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Surface immunoglobulin ligands and cytokines differentially affect proliferation and antibody production by human CD5+ and CD5- B lymphocytes

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Abstract

Normal human peripheral blood B lymphocytes were separated into CD19+ CD5+ and CD19+ CD5- subsets by dual-color FACS sorting. In most experiments the cells were activated with *Staphylococcus aureus* Cowan I (SAC) and cultured in the absence or presence of recombinant human IL-1α, IL-2, or IL-6, or combinations of these cytokines. Unstimulated CD5+ and CD5- B cells showed a comparable, low level of incorporation of [^{3}H]thymidine into DNA. SAC stimulated proliferation of CD5+ and CD5- B cells, and this proliferation was augmented by IL-2 in the case of CD5- B cells. Anti-μ beads stimulated some proliferation of the CD5- subset and augmented SAC-induced proliferation of these cells. In contrast, anti-μ beads did not stimulate proliferation of the CD5+ subset and had no effect on SAC-induced proliferation of these cells. CD5+ B cells activated by anti-μ beads were stimulated to proliferate in the presence of IL-4, but not in the presence of IL-2. These observations support the interpretation that two signals are required for proliferation of CD5+ B cells. Using a two-step culture system, SAC activation itself did not induce Ig production by either subset of purified B cells. However, it primed the cells for antibody production in the presence of IL-2. IL-1 and IL-6 by themselves augmented antibody formation by these cells slightly, if at all. However, IL-6, and to a lesser extent IL-1, augmented antibody production in the presence of IL-2. Under the culture conditions used CD5- B cells produced IgM, IgG, and IgA whereas the CD5+ B cells produced almost exclusively IgM. The expression on B cells of surface activation markers was analyzed after culture for 2 days with SAC or anti-μ beads. In both subsets expression of Leu-23 and Leu-21 was increased, with some differences in intensity (Leu-23 greater in CD5+ cells, Leu-21 greater in CD5- cells). SAC increased IL-2R expression to a greater extent than anti-μ beads. In neither subset was expression of CD23 increased. These observations are discussed in the context of the possible role of the CD5+ subset of B lymphocytes as components of a system of natural immunity.

Introduction

CD5+ B lymphocytes have been well characterized in the mouse as 'Ly-1 B cells' (1). The CD4 molecule (a 67 kd glycoprotein) is expressed on all murine T lymphocytes as the Ly-1 surface antigen (2). This molecule is also detected in murine B cell tumors (3) and a minority of normal murine splenic B lymphocytes (4,5). Recent studies have suggested that CD5- and CD5+ B lymphocytes are developmentally and functionally distinct B cell lineages designated as the 'conventional' and 'Ly-1'

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lineages (1). Conventional CD5- B lymphocytes predominate in adult spleen and lymph nodes whereas CD5+ (Ly-1) B cells constitute 40 – 80% of the B lymphocytes in the peritoneal cavity and in the fetal spleen (6). Similar studies with human lymphocytes have revealed that the homologous cell surface molecule (Leu-1) is also expressed on all normal human T lymphocytes (7), on most B chronic lymphocytic leukemia (B-CLL) cells (8), and on a subpopulation of normal adult circulating (9) and splenic B lymphocytes (10). In humans, as in mice, 40 – 60% of fetal splenic B lymphocytes are CD5+ (Leu-1) B cells (11).

The CD5+ subset of B lymphocytes has attracted interest for three main reasons. First is its frequent involvement in B cell leukemias and lymphomas in both mice (3) and humans (8), which implies a relatively high susceptibility to malignant transformation. Second is the involvement of CD5+ B cells in autoimmunity. This was suggested by the increased frequencies of CD5+ B cells in strains of mice prone to develop autoimmune disease, including NZB, (NSB/NZW)F_1 and 'moth-eaten' mice (4,5). The absolute numbers of CD5+ B cells are somewhat increased in the peripheral blood of patients with rheumatoid arthritis (RA) (9,12), and further increased in their synovial fluid but not in osteoarthritic synovial fluid (13). The CD5+ subset of B cells produces several varieties of IgM with specificity for autoantigens. This was first shown for antibodies against thymocytes and bromelain-treated mouse erythrocytes (14), which were later found to have anti-phosphatidylcholine specificity (15,16). IgM rheumatoid factor (RF, with anti-γ specificity) is mainly produced by the CD5+ subset of B lymphocytes (9,17,18). This is also true of RF with relatively high affinity for IgG, which is found in patients with RA but not in normal humans (18). Other autoantibodies produced by CD5+ B cells include Igs reacting with single-stranded DNA and thyroglobulin; the former are selectively increased in humans with systemic lupus erythematosus and the latter in humans with Hashimoto's thyroiditis (19). Hence CD5+ B cells are certainly involved in autoimmune responses; whether they contribute to the pathogenesis of autoimmune disease is still uncertain.

The third reason why CD5+ B lymphocytes have attracted attention is because of their possible involvement in 'natural immunity' as opposed to 'acquired immunity' (1,19,20). Antibodies produced by CD5+ B cells can react with a variety of microbial antigens and can provide protection against infections. Examples of such antibodies are those with specificities for phosphatidylcholine (15,16), a common component of eukaryotic membranes, including those of fungi and protozoa; and the bacterial antigens lipopolysaccharide (21), α-1,3-dextran (22), and phosphorylcholine (A.M.S., unpublished data). RF-like antiglobulins can amplify immune responses, for instance augmenting neutralization of Herpes simplex virus (23), and can protect newborn rats from infection with *Trypanosoma lewisi* (24). The CD5+ subset is strategically distributed in the body so as to provide a first line of defense against infections, being present, for example, in tonsils (25), intestinal epithelium (26), and the peritoneal cavity (1).

For all these reasons it is important to define factors regulating major functions of the CD5+ subset of human B cells: their proliferation and their differentiation into antibody-forming cells. A great deal of information has accumulated about these responses in the conventional subset of CD5- B lymphocytes, and there is some information on proliferation of, and antibody formation by, CD5+ B-CLL cells in humans and mice (27 – 29). However, the only publication on normal human CD5+ B cells of which we are aware is a report of antibody formation by cells stimulated with pokeweed mitogen (PWM) (30).

We have compared responses of FACS-sorted CD5+ and CD5- B cells from normal human peripheral blood activated by *Staphylococcus auereus* Cowan I (SAC) or beads coated with anti-μ Ig and cultured with or without recombinant human IL-1α, IL-2, IL-4, and IL-6 alone or in combination. Both SAC (containing protein A) and anti-μ react with surface Ig, mimicking interaction with antigen and providing a more physiological stimulus than do lectin mitogens. The observations reported in this paper show similarities between responses of CD5+ and CD5- B cells prepared from the same blood samples and similarly activated and cultured. For example, both are stimulated by SAC to proliferate and, following activation by SAC, to produce Ig in the presence of IL-2. However, there are also major differences. In general, SAC or anti-μ activation increases expression of the same activation markers on the two subsets of B cells, although there are quantitative differences in levels of expression. These observations are discussed in relation to natural immunity.

Methods

Reagents

Human recombinant IL-2 was from Hoffman-La Roche (Nutley, NJ). Human recombinant IL-6 was from Genetics Institute (Cambridge, MA). Human recombinant IL-1α was from Immunex Corporation (Seattle, WA). Human recombinant IL-4 and polyclonal rabbit anti-human rIL-2 antibody were purchased from Genzyme (Boston, MA). Insolubilized rabbit anti-human IgM antibody was purchased from Bio-Rad Laboratories (Richmond, CA). Formalinized SAC was purchased as Pansorbin from Calbiochem-Behring Corporation (San Diego, CA) and used at a final concentration of 0.001% (v/v). Phorbol myristate acetate (PMA; Sigma Chemical Co., St Louis, MO) was dissolved in DMSO and diluted to a final concentration of 10 ng/ml. For B cell cultures RPMI 1640 (GIBCO, Grand Island, NY) was supplemented with penicillin G (100 IU/ml), streptomycin (50 μg/ml), L-glutamine (0.3 mg/ml), and 10% heat-inactivated (56°C, 40 min) FCS (Hyclone, Logan, UT).

PBMC preparation

PBMC were isolated from heparinized peripheral blood of normal healthy volunteers using Ficoll-Paque (Pharmacia, Piscaway, NJ) gradient centrifugation. T lymphocytes were eliminated by rosetting the PBMC with 2-aminoethylisothiouronium bromide (Sigma)-treated SRBC (31). The T lymphocyte-depleted mononuclear cell population (T-depleted PBMC) routinely contained 40 – 50% B lymphocytes (CD19+ or CD20+), 30 – 40% monocytes (CD14+), and 10 – 20% other cells including NK cells (CD56+) and null cells, and <3% T lymphocytes (CD2+) by FACS analysis. This population was used for sorting B cell subsets. To control for effects of staining, in some experiments responses of PBMC depleted of T cells were either stained with the same antibodies or left unstained.

Surface marker analysis

The mAbs conjugated either with FITC or phycoerythrin (PE), used for sorting and phenotyping the B cell preparations, were

purchased from the following manufacturers: Becton-Dickinson Monoclonal Center (Mountain View, CA): Leu-1 (CD5), Leu-12 (CD19), Leu-16 (CD20), Leu-M3 (CD14), IL-2R (CD25), Leu-20 (CD23), Leu-21, and Leu-23; Coulter Immunology (Hialeah, FA): irrelevant mouse IgG1, mouse IgG2a and NKH1 (CD56).

Flow cytometry and cell sorting

Dual-color flow cytometric analysis and cell sorting were performed on a FACStar plus cell sorter (Becton-Dickinson, Palo Alto, CA) (32). T-depleted PBMC were washed with HBSS and suspended in RPMI 1640 at a concentration of 5×10^6/ml. For staining and sorting of CD5+ and CD5− B cell subsets, a combination of FITC-conjugated anti-Leu-1 (CD5) and PE-conjugated anti-Leu-12 (CD19) mAbs were used. In some experiments, a combination of allophycocyanin-conjugated anti-CD5 and FITC-conjugated anti-μ mAbs were used. After incubation on ice for 20 min, stained cells were centrifuged with a heat-inactivated FCS underlayer to remove dead cells and free mAbs. Stained cells were washed twice with cold HBSS (without phenol red) containing 5% FCS and suspended at a concentration of 10×10^6/ml. The stained population was sorted into CD19+ CD5+ and CD19+ CD5− B cell populations using the gates shown in Fig. 1 (alternatively μ+ CD5+ and μ+ CD5− populations were used). Highly granular or large cells were also gated out using side- and forward-scatter gating. Immediately after sorting, the purity of recovered B cells was analyzed. Each population contained >98% pure CD5+ or CD5− B cell subsets and <1% T cells (CD2+ or CD5+) and other cells (Fig. 1).

B cell cultures

Sorted B lymphocytes were washed and suspended in 10% FCS RMPI 1640 at a concentration of 3×10^5/ml and cultured in triplicate wells (200 μl/well) using 96-well V-bottomed microculture plates (Flow Laboratories, Irvine, UK). For most of the experiments a two-step culture system was used. For the first 3 days B lymphocytes were preactivated by a relatively low concentration (0.001%) of SAC. On day 3, to remove SAC and Igs produced during the preculture period, activated B lymphocytes were washed, resuspended in SAC-free fresh medium, and recultured for an additional 7 days in the presence of combinations of cytokines (IL-1α, IL-2, and IL-6). In some experiments the B lymphocytes were cultured continuously for 7 days with or without SAC stimulation. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2 in air. At the end of the culture period, plates were centrifuged and culture supernatants were collected. The amounts of immunoglobulins (IgM, IgG, and IgA) produced during the culture period were measured by an isotype-specific ELISA immediately after harvesting (see below). Activity of IL-2 was inhibited by pre-incubation for 4 h at 37°C with a rabbit anti-human rIL-2 antibody.

Proliferation assay

Some 3×10^4 purified B cells or 5×10^4 T-depleted PBMC were cultured in 200 μl of 10% FCS RPMI 1640 per well with SAC, anti-μ beads, IL-2, IL-4, or IL-6, or combinations of cytokines in flat-bottomed 96-well microculture plates (Costar, Cambridge, MA). To measure tritiated thymidine ([^{3}H]TdR) (Dupont-New England Nuclear, Boston, MA) incorporation, the cells were pulsed by addition of [^{3}H]TdR to the cultures (1 μCi/well) during the final 16 h. After 60 h culture, cells were harvested and assayed for [^{3}H]TdR incorporation by standard liquid scintillation counting in triplicate cultures.

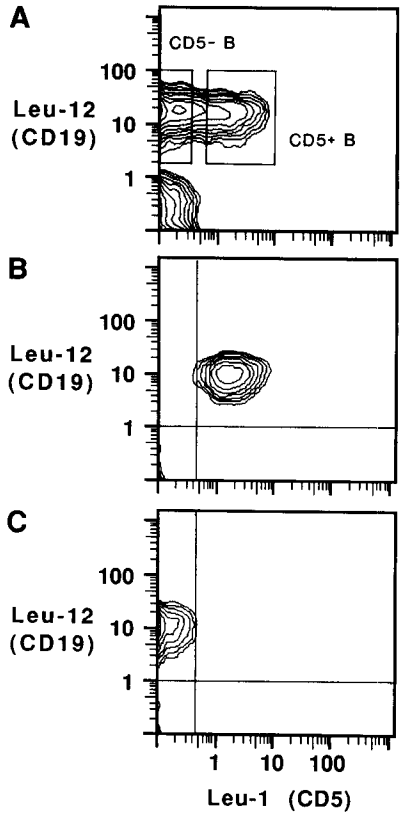

Fig. 1. Two-color FACS analysis and sorting of the CD5+ and CD5− B cell populations from normal human peripheral blood B lymphocytes. T-depleted PBMC were prepared and stained by PE-anti-CD5 mAb and FITC-anti-CD19 mAb as described in Methods. FACS results are presented as 5% probability plots. (A) Gates used for sorting CD5+ and CD5− B cells are indicated by boxes. (B and C) After sorting, each population (B, CD5+; C, CD5−) was analyzed. The lines were used to define the frequency of contaminating cells. The purity of each population was >98%.

Isotype-specific ELISA

The amounts of immunoglobulins (IgG, IgM, and IgA) produced in the culture supernatants were determined by an isotype-specific ELISA. Briefly, 96-well PVC microtiter plates (Costar) were coated with 100 μl of 20 μg/ml of affinity-purified goat anti-human IgG, IgM, or IgA antibodies (Southern Biotechnology, Birmingham, AL) in PBS overnight at 4°C. Wells were blocked by 5% non-fat dry milk (NFDM) PBS for 2 h. After washing with 0.1% BSA – PBS, 100 μl of standards [serial dilutions of human IgG, IgM, and IgA (Tago Diagnostics Inc., Burlingame, CA) in 1% NFDM – PBS] were added to each plate in triplicate. Appropriately diluted culture supernatants were also applied to the plates in triplicate so that sets of CD5+ and CD5− B cell culture supernatants could be assayed in parallel on the same ELISA plate. After 3 h incubation at room temperature, the plates were washed and 100 μl of diluted affinity-purified goat anti-human IgG, IgM, and IgA antibodies conjugated with biotin

(Zymed Laboratories Inc., South San Francisco, CA) were added and incubated for 1 h at room temperature. After washing, 100 μl of diluted streptavidin-alkaline phosphatase (Zymed) was added. After 1 h at room temperature, plates were washed and 100 μl of *p*-nitrophenolphosphate in 0.1 M 2,2-amino-2-methyl-1,3-propandiol buffer, pH 10.3, was added. Plates were kept at room temperature, and the OD was measured at 405/650 nm by using a V_{max} kinetic microplate reader (Molecular Devices, Palo Alto, CA). The concentration of each immunoglobulin in culture supernatants was expressed as a mean concentration (ng/ml or μg/ml) ± SD in triplicate samples of parallel culture supernatants.

Results

Sorting of CD5+ and CD5- B cell populations from normal peripheral blood

The B lymphocyte-enriched population, after depletion of T lymphocytes by AET rosetting, routinely was comprised of 40 – 50% B lymphocytes, 30 – 40% monocytes, and 10 – 20% other cells by FACS analysis. The T-depleted PBMC, stained with PE-conjugated anti-CD19 (Leu-12) and FITC-conjugated anti-CD5 (Leu-1) mAbs, were sorted into CD19+ CD5+ or CD19+ CD5- B cell populations by dual-color FACS sorting using the gates indicated in Fig. 1(A). Typical contour maps of sorted CD5+ and CD5- B cell populations are shown in Fig 1(B and C respectively). The purity of B lymphocytes in the sorted population was >98%, and <1% of T lymphocytes (CD2+ or CD5+) and other cell types was detected. The percentage of CD5+ B cells in peripheral blood varied in different subjects in the range 10 – 40% of B lymphocytes.

Proliferative responses of CD5+ and CD5- B lymphocytes

As shown in Fig. 2, FACS-sorted CD5+ and CD5- B cells were co-cultured with SAC, insolubilized anti-μ beads (10 μg/ml), or a combination of both for 60 h. B lymphocyte proliferation was monitored by [^{3}H]TdR incorporation. There was a low level of spontaneous proliferation of CD5+ and CD5- B cells, with no obvious difference between the subsets. A low concentration of SAC (0.001%) stimulated [^{3}H]TdR uptake in both B cell subsets, but to higher levels in CD5- B cells. Anti-μ beads stimulated proliferation of CD5- cells but not CD5+ cells. When the two activators were added together, anti-μ beads augmented SAC-induced [^{3}H]TdR incorporation in CD5- B cells. In contrast, anti-μ beads had no effect on CD5+ B cells or actually antagonized SAC-induced [^{3}H]TdR uptake. The same findings were also made using CD5+ and CD5- populations of either CD19+ or CD20+ B lymphocytes sorted from the same PBMC preparation (data not shown). Since sorting would deplete the CD5+ and CD5- B cells of accessory cells, the findings in Fig. 2 suggest that SAC is a mitogen acting directly on the two subsets of B cells, while anti-μ beads have direct mitogenic activity on CD5- B cells. In contrast, in the presence of anti-μ beads CD5+ cells require a co-factor for proliferation.

Two co-factors showed activity in our system. IL-4 was used because of its well known B cell stimulatory activity. It is reported to augment proliferation of human B lymphocytes activated with insolubilized anti-μ (33), although this does not appear to be the case with soluble anti-μ (34). As shown in Fig. 3, IL-4 markedly increased the proliferation of both CD5+ and CD5- cells activated by anti-μ beads.

The second lymphokine stimulating proliferation was IL-2. As shown in Figs 3 and 4, IL-2 was a co-stimulator of the proliferation of CD5- B cells activated by anti-μ beads or SAC; the dose dependence of this effect is illustrated in Fig. 4. In contrast, IL-2 had little, if any, effect on the proliferation of CD5+ B cells activated by either anti-μ beads (Fig. 3) or SAC (Fig. 4).

Cytokine-induced immunoglobulin production by CD5+ and CD5- B cells

FACS-sorted CD5+ and CD5- B cells from normal peripheral blood were cultured in 10% FCS – RPMI 1640 using microculture

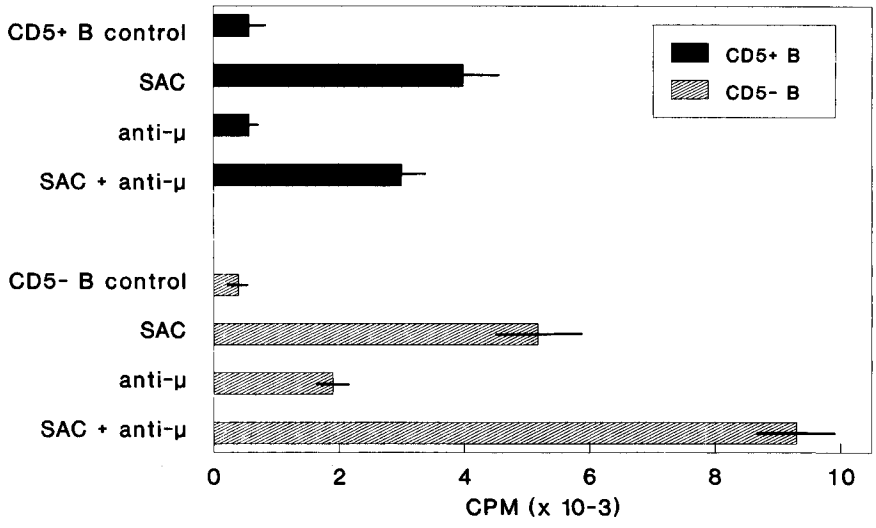

Fig. 2. The proliferative responses of CD5+ and CD5- B cells from normal subjects to SAC or anti-μ beads. 3 × 10^4 CD5+ (solid bar) and CD5- (shaded bar) B cells from a healthy subject were seeded in triplicate microcultures with SAC (0.001%) or anti-μ beads (10 μg/ml). 1 μCi of [^{3}H]TdR was added during the last 16 h of the 60 h incubation and the degree of cellular incorporation was measured. Arithmetic means ± SD of four experiments are shown.

plates. To investigate the effects of cytokines on differentiation of B lymphocytes into antibody-forming cells, we used a two-step culture system. During the first 3 days, B lymphocytes (3×10^4/well) were preactivated using a relatively low concentration (0.001%) of SAC as a polyclonal B cell activator. SAC can activate resting B lymphocytes to enter the cell cycle through binding to surface Ig receptors (35). On day 3, to remove SAC and Igs secreted during the first culture period, SAC-preactivated B lymphocytes were washed and resuspended in SAC-free fresh medium. Then SAC-preactivated B lymphocytes were recultured for an additional 7 days in the presence of IL-1α, IL-2, or IL-6, or combinations thereof. The amounts of IgM, IgG, and IgA secreted into culture supernatants during the second culture period were determined by an isotype-specific ELISA. The low concentration of SAC used was sufficient to pre-activate both CD5+ and CD5− B cells, allowing analysis of the effects of lymphokines on antibody production.

Figure 5 and Table 1 show the results of cytokine-induced IgM, IgG, and IgA secretion by CD5+ or CD5− B cells using two-step cultures. Amounts of Igs ranging from 10 ng/ml to 10 μg/ml were produced during the second culture period by the B lymphocyte subsets. Although the levels of secreted Igs varied from donor to donor, similar observations were repeatedly obtained. The main finding in relation to isotypes is that SAC-activated CD5−

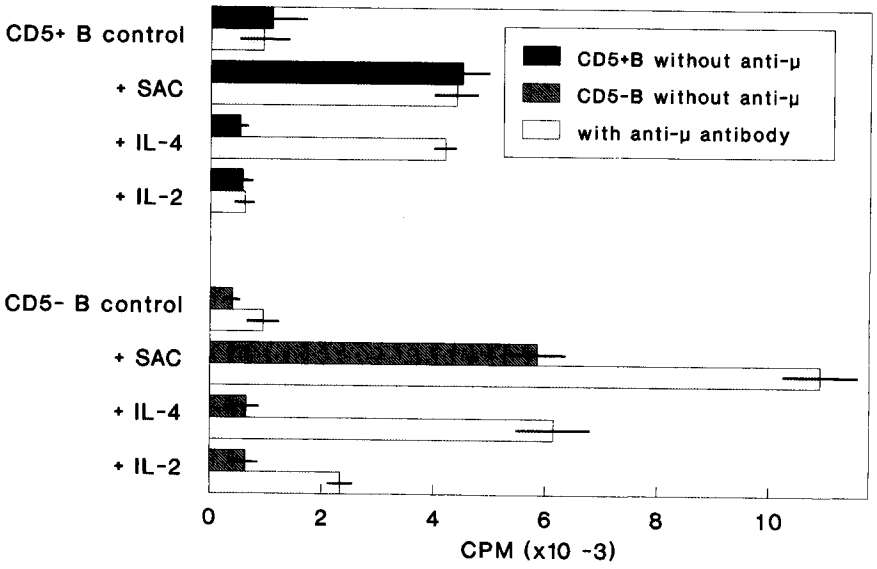

Fig. 3. The effect of rhIL-4 on CD5+ and CD5− B cell proliferation when co-cultured with anti-μ beads. CD5+ (solid bar) and CD5− B cells (shaded bar) were cultured without anti-μ. The open bar represents responses with anti-μ beads (10 μg/ml). 0.001%, SAC, 500 U/ml rhIL-4, and 10 U/ml rhIL-2 were used. Arithmetic means ± SD of triplicate samples are shown.

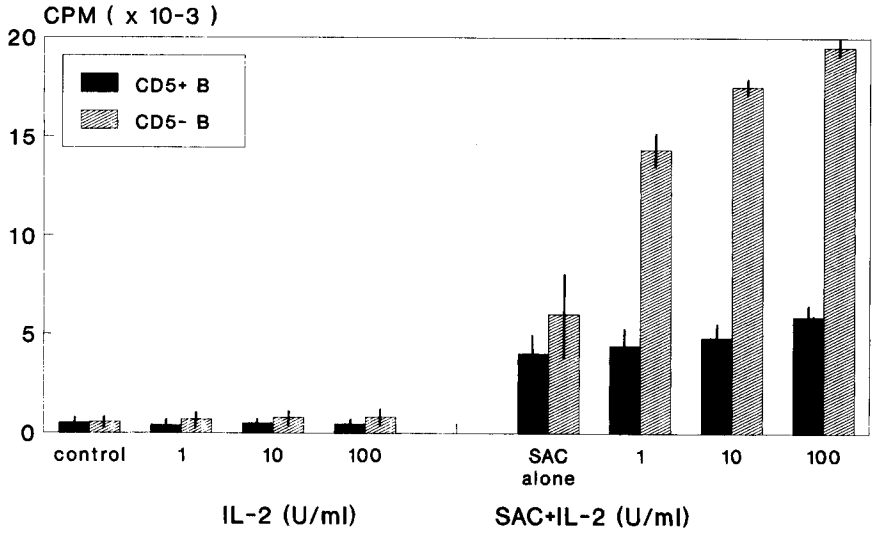

Fig. 4. The different proliferative responses to IL-2 of CD5+ and CD5− B cells. CD5+ (solid bar) and CD5− (shaded bar) were cultured with SAC (0.001%) or rhIL-2 for 60 h. Arithmetic means ± SD of triplicate samples are shown.

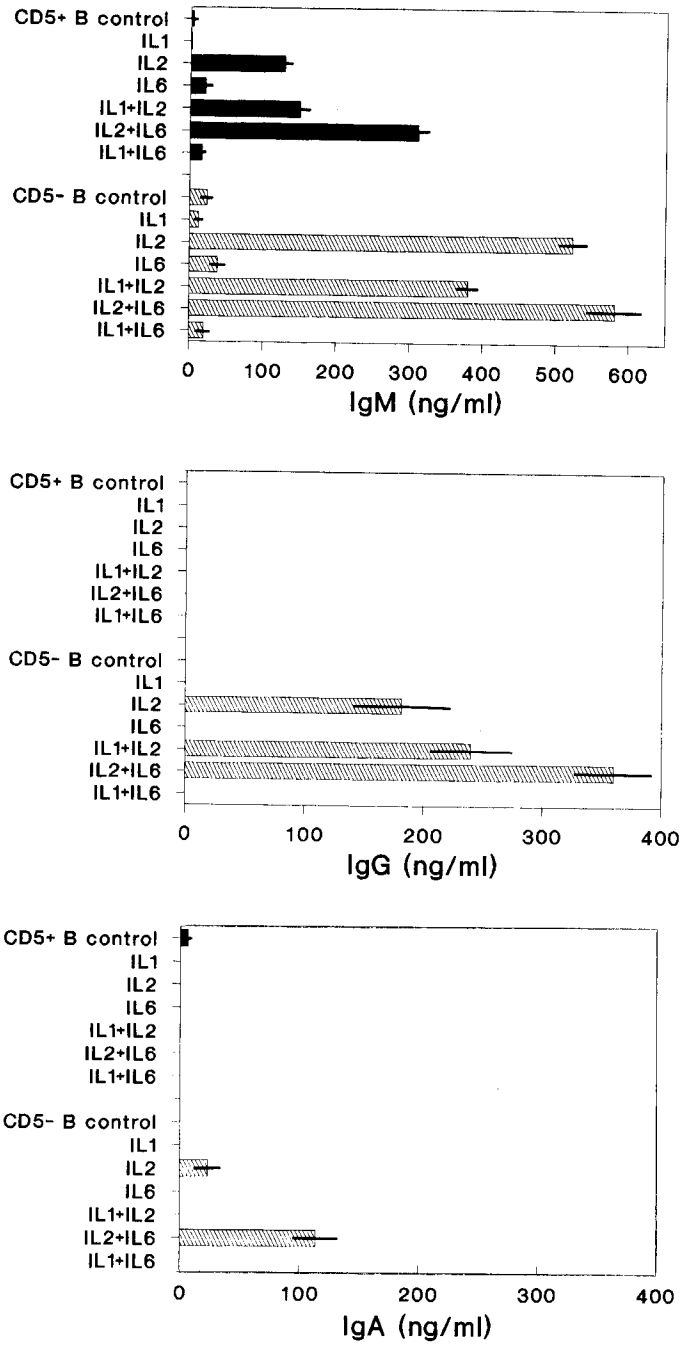

Fig. 5. Lymphokine-induced secretion of IgM, IgG, and IgA by SAC-preactivated CD5+ and CD5- B cells from normal human peripheral blood. 3 × 10^4 sorted CD5+ (solid bar) or CD5- (shaded bar) B cells were cultured using a two-step culture as described in Methods. SAC-preactivated B cells were cultured for 7 days with lymphokines as indicated: rhIL-1α (20 ng/ml), rhIL-2 (10 U/ml), and rhIL-6 (400 U/ml). Each bar shows secreted Igs (ng/ml) during the second culture period. The arithmetic means ± SD of triplicate samples are shown. Representative results from seven subjects are presented.

Table 1. Lymphokine-induced IgM, IgG, and IgA secretion by SAC-preactivated CD5+ and CD5- B cells from normal human peripheral blood using a two-step culture

Experiment no.	ILs[a]	IgM (ng/ml)[b]	IgG (ng/ml)	IgA (ng/ml)
CD5+ B cells[c]				
1	(-)	nd[d]	nd	nd
	IL-1	nd	nd	nd
	IL-2	129 ± 10	nd	nd
	IL-6	20 ± 9	nd	nd
	IL-1 + IL-2	150 ± 11	nd	nd
	IL-2 + IL-6	311 ± 17	nd	nd
	IL-1 + IL-6	16 ± 8	nd	nd
2	(-)	194 ± 24	nd	34 ± 15
	IL-1	154 ± 23	nd	nd
	IL-2	411 ± 27	nd	nd
	IL-6	304 ± 24	nd	10 ± 8
	IL-1 + IL-2	651 ± 39	nd	nd
	IL-2 + IL-6	881 ± 18	22 ± 6	nd
	IL-1 + IL-6	240 ± 18	nd	nd
3	(-)	83 ± 8	14 ± 8	nd
	IL-2	370 ± 20	20 ± 8	nd
	IL-6	291 ± 16	29 ± 9	nd
	IL-2 + IL-6	730 ± 36	86 ± 17	nd
4	(-)	6 ± 1	nd	14 ± 8
	IL-2	21 ± 5	nd	nd
	IL-6	5 ± 1	nd	4 ± 3
	IL-2 + IL-6	59 ± 4	nd	nd
CD5- B cells				
1	(-)	24 ± 7	nd	nd
	IL-1	12 ± 7	nd	nd
	IL-2	504 ± 27	181 ± 51	23 ± 38
	IL-6	38 ± 8	nd	nd
	IL-1 + IL-2	380 ± 19	239 ± 52	nd
	IL-2 + IL-6	582 ± 27	360 ± 55	114 ± 29
	IL-1 + IL-6	19 ± 8	nd	nd
2	(-)	264 ± 24	770 ± 34	nd
	IL-1	250 ± 32	1050 ± 88	nd
	IL-2	673 ± 32	1700 ± 85	nd
	IL-6	935 ± 43	1520 ± 92	74 ± 43
	IL-1 + IL-2	799 ± 43	3530 ± 225	306 ± 37
	IL-2 + IL-6	2070 ± 130	4880 ± 340	520 ± 34
	IL-1 + IL-2	296 ± 18	1785 ± 98	79 ± 19
3	(-)	91 ± 9	1140 ± 105	45 ± 15
	IL-2	699 ± 35	1820 ± 120	220 ± 23
	IL-6	205 ± 13	1700 ± 138	125 ± 27
	IL-2 + IL-6	1540 ± 79	2310 ± 154	591 ± 37
4	(-)	4 ± 1	nd	23 ± 15
	IL-2	67 ± 8	48 ± 14	nd
	IL-6	11 ± 5	nd	13 ± 4
	IL-2 + IL-6	167 ± 8	220 ± 24	57 ± 34

[a]20 ng/ml rhIL-1, 10 U/ml rhIL-2, 400 U/ml rhIL-6 were used.
[b]Arithmetic means ± SD of triplicate samples are shown.
[c]Normal PBL-derived CD5+ and CD5- B cells were precultured with SAC for 3 days and recultured with lymphokines as indicated for 7 days. CD19+ B cells were used in experiments 1-3, sIGM+ B cells were used in experiment 4.
[d]nd, not detected; in experiments 1-3: IgM (<5 ng/ml), IgG (<8 ng/ml), and IgA (<8 ng/ml), and in experiment 4: IgM (<2 ng/ml), IgG (<2 ng/ml), and IgA (<2 ng/ml).

B cells produced considerable amounts of IgM, IgG, and IgA following stimulation with cytokines, whereas SAC-activated CD5+ B cells produced almost exclusively IgM under the conditions used. The levels of IgM produced were higher in CD5- than CD5+ B cells. The small amounts of IgG and IgA produced by the CD5+ population in some experiments may have been due to the presence of a low level of contamination

with CD5- cells. Increased levels of IgG production by CD5- B cells were also observed in those experiments using surface IgM-sorted B lymphocytes as well as CD19+ B lymphocytes (Table 1, exp. 4). Thus the preferential production of IgG by CD5- B cells represents an innate property of this population and is not the result of stimulation of contaminating IgG+ cells within the sorted fraction. For comparison with SAC we examined the effect of PMA, which is reported to activate B cells through stimulation of protein kinase C (36,37). When sorted CD5+ or CD5- B cells were cultured with PMA and cytokines, IgG was produced only by CD5- B cells (data not shown). A second major finding is that IL-2 alone induced considerable levels of Ig production in both CD5+ and CD5- B cell populations. In addition, IL-6 showed synergistic effects with IL-2 on Ig production in both populations. The observations were similar when FACS-sorted CD19+ total B cells were cultured with the same cytokines (Table 2).

Table 2. IgM, IgG, and IgA secretion by total B lymphocytes[a]

ILs[b]	IgM (ng/ml)[c]	IgG (ng/ml)	IgA (ng/ml)
control	223 ± 43[d]	312 ± 23	nd[d]
IL-1 (1)	379 ± 32	372 ± 21	nd
IL-2 (10)	612 ± 39	646 ± 36	nd
IL-6	233 ± 43	728 ± 33	nd
IL-6 + IL-2 (1)	400 ± 43	1503 ± 60	nd
IL-6 + IL-2 (10)	1748 ± 83	2538 ± 115	121 ± 80

[a]FACS-sorted CD19+ total B cells in two-step cultures.
[b]rhIL-2: 1 and 10 U/ml; rhIL-6: 400 U/ml were used.
[c]Arithmetic means ± SD of triplicated samples are shown.
[d]nd, not detected.

To investigate further dose – response effects of these cytokines on antibody formation by CD5+ and CD5- B cells, combinations of different cytokine concentrations were used. Figure 6 shows findings obtained with combinations of IL-2 and IL-6. As before, SAC-preactivated CD5+ B cells exclusively produced antibody of the IgM class at all concentrations of IL-2 and IL-6 tested, whereas CD5- B cells produced relatively high

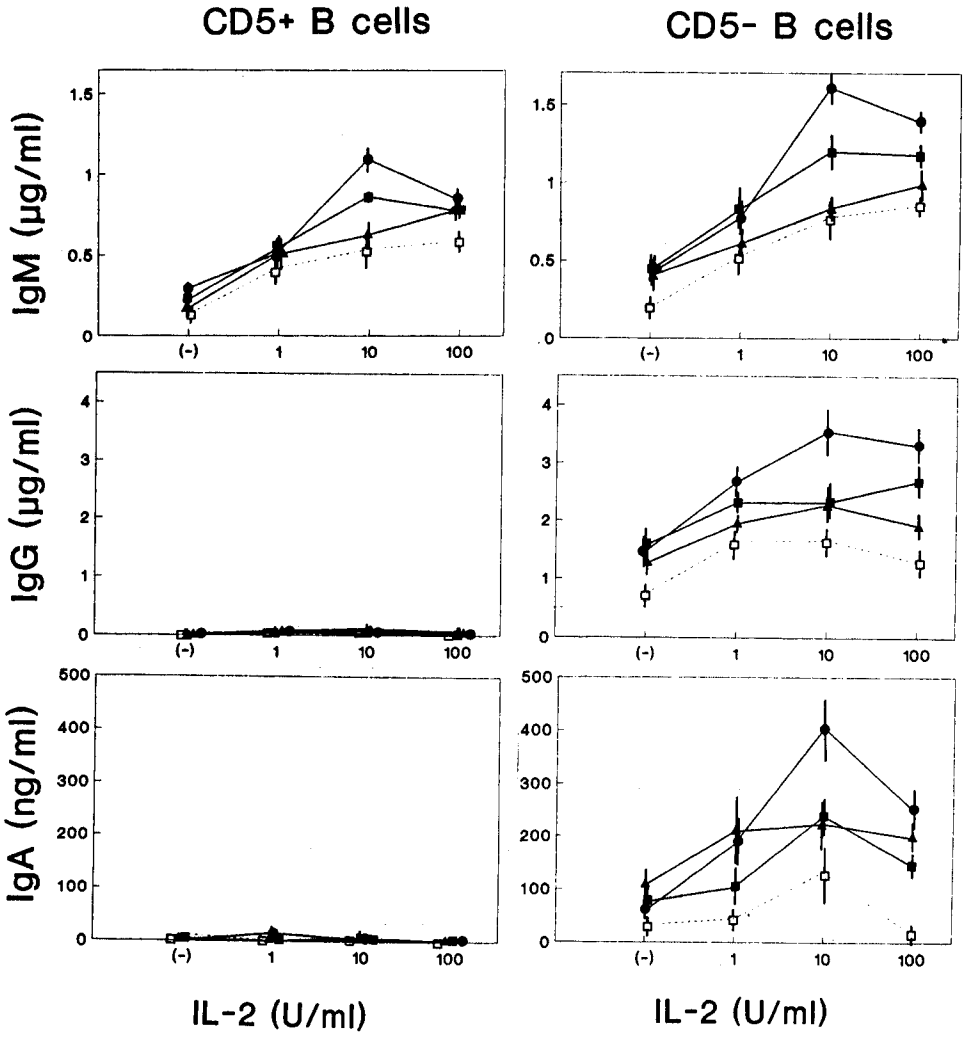

Fig. 6. Dose – response curves of rhIL-2 and rhIL-6 on IgM, IgG, and IgA secretion by CD5+ and CD5- B cells. SAC-preactivated B cells were cultured for 7 days with lymphokines as indicated. Ig production (ng/ml or 4mg/ml) by CD5+ and CD5- B cells are shown as the arithmetic means ± SD of four experiments. (□) control; IL-6: (▲) 4 U/ml; (■) 40 U/ml; (●) 400 U/ml.

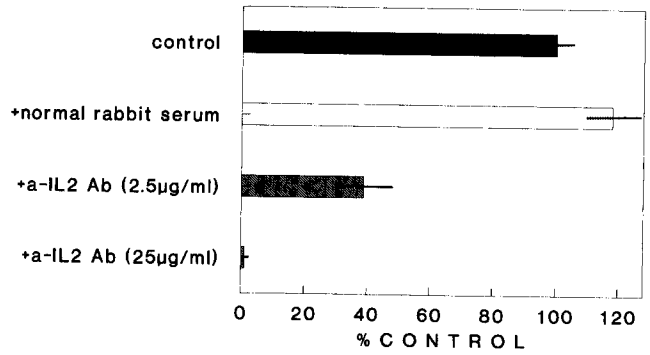

Fig. 7. Control of the specificity of IL-2 stimulation of IgM secretion by CD5+ B cells. Sorted CD5+ cells were cultured with rIL-2 (2 U/ml). Induced IgM production was completely inhibited by a rabbit anti-human rhIL-2 antibody but not by normal rabbit serum containing equivalent Ig. Results are shown as a percentage of control: calculated using rhIL-2-induced IgM as 100%.

levels of IgM, IgG, and IgA. IL-2 by itself induced considerable amounts of IgM production in both SAC-preactivated B cell subpopulations. As shown in Fig. 7, IL-2-induced IgM production by SAC-activated CD5+ B cells was completely inhibited by a rabbit anti-rIL-2 antibody, showing the specificity of IL-2 augmentation of IgM production. Although IL-6 by itself induced only low levels of Ig in both subsets, it augmented IL-2-induced IgM production in a dose-dependent fashion in both CD5+ and CD5- B cells. Maximal Ig production was observed using the combination of 10 U/ml of IL-2 with 400 U/ml of IL-6 (Fig. 6).

IL-1α alone (0.2, 2, or 20 ng/ml), or in combination with IL-6 (4, 40, or 400 U/ml) did not induce Ig secretion from SAC-preactivated sorted B cells (data not shown). However, in several experiments IL-1α augmented IL-2-induced Ig production. As shown in Table 3, IL-1α augmented IgM production in the presence of IL-2 in both CD5+ or CD5- B cells. However, again little IgG and no IgA was secreted from CD5+ B cells with any combination of IL-1 and IL-2 used.

Summarizing cytokine-induced Ig production in CD5+ or CD5- B cells, IL-2 consistently stimulated Ig secretion and IL-6 augmented this IL-2-induced Ig production; IL-1α also increased IL-2-induced Ig production to an extent varying in different experiments. However, in any combination of these lymphokines tested, SAC-preactivated FACS-sorted CD5+ B cells produced almost exclusively IgM, compared to the somewhat greater IgM production, and substantial IgG and IgA production, by CD5- B cells.

In some experiments, CD5+ and CD5- B cells were cultured continuously for 7 days with or without SAC activation (data not shown). Without SAC activation little or no Ig was secreted by either CD5+ or CD5- B cells, even in the presence of cytokines. No spontaneous IgM secretion was observed by either B cell population. However, when SAC and cytokines were present throughout the culture period together, high levels of Igs were secreted by these B cells. A low level of production of IgG and IgA was also observed in populations of CD5+ B cells, which might be due to a small percentage of contaminating CD5- B cells (<1%). When CD5+ and CD5- B cells are precultured with SAC during the first 3 days, similar to the findings shown in Table 1, SAC-activated CD5+ B cells secreted only IgM but no IgG and IgA, whereas SAC-activated CD5- B cells secreted IgM, IgG, and IgA (data not shown).

Table 3. Augmentation of Ig production by rhIL-2 and rhIL-1α in SAC-activated CD5+ and CD5- B cells

ILs[a]	IgM (ng/ml)[b]	IgG (ng/ml)	IgA (ng/ml)
CD5+ B cells[c]			
(-)	194 ± 26	nd[d]	35 ± 20
IL-2 (1)	457 ± 28	nd	nd
IL-2 (10)	411 ± 27	8 ± 8	nd
IL-2 (100)	601 ± 31	35 ± 7	nd
IL-1 (2) alone	176 ± 33	3 ± 8	nd
+ IL-2 (1)	442 ± 35	10 ± 7	nd
+ IL-2 (10)	502 ± 36	11 ± 8	nd
+ IL-2 (100)	756 ± 42	7 ± 9	nd
IL-1 (20) alone	154 ± 34	nd	nd
+ IL-2 (1)	386 ± 34	nd	nd
+ IL-2 (10)	651 ± 39	nd	nd
+ IL-2 (100)	1417 ± 63	nd	nd
CD5- B cells			
(-)	264 ± 26	772 ± 34	nd
IL-2 (1)	672 ± 32	1856 ± 97	91 ± 24
IL-2 (10)	879 ± 37	1700 ± 86	nd
IL-2 (100)	943 ± 38	1521 ± 74	10 ± 5
IL-1 (2) alone	273 ± 32	1121 ± 58	31 ± 50
+ IL-2 (1)	559 ± 37	1893 ± 114	88 ± 47
+ IL-2 (10)	1237 ± 56	2987 ± 168	208 ± 39
+ IL-2 (100)	966 ± 48	2120 ± 133	273 ± 38
IL-1 (20) alone	249 ± 32	1556 ± 88	nd
+ IL-2 (1)	588 ± 38	2012 ± 124	138 ± 40
+ IL-2 (10)	799 ± 43	3528 ± 285	306 ± 38
+ IL-2 (100)	1555 ± 67	1990 ± 121	72 ± 48

[a]rhIL-1-1α: 2 and 20 ng/ml; rhIL-2: 1, 10 and 100 U/ml were used.
[b]Means ± SD of triplicate samples are shown.
[c]CD5+ and CD5- B cells were cultured in two-step cultures.
[d]nd, not detected.

To investigate whether the observations in FACS-sorted B cells were not influenced by the staining and sorting procedures with CD19 and CD5 mAbs, PBMC were depleted of T cells, and responses of stained cells were compared with those of unstained cells. As shown in Table 4, SAC preincubation and cytokine stimulation induced similar levels of Igs in both PBMC populations using two-step cultures. Proliferative responses were also unaffected by staining (Table 5). Therefore it is unlikely that CD5 or CD19 staining altered the production of any immunoglobulin isotypes or proliferative response to factors used in CD5+ or CD5- B cells.

Activation surface marker analysis

As reported above, some differences were observed between CD5+ and CD5- B cells in responses to stimuli of proliferation and antibody production. To follow changes in these B cells induced by surface ligand preactivation we analyzed the expression of B lymphocyte activation surface markers. CD5+ or CD5- B cells were cultured for 2 days with SAC or anti-μ beads and the expression of several B cell activation markers was examined. As shown in Table 6, following SAC activation the expression of Leu-21, Leu-23, and IL-2R was increased,

Table 4. IgM, IgG, and IgA secretion by T-depleted PBMC[a] with or without B cell staining

	Unstained PBMC			Stained PBMC		
	IgM[c]	IgG	IgA	IgM	IgG	IgA
(−)	108 ± 24	287 ± 25	115 ± 52	165 ± 24	580 ± 37	148 ± 35
IL-1[b]	173 ± 24	351 ± 27	265 ± 45	186 ± 25	725 ± 43	172 ± 35
IL-2	364 ± 30	608 ± 38	117 ± 54	298 ± 27	896 ± 50	140 ± 36
IL-6	271 ± 28	298 ± 25	234 ± 50	310 ± 28	418 ± 30	151 ± 43
IL-1 + IL-2	232 ± 24	561 ± 36	189 ± 57	359 ± 29	1702 ± 151	156 ± 36
IL-2 + IL-6	345 ± 27	960 ± 54	187 ± 57	559 ± 29	1045 ± 57	89 ± 44

[a]T-depleted PBMC were stained with PE-anti-CD19 and FITC-anti-CD5 for 20 min. After washing 5 × 10^4 PBMC were SAC-preactivated and recultured with lymphokines as indicated for 7 days.
[b]20 ng rhIL-1, 10 U/mol rhIL-2, and 400 U/ml rhIL-6 were used.
[c]Means ± SD of secreted Igs (ng/ml) of triplicate samples are shown.

Leu-20 (CD23) expression was not induced, and expression of Leu-16 (CD20) was decreased. The findings were similar in both subsets of B cells activated by anti-μ beads. Leu-21 is expressed to a somewhat higher level in the CD5− B cell population whereas Leu-23 is expressed to a somewhat higher level in CD5+ B cells.

Discussion

For reasons outlined in the Introduction, the CD5+ subset of B lymphocytes is attracting attention because of their possible involvement in autoimmunity, natural immunity, and malignancy. Elucidation of mechanisms by which CD5+ B cells can be activated to proliferate and produce Ig may help in the analysis of their role in physiology and pathology. In this study we have activated the cells by ligands of surface Ig, mimicking a likely mechanism of activation *in vivo*. The cells have been cultured in the presence of recombinant human IL-1α, IL-2, IL-4, and IL-6. While this list of cytokines is not comprehensive, it is representative of those reported to have B lymphocyte stimulatory activity, and the use of defined cytokines is preferable to that of undefined supernatants of activated T lymphocytes or monocytes. IL-1α binds to the common IL-1α/IL-1β receptor and activates lymphocytes in an analogous manner (38). We have analyzed in parallel the proliferation of, and antibody production by, CD5+ and CD5− B lymphocytes sorted from the same samples of human peripheral blood. The findings with the CD5− subset correspond well with those published by others, which validates the methods used, and implies that the distinctive pattern of responses of the CD5+ B cells that we have consistently observed reflects intrinsic differences between the two subsets.

Considering first proliferation, two polyclonal stimuli were used, anti-μ beads and SAC. SAC (containing protein A) binds to human B cell surface Ig receptors (35), stimulates PIP2 hydrolysis (39), and activates resting (G_0) peripheral B lymphocytes to enter the cell cycle (40). Spontaneous proliferation in both CD5− and CD5+ B cells in human peripheral blood was low and comparable. SAC induced proliferation of both subsets of B cells, to a higher level in CD5− than CD5+ cells. CD5− cells were also induced to proliferate to some extent by anti-μ beads, this proliferation being augmented by co-stimulation with SAC. In contrast, anti-μ beads induced very little proliferation of CD5+ B cells, nor did they augment the stimulatory effect of SAC. In both CD5+ and CD5− subsets of B cells activated by anti-μ beads, IL-4 strongly stimulated proliferation whereas IL-2 stimulated proliferation only of the CD5− cells.

SAC is a more potent stimulus for IL-2 receptor expression than

Table 5. Proliferative response of T-depleted PBMC[a] with or without B cell staining

Experiment no.		Unstained PBMC[b]	Stained PBMC[b]
1	control	526 ± 16	930 ± 87
	SAC (0.2)	1523 ± 78	1521 ± 45
	SAC (1)	5014 ± 520	4588 ± 91
	SAC (5)	12121 ± 1440	14798 ± 1350
2	control	480 ± 84	537 ± 25
	SAC (1)	2233 ± 175	3800 ± 370
	anti-μ (1)	455 ± 23	773 ± 152
	anti-μ (10)	852 ± 36	918 ± 129
3	control	427 ± 98	358 ± 43
	SAC (1)	3544 ± 450	4338 ± 240
	anti-μ (10)	2307 ± 300	3472 ± 280
	SAC + anti-μ (10)	12712 ± 1200	11500 ± 709

[a]T-depleted PBMC were stained with PE-anti-CD19 and FITC-anti-CD5 for 20 min. After washing 5 × 10^4 PBMC were cultured with SAC (0.2, 1, and 5 × 10^{-3}%) or anti-μ beads (1 and 10 μg/ml) for 60 h. 1 μCi/well of [^{3}H]TdR was pulsed during the last 16 h.
[b] Means ± SD of triplicate samples (c.p.m.) are shown.

Table 6. FACS analysis of B cell surface activation markers after stimulation with SAC or anti-μ beads

	Control		SAC[a]		Anti-μ beads[a]	
	CD5+	CD5−	CD5+	CD5−	CD5+	CD5−
Leu-16 (CD20)	94[b]	87	87	82	90	91
IL-2R	<0.5	<0.5	22	30	6.8	7.2
Leu-20 (CD23)	<5.0	<0.5	<0.5	1.2	<0.5	<0.5
Leu-21	4.5	9.8	35	48	55	63
Leu-23	1.0	0.9	38	27	52	37

[a]3 × 10^4 sorted B cells were cultured for 2 days with SAC (0.001%) or anti-μ beads (10 μg/ml).
[b]Results are shown as percentage positive after subtracting cells stained with irrelevant antibody.

anti-μ beads (Table 6), which may account for the greater sensitivity of SAC-activated cells to co-stimulation with IL-2. Alternatively, SAC may provide two signals to CD5+ B cells, one through binding to surface Ig and a second signal resembling the effect of IL-4. Possibly responses of CD5+ cells were influenced by prior binding *in vivo* of autoantigens or micro-organisms. However, our observations are similar to those reported for B-CLL, which were found to proliferate in response to SAC but not to anti-Ig (35). Addition of $F(ab)_2$ anti-Ig inhibited the proliferative response of B-CLL cells to SAC. Since many B-CLL cells are known to be CD5+ (8,41), this finding may be attributed to the response typical of CD5+ B cells.

When present alone, IL-2 increased Ig production by both the CD5+ and the CD5- SAC-activated B cells. SAC-activated B cells are known to express on their surface IL-2 receptors (p50 and p75, ref. 42; see also Table 6). In SAC-activated B cells, IL-2 stimulates Ig formation as shown by assays for plaque-forming cells (43) and for Ig in the culture medium (40,44,45). Regarding CD5+ B cells, mouse rIL-2 is reported to induce IgM production in CD5+ murine B leukemia cells (46), which is consistent with our observations on SAC-activated human CD5+ B cells. When IL-2 is present from the beginning of the culture period of SAC-activated CD5- cells, more Ig is produced than when the IL-2 is added only during the second step of a two-step culture. This is consistent with observations on unseparated B cells (40, 45), and may be attributable to proliferation induced by IL-2. In the case of CD5+ cells, our findings show that IL-2 does not strongly stimulate proliferation as in CD5- B cells, but is effective in inducing Ig formation.

IL-6, also termed B cell stimulatory factor 2, is reported to increase terminal differentiation by B cells (47). In tonsillar B cells, SAC induces expression of the IL-6 receptor (48). In our SAC-activated CD5+ or CD5- B cells from human peripheral blood, IL-6 alone had little activity in the induction of Ig synthesis. However, IL-6 augmented Ig production induced by IL-2. The IL-2 dependence of the IL-6 effect on Ig production in unseparated cells has also recently been reported (44). By itself IL-1α had little effect on Ig production, but in several experiments IL-1α augmented Ig production in the presence of IL-2. The combination of IL-1 and IL-6 in the absence of IL-2 was relatively ineffective in stimulating antibody formation.

The observations just described suggest that the antibody production by both subsets of B cells, CD5- and CD5+, is similarly regulated by surface Ig ligands and cytokines. However, even when the cells were separated from the same donors and cultured under the same conditions, there were striking differences in the isotypes of the antibodies formed. The CD5- subset produced IgG and IgA as well as IgM, whereas the CD5+ subset produced IgM almost exclusively. Small amounts of IgG were formed in some CD5+ cultures, possibly owing to a low level of contamination with CD5- cells. Human CD5+ B cells activated with pokeweed mitogen were reported to produce IgG as well as IgM (30). It is generally accepted that the major Ig isotype produced by CD5+ B cells is IgM. For example, SAC-activated CD5+ B cells from humans with RA produce IgM RF (9). However, this does not exclude the possibility that under certain conditions CD5+ B cells can produce other isotypes. Although EBV-transformed CD5+ B cell lines originating from human peripheral blood cells produce mainly IgM antibodies, some cell lines producing IgG and IgA antibodies were also obtained (18). In the normal human intestine CD5+ B cells are reported to produce IgA (26). Whether the capacity to produce IgG or IgA is a property of subsets of CD5+ B cells or is related to special factors in their microenvironment remains to be established. For example, IL-5 increases IgA formation in B cells (49), and the same might be true of CD5+ cells in the intestinal epithelium. Since IL-6 is produced by synovial tissue of patients with RA (50), it may be a co-factor in the production of immunoglobulins in that tissue.

The possible role of CD5+ B cells in natural immunity is a large subject (20), and only a few possible examples can be considered in the context of the observations now presented. Natural immunity can be defined as a relatively non-specific defense system that can be rapidly activated to respond to, and contain, infections with a wide range of microorganisms of relatively low virulence. The cells involved should be strategically placed in the body, and a rapid response requires that they can produce antibodies without proliferation; CD5+ B cells are present in lymphoid tissue of the oropharynx (25) and are abundant in intestinal epithelium (26) and the peritoneal cavity (1), all likely sites for infection. Many antibodies produced by CD5+ B cells have broad specificity, reacting with bacterial antigens such as LPS (21), α-1,3-dextran (22), and phosphorylcholine (A.M.S., unpublished data). They also react with phosphatidylcholine (15,16), which is abundant in the outer leaflet of the plasma membrane of eukaryotes, including fungi and protozoa. The phosphatidylcholine is not accessible if the cell is heavily glycosylated; for example, antibodies against phosphatidylcholine do not react with normal mouse erythrocytes but they do after bromelain treatment, which eliminates shielding glycoprotein (15,16).

The possible relevance of this situation to natural immunity can be considered. Strains of *Entamoeba histolytica* with a heavy glycoprotein coat tend to be more virulent than those with less glycoprotein (51). Perhaps when the latter penetrate the intestinal epithelium they encounter anti-phosphatidyl antibodies produced by the CD5+ B cells residing there and are lysed by complement or opsonized for phagocytosis. Antiglobulins produced by CD5+ B cells could amplify effects of low levels of antibodies on the surface of microorganisms. For example, they increase neutralization of Herpes simplex virus (23) and protect newborn rats from *T.lewisi* infections (24).

What is needed to activate CD5+ B cells for a role in natural immunity is a ligand for their surface μ or δ chains (provided by binding to the above-mentioned antigens or immune complexes) and IL-2; their antibodies will then be secreted, as described in this paper. Sources of IL-2 in natural immunity remain to be defined. An interesting possibility is that the subset of T cells with γ/δ receptors for antigen, which have specificity against heat-shock proteins and other widely distributed microbial antigens (25), produce IL-2. The γ/δ T cells are abundant in intestinal epithelium (52), as are CD5+ B cells (26). These two cell types, γ/δ T cells and CD5+ B cells, could function in tandem as components of a natural immunity system, interacting with other components such as complement and leukocytes. They could control most infections; only infections with more virulent organisms would require the proliferation of α/β T cells and CD5- B cells, with later production of antibodies of higher

specificity and affinity. In body fluids of persons with bacterial infections substantial amounts of IL-6 are found (53). This cytokine could augment production of Igs by CD5+ B cells involved in natural immunity.

Proliferation of CD5+ B cells may not be required for rapid responses in natural immunity. That does not, of course, exclude proliferation beforehand, so as to have an adequate number of cells producing antibodies of broad specificity to limit most infections. Antigenic stimulation of mice with bromelain-treated mouse erythrocytes does not increase the number of cells making antibodies against them (54). However, where specificity is narrower, as with CD5- B cell products, proliferation following exposure to organisms or their products is a necessary and typical attribute of acquired immunity. These dual requirements impose interesting restrictions on the V-gene repertoire of CD5+ and CD5- cells which are discussed elsewhere (20).

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Abbreviations

CLL	chronic lymphocytic leukemia
NFDM	non-fat dry milk
PBMC	peripheral blood mononuclear cells
PE	phycoerythrin
RA	rheumatoid arthritis
RF	rheumatoid factor
SAC	*Staphylococcus aureus* Cowan I
TdR	thymidine

References

1 Herzenberg, L. A., Stall, A. M., Lalor, P. A., Sidman, C., Moor, W. A., Parks, D. R., and Herzenberg, L. A. 1986. The Ly-1 B-cell lineage. *Immunol. Rev.* 93:81.

2 Ledbetter, J. A., Rouse, R. V., Micklem, H. S., and Herzeberg, L. A. 1980. T-cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens. Two parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modified current views. *J. Exp. Med.* 152:280.

3 Lanier, L. L., Warner, N. L., Ledbetter, J. A., and Herzenberg, L. A. 1981. Expression of Lyt-1 antigen on certain murine B-cell lymphomas. *J. Exp. Med.* 153:998.

4 Hayakawa, K., Hardy, R. R., Parks, D. R., and Herzenberg, L. A. 1983. The 'Ly-1 B' cell subpopulations in normal, immunodefective and autoimmune mice. *J. Exp. Med.* 157:202.

5 Sidman, C. L., Schultz, L. D., Hardy, R. R., Hayawaka, K., and Herzenberg, L. A. 1986. Production of immunoglobulin isotypes by L-1+ B-cells in viable motheaten and normal mice. *Science* 232:1423.

6 Hayakawa, K., Hardy, R. R., Stall, A. M., Herzenberg, L. A., and Herzenberg, L. A. 1986. Immunoglobulin-bearing B-cells reconstitute and maintain the murine Ly-1 B-cell lineage. *Eur. J. Immunol.* 16:1313.

7 Ledbetter, J. A., Evans, R. L., Lipinski, M., Cunningham-Rundles, C., Good, R. A., and Herzenberg, L. A. 1981. Evolutionary conservation of surface molecules that distinguish T-lymphocyte helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. *J. Exp. Med.* 153:310.

8 Wang, C. Y., Good, R. A., Ammirati, P., Dymbort, G., and Evans, R. L. 1980. Identification of a p69,71 complex expressed on human T-cells sharing determinants with B-type chronic lymphocytic leukemia cells. *J. Exp. Med.* 151:1539.

9 Hardy, R. R., Hayakawa, K., Shimizu, M., Yamasaki, K., and Kishomoto, T. 1987. Rheumatoid factor secretion from human Leu-1+ cells. *Science* 236:81.

10 Caligaris-Cappio, F., Gobbi, M., Bofill, M., and Janossy, G. 1982. Infrequent normal B lymphocytes express features of B-chronic lymphocytic leukemia. *J. Exp. Med.* 155:623.

11 Antin, J. H., Emerson, S. G., Martin, P., Gadol, N., and Ault, K. A. 1986. Leu-1+ (CD5+) B-cells. A major lymphoid subpopulation in human fetal spleen: phenotypic and function studies. *J. Immunol.* 136:505.

12 Plater-Zyberk, C., Maini, R. N., Lam, K., Kennedy, T. D., and Janossy, G. 1985. A rheumatoid arthritis B-cell subset expresses a phenotype similar to that in chronic lymphocytic leukemia. *Arthrit. Rheum.* 28:971.

13 Sowden, J. A., Roberts-Thomson, P. J., and Zola, H. 1987. Evaluation of CD5 positive B-cells in blood and synovial fluid of patients with rheumatic diseases. *Rheumatol. Int.* 7:255.

14 Hayakawa, K., Hardy, R. R., Honda, M., Herzenberg, L. A., Steinberg, A. D., and Herzenberg, L. A. 1984. Ly-1 B-cells: functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc. Natl. Acad. Sci. USA* 81:2494.

15 Mercolino, T. J., Arnold, L. W., Hawkins, L. A., and Haughton, G. 1988. Normal mouse peritoneum contains a large population of Ly-1+ (CD5) B-cells that recognize phosphatidyl choline. *J. Exp. Med.* 168:687.

16 Cox, K. O. and Hardy, S. J. 1985. Autoantibodies against mouse bromelain-modified RBC are specifically inhibited by a common membrane phospholipid, phosphatidylcholine. *Immunology* 55:263.

17 Casali, P., Barastero, S. E., Nakamura, M., Inghirami, G., and Notkins, A. L. 1987. Human lymphocytes making rheumatoid factor and antibodies to ssDNA belong to the Leu-1+ B-cell subset. *Science* 236:77.

18 Burastero, S. E., Casali, P., Wilder, R. L., and Notkins, A. L. 1988. Monoreactive high affinity and polyreactive low affinity rheumatoid factors are produced by CD5+ B-cells from patients with rheumatoid arthritis. *J. Exp. Med.* 168:1979.

19 Casali, P. and Notkins, A. L. 1989. Probing the B-cell repertoire with EBV: polyactive and CD5+ B-lymphocytes. *Annu. Rev. Immunol.* 7:513.

20 Kocks, C. and Rajewski, K. 1989. Stable expression and somatic hypermutation of antibody V regions in B-cell developmental pathways. *Annu. Rev. Immunol.* 7:537.

21 Nakamura, M., Burastero, S. E., Notkins, A. L., and Casali, P. 1988. Human monoclonal rheumatoid factor-like antibodies from CD5 (Leu-1)+ B-cells are polyreactive. *J. Immunol.* 140:4180.

22 Foester, I. and Rajewsky, K. 1987. Expansion and functional activity of Ly-1 + B-cells upon transfer of peritoneal cells into allotype-congenic newborn mice. *Eur. J. Immunol.* 17:521.

23 Asbe, W. K., Daniels, C. A., Scott, G. S., and Notkins, A. L. 1971. Interaction of RF with infectious herpes simplex virus virus – antibody complexes. *Science* 172:176.

24 Clarkson, A. B., Jr and Mellow, G. M. 1981. Rheumatoid factor-like immunoglobulin M protects previously uninfected rat pups and danes from *Trypanosoma lewisi. Science* 214:186.

25 Richard, Y., Leprince, C., Dugas, B., Treton, D., and Galanaud, P. 1987. Reactivity of Leu-1+ tonsillary B-cells to a high molecular weight B-cell growth factor. *J. Immunol.* 139:1563.

26 Peters, M. G., Secrist, H., Anders, K. R., Nash, G. S., Rich, S. R., and MacDermott, R. P. 1989. Normal human intestinal B-lymphocytes. Increased activation compared with peripheral blood. *J. Clin. Invest.* 83:1827.

27 Sthoeger, Z. M., Wakai, M., Tse, D. B., Vinciguerra, V. P., Allen, S. L., Budman, D. R., Lichtman, S. M., Schulman, P., Weiselberg, L. R., and Chiorazzi, N. 1989. Production of autoantibodies by CD5-expressing B lymphocytes from patients with chronic lymphocytic leukemia. *J. Exp. Med.* 169:255.

28 Emilie, D., Karray, S., Merle-Beral, H., Debre, P., and Galanaud, P. 1988. Induction of differentiation in human leukemic B-cells by interleukin 2 alone: differential effect on the expression of μ and J chain genes. *Eur. J. Immunol.* 18:1479.

29 Mercolino, T. J., Arnold, L. W., and Haughton, G. 1986. Phosphatidylcholine is recognized by a series of Ly-1+ murine B-cell lymphomas specific for erythrocyte membranes. *J. Exp. Med.* 163:155.

30 Gadol, N. and Ault, K. A. 1986. Phenotypic and functional characterization of human Leu-1 (CD5) B-cells. *Immunol. Rev.* 93:23.
31 Madsen, M. and Johnson, H. E. 1979. A methodological study of E-rosette formation using AET-treated sheep red blood cells. *J. Immunol. Methods* 27:61.
32 Hardy, R. R. 1986. In Weir, D. M., Herzenberg, L. A., Blackwell, C. C., and Herzenberg, L. A., eds, *Handbook of Experimental Immunology,* 4th edn, Vol. 1, p. 31.1. Blackwell, London.
33 Tadmori, W., Lee, H.-K., Clark, S. C., and Choi, Y. S. 1989. Human B-cell proliferation in response to IL-4 is associated with enhanced production of B-cell-derived growth factors. *J. Immunol.* 142:826.
34 Defrance, T., Vanbervliet, B., Aubry, J.-P., Takebe, Y., Arai, N., Miyajima, A., Yokota, T., Lee, F., Arai, K., DeVries, J. E., and Banchereau, J. 1987. B-cell growth-promoting activity of recombinant human interleukin 4. *J. Immunol.* 139:1135.
35 Romagnani, S., Giudizi, M. G., Biagiotti, R., Almerigogna, F., Maggi, E., Prete, G., and Ricci, M. 1981. Surface immunoglobulins are involved in the interaction of protein A with human B-cells and in the triggering of B-cell proliferation induced by protein A-containing *Staphylococcus aureus. J. Immunol.* 137:1307.
36 Nishizuka, Y. 1984. The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature* 308:693.
37 Francois, D. T., Katona, I. M., June, C. H., Wahl, L. M., Feuerstein, N., Huang, K.-P., and Mond, J. J. 1988. Anti-Ig-mediated proliferation of human B-cells in the absence of protein kinase C. *J. Immunol.* 140:3338.
38 Dower, S. K., Kronheim, S. R., March, C. J., Conlon, P. J., Hoff, T. P., Gillis, S., and Urdol, D. 1985. Detection and characterization of high affinity plasma membrane receptors for interleukin-1. *J. Exp. Med.* 162:501.
39 Walker, L., Guy, G., Brown, G., Rowe, M., Milner, A. E., and Gordon, J. 1986. Control of human B-lymphocyte replication. I. Characterization of novel activation states that precede the entry of G_0 B-cells into cycle. *Immunology* 58:583.
40 Jelinek, D. F., Splowski, J. B., and Lipsky, P. E. 1986. The roles of interleukin-2 and inferferon-γ in human B-cell activation, growth and differentiation. *Eur. J. Immunol.* 16:925.
41 Hayakawa, K. and Hardy, R. R. 1988. Normal, autoimmune, and malignant CD5+ B-cells: the Ly-1 B lineage? *Annu. Rev. Immunol.* 6:197.
42 Saiki, O., Tanaka, T., Doi, S., and Kishimoto, S. 1988. Expression and the functional role of a p70/p75 interleukin 2 binding in human B-cell. *J. Immunol.* 140:853.
43 Nakagawa, T., Nakagawa, N., Goldstein, H., Volkman, D. J., and Fauci, A. S. 1986. Demonstration that human B-cells respond differently to interleukin-2 and B-cell differentiation factor based on their stages of maturation. *J. Immunol.* 137:3175.
44 Splowski, J. B., McAnally, L. M., and Lipsky, P. E. 1990. IL-2-dependence of the promotion of human B-cell differentiation by IL-6 (BSF-2). *J. Immunol.* 144:562.
45 Muraguchi, A., Kehrl, J., Longo, D. L., Volkman, D. J., Smith, K. A., and Fauci, A. S. 1985. Interleukin-2 receptors on human B-cells. Implication for the role of interleukin-2 in human B-cell functions. *J. Exp. Med.* 161:181.
46 Brooks, K. H. and Vitetta, E. S. 1986. Recombinant IL-2 but not recombinant interferon stimulates both proliferation and IgM secretion in a Ly-1+ clone of neoplastic murine B-cells (BCL1). *J. Immunol.* 137:3205.
47 Muraguchi, A., Hirano, T., Tang, B., Matsuda, T., Horii, Y., Nakajima, K., and Kishimoto, T. 1988. The essential role of B-cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B-cells. *J. Exp. Med.* 167:332.
48 Taga T., Kawanishi, Y., Hardy, R. R., Hirano, T., and Kishimoto, T. 1987. Receptors for B-cell stimulatory factor 2. Quantitation, specificity, distribution, and regulation of their expression. *J. Exp. Med.* 166:967.
49 Kunimoto, K. Y., Nordan, R. P., and Strober, W. 1989. IL-6 is a potent cofactor of IL-1 in IgM synthesis and of IL-5 in IgA synthesis. *J. Immunol.* 143:2230.
50 Nawata, Y., Eugui, E. M., Lee, S. W., and Allison, A. C. 1989. IL-6 is the principal factor produced by synovia of patients with rheumatoid arthritis that induces B-lymphocytes to secrete immunoglobulin. *Ann. NY Acad. Sci.* 557:230.
51 Trissl, D., Martinez-Palomo, A., Anguello, C., de la Torre, M., and de la Hoz, R. 1977. Surface properties related to Concanavalin A-induced agglutination. A comparative study of several Entamaeba strains. *J. Exp. Med.* 145:652.
52 Bonnevile, M., Janeway, C. A., Jr, Ito, K., Haser, W., Ishida, I., Nakanishi, N., and Tonegawa, S. 1988. Intestinal intraepithelial lymphocytes are a distinct set of γ/δ T cells. *Nature* 336:479.
53 Helfgott, D. C., Clarick, R. H., May, L. T., and Sehgal, P. B. 1989. Interferon-β2-/interleukin-6 in plasma and body fluids during acute bacterial infection. *Ann. NY Acad. Sci.* 552:562.
54 Von Rooijen, N. 1989. Are bacterial endotoxins involved in autoimmunity by CD5+ (Ly-1) B-cells? *Immunol. Today* 10:334.