Low Molecular Weight B Cell Growth Factor Induces Proliferation of Human B Cell Precursor Acute Lymphoblastic Leukemias

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Experiments were conducted to determine the effect of low mol wt B cell growth factor (L-BCGF) on B cell precursor acute lymphoblastic leukemia (ALL). L-BCGF induced a significant increase in ^{3}H-TdR incorporation in 28 of 37 bone marrow aspirates from patients with B cell precursor ALL, with stimulation indices ranging from 2 to 129. Fluorescence-activated cell sorting confirmed that in five of seven patients the common acute lymphoblastic leukemia antigen (CALLA)/CD10 positive leukemic cells were responding directly to L-BCGF. L-BCGF was capable of inducing, in some patients, an increase in absolute viable cells and could also induce colony formation in vitro. The response of B cell precursor ALL was not attributable to β IL 1, IL 2, or γ interferon. These results indicate that the majority of B cell precursor ALL undergo a proliferative response to L-BCGF, suggesting a regulatory role for this lymphokine in the growth of B cell precursors.

OVER THE PAST several years a number of lymphokines have been described that influence the proliferation and differentiation of human and murine B cells at various stages of development.[1-3] In humans these include low mol wt B cell growth factors (L-BCGF),[4-5] high mol wt BCGF,[6] B cell stimulatory factor 1 (BSF-1)/interleukin 4 (IL 4),[7] B cell differentiation factor (BSF-2),[8] B cell activation factor,[9] pre-B cell growth factor,[10] B cell inhibitory factor,[11] interleukin 2 (IL 2),[12-23] and gamma interferon.[24-26] Analysis of the biochemical and structural characteristics of these lymphokines, their role in B cell activation, and the characterization of their target cells is currently an area of active investigation.

The 12 kd human L-BCGF is a T cell-derived lymphokine that acts on preactivated B cells, ie, activated with anti-IgM antibody.[27] L-BCGF is probably derived from a 60-kd cytosolic precursor protein,[28] and L-BCGF has recently been purified to a single band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis.[29] Studies on L-BCGF-dependent B cell lines and activated B cells show the presence of $\sim 10^4$ high affinity and $\sim 3.5 \times 10^4$ low affinity receptors.[30] In experiments using neoplastic B cells, Ford et al[32] have demonstrated a direct proliferative effect of L-BCGF on hairy cells[31] and on cells from different B cell non-Hodgkins' lymphomas.

The factors influencing the proliferation of malignant human B cell precursors are poorly understood. Experiments using colony assays for growth of B cell precursor acute lymphoblastic leukemias (ALL) suggest a role of soluble growth factors either provided by leukocyte feeder cells[33] or present in leukocyte-conditioned medium,[34,35] and Touw et al[34] suggested that one of the factors was IL 2. As part of an effort to examine factors influencing the growth of B cell precursor ALL (also termed "common" ALL or non-T/non-B ALL), we herein report on experiments using L-BCGF. Our results indicate that the majority of B cell precursor ALL undergo a proliferative response to this lymphokine.

MATERIALS AND METHODS

Leukemic specimens. Bone marrow aspirates from 37 patients with newly diagnosed B cell precursor ALL were analyzed. All specimens were obtained through the Cell Marker Laboratory, Department of Laboratory Medicine and Pathology, University of Minnesota. Only aspirates with >90% blasts were included. Diagnosis of B cell precursor ALL was based on standard morphological, cytochemical, and immunologic criteria. All samples expressed at least two of the three B cell-associated antigens p24/CD9,[36] common acute lymphoblastic leukemia antigen (CALLA)/CD10,[37] and gp42/CD24.[38] No sample contained more than 5% T cells based on expression of CD5[39] and CD7[40]; more than 5% normal, mature B cells based on expression of surface Ig; more than 5% myeloid cells based on expression of CD13[41]; or more than 5% monocytes based on expression of CD11.[42] Eight of the 37 bone marrow aspirates had been cryopreserved in liquid nitrogen for two to 24 months prior to analysis. We have analyzed the influence of freezing and thawing on the response to L-BCGF by comparing the L-BCGF response of seven noncryopreserved specimens to the L-BCGF response after having frozen and thawed the same specimens. Only samples with greater than 85% viable cells after thawing were analyzed, and no significant differences in the response to L-BCGF were observed (data not shown).

Proliferation assay. Bone marrow aspirates were separated on Ficoll-Hypaque (FH) gradients. Two $\times 10^5$ interface cells were incubated in 96 well flat-bottom plates (3596, Costar) in RPMI 1640 containing 10% vol/vol fetal bovine serum (FBS; Gibco Laboratories, Grand Island, NY), in the presence or absence of various concentrations of L-BCGF (Cellular Products Inc, Buffalo, NY). All experiments were set up in triplicate. Cells were harvested after 90 to 96 hours, except in the time course experiments.

Cell proliferation was determined by:

1. ^{3}H-TdR incorporation: ^{3}H-TdR (Amersham, Arlington Heights, IL; specific activity-20 Ci/mmol) was diluted in RPMI 1640/10% FBS and added at a

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concentration of 1 μCi per well for the last eight (patients 9 to 37 in Table 1) or 16 hours (patients 1 to 8). The cells were harvested on glass fiber filters using a cell harvester (M-24, Brandel, Gaithersburg, MD). The filters were dried for at least four hours, and ^{3}H-TdR incorporation was measured by liquid scintillation spectrometry. Stimulation indices are presented as the ratio of ^{3}H-TdR incorporated into cells incubated in the presence of L-BCGF compared to ^{3}H-TdR incorporated into cells incubated with no L-BCGF. We compared the results of 2-, 4-, 8-, and 16-hour incubations with ^{3}H-TdR. As no significant differences in the stimulation indices were observed between the eight- and the sixteen-hour incubations, we used the eight-hour incubation beginning with patient 9 (Table 1).

Table 1. Response of B Cell Precursor ALL Bone Marrow Aspirates to L-BCGF*

Patient No.	10% L-BCGF	No L-BCGF	Stimulation Index†
1	264 ± 117‡	95 ± 72	3 NS
2	145 ± 23	158 ± 51	1
3	101 ± 41	88 ± 14	1
4	138 ± 92	140 ± 76	1
5	98 ± 34	86 ± 70	1
6	2,308 ± 4	1,447 ± 192	2
7	2,270 ± 377	773 ± 3	3
8	7,052 ± 336	401 ± 116	14
9	10,618 ± 608	4,115 ± 456	3
10	12,569 ± 1,549	7,183 ± 775	2
11	22,991 ± 2,908	363 ± 121	63
12	16,708 ± 496	8,868 ± 251	2
13	24,172 ± 4,646	1,435 ± 148	17
14	17,024 ± 1,324	295 ± 126	58
15	853 ± 253	149 ± 91	6
16	5,725 ± 371	449 ± 97	13
17	2,780 ± 259	840 ± 62	3
18	9,622 ± 563	311 ± 21	32
19	55,242 ± 371	11,848 ± 820	5
20	18,962 ± 908	1,514 ± 69	13
21	1,562 ± 517	196 ± 22	8
22	711 ± 179	118 ± 34	6
23	8,330 ± 444	6,725 ± 156	1
24	1,714 ± 24	1,335 ± 73	1
25	626 ± 107	329 ± 75	2
26	130 ± 48	126 ± 25	1
27	4,788 ± 281	409 ± 4	12
28	9,799 ± 475	341 ± 223	29
29	401 ± 120	87 ± 11	5
30	17,850 ± 1,035	289 ± 56	62
31	306 ± 283	350 ± 30	1
32	491 ± 23	205 ± 42	2
33	3,473 ± 1,114	163 ± 76	21
34	15,654 ± 965	121 ± 2	129
35	718 ± 15	116 ± 13	6
36	40,075 ± 5,674	14,406 ± 280	3
37	206,820 ± 21,197	22,181 ± 3,263	9

*Leukemic cells were cultured in the presence or absence of 10% vol/vol L-BCGF for 96 hours and were pulsed with ^{3}H-TdR for the final eight (patients 9 to 37) or 16 (patients 1 to 8) hours.

†Stimulation index: Ratio of ^{3}H-TdR incorporation in the L-BCGF-treated cells compared to controls treated with no L-BCGF.

‡Mean cpm ± SD of ^{3}H-TdR incorporation in triplicate cultures.

2. Cell count: Cells from triplicate wells were vigorously pipetted and pooled. The wells were checked under an inverted microscope to ensure that all cells had been removed. Cell viability and absolute cell counts were analyzed by trypan blue exclusion.
3. Colony assay: One × 10^5 leukemic bone marrow cells were suspended in 1 mL of RPMI 1640 containing 25% FBS, 5 × 10^{-5} mol/L 2-mercaptoethanol, 0.9% methylcellulose in the presence or absence of 10% vol/vol L-BCGF. The cells were cultured in 35-mm petri dishes at 37°C in a humidified 5% CO_2 atmosphere and analyzed by inverted microscopy after ten days.

Lymphokines. Partially purified L-BCGF was obtained from Cytokine Technology, Buffalo, NY. L-BCGF did not contain detectable IL 2 activity based on the inability to support the growth of IL 2-dependent CTLL-20 cells (assay generously conducted by Dr Siew-Lin Wee, Immunobiology Research Center, University of Minnesota). Highly purified L-BCGF was isolated as previously described.[30] Recombinant beta interleukin 1 (β IL 1), recombinant interleukin 2 (IL 2), and recombinant gamma interferon (gamma IF) were purchased from Genzyme Corporation, Boston.

Sorting of CALLA/CD10 positive cells. Bone marrow aspirates were used for cell sorting. In seven of eight samples the cells had been cryopreserved in liquid nitrogen until the results of the effect of L-BCGF on the fresh bone marrow aspirates were known. The cells were thawed from liquid nitrogen, washed twice in fluorescence buffer (PBS containing 2.5% FBS and 0.02% sodium azide), stained with the anti-CALLA/CD10 monoclonal antibody (MoAb) BA-3 for 30 minutes at 4°C, washed once in fluorescence buffer, stained with fluorescein isothiocyanate (FITC) goat antimouse Ig (Cappel Worthington Biochemicals, Malvern, PA, dilution 1:20) for 30 minutes, and washed three times. Three-10 × 10^6 CALLA/CD10 positive cells were then sorted on a FACS IV (Becton Dickinson, Mountain View, CA). Forward and 90° light-angle scatter were used to gate on the viable leukemic cells. Only cells exhibiting fluorescence intensity clearly above background were sorted into the CALLA/CD10 positive fraction. These cells constituted 85% to 90% of the leukemic cell population. To exclude the potential influence of BA-3 on the in vitro L-BCGF response, we incubated and stained cells with BA-3 and FITC goat antimouse Ig, but did not sort them.

Statistics. For analysis of the differences between L-BCGF stimulated and unstimulated cells we used the student's t test on a log scale. Results with $P < 0.05$ were considered statistically significant.

RESULTS

Response of B cell precursor ALL bone marrow aspirates to L-BCGF. We initially analyzed the effect of L-BCGF on the incorporation of ^{3}H-TdR in FH-isolated cells from bone marrow aspirates of 37 patients with B cell precursor ALL. The results obtained when cells were stimulated with 10% vol/vol L-BCGF are presented in Table 1. In 28 of the 37 patients (74%) a significant increase in ^{3}H-TdR incorporation was observed. The individual responses varied widely, with stimulation indices ranging from 2 to 129 and 12 of the patients having a stimulation index >10.

In 15 of 17 responding patients the effect of different concentrations of L-BCGF was studied. The results shown in Fig 1 underscore the variable sensitivity to L-BCGF. Cells from 5 of 15 patients showed a significant increase of ^{3}H-TdR incorporation over background in the presence of only 1% vol/vol L-BCGF. Cells from four patients required

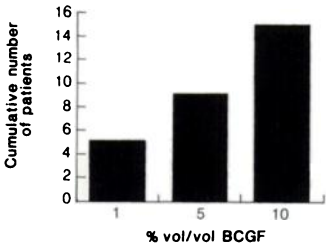

Fig 1. Variability in response of FH-isolated bone marrow cells from 15 patients with B cell precursor ALL who responded to L-BCGF. Cells were incubated with increasing concentrations of L-BCGF or no L-BCGF for 96 hours and pulsed with ^{3}H-TdR for the final eight or 16 hours. Data show the cumulative number of patients that, at a given concentration of L-BCGF, exhibited a statistically significant increase ($P < 0.05$) in ^{3}H-TdR incorporation compared to the controls, as determined by the student's t test.

5% vol/vol L-BCGF, and the remaining six patients required 10% vol/vol L-BCGF to show a response. CALLA/CD10 positive cells were sorted from three patients, and the L-BCGF response of the sorted cells paralleled the results obtained with unsorted cells (data not shown). L-BCGF concentrations of >15% vol/vol inhibited ^{3}H-TdR incorporation in B cell precursor ALL.

Response of CALLA/CD10 positive leukemic cells to L-BCGF. To confirm that the L-BCGF response was not due to ^{3}H-TdR incorporation by nonleukemic cells, we isolated CALLA/CD10 positive leukemic cells from seven patients by cell sorting and examined their response to L-BCGF. We chose this approach, since CALLA/CD10 is not expressed on surface Ig positive normal B cells, and >90% of cells in these aspirates were CALLA/CD10 positive. Cells stained with BA-3 and FITC goat antimouse Ig, but not sorted, were used to control for the effects of surface-bound antibody on L-BCGF-induced ^{3}H-TdR incorporation. The results in Table 2 indicate that in five of seven patients (Nos. 7, 9, 13, 15, and 18), the L-BCGF response of CALLA/CD10 positive cells essentially recapitulated the L-BCGF response of stained, unsorted cells. In patient No. 17 a twofold increase in ^{3}H-TdR incorporation was observed, but this was not statistically significant. In patient No. 8 a comparison of stained, unsorted cells (Table 2) with cells not stained with BA-3 and FITC goat antimouse Ig (Table 1) clearly shows that leukemic cell-bound antibody did not influence the response to L-BCGF. Failure of this patient's CALLA/CD10 positive leukemic cells to respond could be attributable to (1) cell damage during the sorting procedure, (2) ^{3}H-TdR incorporation by surface Ig positive, CALLA/CD10 negative normal B cells in the bone marrow aspirate, or (3) dependency on accessory cells such as monocytes or T cells. In patient No. 7 the L-BCGF response of CALLA/CD10 positive cells was ~tenfold higher than in the stained, unsorted bone marrow aspirate, suggesting an inhibitory effect mediated by factors produced by contaminating normal cells. To formally exclude this as an explanation for the nonresponding leukemic samples, we sorted CALLA/CD10 positive cells from one nonresponding patient (No. 1 in Table 1) and examined their response to L-BCGF. These sorted CALLA/CD10 positive cells did not respond to L-BCGF (Table 2).

Cell proliferation studies. Time sequence studies of ^{3}H-TdR incorporation and absolute viable cell counts of the CALLA/CD10 positive leukemic cells were conducted on several patients. In the first patient (Fig 2A, patient No. 7 in Table 1) an increased incorporation of ^{3}H-TdR was seen after day 2 and continued through day 6 in the presence of L-BCGF. The cell count in the L-BCGF-treated cultures dropped over the first three days to 300×10^3/mL on day 3 and then increased slightly to 400×10^3/mL on day 6 (Fig 2B). The cell count in the control wells plateaued at 150×10^3/mL from days 3 to 6. In the second patient (Fig 2C, No. 18 in Table 1) ^{3}H-TdR incorporation steadily increased from day 2 to day 6 in the L-BCGF-treated cultures. The cell number remained stable from day 2 to day 6 in the presence of L-BCGF, whereas the cell number in the controls dropped to 10% of the starting concentration by day 6 (Fig 2D). In the third patient (Fig 2E, No. 15 in Table 1) an increase in ^{3}H-TdR incorporation in L-BCGF-treated cultures compared to controls was detectable on day 1 and peaked on day 3. The initial cell loss was substantial in the L-BCGF-treated cultures, dropping to 260×10^3/mL within the first 24 hours followed by an increase to 530×10^3/mL by day 3 (Fig 2F). In a fourth patient (No. 37 in Table 1) we analyzed absolute viable cell numbers over a longer period of time (Fig 3). This patient's leukemic cells underwent a rigorous proliferative response to L-BCGF, with the absolute

Table 2. L-BCGF Response of CALLA/CD10 Positive Leukemic Cells*

Patient No.	CALLA/CD10 Positive Cells			Stained/Unsorted Cells		
	10% L-BCGF	No L-BCGF	Stimulation Index†	10% L-BCGF	No L-BCGF	Stimulation Index
7	23,911 ± 3,258‡	2,497 ± 291	10	2,181 ± 195	811 ± 146	3
8	190 ± 77	111 ± 40	2 NS	6,698 ± 701	470 ± 138	14
9	6,212 ± 208	3,362 ± 168	2	8,335 ± 143	3,020 ± 1,347	3
13	21,072 ± 1,749	839 ± 47	25	22,843 ± 1,410	1,370 ± 76	17
15	5,092 ± 558	136 ± 59	37	2,168 ± 520	96 ± 16	23
17	599 ± 216	259 ± 11	2 NS	2,348 ± 208	880 ± 167	3
18	3,166 ± 145	95 ± 3	33	8,830 ± 420	2,954 ± 118	30
1	116 ± 16	92 ± 16	1	188 ± 103	270 ± 185	1

*CALLA/CD10 positive cells isolated by cell sorting, or stained/unsorted cells were cultured in the presence or absence of 10% vol/vol L-BCGF for 96 hours and were pulsed with ^{3}H-TdR for the final eight to 16 hours.

†See Table 1.

‡See Table 1.

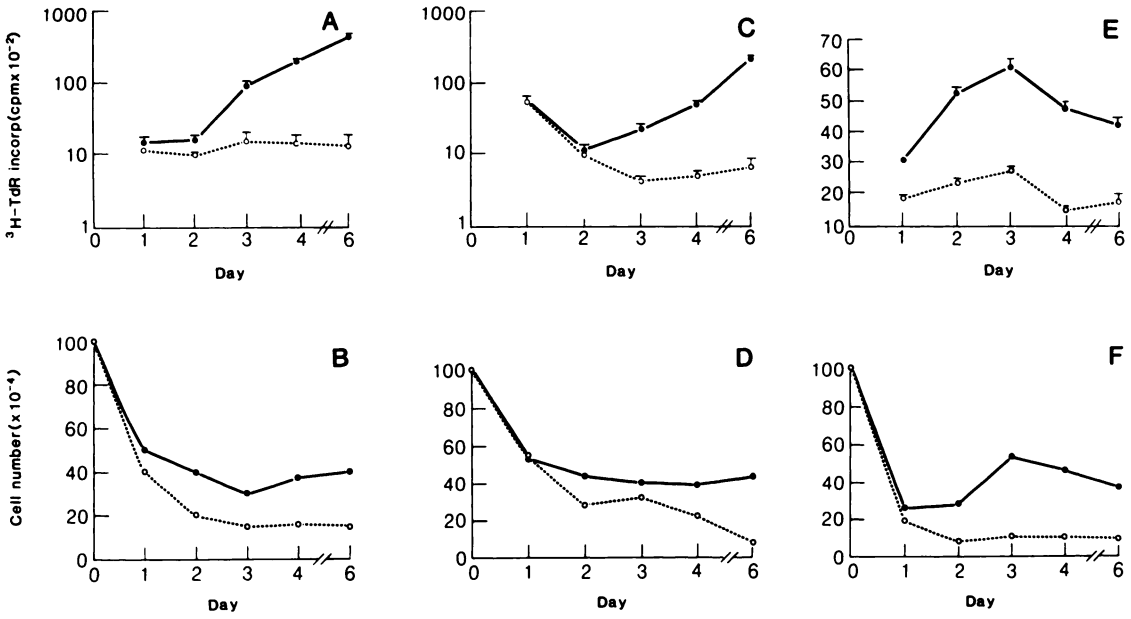

Fig 2. Time sequence of ^{3}H-TdR incorporation (A, C, E) and absolute viable cell numbers as determined by trypan blue exclusion (B, D, F) of CALLA/CD10-postive leukemic cells after incubation in 10% vol/vol L-BCGF (—●—) for zero to six days. Control cultures (--O--) did not contain L-BCGF. The ^{3}H-TdR incorporation data points represent the mean of triplicate values ±1 SD (horizontal bars). The absolute cell number data points were derived by pooling triplicate wells and dividing the total viable cell count by three. A and B are patient No. 7; C and D are patient No. 18; and E and F are patient No. 15 from Table 1. Note that the ordinates in A and C are on a log scale.

viable cell number doubling at five days and tripling at ten days, compared to day 0. Note that control cells cultured with no L-BCGF dropped in absolute viable cell number.

We also used a colony assay to examine the *proliferative* response to L-BCGF. When cells from patient No. 36 (Table 1) were plated in the presence of L-BCGF, dramatic colony formation was observed by day 10 (Fig 4A), whereas no colony formation was observed in cells cultured without L-BCGF (Fig 4B). The cloning efficiency for the L-BCGF cultures was 8.3% for cluster formation (>ten cells) and 0.4% for colony formation (>50 cells). The phenotype of the colony-forming cells was identical to the original, uncultured cells (CD9$^+$, CD10$^+$, CD24$^+$, and cytoplasmic and surface IgM$^-$).

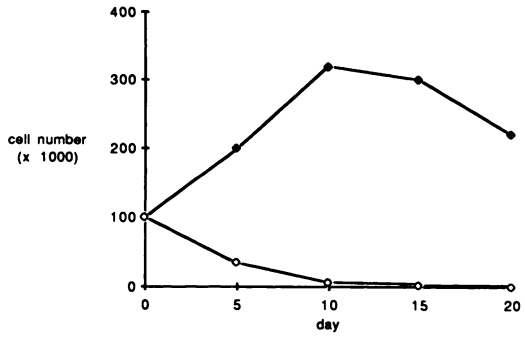

Fig 3. Cell growth of B cell precursor ALL (No. 37 in Table 1) in response to L-BCGF. Cells were incubated in the presence (--●--) or absence (--O--) of 10% vol/vol L-BCGF and quantitated at different time points. Cell viability was assessed by trypan blue dye exclusion.

Response of B cell precursor ALL to highly purified L-BCGF and other recombinant lymphokines. To confirm that the response of B cell precursor ALL was not attributable to a contaminant in the partially purified commercial product, we compared the response of a B cell precursor ALL to partially purified and highly purified L-BCGF. Leukemic cells from patient No. 36 (Table 1) had been maintained in partially purified L-BCGF for 20 days, followed by L-BCGF-free medium for 25 days. Under L-BCGF-free conditions the cells remained viable and showed little change in concentration. Results in Table 3 show the response of these cells to the two sources of L-BCGF. It is very apparent that 0.1% vol/vol and 1.0% vol/vol *highly purified* L-BCGF-triggered ^{3}H-TdR incorporation in the leukemic cells, comparable to the partially purified L-BCGF. Since units of activity are not defined in these preparations, it is not possible to directly equate them. Higher concentrations of highly purified L-BCGF (10% vol/vol) inhibited ^{3}H-TdR incorporation.

We examined the ^{3}H-TdR incorporation of B cell precursor ALL to increasing concentrations of βIL 1, IL 2, gamma IF, and L-BCGF. Whereas the leukemic cells exhibited a dose-dependent response to partially purified L-BCGF, no response occurred to βIL 1 (0.1 to 10 units/mL), IL 2 (10 to 1,000 units/mL), or gamma IF (10 to 10,000 units/mL; data not shown).

DISCUSSION

The growth factors and their receptors, which mediate the proliferation of B cell precursor ALL, are poorly understood. Prior studies reporting colony formation by B cell precursor

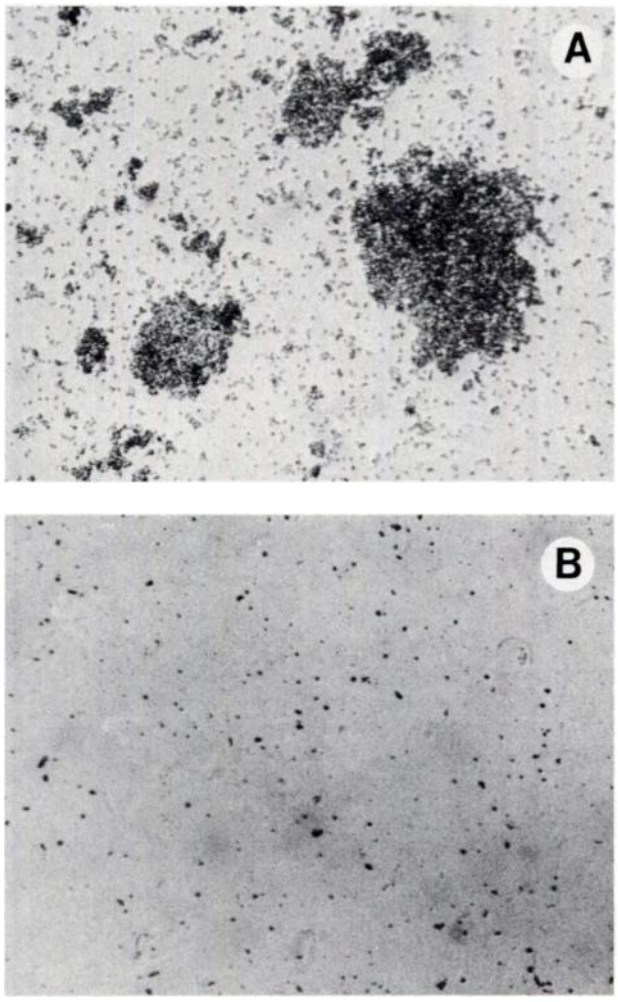

Fig 4. Colony formation of a B cell precursor ALL (No. 36 in Table 1) cultured in the presence (Fig 4A) or absence (Fig 4B) of 10% vol/vol L-BCGF for ten days. Cells from this patient were initially cultured in the presence of L-BCGF for 20 days. The cells were then split, and half of them were maintained in medium containing 25% fetal bovine serum (FBS) with no L-BCGF for 25 days. Under these conditions the cells remained viable and the cell number was stable. The phenotype prior to the colony formation experiment was unchanged as compared to the initial analysis (CD9 positive, CD10/CALLA positive, CD24 positive, negative for surface and cytoplasmic IgM). No colony formation was seen in the cultures without L-BCGF (Fig 4B).

ALL have shown a dependency on soluble factors provided by leukocyte feeders[33] or leukocyte-conditioned medium.[34,35] Although the biochemical nature of the required growth factors were not elucidated, Touw et al[34] showed that B cell precursor ALL colony formation was enhanced by recombinant IL 2 in some cases.

In the present study we have provided evidence that ~75% of B cell precursor ALL respond to L-BCGF. Examination of Table 1 shows that L-BCGF-cultured cells from 28 of 37 patients underwent a significant increase in ^{3}H-TdR incorporation compared to control cells cultured without L-BCGF. Cell sorting experiments confirmed that, in most cases, the CALLA/CD10 positive leukemic cells were responding

Table 3. Comparison of the B Cell Precursor ALL Response to Partially and Highly Purified L-BCGF

Culture Conditions		Response*	Stimulation Index†
No L-BCGF		3,872 ± 69‡	—
Highly purified L-BCGF	0.1%	8,713 ± 1,434	2
	1.0%	29,277 ± 6,065	8
Partially purified L-BCGF	5.0%	14,400 ± 67	4
	10.0%	35,046 ± 2,102	9

*Leukemic cells from patient No. 36 (Table 1) had been maintained in partially purified (Cytokine Technology) L-BCGF for 20 days, followed by L-BCGF-free medium for 25 days. The cells were then cultured with various concentrations of partially or highly purified L-BCGF for 96 hours and were pulsed with ^{3}H-TdR for the final eight hours.

†See Table 1.

‡See Table 1.

directly to L-BCGF (Table 2), although exceptions were noted (patient No. 8). The L-BCGF response of CALLA/CD10 positive cells from patient to patient showed broad variation (Table 2), and explanations for this difference are discussed below. A critical experiment in this study was the direct comparison of the B cell precursor ALL response to partially purified (Cytokine Technology) and highly purified L-BCGF.[29] Data in Table 3 show that the proliferative response of B cell precursor ALL to the two L-BCGF sources was essentially the same. A second critical experiment was the demonstration that L-BCGF promoted colony formation of fresh B cell precursor ALL in vitro (Fig 4A). Increases in absolute cell numbers were also demonstrated in suspension cultures (Fig 2), although the responses were variable. Some leukemic specimens (eg, Figs 2C, 2D) showed an increase in ^{3}H-TdR incorporation with no increase in absolute viable cell number. This suggests that the dividing cells may only be compensating for the cells dying in culture, and L-BCGF is not able to numerically expand these cells in vitro.

Several variables could be invoked to explain the patient-to-patient variability in response to L-BCGF, including: (1) differences in L-BCGF receptor expression/affinity that may reflect distinct stages of development at which a given leukemia is arrested, (2) dependency on external growth factors other than (or in addition to) L-BCGF, (3) dependency on accessory cells such as monocytes or T cells, and (4) dependency on an autocrine growth mechanism. Accessory cell dependency seems unlikely, since highly purified (>99%) CALLA/CD10 positive leukemic cells underwent a rigorous response to L-BCGF (Table 2). Dependency on other growth factors is a plausible explanation. One obvious candidate is IL 2, since this lymphokine has been shown by numerous laboratories to induce normal B cells to proliferate and/or differentiate.[12-23] Touw et al[34] showed that IL 2 enhanced B cell precursor ALL colony formation in the presence of leukocyte feeders. In contrast, we could find no role for IL 2 in the proliferation of B cell precursor ALL. Furthermore we studied 12 L-BCGF responders in Table 1 for their response to L-BCGF and 10 to 100 units/mL of IL 2 but found no evidence for a synergistic effect (data not shown). The discordance between our IL 2 results and those of Touw et al[34] might be attributable to the assay systems under study, ie, colony formation *v* ^{3}H-TdR incorporation.

We feel that L-BCGF receptor expression may well explain the patient-to-patient variability observed in Tables 1 and 2. This variability could manifest itself at the quantitative level of L-BCGF receptor expression or at the level of signal transduction subsequent to binding of L-BCGF to its receptor. A resolution of these various possibilities awaits identification and characterization of the L-BCGF receptor.

In conclusion we have shown that the majority of B cell precursor ALL undergo a direct proliferative response to L-BCGF. We found no general role for IL 2 in B cell precursor ALL proliferation. Thus we believe L-BCGF may be the predominant but not necessarily sole growth stimulus. The possibliity that perturbation of L-BCGF/L-BCGF receptor interaction might disrupt growth of B cell precursor ALL should now be considered.

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