A Case of Human B Cell Leukemia That Implicates an Autocrine Mechanism in the Abnormal Growth of Leu 1 B Cells

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Abstract

Production of B cell growth factor (BCGF) from B-chronic lymphocytic leukemia (B-CLL) cells was demonstrated. Freshly isolated monoclonal B-CLL cells expressed surface μ, δ, B1, and Leu 1, but not Ba (an antigen expressed only on activated B cells). Upon stimulation with anti-IgM, they secreted BCGF, which could act on anti-IgM–stimulated autologous leukemic cells as well as anti-IgM–stimulated normal B cells. Cell lines established from these leukemic cells also constitutively secreted BCGF. The BCGF from B-CLL cells or established cell lines induced neither proliferation nor enhanced HLA-DR expression in resting B cells. These results show the presence of B cell-derived BCGF, which is distinct from BSF-1 and effective only on activated B cells. They also suggest that an autocrine mechanism may operate in the growth of B-CLL cells.

Introduction

Recent studies have demonstrated the involvement of several soluble factors with different functions in the growth and differentiation of antigen-stimulated B cells (1–3). Several of these factors have been well characterized and the gene encoding B cell stimulatory factor-1 (BSF-1),[1] which was originally designated B cell growth factor-1 (BCGF-I) has been cloned (4, 5). BSF-1 shows synergy with anti-IgM in the proliferation of resting B cells. BSF-1 is not strictly a growth factor; however, it is responsible for the activation of resting B cells (6–8). Therefore, a growth factor for B cells comparable to interleukin 2 (IL-2) for T cells has yet to be identified. Our previous report (9) as well as several other studies (10–14) showed that activated B cells or transformed B cell lines secreted B cell growth factor(s) that could induce the proliferation of activated, but not resting B cells, suggesting an autocrine mechanism in B cell proliferation as in the case for T cell growth.

In this study, we report the likelihood that a B cell–derived B cell growth factor (B–BCGF) may be involved not only in the regulation of normal B cells growth but also in the proliferation of neoplastic B cells. The results show that leukemic B cells freshly isolated from a patient with B-chronic lymphocytic leukemia (B-CLL) secrete B–BCGF upon stimulation with anti-IgM and the same leukemic B cells are responsive to their own BCGF only following anti-IgM stimulation. Although the study was conducted on leukemic cells only from a single CLL patient, such results would account for the relatively benign nature of B-CLL, since only in vivo antigen stimulation could induce abnormal proliferation of those B cells. In this regard, the likely autospecificity of Leu 1 B cells and B-CLL is discussed.

Methods

Patient. A patient (K.H.) with B-CLL was studied. According to the staging classification of Rai et al. (15), the patient was in stage 0. At the time of the study, this patient did not receive any treatment. A profile of the blood picture extending from 22 March 1985 to 23 July 1985 is shown in Table I.

Reagents. Staphylococcus aureus Cowan I (SAC) was obtained from Hoechst Japan, Ltd., (Tokyo). Phytohemagglutinin-P (PHA) was purchased from Difco Laboratories (Detroit, MI). $F(ab')_2$ fragment of goat anti-human IgM (anti-μ) was purchased from Cappel Laboratories (Cochranville, PA). Recombinant IL-1 (rIL-1) was a gift of Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Recombinant IL-2 (rIL-2) was provided from Ajinomoto Co., Ltd., Tokyo. 1 U of IL-1 or IL-2 was defined as the activity that gave 50% of the maximum proliferative response. B cell differentiation factor (BSF-2) was purified in this laboratory from a human T cell leukemia virus (HTLV)–transformed T cell clone, Na-I, as previously described (16). 100 pM of purified BCDF gave maximum response in a BCDF assay, in which Ig secretion from EBV-transformed B cell lines or SAC-activated B cells was measured (16). B–BCGF was prepared from a B cell line, RPMI 1788, as described in a previous report (9). PHA-conditioned medium from T cells (PHA-T supernatant) was prepared by a method described previously (17). Biotinated anti-HLA-DR and anti-Leu 1 monoclonal antibodies were obtained from Becton Dickinson Monoclonal Center, Inc. (Mountain View, CA). Purified anti-Tac monoclonal antibody (18) was kindly provided by Dr. T. Uchiyama (Kyoto University). Insoluble anti-Ig (ISαIg) was prepared by coupling 1 mg purified anti-IgM antibody to 1 ml of cyanogen bromide (CNBr)–activated Sepharose 4B beads (Pharmacia Fine Chemicals, Uppsala, Sweden).

Animals and cell lines. Female Balb/c mice were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and bred in our own colony. An IL-2–dependent murine cytotoxic T cell line, MTH41.16, was a kind gift of Dr. Hamuro (Ajinomoto Co. Ltd., Tokyo). The EBV-transformed B cell line, RPMI 1788, was originally obtained from Dr. P. Ralph (Cetus Co., Palo Alto, CA). Three cell lines (KHB1, KHB2, and KHB3) were established from peripheral blood lymphocytes (PBL) of a patient (K.H.) as described in the text, maintained in RPMI 1640 medium (Flow Laboratories, Irvine, CA) supplemented with 10% fetal calf serum (FCS) (645, Gibco, Grand Island, NY), 100 U/ml penicillin,

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1. *Abbreviations used in this paper:* anti-μ, anti-IgM; BCGF, B cell growth factor; BCDF, B cell differentiation factor; BSF-1, B cell stimulatory factor-1; CLL, chronic lymphocytic leukemia; EBV, Epstein Barr virus; EBNA, Epstein Barr nuclear antigen; FACS, fluorescence activated cell sorter; FCS, fetal calf serum; FITC, fluoroisothiocyanate; γ-IFN, gamma interferon; HTLV, human T cell leukemia virus; IL-1, 2, interleukin 1 or 2; ISαIg, insoluble anti-immunoglobulin; MNC, mononuclear cells; PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin; SAC, *Staphylococcus* aureus Cowan I; 6MPDR, 6-methylpurine deoxyriboside; TdR, [^{3}H]thymidine; TR, Texas Red.

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Table I. Analysis of PBL of the B-CLL Patient (K.H.)

Date	3/22/85	5/1/85	7/23/85
RBC ($\times 10^{-4}$)	548	525	518
Platelet ($\times 10^{-4}$)	12.8	14.9	14.5
WBC	33,600	46,700	41,000
Lymph (%)	79	90	77
Neu (%)	19	9	22
Surface phenotypes of leukocytes (3/22/85)*			
T3	8.0%	Ia	91.1%
T4	6.2%	J_5	29.2%
T8	8.0%	B_1	91.5%
T11	16.7%		

* PBL were analyzed by FACS 440 for expression of cell surface antigens.

100 μg/ml streptomycin, 2 mM L-glutamine, and 5×10^{-5} M 2-mercaptoethanol. The medium was replaced every 3–4 d. These cell lines were found to be free from mycoplasma when screened by mycoplasma detection kit (Gen-Probe, San Diego, CA) and the mycoplasma-mediated 6-methylpurine deoxyriboside (6MPDR) toxicity test described by Proust et al. (19).

Cell preparation. Peripheral blood mononuclear cells (MNC) from the B-CLL patient (K.H.) or tonsillar MNC obtained from patients with chronic tonsilitis were separated by standard Hypaque-Ficoll gradient centrifugation. B cell-enriched populations (E^- cells) and T cell-enriched populations (E^+ cell) were separated by rosetting MNC twice with 2-amino-ethylisothiouronium bromide (AET)–treated sheep red blood cells. E^- cells were further treated with anti–monocyte (M206) antibody (20) and newborn rabbit complement. Cells recovered after such treatment usually contained >90% sIg^+ cells, <0.1% E^+ cells and monocytes, and were used as purified B cells. In some experiments, freshly prepared tonsillar B cells or in vitro activated B cells that had been incubated with either SAC or anti-μ antibody for 3 d were separated into high density cells (small B cells) and low density B cells (B cell blasts) by using a discontinuous Percoll gradient centrifugation. About 2×10^7 B cells were layered on the top of the gradients and tubes were centrifuged at 1,280 *g* for 15 min at 4°C. Human B cells with low and high density were recovered in fractions between 40/50% and 60/70%, respectively.

Cell staining and FACS analysis. 1 million cells were incubated with 0.1 to 1 μg of biotinated anti-HLA monoclonal antibody for 20 min, washed three times with staining buffer (RPMI 1640 deficient of biotin, riboflavin, and phenol red), incubated with Texas Red (TR)–conjugated avidin for 20 min, and washed three times with staining buffer. Two-color fluorescence-activated cell sorter (FACS) analysis was carried out by staining cells with fluoroisothiocyanate (FITC)-conjugated antibody and biotinated-antibody followed by TR-avidin. Stained cells were analyzed on a FACS 440 (Becton Dickinson Co.) equipped with both an argon ion laser operating at 448 nm generating forward scatter, fluorescein, propidium iodide (PI), and large angle scatter and a second argon ion laser pumping an organic dye laser circulating rhodamine 6G tuned to emit 595 nm light to excite TR. List mode data was collected on 20–50×10^3 cells for each sample and analyzed on a VAX 11/730 computer using programs originally developed at Stanford University (21).

Southern blotting analysis. High molecular weight DNA was isolated from PBL of the B-CLL patient (K.H.) or established cell lines and digested with the indicated restriction enzymes. Digested DNA fragments were size-fractionated by electrophoresis in 0.5% agarose and transferred to nitrocellulose filters by the Southern blotting technique (22). Filter-bound DNA fragments were then hybridized to nick-translated (^{32}P) probe and visualized on autoradiograms (23). The human Ig gene probe used in this study was the Ig heavy chain joining region J_H probe (3.5 kb embryonic Eco RI-Hind III J_H–containing fragment) provided by T. Honjo (Kyoto University) (24).

In vitro culture. To assay proliferation, cells (usually 10^5/well if not specified) were put in 100 μl of serum-free HB101 medium (Hana Media, Inc., Berkeley, CA) in flat-bottomed microtiter plates (2596, Costar Data Packaging, Cambridge, MA) and 100 μl of various concentrations of IL-1, IL-2, SAC, anti-μ, BCGF, BCDF, or test samples were added. All cultures were incubated for 60–70 h as indicated in the legends. To assay IL-1, thymocytes from 6-wk–old Balb/c mice were cultured for 3 d at a cell density of 2×10^6/well with 1 μg/ml PHA in the presence of test samples. To assay IL-2, an IL-2–dependent murine T cell line, MTH41.16, was cultured at a cell density of 2×10^3/well with test samples for 24 h. All cultures were pulsed with 1 μCi of [^{3}H]thymidine (TdR) (15.1 Ci/mmol: New England Nuclear, Boston, MA) at various periods as indicated in figure legends, followed by harvesting on glass filter paper by an automated cell harvester (Labo Mash Science Co., Tokyo) and incorporation of radioactivity was measured by a Beckman scintillation counter (Beckman Instruments, Inc., Fullerton, CA). All assays were performed in triplicate.

Preparation of culture supernatant. Culture supernatants from PBL of the B-CLL patient (K.H.) were obtained after culturing the cells (10^6/ml) for 48 to 72 h in medium alone, with insoluble anti-Ig (ISα Ig: anti-Ig coupled on beads), or with control beads (CB) in 1 ml of serum free HB101 medium in a 15.5-mm diam dish (3047, Falcon Labware). Culture supernatants from established cell lines (KHB1, KHB2, and KHB3) were obtained by culturing the cells (10^6/ml) for 48 h in HB101 medium. The collected supernatants were passed through a 0.45-μm filter, followed by dialysis against PBS. Samples were frozen at −20°C before use.

Assays for γ-interferon (IFN). γ-IFN activity was assayed by the method of Yip et al. (25) with some modifications, in which inhibition of the cytopathic effect of Sindbis virus in human FL5.1 cells was measured.

Detection of EBNA. Epstein Barr virus nuclear antigen (EBNA) was detected using a standard anti-EBNA sera (26). Freshly separated PBL from K.H. was found to be negative with the anti-EBNA sera, while all three established cell lines from PBL of the patient were found to be positive with the anti-EBNA sera.

Results

Analysis of surface phenotypes and Ig gene configuration of PBL from the B-CLL patient (K.H.). The surface phenotype of the PBL from K.H. is summarized in Table I. More than 90% of PBL ($26,500/mm^3$) were B cells as judged by B1 expression and the percentage of T cells was less than 10% as judged by T3 expression. Two-color FACS analysis, shown in Fig. 1, *A–D*, demonstrated that >90% of PBL expressed the B1 antigen, but did not express the Ba antigen (27). Very few cells expressed the Tac antigen or the Leu 4 antigen. Of note is that the majority of the cells strongly expressed both IgM and IgD on their surface, which is unusual in the case of B-CLL. All surface IgM^+ cells expressed Leu 1, which is a common feature of B-CLL (28).

In order to study the clonality of the B cells, the rearrangement of Ig genes was examined. The DNA from patient PBL or from human placenta was digested with Hind III or Xba I and the fragments were hybridized with the 3.5-kb J_H Hind III/Eco RI probe (Fig. 2, lane 1 and 2). The Hind III restriction fragments of patient PBL gave a 6.0-kb (kilobase) band (lane *2*), while those of placenta showed an expected 10.9-kb band (lane *1*). The Xba I fragments of patient PBL gave two bands of 6.6 and 6.0-kb, respectively, (lane *2*), whereas those of placenta showed the expected 6.6-kb germ line band (lane *1*). The results show the monoclonal nature of the B cells in this B-CLL patient (K.H.).

In vitro proliferative response of the B cells from the B-CLL patient (K.H.) to various stimuli. The B cells from the patient (K.H.) were examined for their response to various B cell mitogens or lymphokines by proliferation. The B cells were cultured

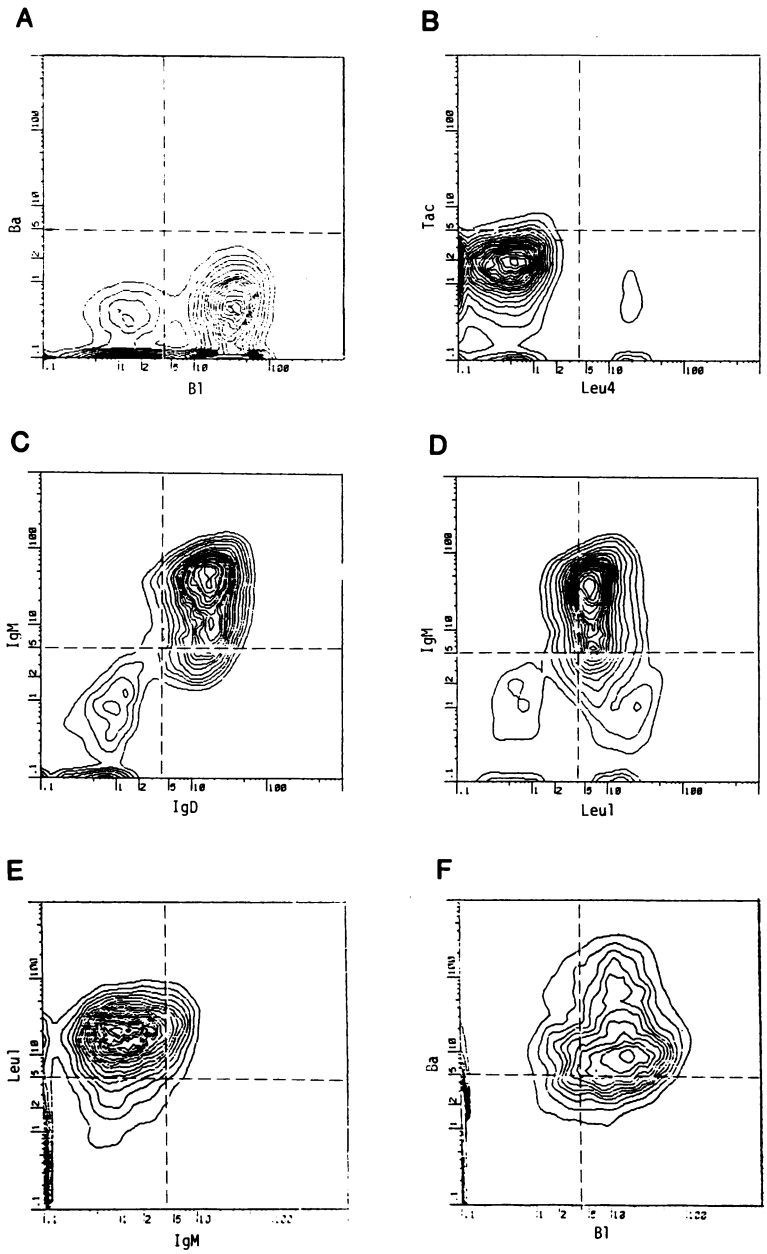

Figure 1. Two-color FACS analysis of PBL of the B-CLL patient (K.H.). PBL separated by Ficoll-Hypaque gradient centrifugation from the blood of a B-CLL patient were stained with biotinated anti-Ba and FITC-anti-B1. (*A*) Biotinated anti-Tac and FITC-anti-Leu 4; (*B*) biotinated anti-IgM and FITC-anti-IgD; (*C*) biotinated anti-IgM and FITC-anti-Leu 1 (*D*) and followed by TR-coupled avidin. An established B cell line (KHB1) was also stained with biotinated anti-Leu 1 and FITC–anti-IgM (*E*); biotinated anti-Ba and FITC–anti-B1 (*F*), followed by TR-coupled avidin. Analysis was performed on a FACS 440 with a dual laser system as described in Methods. Contour plots represent the correlated expression of two-surface antigens by showing peak lines enclosing equal percentages of cells within the two-parameter distribution.

with anti-μ, SAC, rIL-1, rIL-2, or B cell-derived BCGF (9), and the proliferative responses were measured. As listed in Table II, rIL-1, rIL-2, rIL-1 plus rIL-2, SAC, and BCGF did not induce any significant proliferation of those B cells. Some proliferative response was observed by anti-Ig stimulation and the addition of B-BCGF with anti-μ synergistically augmented this proliferation. It is noteworthy that B cells freshly isolated from the patient did not express Ba (Fig. 1 *A*), which is expressed on activated B cells at G1 phase, but not on resting B cells (27). These results indicate that freshly isolated leukemic B cells are at the resting stage and so do not respond to growth factors. However, anti-Ig stimulation is able to activate these leukemic B cells to a stage responsive to BCGF.

Growth factor production by leukemic B cells from the B-CLL patient (K.H.). Anti-μ stimulation of the leukemic B cells in the absence of BCGF induced a significant proliferation (Table II), suggesting the possibility that these cells could secrete BCGF upon stimulation with anti-μ. To study this possibility, highly purified B cells were cultured in serum-free medium with anti-Ig-coupled Sepharose beads (ISαIg) and culture supernatant was examined for the presence of the growth factor activity on autologous leukemic B cells. Supernatant from such ISαIg-stimulated leukemic B cells induced proliferation of anti-μ–stimulated autologous B cells, whereas supernatant from leukemic B cells incubated with control beads or medium alone did not induce any proliferation of anti-μ–stimulated leukemic B cells. Representative data of three different experiments with similar results are shown in Fig. 3. It should be pointed out that supernatant from ISαIg-stimulated leukemic B cells did not induce any proliferation of nonstimulated leukemic B cells. The results indicate that anti-μ stimulation of leukemic B cells induces BCGF production as well as the expression of BCGF receptors, thus leading to proliferation in an autocrine fashion.

In order to examine whether the supernatant from anti-Ig-stimulated leukemic B cells induces proliferation of normal B or T cells, purified B or T cells from tonsils were cultured with

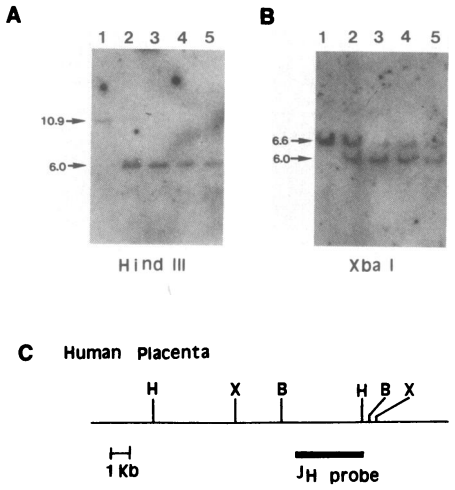

Figure 2. Immunoglobulin heavy-chain gene analysis of the B-CLL–PBL and established cell lines. High molecular weight DNA isolated from patient PBL or established cell lines was digested with Hind III (*A*) or Xba I (*B*). Digested DNA was size-fractionated and hybridized with a ^{32}P-labeled 3.5 kb Eco RI-Hind III J_H probe. Lane *1*, human placenta; lane *2*, B-CLL–PBL; lane *3*, cell line KHB1; lane *4*, cell line KHB2; lane *5*, cell line KHB3. Numbers indicate fragment sizes in kilobases (kb). (*C*) Restriction map of human placental J_H region. The restriction endonuclease cleavage sites are shown as *H* (Hind III), *X* (Xba I), and *B* (Bgl II).

the supernatant in the presence or absence of suboptimal concentrations of mitogens. The supernatant did not induce any proliferation of nonstimulated or PHA-stimulated T cells (data not shown). In contrast, it induced a significant proliferation of anti-μ–stimulated B cells in a dose-dependent fashion, but no response with nonstimulated B cells (Fig. 4). The control supernatant obtained from leukemic B cells that had been incubated with control beads did not induce any proliferation of anti-μ–stimulated B cells. These results again demonstrate that activated leukemic B cells secrete BCGF, which can act only on activated B cells.

It has been reported that IL-1, IL-2, or γ-IFN have significant effect on the proliferation of B cells (29–31). In order to determine whether the supernatant from anti-Ig–stimulated leukemic B cells contained any IL-1 or IL-2 activity, various concentrations of the supernatant from the ISαIg-stimulated leukemic B cells

Table II. Effect of Mitogens and Lymphokines on the Proliferation of Leukemic B Cells

Stimulant*	Proliferation (Stimulation Index)‡
Nil	1.0
IL-1	1.6
IL-2	2.6
IL-1 + IL-2	1.6
anti-μ	*4.6*
BCGF	1.6
anti-μ + BCGF	*24.0*
SAC	1.6

* 1×10^5 cells were stimulated with recombinant IL-1 (1 U/ml), recombinant IL-2 (1 U/ml), anti-μ antibody (10 μg/ml), BCGF (20% vol/vol), or SAC (0.01% vol/vol).
‡ Cells were pulsed with 1 μCi/well of [^{3}H]TdR over the last 16 h of a 60-h culture. Background counts per minute obtained by cells cultured in medium alone was 1,230±56.

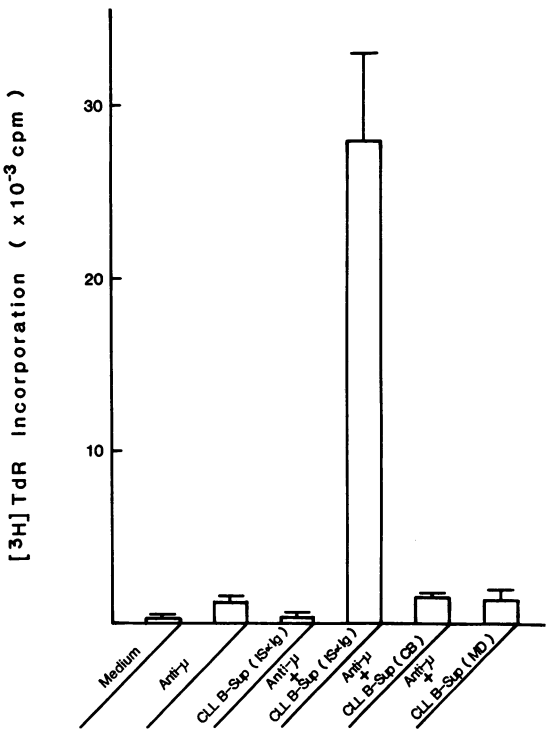

Figure 3. Effect of the supernatant from ISαIg-stimulated leukemic B cells on the proliferation of autologous leukemic B cells. Supernatant was obtained by incubating B-CLL cells with ISαIg or control beads (CB). The supernatant was added to the culture of freshly separated autologous leukemic B cells at a concentration of 30% vol/vol in the presence or absence of anti-μ antibody (10 μg/ml). Cultures were pulsed with 1 μCi [^{3}H]TdR over 16 h of a 68-h incubation and [^{3}H]TdR uptake was determined. All data are shown as means±SEM of the triplicate cultures.

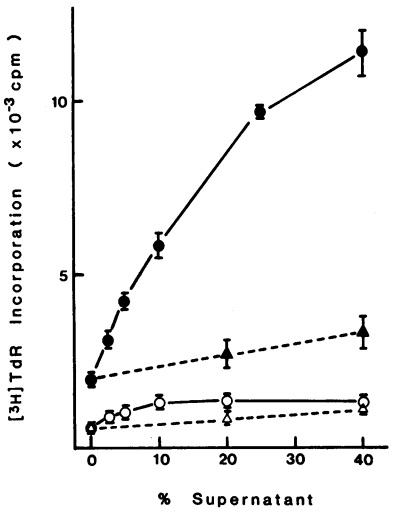

Figure 4. Effect of the supernatant from ISαIg-stimulated leukemic B cells on the proliferation of normal B cells. Highly purified tonsillar B cells (10^5/well) were cultured with (*a*) various concentrations of the supernatant from insoluble anti-Ig–stimulated leukemic B cells in the presence (● —— ●) or absence (▲ – – ▲) of 10 μg/ml anti-μ antibody; (*b*) the supernatant obtained from control beads stimulated leukemic B cells in the presence (○ —— ○) or absence (△ – – △) of 10 μg/ml anti-μ-antibody. Cultures were pulsed with 1 μCi [^{3}H]TdR over 16 h of a 60-h incubation and [^{3}H]TdR uptake was measured. Results are shown as means±SEM of the triplicate cultures.

were added to PHA-stimulated murine thymocytes or to an IL-2–dependent T cell line, MTH 41.16 cells, and proliferative responses were measured (Fig. 5). Virtually no growth activity on thymocytes or a T cell line was detected in the supernatant obtained from the ISαIg-stimulated leukemic B cells. The supernatant did not contain any detectable γ-interferon (data not shown). In addition, no inhibitory activity for IL-1, IL-2, or γ-IFN was detected in the supernatant, since the addition of the supernatant did not diminish those activity (data not shown).

Establishment of cell lines from the patient's PBL. Purified B cells from the patient's PBL were seeded at a cell density of 10^6/ml in 10% FCS-containing RPMI 1640 medium in culture flasks and maintained over several months. Although no increase in cell number was observed in cultured cells over the first 3 wk, a population of cultured cells started to grow with doubling time of 7–10 d hereafter. The growth rate gradually accelerated and the doubling time reached to 48–72 h after 3 mo. Three different cell lines, referred to as KHB1, KHB2, and KHB3, were examined for Ig gene configuration by Southern blot analysis. The rearrangement patterns of the Ig gene in all three cell lines were exactly identical to that of the original leukemic B cells (Fig. 2, lanes *3–5*). FACS analysis revealed that all three cell lines expressed B1 as well as Leu 1 antigens as the original leukemic B cells did, although the expression of IgM became dull (Fig. 1, *E* and *F*). Of note is the fact that established B cell lines expressed the Ba antigen, although the original leukemic B cells did not. From these data, it could be concluded that these cell lines were derived from the original leukemic B cells, but at an activated stage.

Production of BCGF by the established cell lines. Cell-free supernatants from cultures of the established cell lines (KHB1, KHB2, and KHB3) were collected and examined for the presence of BCGF activity. As shown in Fig. 6, the supernatants from the cell lines induced the proliferation of anti-μ–stimulated normal tonsillar B cells in a dose-dependent fashion. It is noteworthy to point out that these culture supernatants did not induce significant proliferation of B cells in the absence of anti-μ stimulation. Furthermore, no IL-1 or IL-2 (Fig. 4) γ-IFN or BCDF (BSF-2) activity (data not shown) was detected. These data demonstrate that these established B cell lines constitutively produce B cell growth factor, which acts on activated B cells. Stimulation of the established B cell lines with ISαIg or SAC did not augment the production of BCGF (data not shown).

To delineate the stage at which KHB1-derived BCGF acts during the cell cycle of normal B cells, small resting B cells or activated B blast cells were cultured with KHB1 supernatant and the proliferative response was measured (Fig. 7). KHB1 supernatant induced the proliferation of B blast cells, which had been obtained by anti-μ or SAC stimulation, but not resting B cells, suggesting that BCGF in KHB1 supernatant served as a progression signal in the B cell cycle. Recent studies have shown that murine BSF-1 induces enhanced Ia expression on resting murine B cells (6). In order to study whether BCGF in KHB1 supernatant could act on resting B cells, changes in HLA-DR expression were investigated. Purified B cells were incubated with KHB1 supernatant or control supernatants and HLA-DR expression was measured by FACS analysis (Fig. 8). There was no increase in the expression of HLA-DR in B cells cultured with KHB1 supernatant, whereas there was a significant enhancement of the HLA-DR expression on resting B cells cultured with anti-μ or PHA-T supernatant as previously reported (9). These data indicate that BCGF in KHB1 supernatant delivers a progression signal for proliferation, but not a competence signal responsible for the activation of resting B cells.

In order to study whether the established cell lines are able to respond to autologous growth factor, KHB1 cells were cultured with various concentrations of KHB1 supernatant and the proliferative responses were measured (Fig. 9). There was no augmentation of DNA synthesis in KHB1 cells by the addition of autologous culture supernatant, whereas the same supernatant could induce or augment the proliferation of anti-μ–stimulated B blast cells or that of a B lymphoblastoid cell line, RPMI 1788.

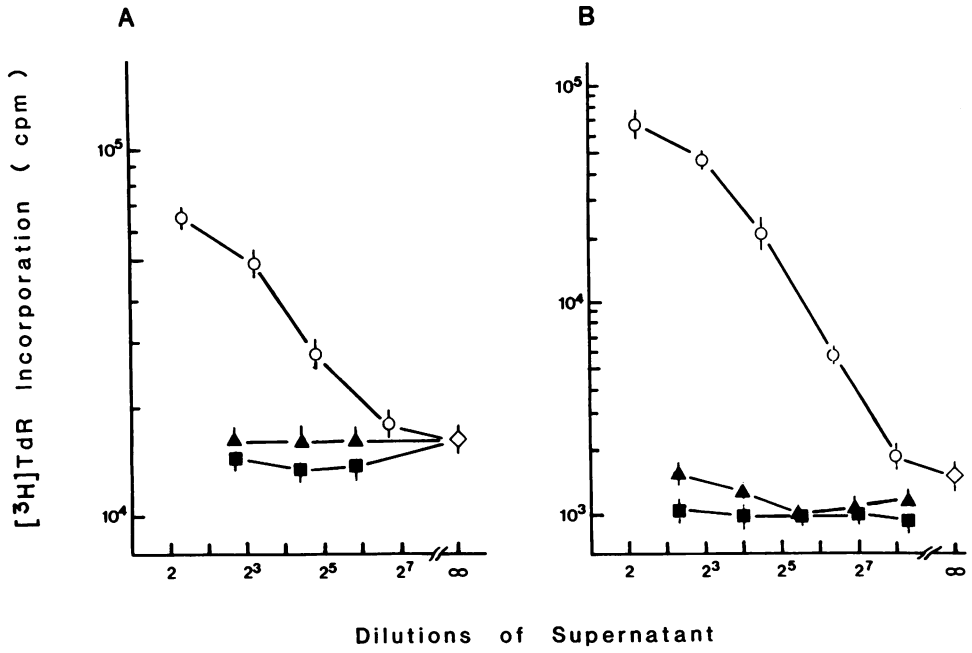

Figure 5. IL-1 and IL-2 activities in the supernatant from ISαIg-stimulated leukemic B cells or KHB1 cells. (*A*) Balb/c thymocytes (2×10^6/well) were cultured with 0.02% PHA-P plus various concentrations of the supernatant from ISαIg-stimulated patient B cells (▲——▲) or KHB1 cells (■——■). Medium containing rIL-1 (30 U/ml) was used as a control (○——○). Cultures were pulsed with 1 μCi [^{3}H]TdR over the last 16 h of a 64-h incubation and [^{3}H]TdR was determined. (*B*) 5×10^3 of an IL-2–dependent T cell line, MTH41.16, were cultured with various concentrations of the supernatant from ISαIg-stimulated leukemic B cells (▲——▲) or KHB1 cells (■——■). Medium containing rIL-2 (2 U/ml) was used as a control (○——○). Cultures were pulsed with 1 μCi [^{3}H]TdR over last 4 h of a 24-h incubation and uptake of [^{3}H]TdR was determined. All data are represented as means±SEM of triplicate cultures.

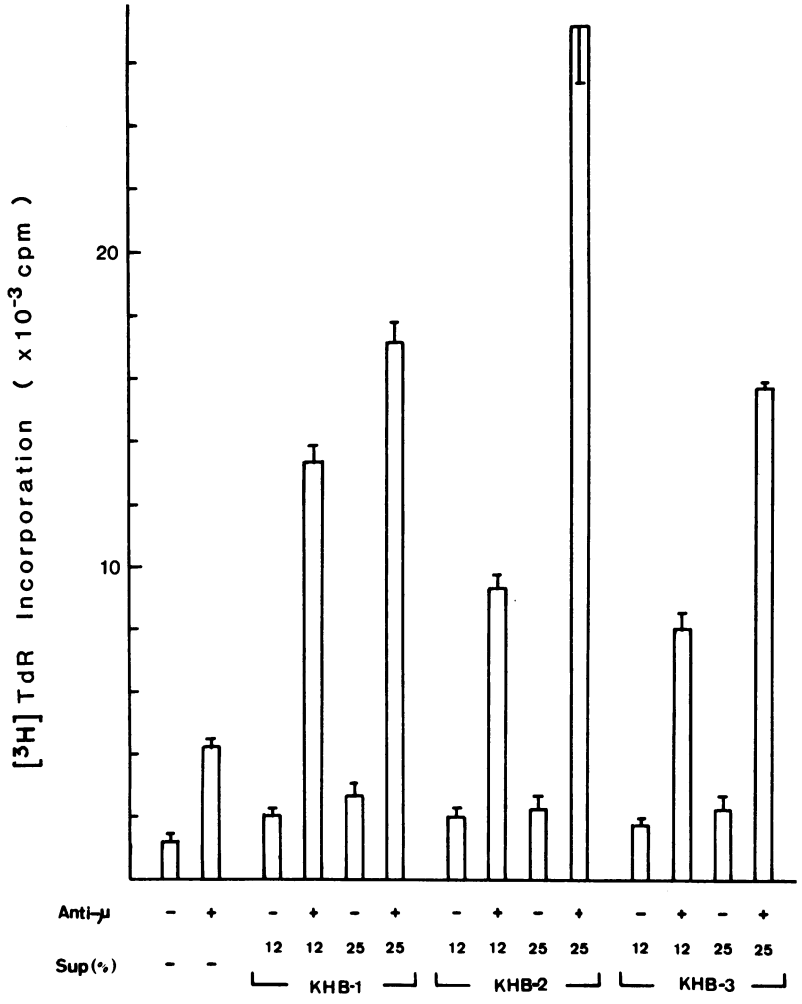

Figure 6. Effect of the supernatants from KHB1, KHB2, and KHB3 cells on the proliferation of normal B cells. Purified tonsillar B cells (10^5/well) were cultured with 12 or 25% of the supernatants from KHB1, KHB2, or KHB3 cells in the absence or presence of anti-μ antibody (10 μg/ml). Cultures were pulsed with [^{3}H]TdR (1 μCi/well) over the last 16 h of a 60-h incubation and [^{3}H]TdR uptake was measured. Results are shown as means±SEM of triplicate cultures.

Discussion

This study demonstrates that monoclonal leukemic B cells derived from a B-CLL patient secrete a BCGF upon stimulation with anti-μ. Moreover, the BCGF derived from leukemic B cells could induce the proliferation of anti-μ–stimulated autologous leukemic B cells as well as anti-μ–stimulated normal B cells. These results may explain the reason why anti-μ stimulation could induce a significant proliferation of the leukemic B cells without addition of growth factors; anti-μ induced BCGF production in the leukemic B cells and also activated the cells to a state responsive to BCGF. The freshly isolated leukemic cells did not respond to any growth factors in the absence of anti-μ stimulation, indicating that the cells were at the G_0 resting stage. Cell surface analysis with FACS also showed that Ba molecules, which are expressed only on activated B cells at G_1 phase (27), were not detectable on these cells. This might explain the relatively benign nature of B-CLL; it is likely that stimulation of B-CLL cells with the appropriate antigen might induce proliferation of these cells in vivo. The present study was conducted on leukemic cells from a single patient. Therefore, while the results are informative for the autocrine mechanism in the growth of B-CLL cells, we have to admit that it is not clear how applicable the results are to other CLLs.

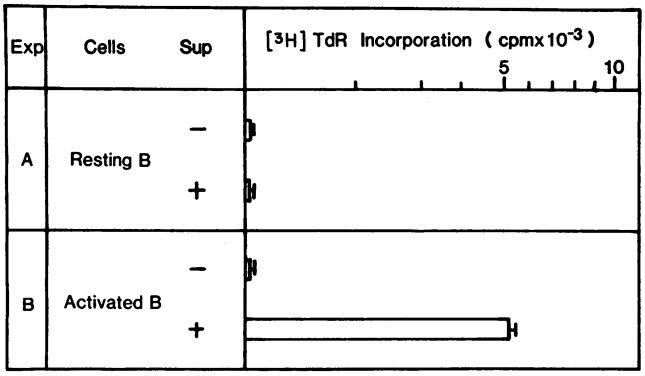

Figure 7. Effect of KHB1-supernatant on the proliferation of resting B cells vs. activated B cells. Small resting B cells separated from tonsillar B cell fraction (Exp *A*), activated blast cells which had been prepared by incubating small resting B cells with anti-μ antibody (Exp *B*), were cultured with or without 50% vol/vol of KHB1-supernatant at a cell density of 10^5/well. Cultures were pulsed with [^{3}H]TdR (1 μCi/well) over last 16 h of a 64-h incubation and [^{3}H]TdR uptake was measured. Results are shown as means±SEM of triplicate cultures.

The B-CLL cells in this study coexpressed B1 and Leu 1 antigens (Fig. 1). The latter is a 65–69-kD glycoprotein that was previously thought to be expressed only on cells of the T lineage (32). However, the presence of Leu 1 positive B cells in normal individuals (33) and the high frequency of Leu 1–positive B cells in certain autoimmune patients (34) has been demonstrated. Most B-CLL cells seem to belong to the category of Leu 1 B cells (28). Ly1 B cells in the mouse, which may be the counterpart of human Leu 1 B cells, secrete autoantibodies in NZB mice

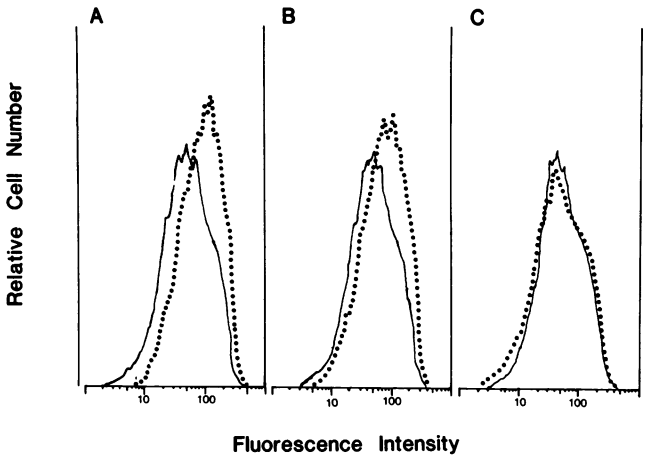

Figure 8. Effect of the KHB1-supernatant on the expression of HLA-DR antigen. Highly purified tonsillar B cells were cultured for 48 h with medium alone (——); 10 μg/ml of anti-μ (– – –) (*A*); 25% vol/vol of PHA-stimulated T-supernatant (– – –) (*B*), or 25% vol/vol of KHB1-supernatant (– – –) (*C*). Cultured cells were stained with biotinated anti-HLA-DR monoclonal antibody, following by TR-conjugated avidine. Stained cells were analyzed by FACS 440. Histograms represent the relative number of cells as a function of fluorescence intensity within a particular gated population.

(35), suggesting that these cells include autoantigen-reactive B cell clones. If this should be the case for human Leu 1 B cells, autoantigens might be responsible for the induction of proliferation of B-CLL cells in an autocrine fashion; the autoantigen activates resting B-CLL cells to the responsive stage to BCGF and induces the production of BCGF, although this is speculative without any evidence. McGrath and Weissman (36) proposed a similar possibility in leukemogenesis with retrovirus; they suggested that leukemic cells have antiviral specificity and the envelope of the retrovirus stimulates those lymphocytes into growth.

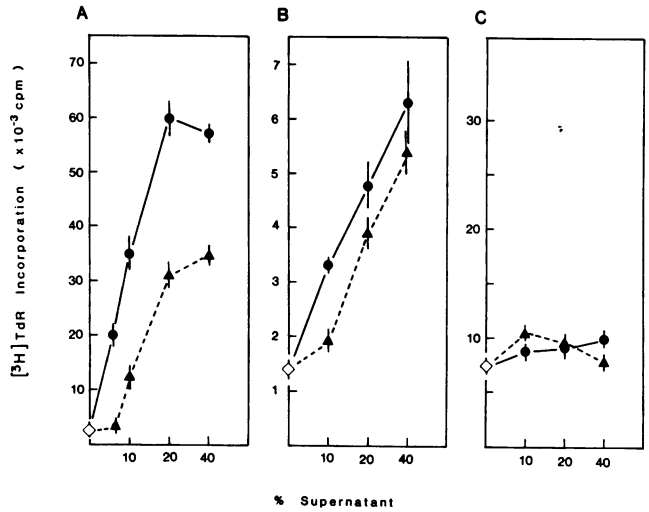

Figure 9. Effect of the KHB1-supernatant on the growth of normal B blast cells, RPMI 1788 cells and KHB1 cells. 10^5 of normal B blast cells that had been incubated with anti-μ for 48 h (*A*); 10^3 of RPMI 1788 cells, (*B*) or 5×10^3 of KHB1 cells, (*C*) were cultured with medium alone (◇); various concentrations of the KHB1-supernatant (● —— ●) or RPMI 1788-supernatant (▲ —— ▲). Cultures were pulsed with [³H]TdR (1 μCi/well) over the last 16 h of a 60-h incubation and [³H]TdR uptake was measured. Results are shown as means±SEM of triplicate cultures.

In contrast to the freshly prepared leukemic B cells, the established cell lines expressed Ba molecules and could constitutively secrete BCGF without any stimulation. However, BCGF did not augment the proliferation of the established cell lines. The result does not prove but strongly suggests that the autocrine mechanism may not operate any longer in the continuous growth of the established cell lines. In an IL-3–dependent myeloid cell line, it was shown that transformation with Abelson virus abrogated the factor dependence of those cells (37). In this study, leukemic B cells appear to be transformed with EBV, since EBNA was detected in these cell lines. Therefore, the situation may be the same as that observed with the IL-3–dependent cell lines. Growth factor independence and autonomous growth of transformed cells might be due to constitutive expression and activation of the membrane receptor that serves as a transducer of the extracellular signal. Alternatively, it might be due to the continuous expression of intracellular signals that eventually leads to cell division.

The BCGF derived from the leukemic B cells appears to be different from IL-1 or IL-2, since there was no stimulatory effect on PHA-stimulated murine thymocytes or IL-2–dependent cell line, MTH 41.16 cells (Fig. 5). No IL-1 or IL-2 inhibitor(s) in the supernatant was detected, since the supernatant did not inhibit IL-1–induced growth of PHA-stimulated thymocytes or IL-2–dependent growth of MTH41.16 cells (data not shown). Moreover, recombinant IL-1 or IL-2 did not induce any proliferation of B-CLL cells in the absence or presence of anti-μ antibody (Table I and unpublished data). A previous study (9) demonstrated the secretion of BCGF from SAC-activated normal B cells or B lymphoblastoid cell lines. It showed that B cell–derived BCGF (B-BCGF) from those cells could induce proliferation of SAC– or anti-Ig–activated B cells and sustain growth of activated B cells. B-BCGF from these cell lines could also augment the growth of autologous cells; it induced colony formation of autologous cells in soft agar as well as augmented DNA synthesis of autologous cells. The present as well as previous results indicate that normal B cells, transformed B cell lines and leukemic B cells can secrete and respond to B cell specific growth factor(s). At present, it is not clear whether the same molecules are secreted from normal activated B cells, transformed B cell lines and the leukemic B cells. BCGF(s) seem to be very heterogeneous with regard to their molecular weight and isoelectric point (9, 11). It is thus unclear whether BCGF activity is associated with a single factor or multiple factors. This may be answered when the gene(s) for BCGF(s) is cloned. However, the immunological functions of these factors are similar; (*a*) they act only on activated B cells and induce proliferation but not Ig secretion, (*b*) they do not induce proliferation or Ia expression of resting B cells, and (*c*) they do not show any activity in BCL_1 assay.

The present as well as previous results (9) obtained with normal and leukemic B cells show that B cells can secrete their own growth factors and strongly support the notion that an autocrine mechanism similar to the growth of T cells might be operating in the growth of B cells.

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