

INTERLEUKIN 2 RECEPTORS ON HUMAN B CELLS

Implications for the Role of Interleukin 2 in Human B Cell Function

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It has been demonstrated (1, 2) that a number of antigen-nonspecific soluble factors exist that can transmit signals for growth and/or differentiation among lymphoid cells, particularly T lymphocytes, and may play a major role in the regulation of the immune response. Recent studies (3–5) in both murine and human systems have revealed that such antigen-nonspecific soluble factors also play a critical role in regulating the proliferation and differentiation of B lymphocytes.

We have attempted to delineate the minimal and optimal signals required for the induction of resting human B cells to proliferate as well as the signals required for the induction of activated B cells to differentiate into immunoglobulin-secreting cells (4, 5). In this regard, we have recently demonstrated (6–8) that *Staphylococcus aureus* Cowan I (SAC)¹ or a high concentration of antiimmunoglobulin antibody that interacts with the B cell surface immunoglobulin (sIg) directly induces the proliferation of resting human B cells, while a less powerful sIg-mediated signal, such as that delivered by low concentrations of anti-Ig antibody, results in the activation of B cells without subsequent proliferation. These activated B cells, presumably arrested in the G1 phase of the B cell cycle, were able to respond to exogenous T cell-derived B cell growth factor (BCGF), now designated B cell stimulatory factor (BSF) (9), to enter the S phase of the cell cycle. These activated B cells can in turn be induced to differentiate into Ig-secreting cells by B cell differentiation factors (BCDF), originally referred to as T cell-replacing factors (10). These BCDF have been shown to be biochemically separable from interleukin 2 (IL-2) and BSF (11–13).

It is currently controversial whether IL-2 exerts a direct effect on B cell function. Certain reports (3, 14) state that it is unlikely that IL-2 directly affects B cells; however, other studies indicate that IL-2 has an important role in the

¹ *Abbreviations used in this paper:* AET, 2-aminoethylisothiuronium bromide; BCDF, B cell differentiation factor; BCGF, B cell growth factor; BSF, B cell stimulatory factor, EBV, Epstein-Barr virus; FACS, fluorescence-activated cell sorter; HTLV, human T cell leukemia virus; IL-2, interleukin 2; MLR, mixed lymphocyte reaction; MNC, mononuclear cell; PMA, phorbol myristate acetate; PHA, phytohemagglutinin; SAC, *Staphylococcus aureus* Cowan I; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

maximal induction of plaque-forming cells (15–18; M. Peters, J. L. Butler, J. B. Margolick, T. L. Gerrard, C. A. Dinarello, and A. S. Fauci, manuscript submitted for publication). Although hairy cell leukemic cells, which have a B cell phenotype, have been reported to express the receptor for IL-2 as defined by the Tac antigen (19), it is still unclear whether normal B cells express IL-2 receptors during activation and/or differentiation. In the present study, by using anti-Tac antibody for immunoprecipitation and radiolabeled IL-2 in binding studies, we have addressed the question of whether IL-2 receptors can be induced on normal human B cells after stimulation and whether freshly established B cell lines express IL-2 receptors. We have also investigated the role of IL-2 receptors on the proliferation and differentiation of B cells using recombinant IL-2. Our results clearly demonstrate that normal activated B cells and some B cell lines express IL-2 receptors that may play a role in the proliferation and differentiation of these cells.

Materials and Methods

Reagents. Anti- μ antibody is the F(ab')₂ fragment of goat heavy chain-specific anti-human IgM (Cappel Laboratories, Cochranville, PA). SAC was obtained from Bethesda Research Laboratories (Gaithersburg, MD); phytohemagglutinin (PHA) was purchased from Wellcome Research Laboratories (Beckenham, England); phorbol myristate acetate (PMA) was obtained from Sigma Chemical Co. (St. Louis, MO). Affinity-purified IL-2 and [³H]leu,lys-IL-2 were prepared as previously described (20). Recombinant IL-2 (lot LP210) was obtained from Cetus Corp. (Emeryville, CA). The recombinant IL-2 (21) was 96% pure by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis and contained <0.04 ng endotoxin/10⁶ U. Mouse ascites containing anti-Tac monoclonal antibody was a generous gift from Dr. Thomas Waldmann, National Institutes of Health, Bethesda, MD. Mixed lymphocyte reaction (MLR) supernatant was prepared as previously described (10). BSF derived from a human T-T hybridoma cell line was the same preparation as previously described in detail (22).

Cell Purification. Human tonsils obtained at tonsillectomy from 6–22-yr-old patients with chronic tonsillitis were dispersed into single-cell suspensions, and mononuclear cells (MNC) were separated by the standard Hypaque-Ficoll gradient method. T cell-enriched populations were obtained by rosetting the cells with 2-aminoethylisothiuronium (AET)-treated sheep erythrocytes. B cell-enriched populations were obtained by depletion of T cells and monocytes (6). These B cell-enriched suspensions were further separated into fractions according to cell volume by the technique of counterflow centrifugation-elutriation as previously described (23). The smallest-sized B cell fractions with a mean volume of 160–180 μm^3 had the following profile: they were >90% sIg-positive as determined by fluorescence-activated cell sorter (FACS) analysis; they contained <0.2% T cells as determined by either erythrocyte receptor-positive cells enumerated by rosetting the cells with AET-treated sheep erythrocytes, or by FACS analysis after staining the cells with anti-OKT3 monoclonal antibody (Ortho Diagnostic Systems, Inc., Raritan, NJ); they contained <0.1% of monocytes as determined by nonspecific esterase staining (23). In some experiments, the B cells were further depleted to T cells by another rosetting technique. Briefly, 5.0×10^7 double AET-rosetted tonsillar cells were pelleted and incubated with 25 μl of a 1:10 dilution of the monoclonal antibody Leu-1 (Becton-Dickinson Monoclonal Center, Inc., Mountain View, CA) for 45 min on ice. The cells were washed twice to remove excess monoclonal antibody and resuspended in 8 ml of RPMI 1640. In a 50-ml conical tube, the cells were combined with 6 ml of 5 gram-percent human albumin and 18 ml of 2% ox erythrocytes that had been previously coated with affinity-purified goat anti-mouse Ig (Boehringer Mannheim Biochemicals, Indianapolis, IN) using chromic chloride. The cells were pelleted, incubated on ice for 45 min, resuspended gently, and centrifuged on Hypaque/Ficoll gradients in a standard fashion. The further purified B cells were

harvested from the interface. The resulting B cells were contaminated by <1/1,000 OKT3-positive cells as determined by immunofluorescence.

Cell Cultures. For binding assays, cells ($2-3 \times 10^6$ /well) were cultured in 1 ml of complete medium that was RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (M. A. Bioproducts, Walkersville, MD), 100 U/ml penicillin, and 100 μ g/ml streptomycin in 16-mm-diam flat-bottomed tissue culture wells (3524; Costar, Data Packaging, Cambridge, MA). For proliferation assays, cells at the densities indicated below were cultured in 0.2 ml of complete medium in flat-bottomed microtiter plates (Costar 3597). Cultures were pulsed after various periods of time as indicated with 1 μ Ci of [3 H]thymidine (6.7 Ci/mM; New England Nuclear, Boston, MA), and incorporation of [3 H]thymidine was measured by standard liquid scintillation counting techniques. For the determination of Ig production, cells were cultured in microtiter plates for various periods of time as indicated, and the amount of Ig in the cell-free supernatants was measured by an enzyme-linked immunosorbent assay (7). In several experiments, cells were cultured with various concentrations of affinity-purified IL-2, recombinant IL-2, or MLR supernatant, as indicated.

Cell Lines. Epstein-Barr virus (EBV)-transformed B cell lines from normal individuals were established in this laboratory as previously described (24). Briefly, MNC were isolated from peripheral blood as described above, and T cells were separated from B cells and monocytes by rosetting with AET erythrocytes. The rosette-negative fraction was then infected with EBV using a modification of the technique previously described (25). Supernatants from the B95-8 marmoset cell line were filtered through a 0.45- μ m filter (Nalge Co., Rochester, NY), diluted fourfold, and incubated with 5×10^6 cells in a sterile 12- \times 74-mm tube (Falcon Labware, Oxnard, CA) for 1 h at 30°C. Cells were then washed three times and incubated in 1.5 ml of 10% FCS and RPMI 1640 containing 80 μ g/ml of gentamicin (Gibco Laboratories, Grand Island, NY). Cyclosporin A (Sandoz, East Hanover, NJ) was added to the incubating cells at a concentration of 0.5 μ g/ml to promote rapid outgrowth of EBV-transformed cells by suppressing T cell inhibitory influences. Cells were fed with fresh medium after 7-10 d, and after ~3 wk they were transferred to 50-ml flasks (Costar) and maintained for 4 mo in RPMI 1640 medium containing FCS. Assays for EBV nuclear antigen were kindly performed by Mr. Gary Armstrong (Food and Drug Administration) using standard antisera. Gross cellular morphology was assessed on Wright's stained cells. Human T cell leukemia virus (HTLV)-transformed B cell lines were established from patients with adult leukemia as previously described (26).

Cell Staining. Cell staining with monoclonal antibodies and data analysis were performed as previously described (6), with certain modifications. To prevent Fc fragment binding, cells were incubated with aggregated human IgG for 45 min at 37°C, and washed cells were allowed to react with mouse ascites containing anti-Tac monoclonal antibody, control ascites (P3), or anti-OKT3, whose IgG isotype is matched to that of anti-Tac antibody (27). Fluorescein-conjugated F(ab')₂ fragment of affinity-purified goat anti-mouse IgG (Tago, Inc., Burlingame, CA) was used as a developing antibody. In addition to staining with monoclonal antibody reagents, cells were also directly stained with fluorescein-conjugated F(ab')₂ fragment of goat anti-human Ig (μ , γ , and α chains) obtained from Cappel Laboratories. Stained cells were analyzed by a FACS II.II (B-D FACS Systems, Mountain View, CA).

Characterization of Tac Antigens. Characterization of Tac antigens on cell surface membranes was performed by electrophoretic transfer blotting. Cells ($\sim 10^7$) were solubilized with 1% Nonidet P-40, and lysates were precleared with an isotype-matched monoclonal antibody (anti-mouse Igh-4a; IgG2a-K; Becton-Dickinson Monoclonal Centers, Inc.). Precleared lysates were immunoprecipitated with either anti-Tac or Leu-1 monoclonal antibody, run on a 10% SDS gel, and electrophoretically transferred to nitrocellulose paper (28). Strips cut from the sheet were reacted with anti-Tac or Leu-1, washed, and incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Bio-Rad Laboratories, Richmond, CA). The strips were then developed with 4-Cl-1 naphthol. Antigens were also characterized by surface labeling in which cells ($\sim 10^7$) were washed and surface-

labeled with ^{125}I by the lactoperoxidase method (19). Labeled cells were extracted, and supernatants were cleared with an isotype-matched monoclonal Ig (anti-mouse Igh-4a) and protein A-coupled Sepharose. Resulting supernatants were immunoprecipitated with anti-Tac antibody-conjugated protein A sepharose. The pellets were then boiled in 20% glycerol/1% 2-mercaptoethanol/1% SDS and analyzed by 10% SDS-PAGE.

Binding Assay of Radiolabeled IL-2. The IL-2 binding assay was performed by the method described by Robb et al. (20) and Smith (29). Briefly, to determine the level of binding, serial dilutions of [^3H]leu,lys-IL-2 were incubated at 37°C with 10^6 – 10^7 cells in a total volume of 100 μl of RPMI 1640 supplemented with 10% FCS using 1.5-ml Eppendorf micro-test tubes (Brinkman Instruments, Westbury, NY). After 25 min, 1 ml of ice-cold RPMI 1640-FCS was added to each tube, and the cells were spun down at 10,000 g for 15 s in an Eppendorf model 5414 centrifuge. The supernatant was removed and counted by liquid scintillation to determine the unbound radioactivity. The cell pellet was resuspended in 100 μl RPMI 1640-FCS at 4°C, and the cell suspension was layered on a mixture of 84% silicone oil (550 fluid; Contour Chemical Co., North Reading, MA) and 16% paraffin oil (Fisher Scientific Co., Pittsburgh, PA) in a soft 400- μl polyethylene tube (Bio-Rad Laboratories). After centrifugation at 10,000 g for 90 s, the tip containing the cell pellet was cut off and placed in scintillation vials, and the cell pellet was solubilized by the addition of 100 μl of 1% SDS, followed by addition of 15 ml Biofluor (New England Nuclear). The level of nonsaturable binding was determined in the presence of 150-fold molar excess of unlabeled IL-2, and specific binding was calculated by subtracting the amount of the nonsaturable binding.

Results

Tac Expression on Normal Activated B Cells. T cells express Tac antigen, which is recognized by a monoclonal antibody, anti-Tac, after activation by lectins or antigen (27, 30). Subsequent studies (31) have revealed that this anti-Tac antibody can recognize, at least in part, the IL-2 receptor expressed on the plasma membrane. To determine whether activated B cells express Tac antigen, freshly separated B cells were incubated with anti- μ antibody for 3 d and subsequently stained with anti-Tac monoclonal antibody. As a positive control for Tac expression, purified T cell populations were incubated with PHA and stained with the same antibody. To define T cell populations, and also as a control for an isotype-matched monoclonal antibody to anti-Tac antibody (IgG2a-K), anti-OKT3 antibody was used for the stainings. As expected, the majority (>80%) of PHA-stimulated T cell blasts were OKT3-positive and Tac-positive (Fig. 1, A and B). Anti- μ -activated B cells were also Tac-positive (30–40%) but OKT3-negative (Fig. 1, C and D). These activated B cells were also stained with anti-human Ig antibody to establish that they were in fact B cells. >90% of cells were stained positively. We then investigated the induction of Tac antigen using other B cell activators, PMA or SAC, and found that almost the same proportions of the activated B cells expressed Tac antigen with optimal concentrations of PMA and SAC (Table I). Kinetic studies revealed that Tac antigen was detected after a 24-h incubation of B cells with anti- μ , PMA, or SAC, which suggests that Tac antigen can indeed be induced on the surface membrane of normal B cells.

Tac Expression on a Panel of Established B Cell Lines. The observation that normal activated B cells express Tac antigen on the cell surface membrane posed the possibility that freshly established B cell lines might also express this antigen. Thus, we examined the reactivity of anti-Tac antibody against a panel of human B cell lines (Table II). We found that anti-Tac showed weak but significant

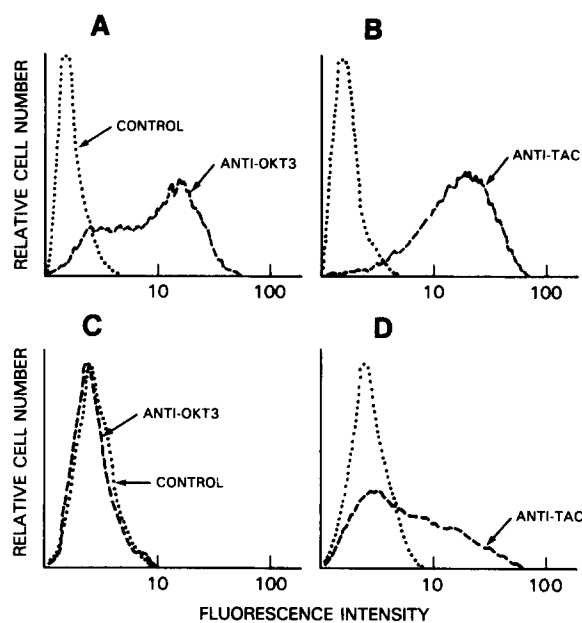


FIGURE 1. FACS profiles of normal activated T cells and B cells stained with monoclonal anti-Tac antibody. T cell blasts were prepared by activating T cells with PHA, and B cell blasts were obtained by activating B cells with anti- μ antibody. Cells were stained by an indirect method: cells were first treated with mouse control ascites, mouse monoclonal anti-OKT3 antibody, or anti-Tac antibody followed by staining with fluorescein isothiocyanate-conjugated F(ab')₂ fragment goat anti-mouse IgG; PHA-activated T cell blasts were stained with anti-OKT3 (A) or anti-Tac (B). Anti- μ -activated B cell blasts were stained with anti-OKT3 (C) or anti-Tac (D). Stained cells were analyzed by FACS II.II. Abscissa represents relative fluorescence intensity and ordinate represents relative cell number in individual fluorescence intensity channels.

TABLE I
Tac Expression on Normal Activated Human B Cells

Group	Cells*	Incubation [‡]	Percent positive cells [§]		
			OKT3	Tac	sIg
A	T	PHA, 72 h	85%	93%	ND [†]
B	B	—, 0 h	<1	<1	92
C	B	Anti- μ , 24 h	<1	25	ND
D	B	Anti- μ , 48 h	<1	38	ND
E	B	Anti- μ , 72 h	<1	42	90
F	B	PMA, 72 h	<1	54	93
G	B	SAC, 72 h	<1	50	91

* T cells and B cells were separated from tonsillar MNC as described in Materials and Methods.

[‡] Cells were cultured with PHA (2 μ g/ml), anti- μ (100 μ g/ml), PMA (50 ng/ml), or SAC (1.5 \times 10⁴ vol/vol).

[§] Percent of positive cells was determined by FACS II.II. Background (cells stained with control ascites) was subtracted.

[†] ND, not done.

TABLE II
Tac Expression on Established B Cell Lines

Cell lines examined	Surface antigens			
	sIg*	Tac [‡]	EBNA [§]	p19 [†]
FB9	++	-	+	-
SR	++	-	+	-
HT	++	-	+	-
AL	++	-	+	-
LE	++	-	+	-
AP	++	+	+	-
CB	++	+	+	-
HS1	++	++	-	+
CS2	++	++	-	+

* Cells were stained with fluorescein isothiocyanate-conjugated (Fab')₂ fraction of anti-human Ig (μ , γ , α) antibody.

[‡] Cells were incubated with mouse anti-monoclonal anti-Tac antibody followed by staining with fluorescein isothiocyanate-conjugated F(ab')₂ fraction of anti-mouse Ig.

[§] EBV nuclear antigen was detected using standard anti-EBV nuclear antigen sera.

[†] p19, an antigen that is expressed on the cell membrane of HTLV-infected cells, was examined by staining cells with anti-p19 antibody.

reactivity to only two out of eight EBV-transformed B cell lines, which had been established from normal donors. Anti-Tac showed definite reactivity to two out of two B cell lines that were infected by HTLV. As reported by Longo et al. (26), the HS1 B cell clone was isolated from the peripheral blood of a patient with adult T cell leukemia and it contained integrated HTLV proviral sequences in its DNA and expressed HTLV-encoded virus protein on its cell membrane. The CS2 B cell clone was obtained from normal cord blood lymphocytes by co-culturing them with irradiated HS1 cells. Representative FACS profiles of B cell lines stained with anti-Tac antibody are shown in Fig. 2. Taken as a whole, these data suggest that (a) some established B cell lines from normal donors express Tac antigen and (b) there is a strong relationship between Tac expression and HTLV infection in human B cells.

Partial Characterization of Tac Antigen on B Cells. Initial characterization of the cell surface Tac antigen on B cells was performed by electrophoretic transfer blotting with anti-Tac antibody as well as immunoprecipitation of surface-iodinated cells with anti-Tac. In electrophoretic transfer blotting with anti-Tac, a broad 50,000–55,000 mol wt band was obtained from both anti- μ -activated B cells and PHA-stimulated T cells (Fig. 3A, lanes a and b). However, in the same type of experiment using anti-Leu-1 antibody, a 65,000 mol wt band was obtained only from PHA-stimulated T cells but not from activated B cells (Fig. 3A, lanes c and d). When anti- μ -activated normal B cells, HS1 cells, and PHA-stimulated T cells were surface-iodinated and immunoprecipitated with anti-Tac (Fig. 3B, lanes a and c), a broad 50,000–58,000 mol wt band was obtained from normal activated B cells as well as HS1 cells. These antigens were found to be essentially identical in size to that obtained from the membranes of normal activated T cells (Fig. 3, lane b).

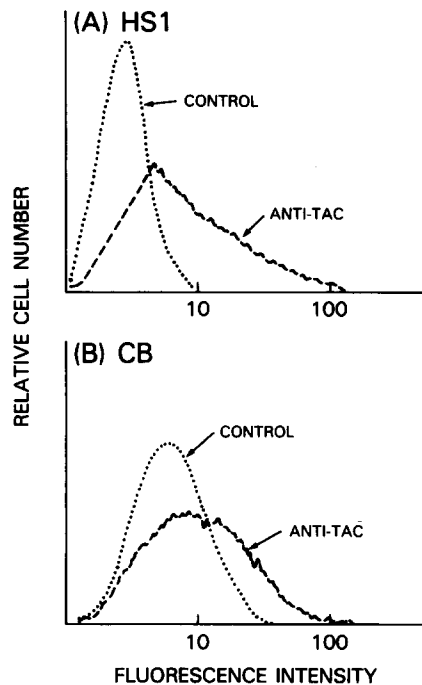


FIGURE 2. FACS profiles of B cell lines stained with monoclonal anti-Tac antibody. B cell lines were incubated with mouse control ascites or mouse anti-Tac antibody followed by the staining with fluorescein isothiocyanate-conjugated F(ab')₂ fraction of goat anti-mouse IgG. Stained cells were analyzed by FACS II.II. Abscissa represents relative fluorescence intensity, and ordinate represents relative cell number. (A) HTLV-infected B cell line, HS1; (B) EBV-infected B cell line, CB.

Binding Assay of IL-2. A binding assay using [³H]leu,lys-IL-2 was performed to determine the number and affinity of IL-2 receptors on B cells and was compared with those expressed on PHA-stimulated T cell blasts. It was demonstrated that the average number of receptors per anti- μ -activated B cell was less than that of PHA-stimulated T cell blasts (320 and 4,050 for B cells and T cells, respectively) (Fig. 4). It was also demonstrated by Scatchard plot analysis that the affinity of IL-2 receptors on anti- μ -activated B cells was lower than that of PHA-T cell blasts; dissociation constants obtained were 457 and 26 pM for B cells and T cells, respectively (Fig. 4). Furthermore, binding data of [³H]IL-2 to cloned HS1 B cells and PHA-T cell blasts demonstrated that the average number of receptor binding sites per cell was 1,000 for HS1 cells and 6,000 for PHA-T cell blasts (Fig. 5). A Scatchard plot of binding of [³H]IL-2 to HS1 cells and PHA-T cell blasts indicated that HS1 cells have much lower affinity binding sites compared with PHA-T cell blasts (55 pM for HS1 cells and 14 pM for PHA-T cell blasts). Thus, these data demonstrate that both normal activated B cells and a B cell line have significantly fewer binding sites and lower affinity of IL-2 receptors as compared with PHA-stimulated T cell blasts.

Effects of IL-2 on Activation and Differentiation of Normal Human B Cells. Recombinant IL-2 (21, 32) was used to investigate the effects of IL-2 on the proliferation and differentiation of highly purified normal tonsillar B

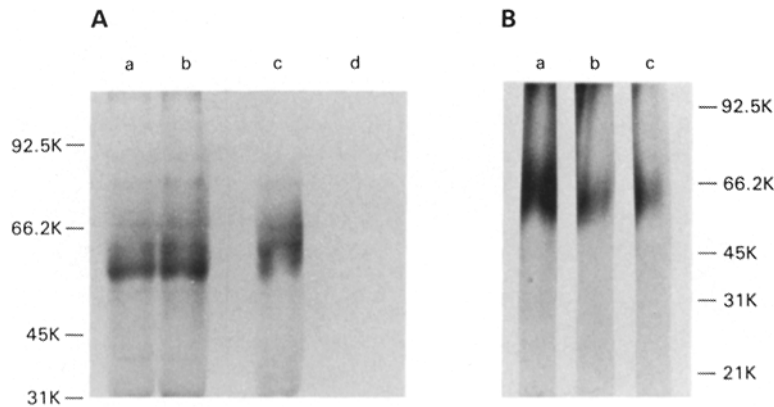


FIGURE 3. (A) Electrophoretic transfer blotting of solubilized PHA-T cell blasts and anti- μ -activated B cells with anti-Tac antibody and Leu-1 antibody. Solubilized cells were immunoprecipitated with an antibody and transferred to the nitrocellulose paper as described in Materials and Methods. Strips cut from the sheet were reacted with the identical antibody, washed, and incubated with goat anti-mouse IgG. Blotting analysis of PHA-stimulated T cells with anti-Tac antibody (lane *a*); anti- μ -activated B cells with anti-Tac antibody (lane *b*); PHA-stimulated T cells with Leu-1 antibody (lane *c*); anti- μ -activated B cells with Leu-1 antibody (lane *d*). (B) SDS gel comparison of immunoprecipitation with anti-Tac antibody from normal activated B cell blasts, a B cell line, and normal activated T cell blasts. Cells surface labeled with ^{125}I were extracted and immunoprecipitated with anti-Tac antibody as described in Materials and Methods. Antigen immunoprecipitated with anti-Tac antibody from PHA-stimulated T cells (lane *a*); with anti- μ -activated B cells (lane *b*); and with a B cell line, HS1 (lane *c*) was analyzed. Markers used in these experiments were phosphorylase *b* (92,500), albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), and soybean trypsin inhibitor (21,500).

cells. Tonsillar B cells were activated *in vitro* for 2 d with SAC, harvested, and recultured with various concentrations of recombinant IL-2. As shown in Fig. 6, recombinant IL-2 enhanced the DNA synthesis of SAC-activated B cells in a dose-dependent manner. In data not shown, IL-2, in the absence of an activating signal, did not induce proliferation of resting tonsillar B cells (J. H. Kehrl, A. Muraguchi, and A. S. Fauci, manuscript in preparation). Thus, IL-2, like BSF, can promote the growth of activated B cells.

The BSF we studied previously promoted the growth but not the differentiation of activated B cells (4). To assess the effects of IL-2 on B cell differentiation, tonsillar B cells were activated for 2 d with SAC and cultured with various concentrations of IL-2, and then the Ig content in the culture supernatants was measured after an additional 4 d of culture (Fig. 6). The addition of recombinant IL-2 to preactivated B cells induced significant IgG and IgM production. These results suggest that IL-2 can act directly on SAC-activated B cells to induce differentiation, although the possibility that IL-2 stimulated residual T cells to induce a pool of lymphokines that secondarily influenced B cell function remained a possibility.

To address this possibility, we cultured highly purified tonsillar B cells (<0.1% OKT3-positive) at various cell densities with or without recombinant IL-2. Even at very low cell densities, IL-2 enhanced both DNA synthesis and Ig production

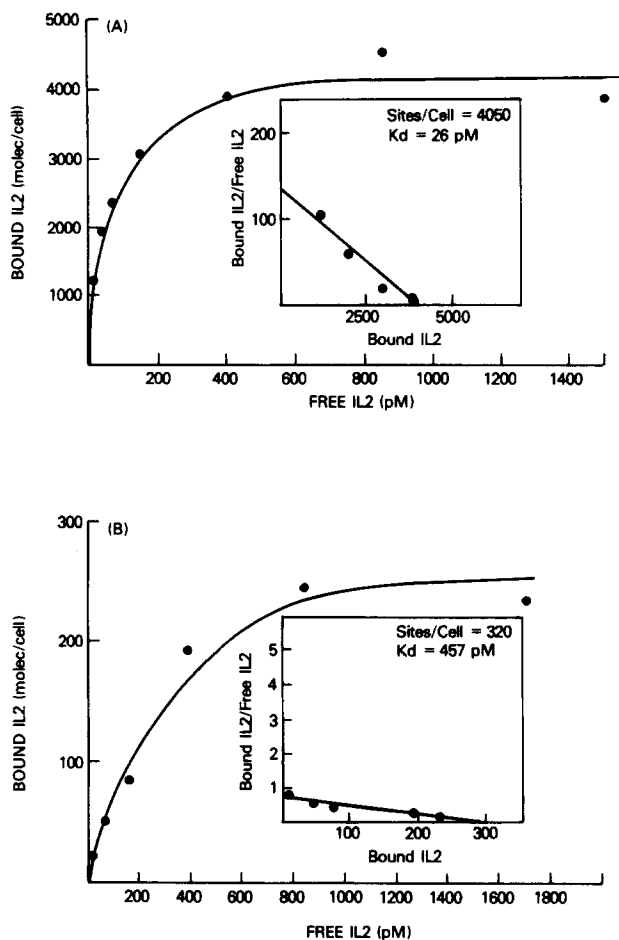


FIGURE 4. Binding of [³H]IL-2 to activated T cell blasts vs. activated B cell blasts. (A) Binding of [³H]IL-2 to PHA-activated T cell blasts. T cell blasts were prepared by incubating T cells with PHA (2 μg/ml) for 3 d, and specific bindings were determined. Insert depicts Scatchard plot from the binding data. (B) Binding of [³H]IL-2 to anti-μ-activated normal B cells. Activated B cells were prepared by incubating B cells with anti-μ (100 μg/ml) for 3 d and specific bindings were determined. Insert depicts Scatchard plot from the binding data.

by tonsillar B cells (Table III). We conclude that IL-2 can directly act on IL-2 receptor-bearing B cells to promote both their growth and differentiation.

Effect of IL-2 on the Proliferation and Differentiation of the IL-2 Receptor-bearing B Cell Line HS1. To study the effect of IL-2 on the proliferation of IL-2 receptor-bearing homogeneous B cells, cloned HS1 cells were incubated with recombinant IL-2 (10^{-2} to 10^4 U/ml) for 3 d, and DNA synthesis was measured by incorporation of [³H]thymidine. IL-2 at high concentrations induced significant enhancement of proliferation of HS1 cells in a dose-dependent manner (Fig. 7A). The decrease in proliferation noted at the highest concentration of recombinant IL-2 may be due to buffer toxicity (K. Koths, Cetus Corp., personal communication). Importantly, this IL-2-induced enhancement of proliferation of HS1 cells was inhibited by the addition of anti-Tac antibody but not control

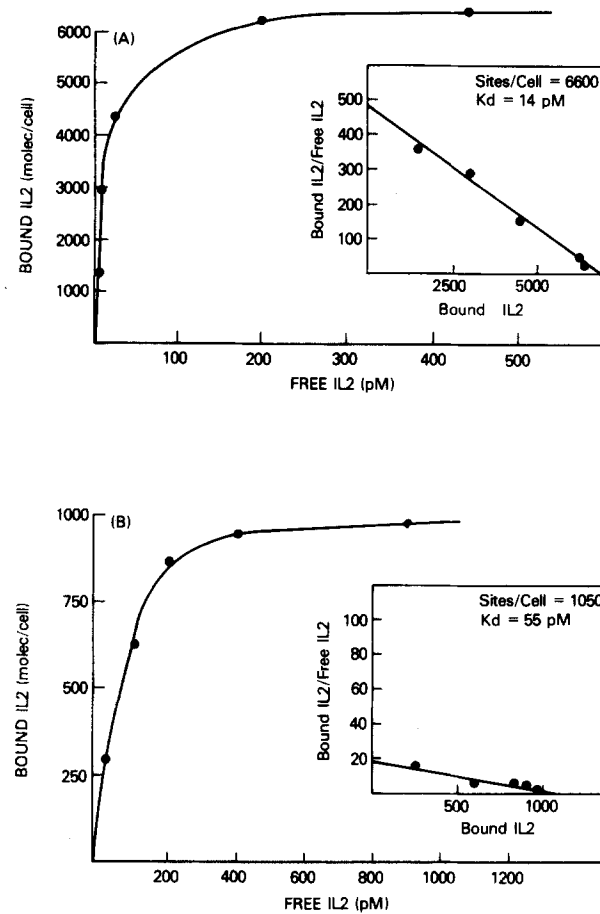


FIGURE 5. Binding of $[^3\text{H}]\text{IL-2}$ to activated T cells vs. a B cell line, HS1. (A) Binding of $[^3\text{H}]\text{IL-2}$ to PHA-activated T cell blasts. T cell blasts were prepared by incubating T cells with PHA for 3 d, and specific bindings were determined. Insert depicts Scatchard plot from the binding data. (B) Binding of $[^3\text{H}]\text{IL-2}$ to a B cell line, HS1. Insert depicts Scatchard plot of the data.

ascites to the culture (Fig. 7B). The effect of IL-2 on Ig production was assessed by incubating HS1 cells with various concentrations of recombinant IL-2 followed by measuring the amount of Ig secreted in the supernatant on day 3. It was found that HS1 cells were spontaneously producing IgM (~ 800 ng/ml per 10^4 cells) but no IgG or IgA in the absence of IL-2 and that only minimal enhancement of IgM production or induction of IgG and IgA was observed in the presence of IL-2 (data not shown). These data demonstrate that IL-2 at high concentrations can enhance the growth of HS1 cells but has limited effects on Ig production in these cells.

Discussion

The present study has demonstrated that IL-2 receptors in the form of Tac antigen expression could be induced in normal human B cells after in vitro

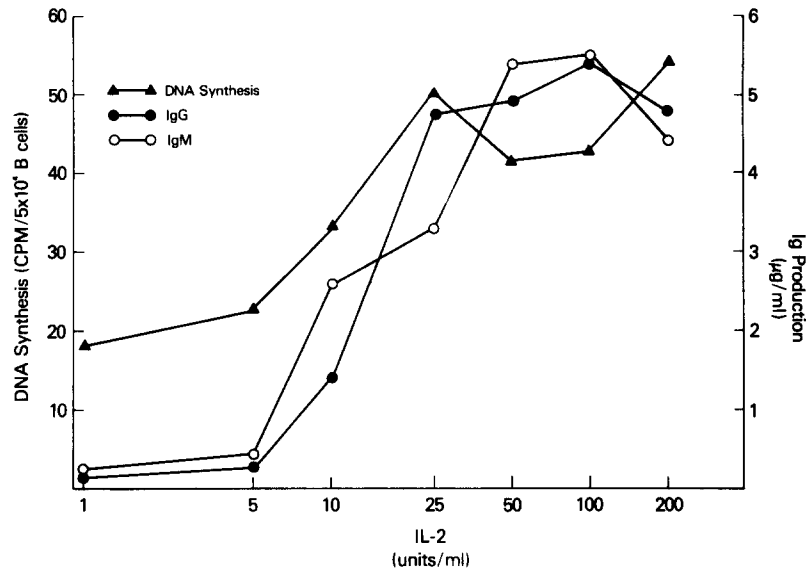


FIGURE 6. Effects of IL-2 on proliferation and differentiation of normal B cells. Highly purified tonsillar B cells, triple rosetted, were cultured with SAC for 2 d and then recultured (5×10^4 /well) with various concentrations of recombinant IL-2. DNA synthesis was measured by pulsing cultures with [^3H]thymidine ($1 \mu\text{Ci}$ /well) over the last 16 h of the 72-h reculture period. Ig production (IgG and IgM) was determined from after 4 d of reculture by enzyme-linked immunosorbent assay. Data represent the mean of two experiments.

TABLE III
*IL-2 Promotes the Growth and Differentiation of In Vitro Activated Human B Lymphocytes at Low Cell Densities**

Cell number ($\times 10^{-3}$ /well)	DNA synthesis [‡]		IgG productions [§]	
	- IL-2	+ IL-2	- IL-2	+ IL-2
	<i>cpm/well</i>		<i>ng/ml</i>	
0.8	265 ± 14	468 ± 55	0	73 ± 40
1.6	390 ± 28	915 ± 58	3 ± 1.5	73 ± 34
3.1	465 ± 14	1,888 ± 330	12 ± 6	156 ± 47
6.2	2,058 ± 197	7,477 ± 654	27 ± 19	393 ± 38
12.5	4,150 ± 386	14,609 ± 504	100 ± 40	1,326 ± 316
25	9,308 ± 494	29,325 ± 1,255	240 ± 117	2,647 ± 901
50	23,051 ± 2,383	44,012 ± 1,995	1,428 ± 700	8,406 ± 1,966

* Tonsillar B lymphocytes were activated for 2 d with SAC, harvested, and replated at the indicated cell densities with 50 U/ml of IL-2.

[‡] DNA synthesis was measured by [^3H]thymidine incorporation. A representative experiment is shown; data represent the means \pm SEM of five cultures.

[§] IgG production was measured by an enzyme-linked immunosorbent assay. Means \pm SEM of four experiments.

activation. In addition, Tac antigen expression was also observed on the surface of some EBV-transformed B cell lines as well as two HTLV-transformed B cell lines. The Tac antigens that were immunoprecipitated from normal activated B cells as well as from a B cell line were shown to have a molecular weight similar to the Tac antigen on PHA-stimulated T cell blasts. These observations raise the

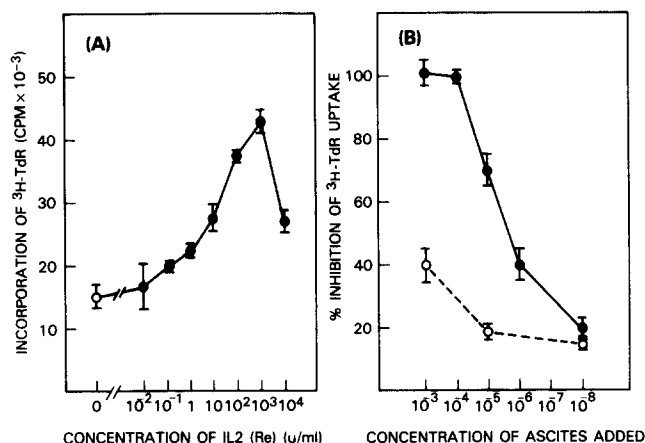


FIGURE 7. (A) Enhancement of proliferation of HS1 cells by recombinant IL-2. HS1 cells (10^4 /well) were cultured with various concentrations of recombinant IL-2 (Re IL-2) from 0.01 to 10,000 U/ml. Cultures were pulsed with [^3H]thymidine ($1 \mu\text{Ci}/\text{well}$) over the last 6 h of a 72-h culture. Data represent the means \pm SEM of triplicate cultures. (B) Effect of anti-Tac on IL-2-induced enhancement of proliferation of HS1 cells. HS1 cells (10^4 /well) were cultured with 100 U/ml of recombinant IL-2 in the presence of control ascites P3 (\circ) or anti-Tac ascites (\bullet). Percent inhibition was determined by $[1 - (b - c)/(a - c)] \times 100$, where a = cpm with 100 U/ml IL-2, b = cpm with IL-2 plus ascites, c = cpm with medium alone. Data represent the means \pm SEM of triplicate cultures.

obvious question of the role of IL-2 in B cell function. In this regard, there is considerable controversy about whether IL-2 in fact has any direct effect on B cell function.

The opinion that IL-2 does not seem to act directly on B cells stems from various lines of evidence. First, activated T cells respond to IL-2 and grow well in *in vitro* culture, while activated B cells do not respond by proliferation to IL-2 and grow poorly in IL-2-containing supernatant (33, 34). Second, B cells and B cell blasts cannot absorb IL-2 activity in the murine system (15). Third, highly purified IL-2 or IL-2-containing supernatants from T-T hybridomas have little effect on B cell proliferation in the absence or presence of polyclonal B cell activators (35-37). Fourth, it has been demonstrated (14) that clonal expansion and differentiation of individual hapten-specific B cells require soluble factors distinct from IL-2. Fifth, many investigators have successfully separated IL-2 by biochemical procedures from BCGF and BCDF in the murine system (38, 39) as well as in the human system (11-13, 35).

On the other hand, in spite of these observations, there are several lines of evidence that IL-2 may act directly on B cells. In studies of the synergistic effects of cofactors on B cell function, depletion of IL-2 from cofactor-containing supernatants by absorption on IL-2-dependent cells also removed factors that influence B cell function (40), which suggests that IL-2 may be one of the cofactors affecting B cell responses. Our recent data also suggested that maximal induction of Ig production in *in vivo* preactivated human B cells can be obtained by the addition of two kinds of B cell helper factors plus IL-2 (Peters et al., manuscript submitted for publication). Thus, the question of the direct effect of IL-2 on B cell function has not been completely resolved.

One way to more directly address this issue would be to study the expression of IL-2 receptors on B cells. In this regard, Korsmeyer et al. (19) have reported recently that Tac antigen is found on hairy cell leukemic cells that also express B cell surface markers. They have also shown rearrangement and expression of Ig genes in these malignant cells. Since Tac antigen has been reported to be at least a part of the IL-2 receptor (31), this finding clearly implies that B cells express IL-2 receptors during a stage of B cell differentiation.

In the present study, we have demonstrated by using monoclonal anti-Tac antibody that B cells stimulated with polyclonal B cell activators (anti- μ , SAC, or PMA) stained positively and thus expressed the IL-2 receptor. It is unlikely that the staining was nonspecific because control ascites (IgG2a-containing ascites, P₃) or the isotype-matched monoclonal antibody OKT3 did not stain those cells. It is also unlikely that positively stained cells were T cells because even after incubation the majority of the cells remained sIg-positive and OKT3-negative. Furthermore, immunoprecipitation experiments, either by the surface-labeling method or the electrophoretic transfer blotting method, revealed that Tac antigens on normal activated B cells had a molecular weight similar to that of the Tac antigens on PHA-stimulated T cell blasts.

In a survey of established B cell lines, two out of eight EBV-transformed B cell lines were found to be Tac-positive. Interestingly, the surface characteristics of these Tac-positive cell lines revealed that these cells expressed IgD molecules on their surface membrane, while other Tac-negative B cell lines lacked sIgD (A. Muraguchi, D. J. Volkman, and A. S. Fauci, unpublished observation). These results suggest that such B cell lines might represent a certain stage of normal B cell differentiation because there are several lines of evidence indicating that B cells express sIgD temporarily during B cell maturation (41, 42). In fact, these two B cell lines were found to be capable of responding to T cell-derived BCDF or PMA by enhancing their Ig secretion (D. J. Volkman, A. S. Fauci, and A. Muraguchi, unpublished observations).

Two HTLV-infected B cell lines were found to be strongly Tac positive. Among adult T cell leukemias, it has been demonstrated (43) that there is a strong correlation between the expression of Tac antigen and the presence of HTLV. Thus, it was felt that human T cell leukemias, like B cell hairy cell leukemia, might result from a transformation-associated activation of a gene for expression of the Tac antigen (19). In this regard, Tac expression on HTLV-infected B cells might be a consequence of HTLV infection. Alternatively, as has been suggested for T cells (44), the IL-2 receptor on B cells might be the binding site for HTLV. If this is the case, we speculate that only activated B cells are targets for HTLV infection. We are now intensively investigating this hypothesis.

The [³H]IL-2 binding study revealed that normal activated B cells and one of the HTLV-transformed B cell lines (HS1) had significantly fewer binding sites (300–1,000/cell) compared with PHA-T cell blasts (4,000–6,600/cell) and lower-affinity IL-2 receptors (dissociation constant of 55–460 pM) compared with PHA-T cell blasts (14–26 pM). Furthermore, in these experiments, there was a significant difference in the numbers of binding sites as well as the affinities of IL-2 receptors between the B cell line and normal activated B cells. The

reason for this discrepancy is unclear at present. However, the values of activated normal B cells may be underestimated because only a fraction of B cells (~20% of cultured B cells) were stained positive with anti-Tac antibody in that experiment. An alternative interpretation may be that HS1 cells do not represent a stage of normal B cell activation and thus may bind radiolabeled IL-2 with higher affinity than normal activated B cells.

Finally, we have addressed the question of the functional properties of the IL-2 receptors on B cells. Within this context, we have studied the function of IL-2 receptors on normal B cells as well as the B cell line using highly purified IL-2 or recombinant IL-2. We found that a moderate concentration of recombinant IL-2 (10–50 U/ml) enhanced the proliferation of in vitro-activated B cells and relatively high concentrations (100–1,000 U/ml) of IL-2 promoted the growth of a B cell line. Enhancement of proliferation in the B cell line was completely blocked by the addition of anti-Tac antibody. Additionally, a moderate concentration of IL-2 induced the differentiation of in vitro activated B cells. To exclude the possibility that IL-2 was mediating its effect solely via contaminating T cells, we used highly purified B cells cultured at low cell densities. Even at these densities, distinct increases in DNA synthesis and Ig production were noted when IL-2 was present. Thus, a single lymphokine was able to promote both the growth and differentiation of a population of normal activated human B lymphocytes. These data indicate that IL-2 receptors, when present on B cells, are indeed functional. Whether human B cells express IL-2 receptors upon activation in vivo is unknown. In this regard, large, presumably in vivo-activated, tonsillar B cells are essentially Tac-negative and, not surprisingly, poorly responsive to IL-2 (Kehrl et al., manuscript in preparation). Studies delineating the expression of Tac receptors on antigen-activated human B cells and on B cells from patients with diseases characterized by B cell hyperreactivity should be of considerable interest.

Summary

In the present study, we examined the expression of interleukin 2 (IL-2) receptors on normal human B cells as well as established B cell lines. Anti-Tac monoclonal antibody did not bind to freshly separated normal human B cells. Unexpectedly, with the appropriate activation of the normal B cells by anti- μ antibody, phorbol myristate acetate, or *Staphylococcus aureus* Cowan I (SAC), Tac antigen was induced on the activated B cells. Anti-Tac antibody showed consistent reactivity with two B cell lines that were infected by human T cell leukemia virus (HTLV) and some reactivity with two out of eight Epstein-Barr virus-transformed B cell lines established from normal adult donors. Immunoprecipitation analysis revealed that antigens of similar size with a molecular weight of 50,000–60,000 can be precipitated with anti-Tac antibody from phytohemagglutinin-stimulated normal T cell blasts and normal activated B cells, as well as a cloned B cell line. Binding assays of IL-2 on normal activated B cells and on the cloned B cell (HS1) revealed that B cells have significantly fewer sites and lower-affinity IL-2 receptors compared with phytohemagglutinin-stimulated normal T cell blasts. Finally, biological properties of the IL-2 receptor on B cells were examined by incubating B cells with recombinant IL-2. It was found that

moderate concentrations of IL-2 induce significant enhancement of proliferation and differentiation in SAC-activated normal B cells. These results suggest that normal B cells may express functional IL-2 receptors or closely related proteins and thus IL-2 may play a significant role in the modulation of B cell function.

We wish to thank Drs. Lê thi Bich-Thuy and H. Clifford Lane for helpful discussions and Ms. Ann C. London for expert editorial assistance.

Received for publication 16 July 1984.

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