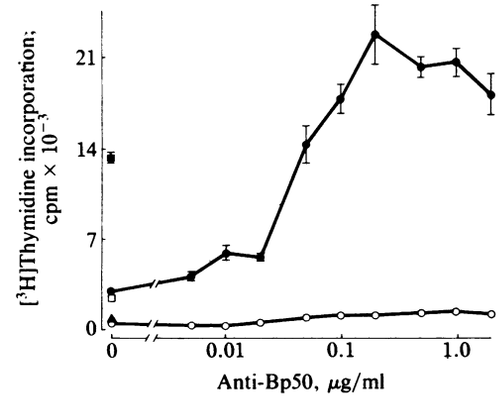

FIG. 3. Dose–response curves for augmentation of proliferation of dense tonsillar erythrocyte-rosette-negative B cells by anti-Bp50. B cells were cultured in medium without additions (○), or containing anti-Bp50 (mAb G28-5) (○), anti-Bp35 (5 μg/ml) (□), BCGF (▲), anti-Bp35 plus BCGF (■), or anti-Bp35 plus anti-Bp50 (●). [^{3}H]Thymidine incorporation (mean ± SEM of 4 cultures) was measured on day 3.

later still augmented proliferation measured at day 4. In contrast, when cells were first treated with anti-Bp50, anti-Bp35 was effective only if added within a few hours after the start of cultures. Similar results were found when anti-μ rather than anti-Bp35 was used (data not shown).

Anti-Bp50 mAb Does Not Activate B Cells to Move Out of G_0 But Does Induce Activated B Cells to Progress Through the Cell Cycle. Previous work showed that anti-Bp35, like low doses of anti-μ antibodies, induces resting tonsillar B cells in G_0 to enlarge (36) and to enter G_1 (15). Thus, it was of interest to compare anti-Bp50 mAb to anti-Bp35 mAb for their effects on B-cell activation. As shown in Fig. 5*A*, unstimulated dense tonsillar B cells, even after 3 days in culture, had a uniform RNA profile characteristic of cells in G_0 (29). However, about 15–30% of cells stimulated with anti-Bp35 or anti-μ had increased RNA content indicative of entry into G_1. In contrast, neither anti-Bp50 (Fig. 5*B*) nor BCGF (Fig. 5*C*) alone induced significant numbers of B cells to enter G_1. For instance, 2 days after activation, anti-Bp35 and anti-μ mAbs induced, respectively, 13.5% and 20.9% of tonsillar cells to enter G_1, whereas cells treated with only anti-Bp50 (2.7%) or BCGF (3.2%) remained at control levels (2.2%). However, when either anti-Bp50 or BCGF was added together with anti-Bp35 or anti-μ, the proportion of cells entering G_1 increased markedly. Similarly, anti-Bp50 and BCGF alone did not induce B cells to enter S phase (Table 2) but together with either anti-Bp35 or anti-μ did increase the number of S-phase cells 2- to 3-fold.

DISCUSSION

Previous studies have suggested that the factors involved in the induction of B cells from G_0 into G_1 are distinct from the factors or requirements for transit into S phase (3, 5–8). This model is based principally on studies showing that agents such as low doses of anti-immunoglobulin (5, 6, 8, 9), B-cell activation factors (7), or anti-Bp35 (15) alone have little or no effect on B-cell proliferation. Yet these same agents can drive B cells to a point in cell activation where they are susceptible to growth factors. In contrast, growth factors such as BCGF (9) or IL-2 (10, 11) alone have no effect on resting B cells but do augment growth of activated B cells.

The results of this study provide additional support for a model of distinct regulation of B-cell activation and growth steps. Here we have shown that activation and proliferation signals in human B cells may be transmitted through distinct cell surface structures. Although anti-Bp35 mAb activated B cells to enter G_1, the mAb alone induced little or no proliferation. Anti-Bp50 mAb had the opposite effect: it could not activate B cells but could, when added even as late as 12–24 hr after activation, induce B-cell growth.

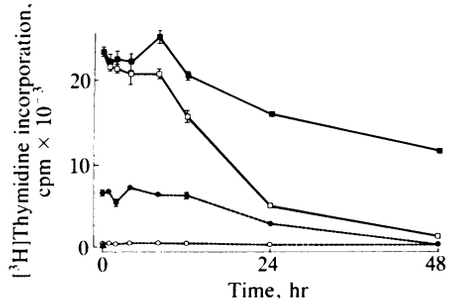

FIG. 4. Anti-Bp50 mAb is most effective at augmenting proliferation when added after a B-cell activation signal. Dense tonsillar erythrocyte-rosette-negative B cells were incubated for 4 days with medium only (▲), anti-Bp50 (0.5 μg/ml) added at different times (○), anti-Bp35 (5 μg/ml) added at different times (●), anti-Bp50 kept constant and anti-Bp35 added later at different times (□), or anti-Bp35 kept constant and anti-Bp50 added at different times (■). [³H]Thymidine was present during the last 10 hr. Data represent mean incorporation (±SEM) for four cultures.

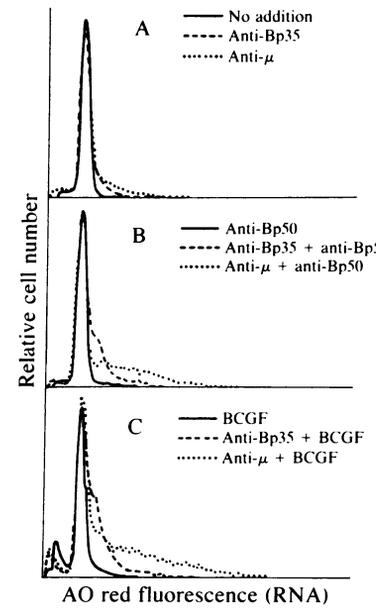

FIG. 5. Comparison of the ability of anti-Bp35 and anti-Bp50 to induce resting tonsillar B cells to leave the G_0 stage of the cell cycle. Cells were analyzed by flow cytometry after 3 days of incubation in medium with the indicated additions. Concentrations were as follows: anti-Bp35, 5 μg/ml; anti-μ (conjugated to Sepharose), 50 μg/ml; anti-Bp50, 5 μg/ml; BCGF preparation, 5% (vol/vol). Data are plotted as relative cell number vs. log of acridine orange (AO) red fluorescence (RNA).

The Bp50 molecule presumably could normally function as a receptor either for a soluble growth factor or for a signal mediated through cell–cell contact. Previous studies have identified several T cell-derived BCGFs that, like anti-Bp50, augment B-cell proliferation. Both high and low molecular weight forms of B-cell growth factors have been identified, and different types have been shown to have additive effects (2, 3, 37–39, 40). Thus, Bp50 might be a receptor for one of these factors. With the exception of IL-2 receptors (10, 11) and the C3d receptor (16–18), the receptors on B cells for growth signals have not yet been identified. The mAb AB-1 reacts with a B-cell marker expressed only on activated B cells (20) and blocks BCGF-dependent proliferation and thus might recognize the BCGF receptor or a related structure. Bp50 appears to be distinct from the AB-1 marker, since mAb

Table 2. Effect of anti-Bp50 and BCGF on cell cycle progression in tonsillar lymphocytes

Competence signal	Progression signal	% cells*		
		G_0	G_1	$S/G_2/M$
Medium	None	89.9	7.1	2.5
Anti-Bp35		80.4	14.5	3.7
Anti-Ig		65.6	27.6	5.7
Medium	Anti-Bp50	83.6	12.0	3.3
Anti-Bp35		54.1	35.5	9.7
Anti-Ig		43.6	36.2	16.2
Medium	BCGF	85.4	11.7	2.2
Anti-Bp35		56.6	32.6	11.6
Anti-Ig		48.4	36.1	14.1

Dense tonsillar lymphocytes (10^6) were incubated with anti-Bp35 (5 μg/ml), anti-μ conjugated to Sepharose (50 μg/ml), anti-Bp50 (0.4 μg/ml), BCGF (5%), or combinations as shown.
*Determined by the acridine orange staining procedure (29).

AB-1 does not block the binding of the G28-5 anti-Bp50 antibody (data not shown) and, unlike the G28-5 mAb, reacts only with activated B cells (20). Bp50 is on all B cells, which, based on adsorption analysis and direct binding assays, appears not to be the case for BCGF receptors (40, 41). As will be described in detail elsewhere, our current data suggest that Bp50 and the receptor for low molecular weight BCGF are distinct structures.

Using a rabbit polyclonal antiserum, Wang *et al.* (19) detected a 54-kDa glycoprotein, gp54, that like Bp50 is expressed on all B cells but at lower levels on blood B cells than tonsillar B cells (Fig. 1 and ref. 19). It is possible that the rabbit antiserum and anti-Bp50 recognize the same or related structures. However, unlike anti-Bp50 mAb, the rabbit antiserum to gp54 alone was sufficient to stimulate B-cell proliferation.

Anti-Bp35 alone, unlike anti-Bp50, can activate B cells from G_0 to G_1 and thus can be referred to as an "activation" signal (ref. 15; Fig. 5). Whether or not Bp35 functions only in early B-cell activation is not clear, since anti-Bp35 can stimulate some B cells to divide (ref. 14; Table 2). Similarly, Bp50 may not strictly function only as a "growth" signal: anti-Bp50 antibodies together with activation signals (anti-Bp35 or anti-μ) augment not only proliferation but also the total number of B cells entering G_1 (Table 2). In other words, anti-Bp50 as a costimulant acts to promote the progression of both the activation ($G_0 \rightarrow G_1$) and growth ($G_1 \rightarrow S$) phases of the cell cycle. The BCGF used in these studies had similar activity (Fig. 5*C*). Thus, anti-Bp35 and anti-Bp50 (or BCGF) appear to be most analogous to the "competence" and "progression" factors described in studies of fibroblast growth regulation (42, 43). How B cells respond to anti-Bp35 or anti-Bp50 clearly may depend on their state of differentiation or activation.

Here we have shown that two mAbs, anti-Bp35 (a "competence" signal) and anti-Bp50 (a "progression" signal), together can induce substantial proliferation of highly purified B cells in the absence of antigen or other known factors. The natural ligands for these structures are not known. However, since mAb to appropriate epitopes can mimic both soluble factors and signals mediated by cell–cell interactions, it may be possible to use appropriate combinations of mAb to direct and regulate human B-cell proliferation or differentiation. This work in turn may help in devising strategies *in vivo* for the control of human diseases such as B-cell malignancies, immunodeficiencies, and certain autoimmune diseases.

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