

EXPRESSION OF INTERLEUKIN 2 RECEPTORS ON ACTIVATED HUMAN B CELLS

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Several antigen-nonspecific, genetically unrestricted factors derived from T cells have been shown to play a role in the regulation of B cell responses (1–9). One such factor, designated B cell growth factor (BCGF-1 or BSF-1),¹ appears to be required for the proliferation of a subset of B cells after interaction with antigen or antiimmunoglobulin molecules. A second series of factors, called B cell differentiation (BCDF) or T cell-replacing factors (TRF), is involved in the terminal maturation of proliferating B cells into immunoglobulin-secreting cells. There has been controversy regarding the possible involvement of T cell growth factor or interleukin 2 (IL-2) in B cell responses and specifically the ability of this growth factor to act directly on B lymphocytes. The proponents of a direct action of IL-2 on B cells showed that depletion of IL-2 from cofactor-rich supernatants by absorption on IL-2-dependent T cell lines also removed a factor required for B cell differentiation (4, 5, 10). Furthermore, there was a strict correlation between the levels of IL-2 and one of the B cell-specific factors required for antibody production (4). This view that IL-2 acts directly on B cells has been challenged, since both mouse and human IL-2 have been distinguished from B cell growth and differentiation factors (7–11). The IL-2-containing supernatants generally used in the previous studies (the supernatant of the FS6-14.13 line) also contained BCGF and one or more TRFs (9). Furthermore, IL-2 was not absorbed from cofactor-rich supernatants by incubation with lipopolysaccharide (LPS)-stimulated splenic lymphoblasts (4). Finally, radiolabeled IL-2 did not bind to LPS-stimulated B cells or to either of the two Burkitt's lymphoma B cell lines examined (Raji and Daudi) suggesting that B cells do not manifest receptors for IL-2 (12).

We have reexplored the possibility that certain activated B cells display receptors for IL-2 using the anti-Tac monoclonal antibody produced in our laboratory (13, 14). This monoclonal antibody identifies the human membrane receptor for IL-2 (15, 16, 17). The observations that led to this conclusion include: (a) anti-

¹ *Abbreviations used in this paper:* BCDF, B cell differentiation factor; BCGF, B cell growth factor; EBV, Epstein-Barr virus; IL-2, interleukin 2; LPS, lipopolysaccharide; NWSM, *Nocardia* water-soluble mitogen; PWM, pokeweed mitogen; SDS, sodium dodecyl sulfate; SLO, Streptolysin-O; WGA, wheat germ agglutinin.

Tac blocks the proliferation of IL-2-dependent T cell lines, but does not affect the proliferation of IL-2-independent T cell lines; (b) anti-Tac blocks the binding of radiolabeled IL-2 to its specific receptor on activated T cells; and (c) IL-2 blocks the binding of radiolabeled anti-Tac to its antigenic target on activated T cells and T cell lines. Furthermore, all membrane-associated, IL-2-binding molecules were removed by passage through an anti-Tac Sepharose column and, in parallel studies, all molecules that bound to an anti-Tac Sepharose column were removed by prior passage through an IL-2 Affigel affinity support (16). Thus, under the conditions examined, all anti-Tac-reactive molecules appeared capable of binding IL-2 and the ability to bind IL-2 was limited to the Tac protein.

Previously (17, 18), we made two observations using the monoclonal anti-Tac that suggested that IL-2 might play a role in B cell maturation. First, anti-Tac inhibited the maturation of B cells into immunoglobulin-secreting cells as measured in a reverse hemolytic plaque assay when peripheral blood mononuclear cells were stimulated with pokeweed mitogen (PWM) (17). Secondly, virtually all hairy cell leukemic populations that were demonstrated to be B cells on the basis of immunoglobulin gene rearrangements displayed the Tac antigen on the cell surface (18).

In the present study we demonstrate that, while normal resting B cells are Tac antigen negative, these cells can be activated to express the Tac antigen. Furthermore, we demonstrate that certain leukemic B cell populations are Tac antigen positive. In addition, cloned lines of normal, Epstein-Barr virus (EBV)-transformed B cells display the Tac antigen and high affinity IL-2 receptors detected with radiolabeled IL-2. We also demonstrate that the receptor identified by anti-Tac on activated B cells is similar in size (M_r 55,000) to the IL-2 receptor present on activated T cells. Finally using a Tac-positive cloned normal B cell line, we demonstrate that purified IL-2 is capable of amplifying the level of Tac antigen expression and inducing augmented immunoglobulin production. Thus, IL-2 receptor expression does not appear to be confined to cells of the T cell lineage, but can also be induced on normal B lymphocytes.

Materials and Methods

Cells Analyzed for IL-2 Receptor Expression. Purified B cells prepared from heparinized peripheral blood from five normal individuals and EBV-transformed B cell lines from six normal individuals, from six patients with adult T cell leukemia, and from 10 patients with Burkitt's lymphoma were analyzed for surface IL-2 receptor expression by indirect immunofluorescent staining with anti-Tac. The purified B cells were prepared from normal, heparinized venous blood using Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation to isolate mononuclear cells, followed by centrifugal elutriation to remove monocytes. The T cells were removed from these lymphocyte preparations by rosette formation with neuraminidase-treated sheep erythrocytes. The Burkitt's lymphoma lines were established from the involved lymph nodes of patients with the clinical features of Burkitt's lymphoma. Each of the lines demonstrated the reciprocal translocation between chromosome 8 and chromosomes 14, 2, or 22 characteristic of Burkitt's lymphoma. The adult T cell leukemia patients had circulating antibodies to the p19 and p24 structural (gag) proteins of human T cell leukemia/lymphoma virus I (HTLV-I) (19). Four of the five B cell lines examined from these patients contained HTLV-I provirus integrated into their genome as identified by molecular hybridization studies. The EBV-transformed, B cell lines were established from normal individuals using heparinized peripheral blood mononuclear cells depleted of T

cells by rosetting with neuraminidase-treated sheep erythrocytes and transformed with EBV (culture supernatant of line B95-8). These B cell preparations contained <4% T cells as defined by flow microfluorometry after staining with Leu-4 (pan T cell) monoclonal antibody.

To induce Tac antigen expression in these freshly obtained normal purified B cells prepared as described above, 1×10^6 purified B cells were co-cultured with 1×10^5 irradiated (2,000 rad) T cells and 1×10^4 irradiated (10,000 rad) monocytes in the presence of PWM ($10 \mu\text{g}$) for 6 d in RPMI 1640 media supplemented with 10% fetal calf serum. At the end of the culture period, the T cells were removed by rosetting with neuraminidase-treated sheep erythrocytes, and the residual B cells were assessed by flow microfluorometry for reactivity with Leu-4, Leu-10, and anti-Tac monoclonal antibodies.

To establish Tac-positive B cell lines from normal individuals, B cells activated by the procedure described above were incubated with anti-Tac for 30 min and then rosetted in the presence of ox erythrocytes coated with an anti-mouse immunoglobulin reagent. Cells rosetted in this manner were separated from the nonrosetting cells by Ficoll-Hypaque centrifugation and then cultured in RPMI 1640 media supplemented with 20% fetal calf serum in the presence of EBV (B95-8 supernatant) for 4 wk. The resulting lines were then reassessed for reactivity with anti-Tac. A line (designated B22) demonstrating 25% reactivity with anti-Tac was cloned by limiting dilution at a density of 0.5 B cells/well in 96-well, round-bottom Costar plates in the presence of 2×10^4 allogeneic, irradiated (5,000 rad) peripheral blood lymphocytes as a feeder layer. Two clones, 1C9 and 5B4, were expanded for more extensive analysis. Both clones secreted immunoglobulin; clone 1C9 produced IgG κ while 5B4 produced IgM κ .

Monoclonal Antibodies. The OKT3, T4, T8, T9, and T10 antibodies were obtained from Ortho Diagnostic Systems Inc., Westwood, MA. The Leu-4 and Leu-10 antibodies were obtained from Becton, Dickinson & Co., Mountain View, CA. The B1 and fluorescein-conjugated F(ab')₂ goat anti-mouse immunoglobulin antibodies were obtained from Coulter Electronics, Hialeah, FL.

The preparation of the murine hybridoma-producing monoclonal anti-Tac antibody, an anti-IL-2 receptor antibody, has been previously described (13–15). In this study, anti-Tac antibody used in *in vitro* immunoglobulin biosynthesis experiments was purified from mouse ascites by DEAE cellulose (Whatman Laboratory Products, Inc., Clifton, NJ) ion exchange chromatography using gradient elution in Tris buffer, pH 8.0, ranging in molarity from 0.01 to 0.3 M. Anti-Tac antibody conjugated for fluorescence studies was purified from ascites by affinity chromatography with staphylococcal protein A-Sepharose (Pharmacia Fine Chemicals) according to the method of Ey et al. (20). The purified antibody was conjugated to biotin succinamide (Bioresearch, San Rafael, CA). Avidin (Pierce Chemical Co., Rockford, IL) was conjugated to fluorescein isothiocyanate (Sigma Chemical Co., St. Louis, MO) using the method of Rinderknecht (21) or to Texas Red (Molecular Probes, Junction City, OR) using the method of Titus et al. (22).

Immunofluorescence Staining. The cells were analyzed using immunofluorescence and flow microfluorometry analyses by a modification of a procedure previously described (23). Cell suspensions for immunofluorescent staining were prepared in Hanks' balanced salt solution (without phenol red) containing 3% fetal calf serum and 0.05% sodium azide. For indirect one-color staining, 1×10^6 cells were incubated for 30 min at 4°C with saturating amounts of the appropriate monoclonal antibody or mouse serum as a control, washed twice, incubated 30 min at 4°C with fluorescein-conjugated anti-mouse immunoglobulin reagent, rewashed twice, and finally resuspended and analyzed for fluorescence. For dual fluorescence studies, cells were stained initially with biotin-conjugated anti-Tac antibody followed by Texas Red-conjugated avidin and then stained with fluorescein-conjugated antibody with appropriate washing between incubations.

For inhibition experiments, 1×10^6 cells were incubated for 60 min at 4°C with the material being tested for blocking activity. Either fluorescein-conjugated antibody or biotin-conjugated anti-Tac antibody were then added directly to the cells with no intervening washing and incubated for 20 min at 4°C. Suspensions were then washed twice and analyzed for fluorescence or further incubated with Texas Red or fluorescein-

conjugated avidin before analysis as indicated. Flow microfluorometry analyses were performed using a Dual Laser FACS II (B-D FACS Systems, Sunnyvale, CA) and data were collected and analyzed using a PDP 11/34 computer (Digital Equipment Corp., Maynard, MA) interfaced to the FACS II. Fluorescence data were collected using logarithmic amplification on 5×10^4 viable cells as determined by forward light scatter intensity. Data from inhibition experiments were calculated as previously described (23).

Source of Interleukin 2 and Polyclonal Activators. Two preparations of IL-2, immunoaffinity-purified, JURKAT-derived IL-2 and recombinant IL-2, were used in the present study to assure that IL-2 itself rather than any potential contaminant was the active agent. The procedures used to purify and characterize the JURKAT-derived IL-2 have been described previously (24). The IL-2 was prepared by column affinity chromatography using an IL-2-binding monoclonal antibody, 1H11-1AS, immobilized on Sepharose 4B. The bound IL-2 was eluted with 1.5% acetic acid, pH 2.5. When analyzed by two-dimensional gel electrophoresis using silver staining for detection, the contents of the pH 2.5 eluate migrated as a single spot. The recombinant-purified IL-2 was obtained from Cetus Corp., Emeryville, CA from the JURKAT IL-2 gene expressed in *E. coli* (25). The protein was 96% pure by SDS-PAGE analysis and contained <0.3 ng of LPS/1,000,000 U of IL-2 as assessed by the limulus amoebocyte assay. The IL-2 concentrations used in the present study are expressed as nanograms per milliliter. There are 0.31 U/ng of IL-2, where the units are those described previously by Robb and co-workers (24). One such unit of IL-2 activity is equivalent to 41.5 U as recently defined by the Biological Response Modifiers program of the National Cancer Institute.

A series of materials were used in the *in vitro* studies to activate B lymphocytes to synthesize and secrete immunoglobulin molecules. PWM was obtained from Gibco Laboratories, Grand Island, NY. *Nocardia* water-soluble mitogen (NWSM) was the kind gift of Dr. Constantin Bona. Wheat germ agglutinin (WGA) was obtained from Miles Laboratories, Elkhart, IN. Streptolysin-O (SLO) was obtained from Difco Laboratories, Detroit, MI.

Measurement of Immunoglobulin Synthesis by Lymphocytes Cultured In Vitro. To study the effect of the anti-Tac monoclonal antibody on the differentiation of circulating B lymphocytes into immunoglobulin-synthesizing and -secreting cells, normal peripheral blood B cells and monocytes free of T cells were co-cultured with autologous, irradiated (2,000 rad) T cells. Polyclonal activators were added in the presence of anti-Tac or a control monoclonal immunoglobulin (RPC-5; Litton Bionetics, Inc., Kensington, MD) of the same IgG2a isotype. The B cell and T cell populations for these studies were extensively washed to remove serum immunoglobulins and then prepared according to techniques described previously (26) using a two-step procedure that involves passage of peripheral blood mononuclear cells through an anti-human F(ab')₂ immunoabsorbent column followed by a rosetting step with neuraminidase-treated sheep erythrocytes. In the biosynthesis experiment 1×10^6 B cells and 1×10^6 irradiated (2,000 rad) T cells were co-cultured in the presence of optimal concentrations of PWM, WGA, SLO, or NWSM (26) for 12 d in RPMI 1640 media supplemented with 10% fetal calf serum (Armour Pharmaceutical Co., Phoenix, AZ), 2 mM L-glutamine (Gibco Laboratories), and 50 μ g/ml penicillin and streptomycin solution (Gibco Laboratories).

Scatchard Analysis of ³H-Anti-Tac Binding to Tac-positive B Cell Lines. To estimate receptor number and ligand affinity, purified anti-Tac was tritiated to high specific activity by reductive methylation (910 Ci/mmol) (27). Cell populations to be examined were washed three to four times in balanced salt solution and suspended at 20×10^6 viable cells/ml in binding medium [RPMI 1640, 1% bovine serum albumin (Sigma Chemical Co.), 1 mg/ml human IgG (Cutter), 25 mM Hepes (Gibco Laboratories), and 0.1% sodium azide (Sigma Chemical Co.)]. Aliquots of 10^6 cells were incubated with labeled antibody (1–11 ng, depending on the assay) for 45 min at room temperature in the presence of graded amounts (0–1,600 ng) of unlabeled anti-Tac in a final volume of 90–200 μ l in 1.5-ml Eppendorf tubes. Cell-associated and free radiolabeled antibodies were separated by centrifuging the reaction mixture through 750- μ l cushions of binding medium containing 1 M sucrose. Supernatants were aspirated and the cell pellets resuspended in balanced

salt solution and transferred to glass vials for liquid scintillation counting. Specific binding was determined by incubating cells and labeled antibody with a >1,000-fold excess of unlabeled anti-Tac, generally 28 μg . Scatchard analyses (28, 29) were performed by plotting the values for bound/free vs. bound radioactivity.

Radiolabeled IL-2-binding Assay. Biosynthetically radiolabeled human IL-2 was prepared by stimulation of JURKAT cells ($4 \times 10^6/\text{ml}$) in serum-free Dulbecco's modified Eagle's medium containing 40 μM unlabeled leucine, 65 μM unlabeled lysine, 0.5 mCi/ml of [^3H]leucine (52 Ci/mmol; ICN Nutritional Biochemicals, Cleveland, OH), 0.5 mCi/ml [^3H]lysine (39 Ci/mmol), 1.5 $\mu\text{g}/\text{ml}$ phytohemagglutinin (HA-16; Wellcome Reagents), and 50 ng/ml phorbol-12-myristic-13-acetate (Consolidated Midlands Corp.) (12). After 16 h at 37°C, the cell supernatant was harvested and the radiolabeled IL-2 was extracted by immunoaffinity chromatography (24). The labeled IL-2 (23×10^6 dpm/ μg) migrated as a single band at 15,500 M_r on SDS-PAGE.

The level and affinity of [^3H]IL-2 binding to cells was determined as previously described (12) by incubating a known quantity of cells (0.5 to 1.5×10^6) with serial dilutions of [^3H]IL-2 in RPMI 1640, 10 mg/ml bovine serum albumin, 25 mM Hepes, pH 7.2 (RPMI-BSA), in a final volume of 0.1 ml. After 20 min at 37°C, the cells were washed twice with ice-cold RPMI-BSA and centrifuged through a 400- μl layer of 85% silicon oil/15% paraffin oil. The tip of the tube containing the cell pellet was transferred to a scintillation vial where the cells were suspended in 200 μl PBS, then lysed and dissolved by the addition of 200 μl 1% SDS.

Structure of Receptor on B Cells Identified by Anti-Tac. 10 – 20×10^6 cells were incubated and biosynthetically labeled for 18 h with 250–500 μCi of [^3H]D-glucosamine (32.5 Ci/mmol; New England Nuclear, Boston, MA). Cells were then washed and solubilized for 30–60 min at 4°C in 0.14 M NaCl, 1% Triton X-100, 10 mM Tris HCl (pH 7.4), and 50 $\mu\text{g}/\text{ml}$ phenylmethylsulfonyl fluoride. Nuclei and cell debris were removed by centrifugation (12,000 g for 15 min) and the supernatants immunoprecipitated with anti-Tac and Cowan I strain staphylococci as previously described (15, 30). Immunoprecipitates were analyzed by 8.75% SDS-PAGE performed under reducing conditions. Gels were enhanced (Enlightning; New England Nuclear), dried under vacuum, and analyzed after fluorography. In parallel studies, surface proteins of the B cell lines were labeled by lactoperoxidase-catalyzed iodination. ~ 5 – 10×10^6 cells were washed and suspended in 100 μl of balanced salt solution and 1 mCi of carrier-free Na^{125}I was added. 4 μl of lactoperoxidase solution (100 U/ml) and 7.5 μl of H_2O_2 solution (0.03%) were then added and the cells incubated for 4 min at room temperature. An additional 2 μl of lactoperoxidase solution and 7.5 μl of H_2O_2 were then added and cells incubated for an additional 4 min. Cells were then washed in balanced salt solution and immunoprecipitated as described above.

Results

Anti-Tac Inhibits Helper T Cell-dependent Immunoglobulin Synthesis. The ability of anti-Tac to inhibit helper T cell-dependent immunoglobulin synthesis by normal B cells was evaluated using an in vitro biosynthesis system. B cells and irradiated (2,000 rad) T cells isolated from normal individuals were combined and cultured at 5×10^5 cells/ml for 12 d with PWM, WGA, SLO, or NWSM. The immunoglobulins synthesized and secreted into the media during the 12-d culture period were assayed using sensitive double antibody radioimmunoassays for IgM, IgG, and IgA (31, 32). The geometric mean values for IgM synthesis by B and T cells from 10 normal individuals after stimulation for 12 d with PWM, WGA, SLO, and NWSM were 4,180 (1.4)² ng, 7,950 (2.5) ng, 7,612 (1.6) ng, and 3,879 (1.5) ng, respectively, per 10^6 cells in 1-ml cultures. Purified anti-Tac, when added at a final concentration of 2 $\mu\text{g}/\text{ml}$, inhibited IgM synthesis by 57, 64, 47, and 53% for cultures stimulated by PWM, WGA, SLO, and NWSM,

² Standard error of the log of geometric mean.

respectively (Fig. 1). Anti-Tac also comparably inhibited IgG and IgA synthesis (data not shown). A control monoclonal antibody (RPC-5) of the same isotype (IgG2a) as anti-Tac did not inhibit the immunoglobulin synthesis induced by these stimulants (Fig. 1). The suppression of immunoglobulin biosynthesis by 2 μ g of anti-Tac was overcome by the addition of 50 ng of purified JURKAT-derived IL-2 to the biosynthesis system, supporting the view that anti-Tac inhibits through binding to the IL-2 receptor. However, the maturation of B cells to immunoglobulin-synthesizing cells requires the participation of helper T cells with the polyclonal activators used (32). Thus, the site of action of anti-Tac in this system cannot be completely defined; both an effect on T cells to inhibit their secretion of B cell growth and differentiation factors or a direct action at the B cell level are possible.

Interleukin 2 Receptors on B Cells. For anti-Tac to act directly on B cells, the B cells would be expected to display the Tac antigen (i.e., IL-2 receptors). To determine whether B cells manifest IL-2 receptors, purified B cells from five normal individuals and from individuals with B cell leukemias were examined by indirect immunofluorescence using anti-Tac. Anti-Tac did not react with rigorously purified resting normal B cells (Fig. 2). Furthermore, five of six unselected B cell lines from normal individuals, established using EBV, were Tac antigen negative. In contrast, the Tac antigen was demonstrable on the cell surface of 6 of 10 B cell lines derived from patients with Burkitt's lymphoma and on 5 of 6 EBV-transformed B cell lines from patients with human T cell leukemia/lym-

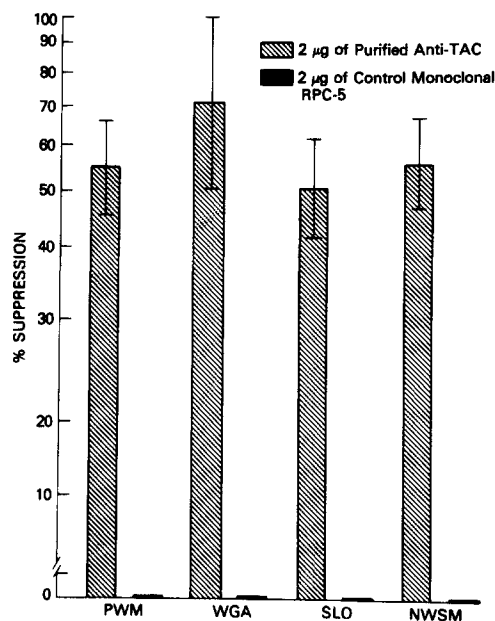


FIGURE 1. Effects of the addition of monoclonal anti-Tac or control monoclonal RPC-5 antibodies on IgM synthesis and secretion of cultured normal B and irradiated (2,000 rad) T cells stimulated by pokeweed mitogen (PWM), wheat germ agglutinin (WGA), streptolysin-O (SLO), and *Nocardia* water-soluble mitogen (NWSM). Purified anti-Tac, when added at a final concentration of 2 μ g/ml, inhibited polyclonal IgM synthesis by 47–64%, whereas control RPC-5 monoclonal antibody did not inhibit immunoglobulin synthesis.

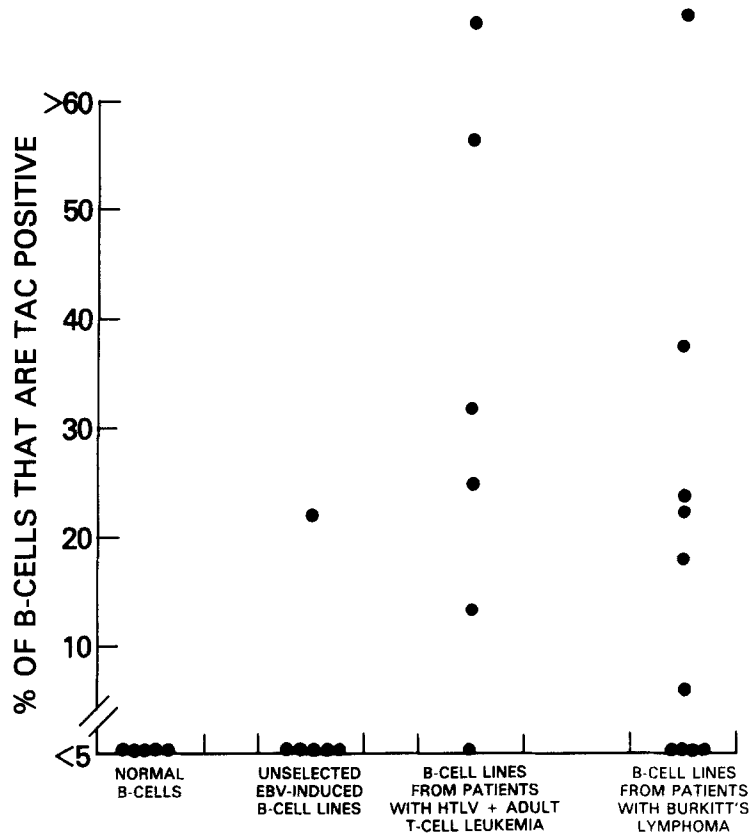


FIGURE 2. Activated B cells from patients with certain lymphoid malignancies manifest IL-2 receptors identified by the anti-Tac monoclonal antibody. The percent of Tac-positive B cells are plotted for individuals with different disease states. The purified B cell populations were analyzed by indirect immunofluorescence flow microfluorometric analysis with anti-Tac and fluorescein-conjugated anti-mouse antibody reagents.

phoma virus-associated adult T cell leukemia. None of the B cell populations exhibiting anti-Tac reactivity by indirect fluorescence were reactive with the control monoclonal RPC-5 antibody or with the Leu-4 antibody, which reacts with virtually all mature T cells.

Although normal resting B cells and the majority of unselected EBV-transformed B cell lines from normal individuals were Tac antigen negative, we next demonstrated that Tac antigen expression can be induced with normal B cells. Furthermore, we have successfully established cloned normal B cell lines that express Tac antigen. Populations of 1×10^6 purified B cells from normal individuals and 1×10^5 autologous (irradiated at 2,000 rad) T cells and 1×10^4 irradiated (1,000 rad) monocytes were stimulated with PWM for 6 d. At the end of the culture period the T lymphocytes were removed by a neuraminidase sheep erythrocyte rosetting procedure. The residual population of B lymphocytes were Leu-4 negative, Leu-10 positive, yet contained a proportion (21–43%) of Tac-positive cells, as evaluated by indirect immunofluorescence. Thus, normal primary B cells could be induced to display the Tac antigen after PWM activation

in the presence of T cells. To establish Tac-positive B cell lines, the activated B cells were incubated with the anti-Tac monoclonal antibody and then rosetted in the presence of ox erythrocytes coated with an antibody to mouse immunoglobulin. Rosetted Tac-positive B cells were cultured in the presence of EBV for 4 wk. 5–39% of the cells in 9 of the 10 EBV-transformed B cell lines established by this method were Tac positive. Cloned lines were established from one of the Tac-positive B cell lines, B22, by limiting dilution at a density of 0.5 B cells/well. Two of the cloned lines, 1C9, which constitutively expressed the Tac antigen, and 5B4, which could be induced to express Tac antigen, were further studied. Both the original line B22 and the cloned daughter lines 1C9 and induced 5B4 contained a proportion of Tac-positive cells identified by microfluorometric analysis when they were stained with biotinylated anti-Tac and then incubated with fluoresceinated avidin. The cloned lines were not reactive with the monoclonal antibodies Leu-4, T4, T8, 3A-1, and T-11, which react with T cells. Furthermore, the Tac-positive cells also displayed surface immunoglobulin and the Leu-10 antigen characteristic of B cells when assessed by two-color fluorescence studies. The addition of 500 ng of purified IL-2, derived from induced JURKAT leukemic cells, blocked the binding of anti-Tac to these lines (Table I). In contrast, the addition of 5,000 ng of IgM to the incubation system did not block the binding of anti-Tac to its receptor on line B22 although it blocked the binding of fluoresceinated anti-IgM to this line (Table I).

The binding of ^3H -anti-Tac to line 1C9 was examined by Scatchard analysis as shown in Fig. 3. Approximately 7.4×10^3 antibody molecules were bound per cell with an apparent K_d of 1.04×10^{-10} M. In parallel studies using biosynthetically labeled IL-2 (JURKAT), line 1C9 expressed $\sim 1,400$ high affinity (K_d , 2.7×10^{-11} M) IL-2 binding sites per cell (average for the entire population in six experiments). Furthermore, binding of the [^3H]IL-2 to 1C9 cells was inhibited by the anti-Tac antibody, demonstrating that, as on activated T cells, the IL-2-binding sites contain the Tac protein. These findings support the conclusion that normal B cells can be activated to express receptors for IL-2.

Induction of IL-2 Receptors on a Normal B Cell Line by IL-2. The cloned normal B cell line 5B4 prepared as described above manifested only small numbers of cell surface molecules identified by anti-Tac. 5% of these cells were Tac positive by flow microfluorometry and displayed only 520 Tac molecules per cell (average for the entire population) as determined by Scatchard analysis of ^3H -anti-Tac

TABLE I
Inhibition of Binding of Anti-Tac on the B Cell Line, B22, by Purified JURKAT IL-2 Assessed by Flow Cytometry

Inhibitor added	Inhibition of anti-Tac binding	Inhibition of anti-human IgM binding
	%	%
IL-2 (500 ng)	100	7
IL-2 (50 ng)	47	8
IL-2 (5 ng)	38	14
Human IgM (5,000 ng)	15	97

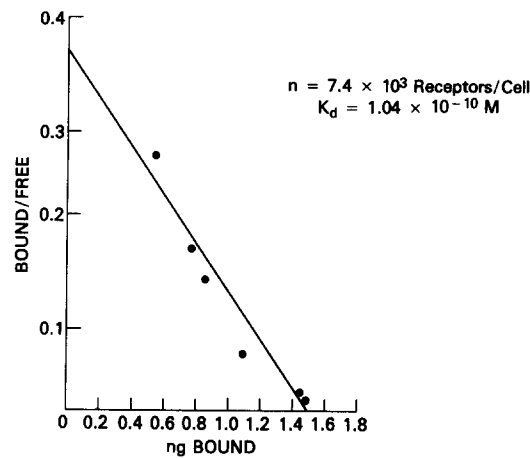


FIGURE 3. Scatchard analysis of ^3H -anti-Tac binding to a cloned normal B cell line. ^3H -anti-Tac binding was performed as described in the Materials and Methods section. Transformation of binding data indicated that 1C9 cells displayed $\sim 7,400$ receptors per cell with an apparent K_d of 1.04×10^{-10} M.

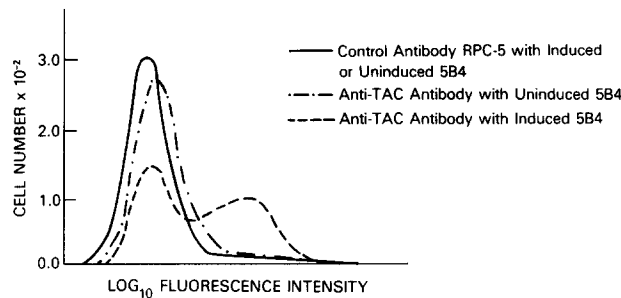


FIGURE 4. Purified IL-2 induces augmented IL-2 receptor expression in cloned normal B cell line 5B4. Binding of anti-Tac or RPC-5 was measured by flow microfluorometry analysis of fluorescence intensity generated by reacting different cell populations with antibody conjugated to biotin succinimide and subsequently with avidin conjugated to fluorescein isothiocyanate. Before flow microfluorometric analysis induced 5B4 cells had been incubated for 48 h with 50 ng/ml of immunoaffinity-purified JURKAT IL-2.

binding. The ability of this population to be induced to express larger numbers of Tac receptors was explored after an initial observation that exposure of this line for 48 h to lectin-depleted conditioned medium containing IL-2 led to an increase in the proportion of Tac-positive cells from 5 to $>30\%$. The addition of either immunoaffinity-purified JURKAT IL-2 or recombinant IL-2 to the culture in the range of $10\text{--}100$ ng/ 10^6 cells increased the receptor number from 520 to 4,500 per cell after 48 h of culture (Figs. 4–6). The addition of 1 ng/ml purified JURKAT IL-2 led to an intermediate 50% increase in the number of receptors per cells. This concentration (6×10^{-11} M) required for one-half-maximal induction is comparable to the K_d (2.7×10^{-11} M) for binding of [^3H]IL-2 to its receptor on the cloned B cell line. An increase in specific binding was seen as early as 12 h after exposure to the purified JURKAT IL-2 preparation and reached a maximum at 48 h (Fig. 5). The K_d for binding of ^3H -anti-Tac to

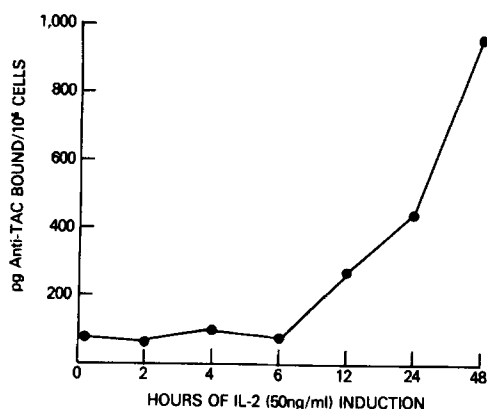


FIGURE 5. Time course of IL-2 induction of Tac antigen expression on 5B4 cells. Specific binding of ³H-anti-Tac is plotted as a function of the time of JURKAT IL-2 induction of 5B4 cells cultured with 50 ng/ml purified IL-2.

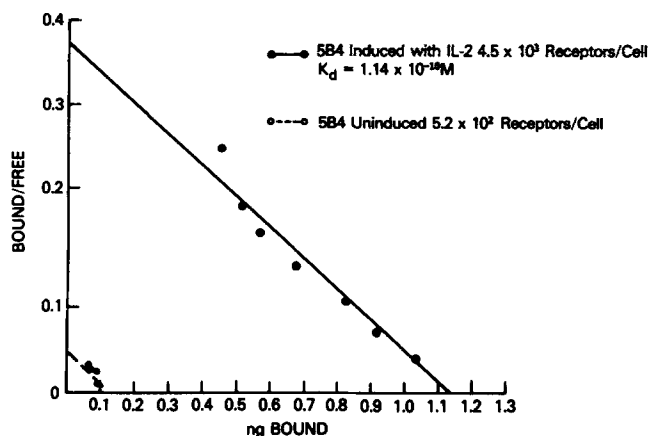


FIGURE 6. IL-2 induction of 5B4 cells increases the binding of ³H-anti-Tac. Uninduced 5B4 line bound 0.13 ng of ³H-anti-Tac/10⁶ cells, indicating an average of 520 receptors per cell whereas 5B4 cells incubated with 50 ng of purified JURKAT IL-2 for 48 h bound 1.12 ng of anti-Tac/10⁶ cells indicating an average of 4,500 receptors per cell with an apparent K_d of 1.14×10^{-10} M.

the induced cell population was 1.14×10^{-10} M (Fig. 6). The addition of cycloheximide (5×10^{-6} M) abrogated the increase in IL-2 receptors after exposure to purified IL-2. The concentration of cycloheximide used blocked ³H-leucine incorporation by 90%, but did not produce immediate cell death as assessed by trypan blue dye exclusion. Furthermore, actinomycin D (3×10^{-7} M and 3×10^{-8} M) produced >95% inhibition of IL-2 receptor induction. In contrast, inhibition of DNA synthesis by irradiation (2,000, 4,000, and 8,000 rad) sufficient to inhibit thymidine uptake by 85% did not inhibit the IL-2-induced IL-2 receptor expression. With the 4,000 rad dose of irradiation, there was only a modest reduction in cell viability, with 91% of the cells still excluding trypan blue. Thus, the induction of IL-2 receptor expression after exposure of

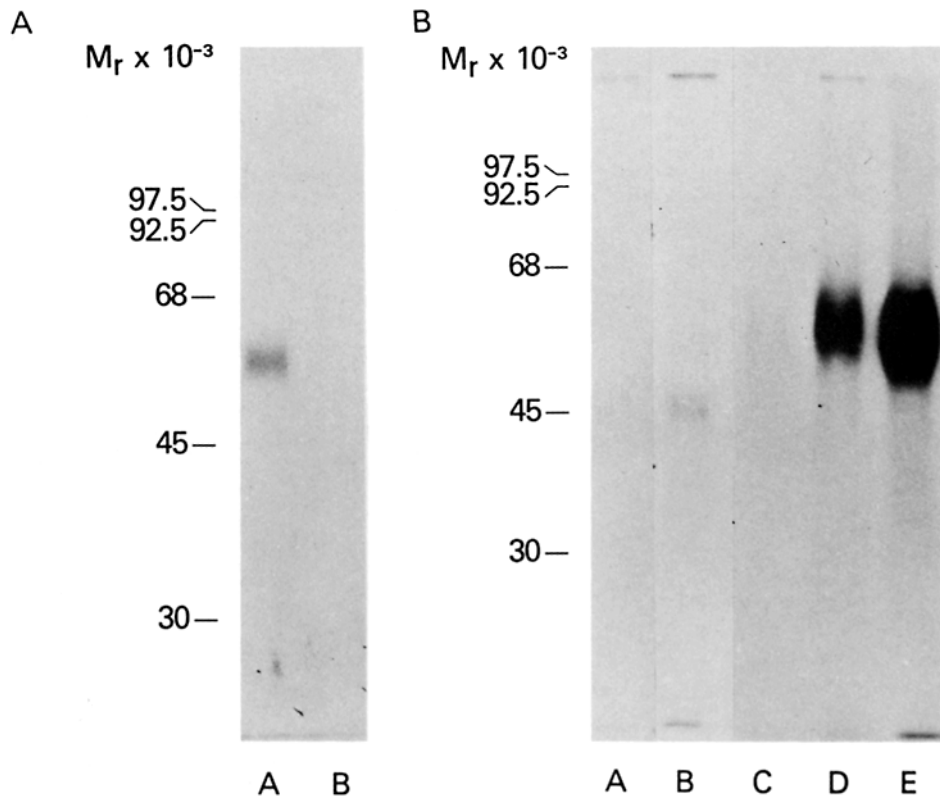


FIGURE 7. Size of IL-2 receptors on cloned normal B cell lines 1C9 and 5B4. (A) 1C9 cells were biosynthetically labeled with [^3H]D-glucosamine for 18 h solubilized in non-ionic detergent and immunoprecipitated with anti-Tac (lane A) or control UPC-10 antibody (lane B) as previously described (15). Immunoprecipitates were analyzed by SDS-PAGE on 8.75% gels performed under reducing conditions. Migration of known molecular weight markers is shown. (B) The 5B4 cell line and activated T cells were surface iodinated and analyzed as in A. No radiolabeled proteins were precipitated by UPC-10 from uninduced (lane A) or induced (lane B) 5B4 cells. Anti-Tac immunoprecipitated a faint band from uninduced 5B4 cells (lane C) and a much more intense band from 5B4 cells induced for 48 h with 50 ng/ml of JURKAT IL-2 (lane D). Anti-Tac immunoprecipitation of surface-labeled, PHA-activated T lymphoblasts is shown in lane E.

the 5B4 line to IL-2 requires RNA transcription and protein synthesis but not DNA replication.

Structure of IL-2 Receptors on B Cells. On the basis of our previous studies (15, 31), the Tac antigen (IL-2 receptor) present on activated normal T cells was shown to be a glycoprotein with an M_r of 53,000–57,000. The Tac antigen on cloned normal B cell line 1C9, biosynthetically labeled with [^3H]D-glucosamine and immunoprecipitated with anti-Tac but not control UPC-10 antibody, was similar in size to the receptor on T cells (Fig. 7A). In addition, the size of the Tac antigen on 5B4 cells induced with IL-2 and labeled with ^{125}I was similar in size to the receptor precipitated by anti-Tac on simultaneously studied PHA-stimulated T cell lymphoblasts (Fig. 7B).

Functional Effects of IL-2 and Anti-Tac on the B Cell Line 5B4. The synthesis

TABLE II
*The Effect of Purified JURKAT IL-2 and Anti-Tac on
 Immunoglobulin Synthesis by Line 5B4*

Growth factor added	Monoclonal antibody added	Immunoglobulin synthesized by 10^6 5B4 cells in culture for 14 d
		ng
1. None	None	2,790
2. IL-2 (100 ng)	None	10,754
3. IL-2 (20 ng)	None	9,323
4. None	Anti-Tac (20 μ g)	3,629
5. None	RPC-5 (20 μ g)	3,842
6. IL-2 (20 ng)	Anti-Tac (20 μ g)	3,015
7. IL-2 (20 ng)	RPC-5 (20 μ g)	7,137

and secretion of IgM into the media in these cultures was increased two- to fourfold by the addition of 1, 10, 20, 50, or 100 ng of purified JURKAT IL-2/ 10^6 5B4 cells per milliliter ml in culture (Table II). This IL-2-inducible component of IgM synthesis was completely blocked by the addition of 20 μ g of anti-Tac. In contrast, the addition of the control monoclonal (RPC-5) did not alter IL-2-stimulated immunoglobulin synthesis. Furthermore, the addition of various concentrations of purified JURKAT IL-2 or anti-Tac had no effect on the cell number at the termination of the culture or the proliferation ($[^3\text{H}]$ thymidine uptake) of 5B4 cells, either when added before or after induction of IL-2 receptor expression (data not shown). Thus, IL-2 did not alter the rate of proliferation of the 5B4 line, but appeared to facilitate the terminal differentiation of these cloned normal B cells into immunoglobulin-synthesizing and -secreting cells.

Discussion

IL-2 Receptor Expression on Activated B Cells. In previous reports (4, 12), no receptors for IL-2 were demonstrated on resting B cells or on the activated B cells examined. These activated B cells include LPS-stimulated mouse spleen cells as well as the Burkitt lines Raji and Daudi. In this report, we demonstrate that some activated B cells express membrane receptors for IL-2. Monoclonal anti-Tac, which identifies the human receptor for IL-2, binds to certain malignant B cell populations including 6 of 10 patients with Burkitt's lymphoma, and the B cell lines derived from 5 of 6 patients with the HTLV-I-associated adult T cell leukemia. Furthermore, B cells from normal individuals activated by PWM in the presence of T cells expressed the Tac antigen. In addition, cloned EBV-transformed B cell lines derived from Tac-positive activated normal B cells continued to express Tac antigen in long-term cultures. The binding of anti-Tac to B cells rather than T cells in each of these systems was confirmed by demonstrating that the purified cells and cell lines examined did not react with Leu-4 and that the Tac-positive cells assessed by two-color fluorescence analysis concomitantly expressed the B cell-associated antigen identified by the monoclonal antibody Leu-10. Furthermore, both immunoaffinity-purified, JURKAT-

derived IL-2 and recombinant IL-2 blocked the binding of anti-Tac to the Tac-positive B cell lines. In addition, ~1,400 high affinity IL-2 receptors were detectable on 1C9 cells when studied with purified radiolabeled IL-2. Finally, the size of the receptor identified by anti-Tac on B cells was similar to that present on activated T cells. These data are in accord with the view that some activated B cells express membrane receptors for IL-2.

There are possible explanations for the discordance between this conclusion and the view expressed in previous studies that B cells do not bear IL-2 receptors. We have shown that the Burkitt's lymphoma line studied previously, the Daudi line (12), which was reported to be IL-2 receptor negative, is also unreactive with anti-Tac. Furthermore, the level of Tac antigen expression on activated B cells is 5–10-fold less than that characteristic of activated T cells (33). Even the Tac-positive B cell lines, 1C9 and induced 5B4, which have the most receptors we have observed on normal B cells, manifested only 7,500 and 4,500 Tac receptors, respectively, as assessed by Scatchard analysis of ³H-anti-Tac binding, whereas PHA-activated T cells expressed 30,000–60,000 receptors per cell, and HTLV-positive adult T cell leukemia T cell lines such as HUT102 expressed 150,000–600,000 Tac receptors per cell (33). Thus, the number of high affinity IL-2 receptors on the activated B cells studied previously may have been too low to be detected by some of the techniques used. Taken as a whole, the data support the hypothesis that IL-2 receptors are induced on some but not all activated B cell populations.

IL-2 Induction of IL-2 Receptors on B Cells. The line 5B4 reported in the present study can be induced with IL-2 to express augmented numbers of receptors identified by the anti-Tac antibody (an increase from 520 to 4,500 receptors per cell). These studies suggest that IL-2 may up-regulate the number of IL-2 receptors expressed by this B cell line. The fact that both immunopurified JURKAT IL-2 and recombinant IL-2 could augment receptor expression suggests that IL-2 itself rather than any potential contaminant was the active agent. The requirements for RNA transcription and protein synthesis suggests that *de novo* receptor synthesis is occurring rather than the unmasking of cryptic receptors. The view that IL-2 induces cells with low numbers of receptors to synthesize and express high numbers of cell surface IL-2 receptors rather than causing the outgrowth of cells with high numbers of receptors is supported by the observations that the Tac receptors were induced within 12–24 h and that this induction was not inhibited by doses of gamma irradiation that profoundly inhibited DNA synthesis. Although in many receptor-ligand systems the addition of the ligand leads to down-regulation of the receptor numbers, there is precedence for up-regulation of the number of surface receptors by certain ligands. For example, surface Fc epsilon receptors were induced on activated T cells after the addition of IgE (34). Similarly, Fc alpha receptors were induced on T lymphocytes by addition of IgA to in vitro cultures of these cells (35). Furthermore, an increase in the number of insulin receptors was observed with 3T3 (36) and chondrosarcoma cell lines (37) in response to insulin. In 3T3 cells this induction was slow and was paralleled by the maturation of the 3T3 cells into more mature adipocytes (36). In the case of the chondrosarcoma cells there was no change in apparent state of maturation or phenotype of the cells (37). With the present

line, 5B4, there was no change in the proportion of cells that reacted with a series of monoclonal antibodies including B1, B2, T9, and BA1. Since the line 5B4 is an EBV-transformed line that does not require exogenous IL-2 for its proliferation, it provides a valuable line for the study of the factors involved in the induction of IL-2 receptors on B lymphocytes.

Functional Role of IL-2 in B Cell Differentiation. Although it appears that IL-2 receptors develop on certain B cells during their activation, the functional significance of the interaction of IL-2 with this receptor is not clear. It is evident, for example, in studies of Tac-negative, EBV-transformed B cell lines that this interaction is not absolutely required for immunoglobulin synthesis, nor is the addition of IL-2 alone to B cells sufficient for B cell proliferation or differentiation. Furthermore, it is conceivable that the IL-2 binding receptors on B cells that we have identified might also be receptors for another B cell growth or differentiation factor. However, the high affinity (K_d , 2.7×10^{-11} M) of the binding of IL-2 to these receptors on B cells would be more in line with primary specificity for IL-2 rather than for some other ligand.

Anti-Tac inhibited polyclonal immunoglobulin biosynthesis in response to the helper T cell-dependent polyclonal activators, PWM, SLO, NWSM, and WGA. However, in these cases, the effect could either be on the B cells directly or on the production of B cell growth and differentiation factors by T cells. Howard and co-workers (38) have indeed demonstrated the requirement for IL-2 for the synthesis of B cell growth factor by T cells. However, IL-2 may have a more direct effect on B cells as well. In additional studies we have shown that the immunoglobulin biosynthesis response of rigorously T cell-depleted B cells to a crude T cell-replacing factor (the supernatants of T cells stimulated by PWM) was abrogated by the addition of 20 μ g of anti-Tac. The B cells utilized in this latter study were prepared by adherence to anti-immunoglobulin columns followed by removal of any residual T cells by rosetting with neuraminidase-treated sheep erythrocytes and did not proliferate to T cell-mitogens. Furthermore, they did not synthesize immunoglobulins when stimulated with PWM. However, these B cells synthesized IgM, IgG, and IgA when cultured with the supernatants of T cells cultured for 48 h with PWM. This TRF-stimulated synthesis was abrogated when 20 μ g of anti-Tac was added.

To avoid any potential effects of anti-Tac on such contaminating T cells that might be present in any preparation of freshly obtained mononuclear cells, we have also studied the effect of anti-Tac on immunoglobulin synthesis by a cloned B cell line from a normal individual, line 5B4. Immunoglobulin synthesis by this line was increased two- to fourfold by the addition of purified IL-2 in the absence of a concomitant increase in cell number. This increase in immunoglobulin synthesis could be completely blocked by the addition of anti-Tac (Table II). Augmentation of immunoglobulin synthesis in conjunction with the observation that neither IL-2 nor anti-Tac affected DNA replication by these B cells suggests that IL-2 can facilitate the terminal differentiation of B cells. Clearly, further studies are required to address the significance of the role of IL-2 in B cell proliferation and differentiation. However, these observations, taken in conjunction with studies summarized by Howard and Paul (9), would support the following model of B cell activation, growth, and differentiation: B cells are

activated by the binding of appropriately presented antigen to its specific receptor, cell surface immunoglobulin. B cell activation progresses and the B cells enter a proliferative cycle after the interaction of IL-1 and BCGF with receptors for these ligands that appear on the B cell surface. The terminal maturation of these proliferating B cells involves the binding of a series of antigen-nonspecific B cell differentiation factors and, in certain cases, IL-2 with specific receptors inducibly expressed on the activated proliferating cells. Thus, IL-2 would join other antigen-nonspecific factors produced by T cells as a potential regulator of the differentiation of B cells into immunoglobulin-synthesizing and -secreting cells.

Summary

Using anti-Tac, a monoclonal anti-interleukin 2 (IL-2) receptor antibody, we have explored the possibility that certain activated B cells display receptors for IL-2. Resting normal B cells and unselected B cell lines established from normal individuals were Tac antigen negative. In contrast, the cell surface Tac antigen expression was demonstrable on 6 of 10 B cell lines from patients with Burkitt's lymphoma, 5 of 6 B cell lines derived from patients with HTLV-I-associated adult T cell leukemia (including all four that had integrated HTLV-I into their genome), and on certain normal B cells activated with pokeweed mitogen. Furthermore, cloned Epstein-Barr virus-transformed B cell lines derived from Tac-positive normal B cells continued to express the Tac antigen in long-term cultures and manifested high affinity IL-2 receptors identified in binding studies with purified radiolabeled IL-2. The line 5B4 developed in the present study could be induced with purified JURKAT-derived or recombinant IL-2 to express a larger number of IL-2 receptors. Furthermore, the addition of IL-2 to the 5B4 B cell line augmented IgM synthesis, which could be blocked by the addition of anti-Tac. The size of the IL-2 receptors expressed on the cloned normal B cell lines was similar (53,000–57,000 daltons) to that of receptors on phytohemagglutinin-stimulated T cell lymphoblasts. Thus, certain malignant and activated normal B cells display the Tac antigen and manifest high affinity receptors for IL-2. These data suggest that IL-2 may play a role in the differentiation of activated B cells into immunoglobulin-synthesizing and -secreting cells.

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