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Immunobiologic differences between normal and leukemic human B-cell precursors

(B-cell ontogeny/B-cell precursor acute lymphoblastic leukemia/hematopoietic growth factors/signal transduction/proliferation)

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ABSTRACT **The early stages of normal human B-cell differentiation were studied by flow cytometry and cell sorting based on expression of CD10 (CALLA) and CD19 antigens in fetal liver. Both $CD10^+CD19^+$ and $CD10^+CD19^-$ precursor populations proliferated *in vitro* to form B-cell precursor colonies under stimulation from low molecular weight B-cell growth factor (L-BCGF) or recombinant interleukin 3 but did not respond to high molecular weight B-cell growth factor (H-BCGF). The colonies derived from the $CD10^+CD19^-$ fraction showed induction of CD19 expression in 10–50% of growing cells, suggesting that CD10 expression precedes CD19 expression in B-cell ontogeny. This hypothesis was corroborated by less-differentiated marker profiles of the progeny of $CD10^+CD19^-$ B-cell precursors as compared to $CD10^+CD19^+$ B-cell precursors in BCGF-stimulated cultures and by higher percentages of $CD10^+CD19^-$ versus $CD10^-CD19^+$ B-cell precursors. CD19 crosslinking on normal fetal liver or bone marrow B-cell precursors was associated with an increase in cytoplasmic calcium concentration, but was inhibitory for colony formation. Leukemic B-cell precursors from acute lymphoblastic leukemias (ALLs) differed from normal B-cell precursors in their *in vitro* proliferative responses, since (*i*) they responded not only to L-BCGF and rIL-3 but also to H-BCGF and (*ii*) their proliferation was stimulated rather than inhibited by CD19 crosslinking. A clonogenic leukemic counterpart for the $CD10^+CD19^-$ normal B-cell precursor population does not exist among malignant cells from B-cell precursor ALL patients, suggesting that the CD19 receptor may be involved in leukemogenesis of human B-cell precursor ALL.**

Differentiation of human B-cell precursors (BCPs) into immunoglobulin-producing B lymphocytes represents a multistep maturation process that is accompanied by a coordinated acquisition and loss of B-lineage lymphoid differentiation antigens (1). The current hypothetical models of human B-lymphocyte development and coordinate sequence of antigen expression are largely based on studies performed on leukemic BCPs from B-lineage acute lymphoblastic leukemia (ALL) patients, which are thought to originate from normal BCPs arrested at discrete stages of maturation within the B-cell precursor pathway (1–4). With the exception of a few controversial reports (5–8) about the immunological surface marker profiles of normal and leukemic BCPs, critical information regarding the immunobiologic features of BCPs, mechanisms of growth regulation during early stages of human B-lymphocyte development, or the differences between distinct populations of normal versus leukemic BCPs has not been published.

In this report, we used multiparameter flow cytometry and fluorescence-activated cell sorting (FACS) to identify as well as to isolate distinct subpopulations of normal human BCPs from fetal livers and leukemic BCPs from B-lineage ALL bone marrow samples. BCP colony assays were performed to elucidate and compare the immunobiologic features of purified subpopulations of normal and leukemic BCPs.

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MATERIALS AND METHODS

Human Fetal Liver, Fetal Bone Marrow, and Leukemic Bone Marrow Samples. These tissues were obtained and used by following the guidelines of the University of Minnesota Committee on the Use of Human Subjects in Research for secondary use of pathological or surgical tissue. Fetal livers and fetal bone marrow were obtained from prostaglandin-induced human abortuses of 16–22 weeks gestation. Fresh bone marrow from four BCP ALL patients was procured by routine procedures. Fetal liver mononuclear cells and ALL blasts were isolated by centrifugation of fetal liver/leukemic bone marrow cell suspensions on Ficoll/Hypaque gradients.

Monoclonal Antibodies (mAbs) and Growth Factors. mAbs 24.1, B43, G28-7, and G3.7 are directed against human antigens CD10, CD19, CD22, and CD7, respectively. Fluorescein isothiocyanate (FITC) and B43–phycoerythrin (PE) were prepared as described (9, 10). Control mAbs included MsIgG1-RD1 (PE-labeled mouse IgG1 purchased from Coulter), MsIgG2a–FITC (FITC-labeled mouse IgG2a purchased from Coulter), and a locally prepared control ascites fluid. Biochemically purified low molecular weight B-cell growth factor (L-BCGF) and high molecular weight BCGF (H-BCGF) were prepared as described (11).

Multiparameter Flow Cytometry Analyses and FACS. Fetal liver mononuclear cells or leukemic BCPs (1×10^7 cells per ml) were put into a cold mixture of B43/CD19–PE (10 μg/ml) and 24.1/CD10–FITC (10 μg/ml), incubated 30 min on ice, washed three times in phosphate-buffered saline, and analyzed for two-color immunofluorescence on a five-parameter FACS 440, and immunologically distinct populations were sorted as described (8, 9, 11).

BCP Colony Assay. FACS-sorted normal and leukemic BCPs were assayed for colony formation *in vitro* by using a colony assay system as described (11–13). Cells were suspended in α minimal essential medium (GIBCO) supplemented with 0.9% methylcellulose, 30% calf bovine serum, 1% penicillin/streptomycin, and L-BCGF (2 ng/ml), H-BCGF (5 ng/ml), recombinant interleukin 2 (rIL-2) (100 units/ml) (kindly provided by Cetus Corp., Emeryville, CA), or rIL-3 (8 units/ml) (kindly provided by Steven Clark,

Abbreviations: BCP, B-cell precursor; FACS, fluorescence activated cell sorting/sorter; L-BCGF, low molecular weight (M_r, 12,000) B-cell growth factor; H-BCGF, high molecular weight (M_r, 60,000) BCGF; rIL-2 and -3, recombinant interleukins 2 and 3; sIg, surface immunoglobulin; C_μ, cytoplasmic immunoglobulin μ heavy chains; ALL, acute lymphoblastic leukemia; TdT, terminal deoxynucleotidyltransferase; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate; PE, phycoerythrin; $[Ca^{2+}]_i$, cytoplasmic calcium concentration.

Genetics Institute, Boston). Duplicate 1-ml samples of 50,000 (normal BCPs) or 100,000 (leukemic BCPs) cells were cultured in 35-mm Petri dishes for 7 days at 37°C in humidified 5% CO_2/95% air. On day 7, colonies containing >20 cells were enumerated with an inverted phase-contrast microscope. Subsequently, morphological–immunological characterization of colony cells was performed as described (11–13).

Measurement of Cytoplasmic Free Calcium Concentration ($[Ca^{2+}]_i$) in Fetal Liver BCPs. $[Ca^{2+}]_i$ was measured with the dye Indo-1 (Molecular Probes, Eugene, OR) and a model 50HH/2150 cell sorter (Ortho Diagnostics) as described (9, 14, 15).

RESULTS

Immunobiologic Features of Normal BCP Populations in Human Fetal Liver. To study the sequence of acquisition for CD10 and CD19 antigens during B-lymphocyte development, we performed multiparameter immunofluorescence analyses on lymphoid cells from 12 freshly obtained fetal livers of different gestational ages (mean gestational age, 19.3 ± 0.6 wk; see Table 1). The mean percentage of $CD10^+$ cells was significantly higher than the mean percentage of $CD19^+$ cells (17.0% ± 3.7% versus 7.2% ± 1.5%; $P = 0.022$). Furthermore, the percentage of $CD10^+CD19^+$ cells was significantly higher than the percentage of $CD10^-CD19^+$ cells (5.9% ± 1.6% versus 1.3% ± 0.3%; $P = 0.008$) since the majority of $CD19^+$ cells (77.8% ± 4.9%) also expressed CD10. By comparison, a significant fraction of $CD10^+$ cells (mean ± SEM = 65.6% ± 3.7%; range, 48.2–82.2%) were $CD19^-$ (Table 1, Fig. 1). The proportion of this $CD10^+CD19^-$ cell population was significantly higher than the proportion of $CD10^-CD19^+$ cells (11.1% ± 2.4% versus 1.3% ± 0.3%; $P < 0.001$).

Based on this correlation of CD10 and CD19 antigens on fetal liver lymphoid cells, we postulated that (*i*) the expression of CD10 antigen precedes CD19 expression during human B-cell ontogeny and (*ii*) $CD10^+CD19^-$ lymphoid cells represent an early BCP population. To test this hypothesis, fetal liver cells were stained and sorted based on coordinate expression of CD10 and CD19 antigens (Fig. 1, Table 2). FACS-sorted $CD10^+CD19^-$ as well as $CD10^+CD19^+$ fetal liver lymphoid cells had lymphoblast morphology and >75% were terminal deoxynucleotidyltransferase (TdT) positive. BCP colony assays were used to study the immunobiological features of sorted $CD10^+CD19^+$ and $CD10^+CD19^-$ fetal liver lymphoid cells. Notably, L-BCGF stimulated *in vitro* colony formation by $CD10^+CD19^+$ as well as $CD10^+CD19^-$ lymphoid cells. The mean number of colonies derived from $CD10^+CD19^-$ cells was 2.6-fold higher than the mean number of colonies derived from $CD10^+CD19^+$ cells (219 colonies versus 83 colonies; $P = 0.013$). B43/CD19 mAb (1 μg/ml)

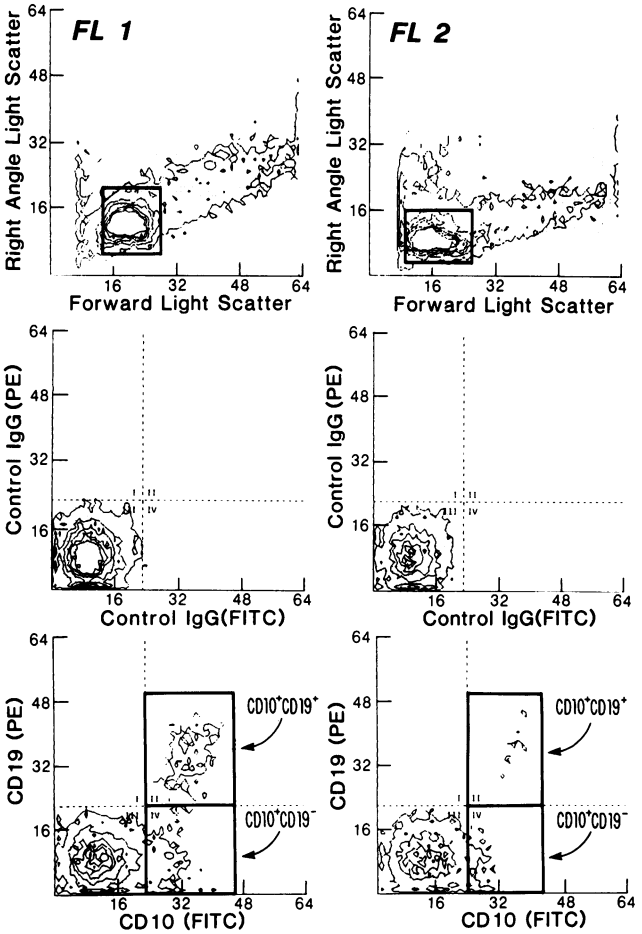

FIG. 1. Multiparameter flow cytometry analysis of fetal liver cells two-color-stained with 24.1/CD10–FITC and B43/CD19–PE. Boxes in the light scattering contour plots identify the gates used to reanalyze the list mode data for determining the correlations between CD10 and CD19 expression on lymphoid cells. Boxes on the FACS-correlated two-color displays of gated fetal liver lymphoid cells identify the windows used for sorting the $CD10^+CD19^-$ and $CD10^+CD19^+$ BCP populations. Control samples were stained with the control IgG MsIgG1–RD1 and MsIgG2a–FITC.

Table 1. Normal B-cell precursor subpopulations in human fetal liver

Fetal liver	Gestational age, wk	Percentage of positive fetal liver lymphoid cells: $CD10^+$ (% of total)	$CD19^+$ (% of total)	$CD10^+CD19^+$ (% of $CD19^+$)	$CD10^+CD19^-$ (% of $CD10^+$)	$CD19^+CD10^-$ (% of total)
1	16	19.9 (16.1)	10.9 (8.8)	10.3 (94.5)	9.6 (48.2)	0.6 (5.5)
2	17	30.2 (14.8)	7.8 (3.8)	6.5 (83.3)	23.7 (78.5)	1.3 (16.7)
3	17	52.3 (12.5)	22.5 (5.4)	21.5 (95.6)	30.8 (58.9)	1.0 (4.4)
4	18	13.8 (6.5)	6.2 (2.9)	5.7 (91.9)	8.1 (58.7)	0.5 (8.1)
5	18	7.0 (4.0)	3.1 (1.8)	2.6 (83.9)	4.4 (62.9)	0.5 (16.1)
6	19	14.0 (2.7)	3.9 (0.7)	2.9 (74.4)	11.1 (79.3)	1.0 (25.6)
7	20	8.0 (3.3)	6.5 (2.7)	3.7 (56.9)	4.3 (53.8)	2.8 (43.1)
8	20	12.4 (6.1)	7.4 (3.6)	6.2 (83.8)	6.2 (50.0)	1.2 (16.2)
9	21	7.9 (2.9)	3.9 (1.4)	3.3 (84.6)	4.6 (58.2)	0.6 (15.4)
10	21	15.0 (3.8)	4.2 (1.1)	3.7 (88.1)	11.3 (75.3)	0.5 (11.9)
11	22	15.2 (4.4)	6.2 (1.8)	2.7 (43.5)	12.5 (82.2)	3.5 (56.5)
12	22	8.8 (1.4)	3.2 (0.5)	1.7 (53.1)	7.1 (80.7)	1.5 (46.9)

Fetal liver mononuclear cells were two-color-stained for CD10 and CD19 antigens using 24.1/CD10–FITC and B43/CD19–PE and analyzed for immunofluoresence on a five-parameter FACS 440. Results are shown as immunologically distinct proportions of fetal liver lymphoid populations.

Table 2. Proliferative responses of FACS-sorted normal fetal liver and leukemic bone marrow B-cell precursor populations to hematopoietic growth factors

Fetal liver	FACS-sorted fraction	Mean B-cell precursor colonies/50,000 FACS-sorted fetal liver cells: No growth factor	L-BCGF − B43	L-BCGF + B43	H-BCGF
1	$CD10^+CD19^-$	0	125	134	0
	$CD10^+CD19^+$	0	75	11	0
2	$CD10^+CD19^-$	0	153	149	0
	$CD10^+CD19^+$	0	15	1	0
3	$CD10^+CD19^-$	0	487	ND	0
	$CD10^+CD19^+$	0	28	3	0
4	$CD10^+CD19^-$	0	195	ND	0
	$CD10^+CD19^+$	0	88	55	0
8	$CD10^+CD19^-$	0	291	286	0
	$CD10^+CD19^+$	0	275	27	0
10	$CD10^+CD19^-$	0	62	ND	12
	$CD10^+CD19^+$	0	14	0	0

Patient	FAC-sorted fraction	Mean B-cell precursor colonies/100,000 FACS-sorted ALL cells: No growth factor	L-BCGF − B43	L-BCGF + B43	H-BCGF
1	$CD10^+CD19^-$	0	0	0	0
	$CD10^+CD19^+$	0	575	1023	119
	$CD10^-CD19^+$	0	475	1208	52
2	$CD10^+CD19^-$	0	0	0	0
	$CD10^+CD19^+$	0	211	298	178
	$CD10^-CD19^+$	0	235	284	150
3	$CD10^+CD19^-$	0	0	ND	0
	$CD10^+CD19^+$	0	64	214	15
	$CD10^-CD19^+$	0	68	155	23
4	$CD10^+CD19^-$	0	0	ND	0
	$CD10^+CD19^+$	0	21	73	0
	$CD10^-CD19^+$	0	39	112	0

The proliferative responses of FACS-sorted normal and leukemic BCP populations to L-BCGF in the presence (+) and absence (−) of CD19 mAb B43 and to H-BCGF were analyzed in BCP colony assays. ND, not determined.

inhibited L-BCGF-stimulated proliferation of $CD10^+CD19^+$ fetal liver BCPs but it did not affect the proliferative activity of $CD10^+CD19^-$ fetal liver BCPs (Table 2). rIL-3 was also able to stimulate colony formation by $CD10^+CD19^+$ as well as $CD10^+CD19^-$ fetal liver lymphoid cells (data not shown). The ability of L-BCGF and rIL-3 to stimulate BCP colony formation provided evidence that $CD19^+$ and $CD19^-$ fractions of $CD10^+$ BCPs constitutively express functional receptors for both L-BCGF and IL-3. In contrast to L-BCGF or rIL-3, H-BCGF (Table 2) and rIL-2 (data not shown) did not elicit a detectable stimulatory activity on $CD10^+CD19^+$ or $CD10^+$-$CD19^-$ BCPs.

Immunophenotypic Evidence for the B-Lineage Affiliation of the Progeny of $CD10^+CD19^+$ and $CD10^+CD19^-$ Fetal Liver BCPs. $CD10^+CD19^+$ cells yielded colonies of TdT^+CD10^+-$CD19^+$ surface immunoglobulin-negative (sIg^-) $CD7^-$ BCPs (Table 3, Fig. 2): The expression of nuclear TdT and surface CD10 confirmed that they were lymphocyte precursors; the surface expression of the B-lineage-specific CD19 antigen in the absence of the mature B-cell marker sIg confirmed that they were BCPs; and the lack of the prethymic early T-lineage marker CD7 confirmed that they were not T-cell precursors. The majority of these cells expressed the B-lineage-specific marker CD22 in their cytoplasm, providing additional evidence for their B-lineage affiliation (Table 3). Notably, 22–38% of cells were also C_μ^+ (positive for cytoplasmic immunoglobulin μ heavy chains) consistent with pre-B stage of B-lineage lymphoid differentiation. By comparison, colonies derived from $CD10^+CD19^-$ FACS-sorted cells were composed of $TdT^+CD10^+sIg^-CD7^-$ lymphoid cells that expressed the B-lineage-specific early antigen CD22 in their cytoplasm, confirming their B-lineage affiliation. The expression of TdT and CD10 and in the absence of sIg was consistent with BCP stage. Furthermore, 10–50% of cells were also $CD19^+$, suggesting acquisition of CD19 antigen by $CD19^-$ BCP during the 7 days of culture in the presence of L-BCGF (Table 3). Only 7.6% ± 2.6% (range, 0–15%) of these cells were C_μ^+ pre-B cells, which is >4 times lower than the

Table 3. Immunophenotypic features of colony cells in L-BCGF-stimulated cultures of FACS-sorted normal fetal liver and leukemic bone marrow B-cell precursor subpopulations

	FACS-sorted fraction	% cells positive: TdT	CD10	CD19	CD22	C_μ	sIg	CD7
Fetal liver								
1	$CD10^+CD19^+$	93	83	95	ND	35	0	0
	$CD10^+CD19^-$	95	89	13	75	11	0	0
2	$CD10^+CD19^-$	100	98	25	60	5	1	0
3	$CD10^+CD19^+$	100	85	75	ND	ND	ND	ND
	$CD10^+CD19^-$	99	100	10	25	0	0	0
4	$CD10^+CD19^+$	85	89	100	90	22	0	0
	$CD10^+CD19^-$	100	95	50	95	15	0	0
8	$CD10^+CD19^+$	98	95	95	92	38	0	0
	$CD10^+CD19^-$	100	94	18	95	7	0	0
Patient								
1	$CD10^+CD19^+$	100	75	89	100	0	0	0
	$CD10^-CD19^+$	75	0	85	85	0	0	0
2	$CD10^+CD19^+$	95	75	88	100	0	0	0
	$CD10^-CD19^+$	87	2	94	95	0	0	0
3	$CD10^+CD19^+$	85	74	100	ND	0	0	0
	$CD10^-CD19^+$	86	0	95	ND	0	0	0

The immunological marker profiles of BCP colony cells derived from L-BCGF-stimulated normal and leukemic BCP subpopulations were determined by immunofluorescence staining techniques and immunofluorescence microscopy. Quantitative data were obtained from examination of 100–200 cells. Data are expressed as the percentage of cells expressing each immunological marker. ND, not determined.

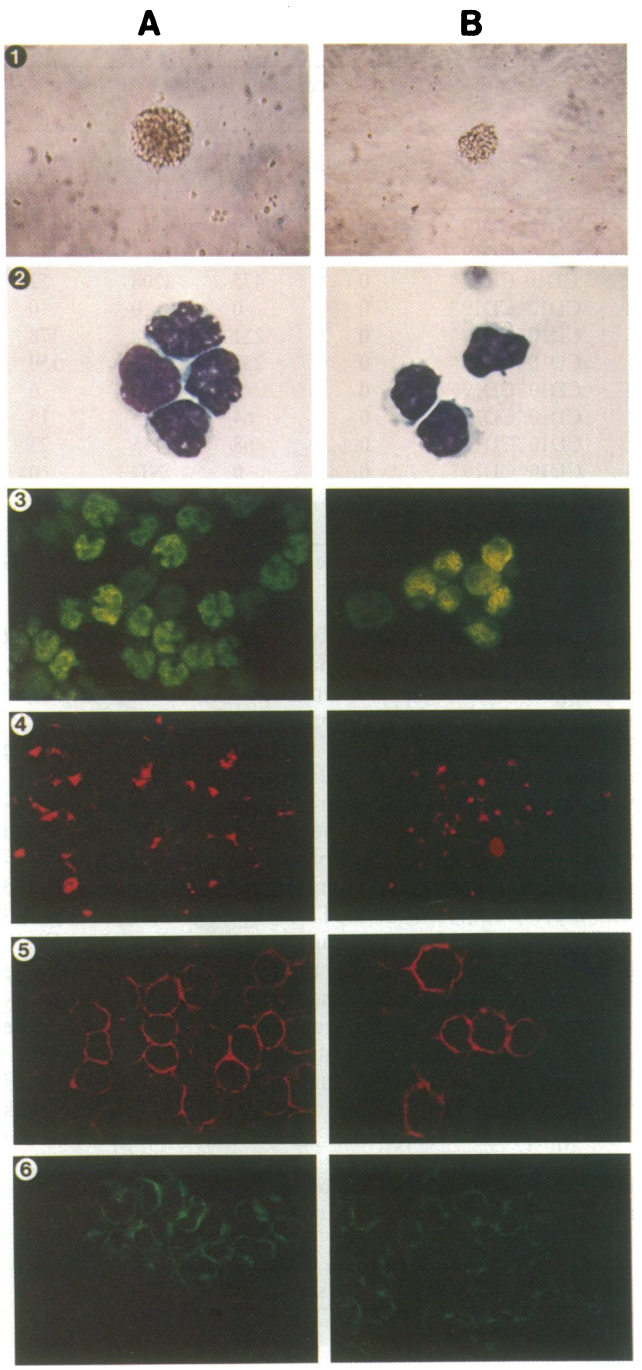

FIG. 2. Immunophenotypic analyses of BCP colony cells. (*A*) $CD10^+CD19^-$ BCPs; (*B*) $CD10^+CD19^+$ BCPs from fetal liver 1. (*1*) A day-7 BCP colony in L-BCGF-stimulated cultures. (*2*) Wright–Giemsa-stained cells pooled from BCP colonies had a lymphoid morphology. (*3–6*) Immunophenotypic features of BCP colony cells are illustrated: (*3*) nuclear TdT, (*4*) surface CD10, (*5*) surface CD19, (*6*) cytoplasmic CD22.

percentage of C_μ^+ pre-B cells in cultures of $CD10^+CD19^+$ FL BCPs (31.7% ± 4.9%; range, 22–38%; $P = 0.003$).

Immunobiologic Features of Leukemic BCP Populations. In parallel experiments, $CD10^+CD19^+$, $CD10^+CD19^-$, and $CD10^-CD19^+$ FACS-sorted ALL blasts from four BCP ALL patients were assayed for BCP colony formation in the presence of hematopoietic growth factor (Table 2). L-BCGF, rIL-3 (data not shown), as well as H-BCGF stimulated colony formation by $CD10^+CD19^+$ as well as $CD10^-CD19^+$ blasts, but they failed to stimulate $CD10^+CD19^-$ blasts (Table 2). rIL-2 stimulated leukemic BCP cultures did not yield any colonies (data not shown). B43/CD19 mAb augmented the proliferative response of leukemic BCPs to L-BCGF (Table 2). As shown in Table 3, colony cells in L-BCGF stimulated cultures of leukemic BCPs were TdT^+, $CD10^\pm$ ($CD10^+$ cells yielded $CD10^+$ colonies, $CD10^-$ cells yielded $CD10^-$ colonies), $CD19^+$, $CD22_c$ (cytoplasmic)$^+$, and sIg^-, consistent with BCP ALL.

CD19 Ligation Increases $[Ca^{2+}]_i$ in Normal and Leukemic BCPs. CD19 crosslinking on mature B cells and leukemic BCPs was previously shown to increase $[Ca^{2+}]_i$ (9, 15). We therefore examined Indo-1-loaded normal BCPs for CD19-induced alterations in $[Ca^{2+}]_i$. When resting $[Ca^{2+}]_i$ was measured after CD19 or CD10 staining of fetal liver mononuclear cells, it was apparent that $CD19^+$ cells that bound the CD19 mAb had a higher $[Ca^{2+}]_i$ than $CD19^-$ cells or cells stained with CD10 mAb (Fig. 3*A*). This same pattern of increased $[Ca^{2+}]_i$ in cells after binding of CD19 mAb was seen in three separate fetal livers (data not shown). Fetal bone marrow cells were also Indo-1-loaded and examined for $[Ca^{2+}]_i$ responses after CD19 staining (Fig. 3*B*). $CD19^+$ and $CD19^-$ cells were simultaneously analyzed while the surface CD19 mAb was further crosslinked by addition of an anti-mouse immunoglobulin second-step reagent. While $CD19^+$ cells responded to crosslinking, $CD19^-$ cells did not. The increase in $[Ca^{2+}]_i$ depended on the CD19 mAb, since CD10-stained cells did not respond, nor was there a response from the anti-mouse immunoglobulin alone (data not shown).

DISCUSSION

In the present study, three immunologically distinct normal BCP subpopulations were identified among the lymphoid cells from human fetal livers based on correlated expression of CD10 and CD19 antigens. $CD10^+CD19^-$ BCPs were found in larger numbers than $CD10^+CD19^+$ or $CD10^-CD19^+$ BCPs, they acquired CD19 antigen *in vitro*, and their day 7

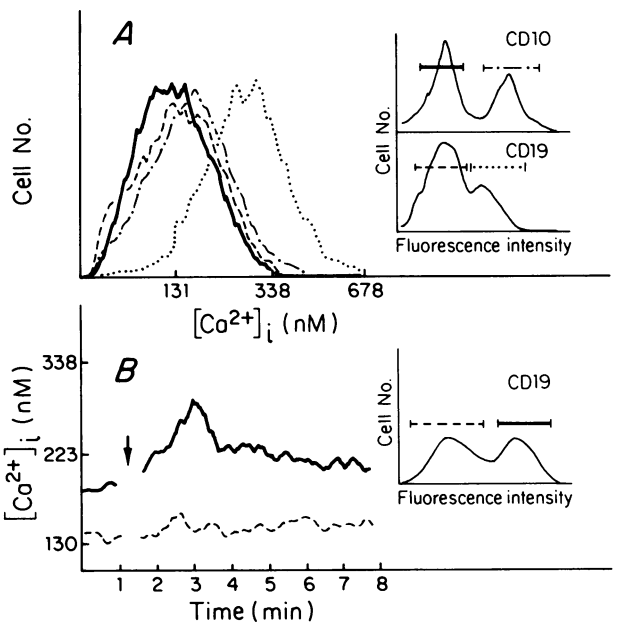

FIG. 3. $[Ca^{2+}]_i$ responses to CD19 ligation by fetal liver and fetal bone marrow BCP. Fetal liver cells were Indo-1-loaded and assayed by flow cytometry for $[Ca^{2+}]_i$ levels (*A*). Cells were stained with B43–PE (anti-CD19) or 24.1–FITC (anti-CD10) and gated as indicated. The $[Ca^{2+}]_i$ levels of cell populations within the gates are indicated by the corresponding lines. Fetal bone marrow cells (18 wk old) were Indo-1-loaded, stained with B43–PE, and gated as indicated in *B*. At $t = 1.5$ min, an anti-mouse immunoglobulin was added in excess and the responses of $CD19^+$ and $CD19^-$ cells were simultaneously analyzed.

progeny in BCP colonies displayed a more immature immunophenotype than the progeny of $CD10^+CD19^+$ BCPs. These findings indicate that (*i*) CD10 antigen expression precedes the acquisition of CD19 antigen and (*ii*) CD19 expression is developmentally programmed in B-cell ontogeny. Based on the data on the surface antigen profiles of $CD10^+$ fetal liver lymphoid cell populations and their progeny in BCGF-stimulated cultures, we propose a hypothetical model of early B-lineage lymphoid differentiation: According to this model, the most immature BCPs (stage 0) express nuclear TdT and surface CD10 but lack the B-lineage-specific antigens CD19, CD22, and cytoplasmic/sIg. During the subsequent stage of B-lymphocyte development, TdT^+CD10^+ BCPs become committed to B-lineage differentiation and express CD22 in their cytoplasm (stage I). The acquisition of CD19 identifies the next stage of differentiation (stage II) within the BCP pathway. $TdT^+CD10^+CD19^+CD22_c^+$ BCPs subsequently lose CD10 yielding $TdT^+CD10^-CD19^+CD22_c^+$ stage III BCPs. Stage II as well as stage III BCPs can differentiate into pre-B cells (stages IVa and IVb), which are identified by expression of μ heavy chains in their cytoplasm. In the present study, we identified the majority of BCPs in fetal liver samples as stage 0–I and stage II BCPs. $CD10^-CD19^+$ stage III BCPs constituted only $1.3\% \pm 0.3\%$ of fetal liver lymphoid cells (Table 1).

There is now considerable evidence that the CD19 receptor is an important functional regulator of normal and malignant B-cell proliferation. This evidence includes: (*i*) CD19 expression correlates with the cloning efficiency and with DNA synthesis activity of leukemic BCPs (13); (*ii*) CD19 receptor density on leukemic BCPs is augmented during growth stimulation by L-BCGF (9); (*iii*) CD19 receptor is expressed in all BCP leukemias, and B-lineage leukemic progenitor cells in BCP ALL patients are $CD19^+$ (9, 11–13). In a recent study, we found that crosslinking the CD19 receptor with a mAb induces increases in $[Ca^{2+}]_i$ in leukemic BCPs and mediates a positive signal for proliferation (15). The CD19-induced $[Ca^{2+}]_i$ responses were observed in the absence of extracellular calcium, suggesting that these signals are likely mediated by activation of inositol phospholipid metabolism that results in inositol trisphosphate formation and mobilization of cytoplasmic calcium (15). In the present study, we provide evidence that CD19 ligation induces a distinct $[Ca^{2+}]_i$ signal in normal BCPs as well. Notably, two hitherto unrecognized differences were found between normal and leukemic BCPs relative to the function of their CD19 receptors. (*i*) The $CD19^-$ normal BCPs but not the $CD19^-$ leukemic BCPs were capable of self-renewal and clonogenic proliferation in response to L-BCGF or rIL-3. The lack of clonogenic leukemic counterparts for $CD10^+CD19^-$ stage 0–I normal BCPs suggests that the B-lineage-specific CD19 receptor may play an important role in leukemogenesis of BCP leukemias. (*ii*) Crosslinking the CD19 receptor with B43 mAb augmented the L-BCGF responses of $CD19^+$ leukemic BCPs but inhibited the L-BCGF-induced proliferation of normal $CD19^+$ BCPs. The observation that the CD19 molecule mediates stimulatory signals for leukemic BCPs and inhibitory signals for normal BCPs (Table 2) and mature B cells (9) prompts the hypothesis that the function of the CD19 receptor and/or the CD19-linked signal transmission pathways may be altered during leukemogenesis in human BCP leukemias. Intriguingly, recent cDNA cloning of CD19 has shown a significant homology in the cytoplasmic domain of CD19 with the *INT-1* oncogene and an Epstein–Barr virus protein (16).

L-BCGF and rIL-3 (data not shown) were capable of stimulating the proliferative activity of immunologically distinct subpopulations of normal as well as leukemic BCPs. Thus, L-BCGF and IL-3 may play an important growth regulatory role in early stages of normal B-cell ontogeny as well as clonal expansion of leukemic BCP populations. By comparison, H-BCGF was unable to stimulate normal BCP but it did stimulate *in vitro* colony formation by leukemic BCPs (11). This difference may be a consequence of altered/amplified H-BCGF receptor gene expression in leukemic BCPs. Synergistic interactions between L-BCGF and H-BCGF (11) may contribute to a rapid clonal expansion of leukemic BCP populations. While expanding the information from our previous studies (9, 11–13, 15, 17–19) on the surface growth factor receptors of human lymphoid precursor cells, the presented data should also promote future analyses of growth factor interactions at the level of normal and leukemic BCPs.

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