JCI The Journal of Clinical Investigation

Interleukin 4 inhibits in vitro proliferation of leukemic and normal human B cell precursors.

D Pandrau, … , N Philippe, J Banchereau

J Clin Invest. 1992;90(5):1697-1706. https://doi.org/10.1172/JCI116042.

Research Article

In the present study, we have investigated the effects of IL-4 on the proliferation and differentiation of leukemic and normal human B cell precursors (BCP). We have demonstrated that IL-4 significantly inhibited spontaneous [3H]thymidine ([3H]-TdR) incorporation by leukemic blasts from some B lineage acute lymphoblastic leukemia (BCP-ALL) patients (8 of 14). Furthermore, IL-4 was found to suppress the spontaneous and factor-dependent (IL-7 and IL-3) proliferation of normal BCP (CD10+ surface [s] IgM- cells) isolated from fetal bone marrow. Maximum growth inhibition of either leukemic or normal BCP was reached at low IL-4 concentrations (10 U/ml), and the effect was specifically neutralized by anti-IL-4 antibody. IL-4 was further found to induce the expression of CD20 antigen on BCP-ALL cells from a number of the cases examined (5 of 8), but in contrast to leukemic cells, IL-4 failed to induce CD20 antigen on normal BCP. Finally, IL-4 was found to induce neither the expression of cytoplasmic mu chain, nor the appearance of sIgM+ cells in cultures of normal or leukemic BCP. Our data indicate that IL-4 has the potential to inhibit cell proliferation in leukemic and normal human B lymphopoiesis but is unable to drive the transition from BCP to mature B cells.

Find the latest version:

https://jci.me/116042/pdf

Interleukin 4 Inhibits In Vitro Proliferation of Leukemic and Normal Human B Cell Precursors

Dominique Pandrau, Sem Saeland, Valérie Duvert, Isabelle Durand, Anne-Marie Manel,* Marie-Thérèse Zabot,* Noël Philippe,* and Jacques Banchereau

*Laboratory for Immunological Research, Schering-Plough, 69571 Dardilly Cedex, France; and *Debrousse Pediatric Hospital, 69332 Lyon Cedex 05, France*

Abstract

In the present study, we have investigated the effects of IL-4 on the proliferation and differentiation of leukemic and normal human B cell precursors (BCP). We have demonstrated that IL-4 significantly inhibited spontaneous [^{3}H]thymidine ([^{3}H]-TdR) incorporation by leukemic blasts from some B lineage acute lymphoblastic leukemia (BCP-ALL) patients (8 of 14). Furthermore, IL-4 was found to suppress the spontaneous and factor-dependent (IL-7 and IL-3) proliferation of normal BCP (CD10$^+$ surface [s] IgM$^-$ cells) isolated from fetal bone marrow. Maximum growth inhibition of either leukemic or normal BCP was reached at low IL-4 concentrations (10 U/ml), and the effect was specifically neutralized by anti-IL-4 antibody. IL-4 was further found to induce the expression of CD20 antigen on BCP-ALL cells from a number of the cases examined (5 of 8), but in contrast to leukemic cells, IL-4 failed to induce CD20 antigen on normal BCP. Finally, IL-4 was found to induce neither the expression of cytoplasmic μ chain, nor the appearance of sIgM$^+$ cells in cultures of normal or leukemic BCP. Our data indicate that IL-4 has the potential to inhibit cell proliferation in leukemic and normal human B lymphopoiesis but is unable to drive the transition from BCP to mature B cells. (*J. Clin. Invest.* 1992. 90:1697–1706.) Key words: B cell ontogeny • B lineage acute lymphoblastic leukemia • interleukin 4 • maturation • proliferation

Introduction

B cells are continuously generated in mammalian bone marrow, as a result of the expansion and differentiation of primitive hematopoietic cells. As in the other hematopoietic lineages, B cell ontogeny follows an ordered progression, with discrete well-characterized maturation stages (for reviews on B lineage development, see references 1–4). Aberrant events in this scheme may lead to the generation and expansion of malignant B cell precursors (BCP)[1] in B lineage acute lymphoblastic leukemia (ALL). Much effort has been devoted to understanding the factors involved in the regulation of B cell ontogeny and a number of cytokines have been shown to play a central role in controlling BCP development. Among other cytokines, IL-4 has been shown to display a wide variety of biological effects on various cell types. In particular IL-4 plays an important role in the activation, proliferation and differentiation of mature B lymphocytes (5–7). A number of studies have addressed the role of IL-4 in murine BCP development. Thus, IL-4 has been described to stimulate the proliferation of pro B cells (i.e., which display germ-line immunoglobulin genes) (8, 9). During the less primitive stages of murine B cell ontogeny, IL-4 has been described to exert either a suppressive (10, 11) or a potentiating effect on the generation of cytoplasmic (c) μ (pre-B) cells (12). Finally, IL-4 has been reported to induce the appearance of surface (s) IgM$^+$ murine B cells in culture (13, 14). In humans, only limited information is available on the role of IL-4 in early B cell ontogeny. One report has claimed that IL-4 suppresses BCP proliferation and increases the proportion of cμ^+ and sIgM$^+$ cells in cultures of CD24$^+$ fetal bone marrow cells (15). However, the notion that IL-4 induces the emergence of sIgM$^+$ cells has been subsequently challenged (16). Finally, IL-4 has recently been described to inhibit the proliferation of Philadelphia (Ph^1)-positive ALL cells (17). In view of these data, we have extended the investigation of the effects of IL-4 on growth and maturation of leukemic and normal human BCP. Here, we describe that IL-4 inhibits cell proliferation in some, but not all, cases of Ph^1-negative BCP-ALL and suppresses growth of normal fetal BCP. However, although capable of inducing expression of CD20 antigen in BCP-ALL, IL-4 failed to deliver a maturation signal for the transition to IgM$^+$ leukemic or normal B cells.

Methods

Collection and isolation of cells

Bone marrow cells were obtained according to institutional guidelines.

Normal BCP. Fetal femurs were obtained after therapeutic or spontaneous abortions between 18 and 24 wk of pregnancy, and were kindly provided by Prof. J. L. Touraine (Lyon, France). Fetal bone marrow was collected by scraping and flushing the femurs, and was subsequently filtered on nylon wool to remove bone fragments. Mononuclear fractions isolated by Ficoll-Hypaque gradient centrifugation were depleted of adherent cells as previously described (18). Cells bearing CD10 antigen were subsequently isolated from nonadherent mononuclear fractions by immune "panning" with the ALB-1 anti-CD10 mAb (Immunotech, Marseille, France), using a technique reported in detail elsewhere (19). As LeBien et al. (20) have reported that CD10 is a pan B marker in fetal bone marrow sIgM$^+$ cells were subsequently depleted from the CD10 population by use of an anti-IgM mAb (Immunotech) and immunomagnetic beads coated with anti-mouse Ig (Dynabeads M-450, Dynal, Oslo, Norway).

Part of this work was presented as a communication at the Keystone Symposium on Molecular and Cellular Biology, Keystone, CO, April 1991 (1991. *J. Cell. Biochem.* 15F:P201a [Abstr.]).

Address reprint requests to Dr. Sem Saeland, Schering-Plough, Laboratory for Immunological Research, 27 chemin des Peupliers, BP 11, 69571 Dardilly Cedex, France.

Received for publication 1 July 1991 and in revised form 15 June 1992.

1. *Abbreviations used in this paper:* ALL, acute lymphoblastic leukemia; BCP, B cell precursors; c, cytoplasmic; s, surface.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/92/11/1697/10 $2.00
Volume 90, November 1992, 1697–1706

Purity of the recovered cells was > 98% as indicated by staining with anti-CD10 mAb (see below). Furthermore, all cells expressed B lineage CD19 antigen but lacked sIgM, indicating that they represented a virtually pure normal BCP population. In some experiments, $CD20^+$ cells were removed from the $CD10^+$ $sIgM^-$ population by sorting with a $FACstar^+$ flow cytometer (Becton, Dickinson & Co., Sunnyvale, CA).

Leukemic BCP. Blast-invaded (> 80%) bone marrow samples were obtained by iliac crest aspiration from 14 pediatric patients at initial diagnosis or relapse of Ph^1-negative B lineage ALL. Light-density mononuclear cells were isolated by Ficoll-Hypaque ($d = 1.077$) (Eurobio, Paris, France) gradient centrifugation.

Leukemic blasts were subsequently depleted of contaminants by immunomagnetic bead depletion (see above) of cells reactive with a cocktail of mAbs against T cells (anti-CD2, CD3) (Aster, La Gaude, France); anti-CD4, CD8 (BL4, BL15, Institut Mérieux, Lyon, France), mature B cells (anti-κ and -λ chains, Immunotech), and myeloid cells (anti-CD33 [My9, Coulter Immunology, Hialeah, FL]; anti-CD14 [Immunotech]). In addition, in some experiments, leukemic samples were depleted of $CD20^+$ cells by labeling with B1 anti-CD20 mAb (Coulter Immunology) and immunomagnetic beads (see above).

The characteristics of the isolated leukemic BCP are summarized in Table I.

Cell cultures

Suspension cultures of leukemic and normal BCP were established in RPMI 1640 medium supplemented with 10% vol/vol heat-inactivated FCS (Flow Laboratories, Irvine, UK) 10 mM Hepes, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, penicillin (100 U/ml), and streptomycin (100 μg/ml) (complete medium).

For proliferation assays, leukemic BCP were seeded in 96-well flat-bottom microtest tissue culture plates (Falcon Plastics, Oxnard, CA) at 4×10^4 cells/100 μl, whereas normal BCP were seeded in 96-well round-bottom plates (Nunc, Roskilde, Denmark) at 10^4 cells/100 μl. Cells were cultured at 37°C and 5% CO_2 in complete medium alone or in the presence of cytokines (see below), and were subsequently pulsed with 1 μCi methyl-thymidine ([^{3}H]TdR) (Commissariat à l'Energie Atomique, Saclay, France, sp act 25 Ci/mmol) for the last 8 h of culture. [^{3}H]TdR incorporation data were expressed as counts per minute±standard deviation (SD) as measured in triplicate culture wells.

In parallel, normal and leukemic BCP were seeded in 24-well culture plates (Flow Laboratories, Inc., McLean, VA) at 4×10^5 cells/ml for cell counts and phenotype analysis.

Human cytokines

Purified recombinant (r) IL-4 was kindly provided by Dr. P. Trotta (Schering-Plough Research, Bloomfield, NJ) (sp act 10^7 U/mg). Unless otherwise indicated, experiments were performed with 250 U/ml IL-4, as a standard dose found to be saturating in all assays described in the present study. In this respect, a working concentration of 250 U/ml IL-4 is routinely reported for in vitro studies. In addition, IL-4 failed to exhibit any toxic effect on unresponsive cells, even when tested at concentrations as high as 10^4 U/ml (data not shown).

Specificity of the IL-4 effects was evaluated in the presence of a neutralizing heat-inactivated (56°C) anti-IL-4 rabbit antiserum (21) added concomitantly with IL-4 at a dilution of 1/150. A normal heat-inactivated rabbit serum was used as control. Purified rIL-3 (kindly provided by Dr. S. Tindall, Schering-Plough Research; specific activity 5×10^6 U/mg) was used at a concentration of 10 ng/ml, previously described to be optimal for the proliferation of hematopoietic progenitor cells (22). Purified rIL-7, kindly provided by Dr. F. Lee (DNAX, Palo Alto, CA), was used at 20 U/ml (1 U defined as inducing a half-maximal proliferative response of clone K cells, an IL-7-dependent murine BCP cell line [23]), a concentration previously determined to be optimal for inducing the proliferation of fetal BCP (24).

Phenotype analysis

Cell-surface phenotyping was performed by standard immunofluorescence with the following mAbs: HPCA-1 (anti-CD34), anti-HLA-DR, Leu 14 (anti-CD22), CALLA (anti-CD10), Leu 12 (anti-CD19), Leu 16 (anti-CD20) (Becton, Dickinson & Co., Mountain View, CA), BA1 (anti-CD24) (Boehringer-Mannheim, GmbH, Mannheim, FRG), all revealed by FITC-goat $F(ab')_2$ anti-mouse Ig (Bioart, Meudon, France); or FITC-conjugated IOB1a (anti-CD21) (Immunotech), and anti-IgM (Kallestad, Austin, TX). For detection of intracytoplasmic μ chain, cell membranes were permeabilized by 0.3% (wt/vol) saponin (Sigma Chemical Co., St. Louis, MO) before

Table I. Characterization of Leukemic Blasts from BCP-ALL Patients

			Phenotype								
Case	ID/R	FAB	CD 10	CD 19	CD 20	CD 22	CD 24	HLA-DR	CD 34	cμ	sμ
							%*				
1	ID	L1	15	87	<1	85	90	90	87	<1	<1
2	ID	L1	53	90	18	78	88	93	24	<1	<1
3	ID	L1	90	91	4	94	84	79	74	76	<1
4	R	L1	92	95	10	94	96	86	89	<1	<1
5	R	L1	93	91	3	82	94	95	92	<1	<1
6	ID	L1	91	91	76	90	90	93	0	<1	<1
7	ID	L1	95	94	3	80	85	95	92	<1	<1
8	ID	L1	80	80	11	56	ND	85	80	<1	<1
9	R	L1	83	91	3	89	89	89	71	<1	<1
10	ID	L1	94	96	2	96	96	96	52	<1	<1
11	ID	L1	97	98	1	97	97	97	35	<1	<1
12	R	L1	70	77	3	80	80	80	43	<1	<1
13	ID	L1	86	84	84	ND	ND	80	30	<1	<1
14	ID	L1	95	97	65	93	98	98	95	<1	<1

Abbreviations: ID/R, initial diagnosis/relapse; FAB, French American British cytologic classification.
* Percentage positive cells as determined by immunofluorescence on Ficoll-Hypaque isolated mononuclear bone marrow cells.

and during labeling. Fluorescence was analyzed with a FACScan flow cytometer. Positive cells were determined by reference to nonspecific staining by control mAbs of the same isotype but unrelated specificity.

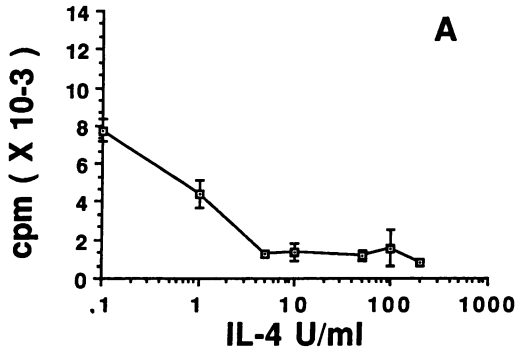
Statistical analysis

Statistical significance of inhibition of DNA replication was evaluated by comparing the means according to the formula, $t = m_A - m_B/(s^2/n_A + s^2/n_B)^{1/2}$, where m_A and m_B are calculated means of compared values, n_A and $n_B = 3$ (number of [^{3}H-TdR] incorporation determinations), and $s^2 = \Sigma (x - m_A)^2 + \Sigma (x - m_B)^2/n_A + n_B - 2$. Inhibition was significant ($P < 0.05$) for calculated values of $t > 2.776$ as determined from a standard t table.

Results

IL-4 inhibits spontaneous DNA replication in some cases of BCP-ALL. BCP-ALL cells, freshly isolated from diagnostic or relapse specimens, consistently displayed spontaneous [^{3}H]-TdR uptake in the absence of exogenously added cytokines, as measured between days 3 and 7 of culture in liquid medium (Table II). This spontaneous DNA replication was found to be inhibited in a dose-dependent manner by IL-4, with a maximum effect reached at ~ 10 U/ml (Fig. 1 *A*) (cells from case 5 presented in Table I).

We next tested the effects of IL-4 on the spontaneous proliferation of cells from 14 cases of B lineage ALL described in Table I. IL-4 was found to significantly ($t > 2.77$, see Methods) inhibit spontaneous DNA replication in 8 out of the 14 cases of BCP-ALL tested (Table II). Notably, however, IL-4–dependent inhibition of [^{3}H]TdR incorporation was found to be of variable magnitude, ranging between 25% and 80% (Table II). In some of the responsive cases, inhibition of DNA replication was paralleled by a decreased cell recovery in the presence of IL-4 (Table II) (particularly in cases 2 and 12). However, in



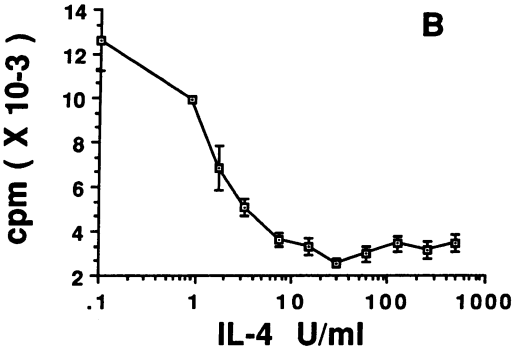


Figure 1. IL-4 inhibits DNA replication of leukemic and normal BCP. (*A*) Freshly isolated BCP-ALL cells (case 5) were cultured for 5 d in the presence of varying concentrations of IL-4. (*B*) Freshly isolated fetal BCP (CD10$^+$ sIgM$^-$ cells) were cultured for 6 d in a combination of saturating concentrations of IL-3 (10 ng/ml) + IL-7 (20 U/ml) in the presence of varying concentrations of IL-4. Data represent [^{3}H]TdR incorporation±SD of cells pulsed with 1 μCi for the final 8 h of culture.

Table II. IL-4 Inhibits Spontaneous DNA Replication in Some Cases of BCP-ALL

Case	Day of culture	[^{3}H]TdR incorporation		Percent* inhibition [^{3}H]TdR incorporation	Percent* inhibition cell numbers
		Medium	IL-4 (250 U/ml)		
		cpm × 10^{-3}			
1	7	5.9±1.1	2.7±0.3	54	ND
2	4	8.1±0.8	2.0±0.3	75	30
3	4	3.7±0.3	3.6±0.3	3(NS)‡	ND
4	5	4.8±0.6	1.4±0.2	71	ND
5	4	5.7±0.2	2.9±0.6	51	ND
6	5	1.3±0.2	1.2±0.2	8(NS)‡	ND
7	3	1.0±0.2	0.2±0.0	80	ND
8	4	1.9±0.2	1.6±0.1	16(NS)‡	5
9	4	2.2±0.2	2.7±0.3	0(NS)‡	4
10	4	0.7±0.1	0.2±0.0	71	16
11	4	2.8±0.4	2.1±0.3	25	0
12	4	0.5±0.1	0.2±0.0	60	59
13	4	1.5±0.1	1.3±0.2	13(NS)‡	28
14	3	8.4±1.0	7.8±1.5	6(NS)‡	11

Freshly isolated BCP-ALL cells were cultured for 3–7 d in the presence of IL-4 or in medium alone. Cells were either pulsed with [^{3}H]TdR (1 μCi) for the final 8 h of culture or counted by using a hemocytometer. [^{3}H]TdR incorporation figures represent mean±SD of triplicate wells. Samples analyzed correspond to the cases described in Table I.

* Percent inhibition was calculated as: 1 − (value in IL-4/value in medium) × 100. ‡ NS, nonsignificant inhibition of [^{3}H]TdR incorporation as evaluated by statistical analysis (described in Methods).

Table III. IL-4–induced Growth Inhibition of BCP-ALL Is Reversed by Anti-IL-4 Antibody

IL-4	[^{3}H]TdR incorporation		
	Medium	+ anti-IL-4	+ control serum
U/ml		*cpm × 10^{-3}*	
0	7.8±0.5	8.4±0.7	6.6±0.3
1	4.4±0.7	8.4±0.6	4.6±1.3
10	1.4±0.5	7.6±0.6	1.0±0.3

Freshly isolated BCP-ALL cells were cultured for 5 d in IL-4 or in medium alone, supplemented or not with a neutralizing rabbit anti–IL-4 antiserum (1/150 dilution) or a control serum. Cells were pulsed with [^{3}H]TdR (1 μCi) for the final 8 h of culture. Results represent mean±SD of triplicate determinations.

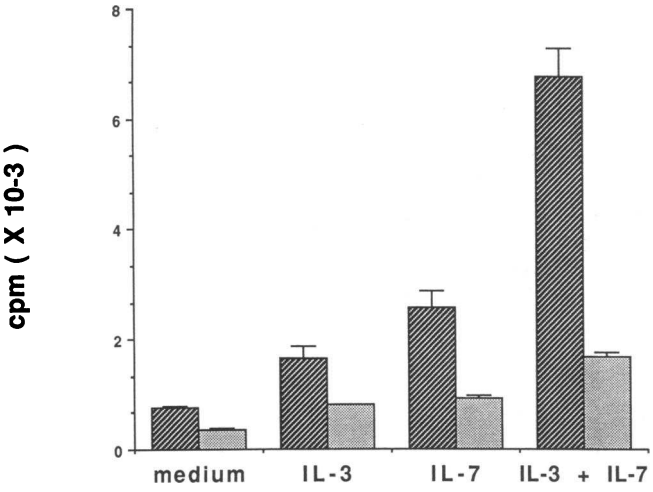


Figure 2. IL-4 inhibits the proliferative response of fetal BCP ($CD10^+sIgM^-$ cells) cultured for 6 d in the presence of medium alone or in a combination of IL-3 (10 ng/ml) and IL-7 (20 U/ml). (□) Cultures in the presence of IL-4 (250 U/ml); (■) cultures in the absence of IL-4. Data are expressed as [^{3}H]TdR incorporation±SD of cells pulsed with 1 μCi for the final 8 h of culture.

several cases of BCP-ALL tested, the relationship between the two effects was not evident (such as in cases 11 and 13). Finally, inhibition of DNA replication was specifically due to IL-4 because it was abrogated by the addition of a neutralizing anti–IL-4 rabbit antibody, whereas normal rabbit serum used as control had no significant effect (Table III).

Of interest, IL-4 failed to inhibit spontaneous [^{3}H]TdR uptake by the $CD20^+$ cases tested (Table II: cases 6, 13, and 14). However, some $CD20^-$ cases were also refractory (Table II: cases 3 and 9). These data indicate that lack of CD20 expression is not a sufficient condition for IL-4–dependent inhibition of DNA replication in BCP-ALL.

IL-4 inhibits the proliferation of normal BCP. In that IL-4 was observed to inhibit the growth of leukemic BCP, we investigated whether this cytokine would also affect the proliferation of normal human BCP. Thus, we cultured highly purified fetal bone marrow $CD10^+$ $sIgM^-$ cells in the presence of various combinations of IL-7, IL-3, and IL-4.

Fig. 2 illustrates that fetal $CD10^+$ $sIgM^-$ cells spontaneously incorporated low levels of [^{3}H]TdR as measured on day 6 of culture. Exogenously added IL-7 (20 U/ml) and IL-3 (10 ng/ml) were both able to induce a proliferative response of the $CD10^+$ $sIgM^-$ cells (stimulation index = 3.4 and 2.2, respectively). The combination of IL-7 (20 U/ml) and IL-3 (10 ng/ml) was found to result in the highest levels of [^{3}H]TdR incorporation (stimulation index = 9.0). Fig. 2 further illustrates that IL-4 (250 U/ml) inhibited spontaneous [^{3}H]TdR uptake by the fetal BCP and strongly reduced the growth response to IL-7 (inhibition 65%), to IL-3 (inhibition 51%), and to the combination of IL-7 + IL-3 (inhibition 75%).

As depicted in Fig. 1 *B*, IL-4 inhibited the proliferative response of $CD10^+$ $sIgM^-$ cells to the combination of IL-7 and IL-3 in a dose-dependent manner, with a maximum effect reached at ~ 10 U/ml. Furthermore, the IL-4–induced inhibition of [^{3}H]TdR uptake by $CD10^+$ $sIgM^-$ cells cultured in the presence of IL-7 + IL-3 was associated with reduced cell recovery, which led to a premature exhaustion of viable cells (Fig. 3). Finally, as illustrated in Table IV, growth-inhibition by IL-4 could be specifically reversed by addition of a neutralizing anti–IL-4 antibody at the onset of the culture.

Effects of IL-4 on the phenotype of BCP-ALL cells. Because inhibition of cell proliferation is often linked to cell differentiation, we investigated whether IL-4 would induce the maturation of leukemic BCP. We observed that IL-4 induced a dose-dependent increase in the percentage of cells expressing CD20

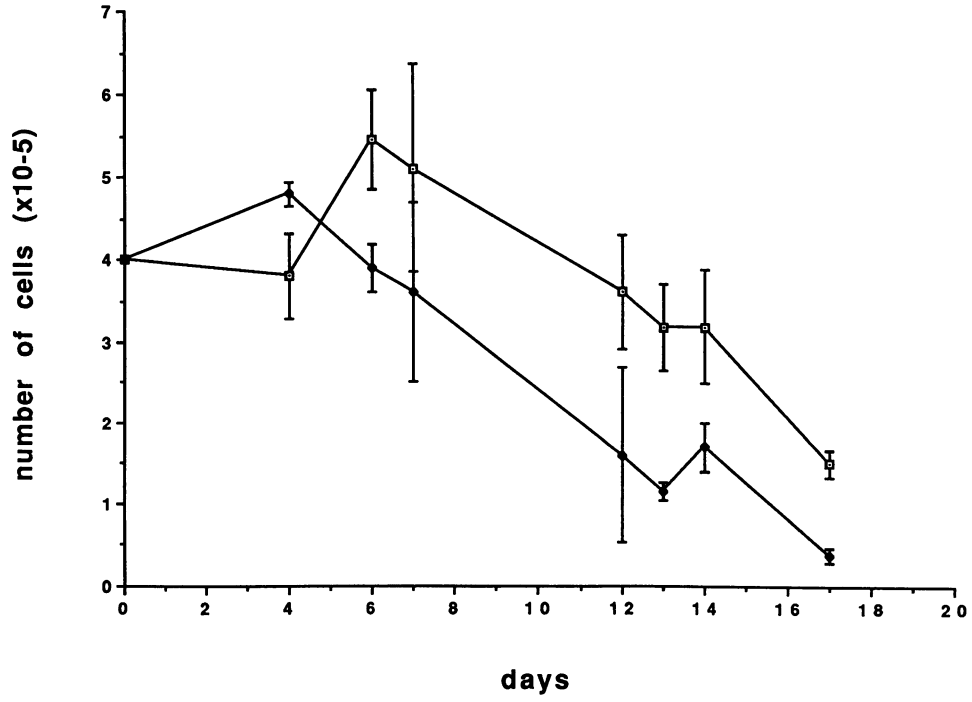


Figure 3. IL-4 decreases the number of viable cells recovered in cultures of fetal $CD10^+$ $sIgM^-$ BCP. 4×10^5 cells were exposed to saturating concentrations of IL-3 (10 ng/ml) and IL-7 (20 U/ml) in the presence (♦) or absence (□) of IL-4 (250 U/ml). Data represent mean cell numbers (trypan blue negative)±SD as determined from three independent cell-counts for each culture.

antigen, with a maximum (50% $CD20^+$ cells) at ~ 10 U/ml IL-4 (Fig. 4) (case 5 cells).

Inasmuch as BCP-ALL populations constitutively contain a variable proportion of $CD20^+$ cells (Table I), we systematically depleted $CD20^+$ cells before culture, in order to detect de novo expression of the antigen. Thus, we found that IL-4 induced CD20 expression on CD20-depleted cells (Table V) from five of eight cases presented in Table I. The responsive cases displayed heterogeneity with respect to the overall numbers of $CD20^+$ cells appearing in culture (for example: case 11 vs. case 10), (Table V), although this variability can be attributed in part to differences in overall cell recovery in the presence of IL-4 (Table II). As further illustrated in Table V, all cases displaying induction of CD20 (specific increase in numbers of $CD20^+$ cells) (cases 4, 5, 10, 11, and 12) were also susceptible to growth inhibition by IL-4.

A representative experiment demonstrating CD20 induction (case 4 cells), illustrates that ~ 50% of the cells cultured in the presence of IL-4 (250 U/ml) expressed CD20 antigen after 72 h (87×10^3 $CD20^+$ cells) (Fig. 5 *C*), whereas spontaneous appearance of CD20 was only observed on a minor proportion (6%) (Fig. 5 *B*) of the cells cultured in medium alone (10.5×10^3 $CD20^+$ cells). The $CD20^+$ cells were $CD10^+$ (Fig. 5 *D*), indicating that they represent the leukemic population that initially expressed this marker.

In contrast to its effect on CD20 antigen, addition of IL-4, whether to CD20-depleted or total BCP-ALL populations, consistently failed to result in the emergence of $c\mu^+$ or $sIgM^+$ ALL cell in all cases examined ($n = 11$), as illustrated for a representative example (case 4) (Fig. 5). In this context, IL-4 did not permit the expression of sIgM in cases of pre B ($c\mu^+$) ALL (case 3, and $c\mu^+$ BCP-ALL lines) (data not shown). Finally, concomittant with a lack of Ig induction, IL-4 did not induce CD21 antigen on the leukemic BCP (not shown). These results indicate that, in spite of induction of CD20, IL-4 is unable to drive the maturation of leukemic BCP into Ig^+ cells.

Effects of IL-4 on the phenotype of normal BCP. Because we showed that IL-4 inhibits the proliferation of normal B lineage precursors, we investigated whether IL-4 would induce their maturation. Thus, fetal $CD10^+$ $sIgM^-$ cells were cultured in the presence or absence of IL-4 (250 U/ml), and cell phenotype was subsequently examined. As illustrated in Fig. 6, a higher proportion of $CD20^+$ cells (45%, corresponding to 4.5×10^5 $CD20^+$ cells) were recovered when $CD10^+$ $sIgM^-$ cells were cultured for 5 d in the presence of IL-4 as compared to medium alone (28%) (1.9×10^5 $CD20^+$ cells). However, numbers of $CD20^+$ cells recovered in the presence of IL-4 were not significantly increased as compared to input values (4.3×10^5 $CD20^+$ cells at day 0). As also illustrated in Fig. 6, the intensity of CD20 expression was higher after culture in IL-4, as compared with day 0 cells. These results indicate either IL-4–dependent induction of CD20 on $CD20^-$ cells or a selective survival of day 0 $CD20^+$ cells and hyperexpression of CD20 in the presence of IL-4. To address this question, $CD20^-$ cells were sorted from the $CD10^+$ $sIgM^-$ population before culture. Data from such an experiment, illustrated in Fig. 7, demonstrate that, although CD20 spontaneously reappears on a subset of

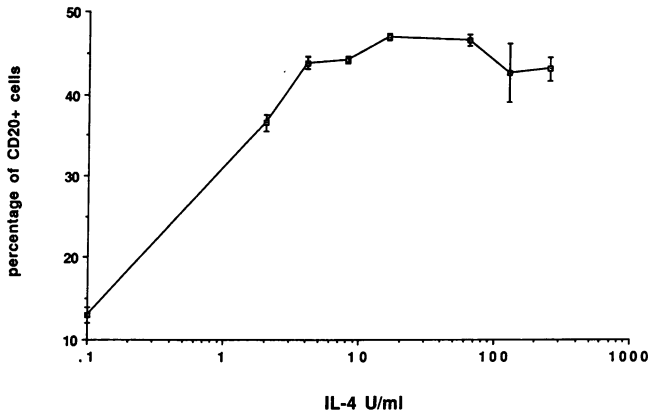


Figure 4. IL-4 induces dose-dependent expression of CD20 antigen on leukemic $CD20^-$ BCP. Case 5 BCP-ALL cells were cultured in triplicate in the presence of varying concentrations of IL-4. Data are expressed as mean±standard deviation of percentage $CD20^+$ cells present in the cultures after 72 h, as determined by FACScan flow cytometry analysis.

Table IV. IL-4–induced Inhibition of Normal BCP Growth Is Reversed by Anti–IL-4 Antibody

	[^{3}H]TdR incorporation		
IL-4	IL-3 + IL-7	IL-3 + IL-7 + anti–IL-4	IL-3 + IL-7 + control serum
U/ml		*cpm × 10^{-3}*	
0	12.6±1.4	ND	ND
7.5	3.6±0.3	14.5±0.9	4.1±0.4
30	2.6±0.2	14.0±1.2	2.9±0.6

Freshly isolated fetal $CD10^+$ $sIgM^-$ cells were cultured for 6 d in the presence of saturating concentrations of IL-3 (10 ng/ml) + IL-7 (10 ng/ml), and various combinations of IL-4, neutralizing anti–IL-4 rabbit antiserum (1/150 dilution), and control nonimmune rabbit serum (1/150 dilution). Cells were pulsed with [^{3}H]TdR (1 μCi) for the final 8 h of culture. Results represent mean±SD of triplicate determinations.

Table V. Relationship between Induction of CD20 Expression and Inhibition of [^{3}H]TdR Incorporation in $CD20^-$ B Lineage ALL Cells in Response to IL-4

Case	Induction of CD 20 expression*	Inhibition of [^{3}H]TdR incorporation‡
2	4	75
4	62	71
5	156	51
8	<2	16
9	5	0
10	15	71
11	258	25
12	26	60

* Numbers of $CD20^+$ cells ($\times 10^{-3}$) specifically appearing in cultures of 4×10 $CD20^-$ BCP-ALL cells in the presence of IL-4 (250 U/ml). Cells seeded were ≤ 1% $CD20^+$ as determined by sensitivity of FACScan analysis. Accordingly, data from cases 2, 8, and 9 were not considered to represent induction of $CD20^+$ cells. ‡ Percent inhibition was calculated as described in Table II.

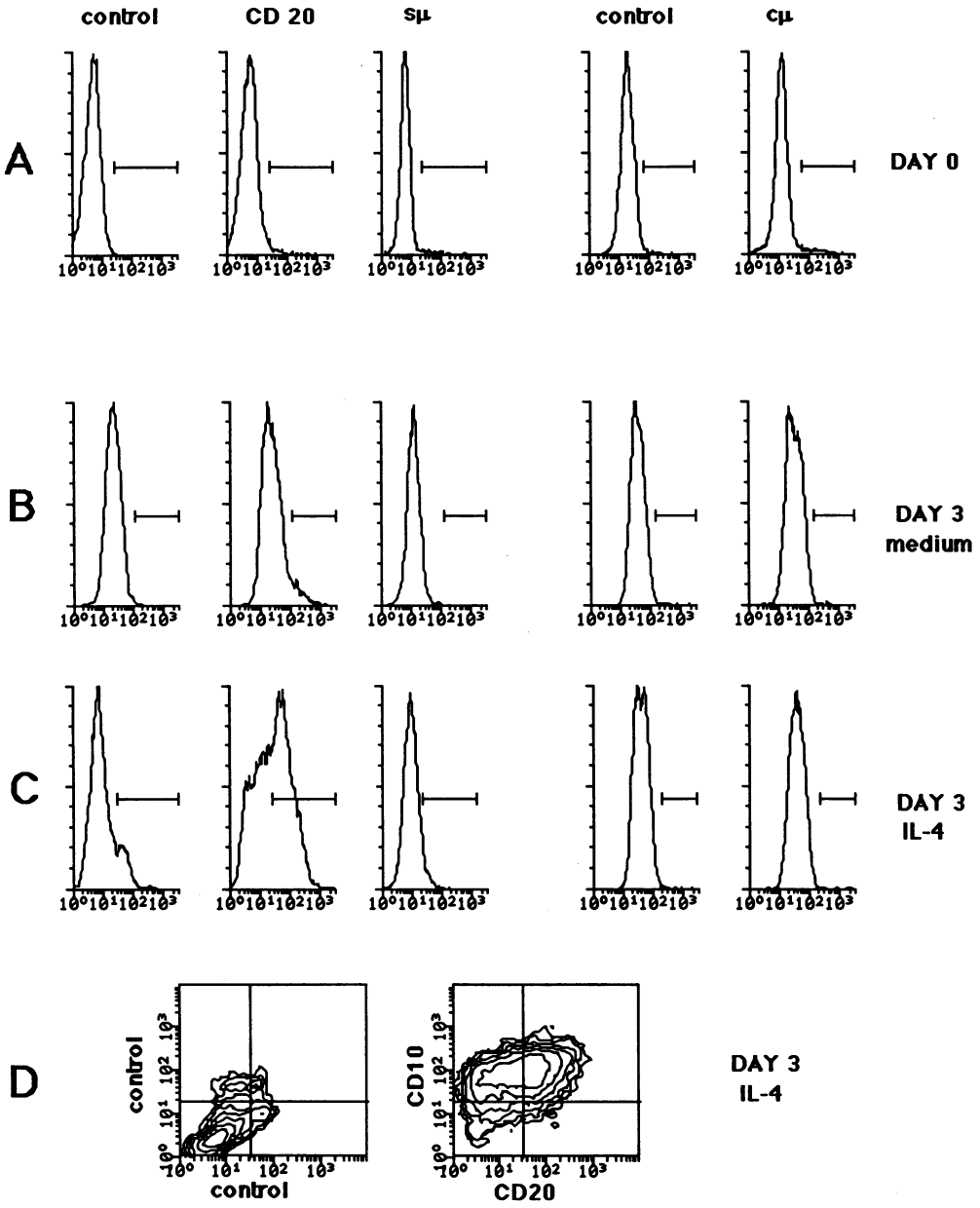


Figure 5. IL-4 induces the expression of CD20 antigen but not cμ or sIgM in cultures of leukemic BCP (case 4). (*A*) Phenotype of freshly isolated CD20-depleted cells. (*B*) Phenotype of cells cultured for 3 d in medium alone. (*C*) Phenotype of cells cultured for 3 d in the presence of IL-4 (250 U/ml). Data in *A–C* represent single fluorescence histograms obtained by FACScan flow-cytometry analysis (see Methods). (*D*) Double-color fluoresence analysis of cells cultured for 3 d in the presence of IL-4 (250 U/ml), demonstrating that CD20 is borne by $CD10^+$ cells. Data represent contour FITC and PE fluorescence plots obtained by FACScan analysis (see Methods).

cells (18×10^3 $CD20^+$ cells recovered), IL-4 does not significantly promote the induction of CD20 antigen expression on normal BCP (15×10^3 $CD20^+$ cells recovered).

As previously reported (24, 25), $sIgM^+$ cells emerged in culture of $CD10^+$ $sIgM^-$ cells in the absence of exogenous factors. Thus, in a representative experiment, after 5 d of culture of $CD10^+$ $sIgM^-$ cells in medium alone, we recovered 0.20×10^6 $sIgM^+$ cells (Fig. 6). This figure did not differ significantly from the number of $sIgM^+$ cells recovered in the presence of IL-4 (250 U/ml) (0.21×10^6 cells), although a minor cell subset expressed higher levels of sIgM in the IL-4–supplemented cultures (Fig. 6).

Of interest, spontaneous emergence of $sIgM^+$ cells was considerably inhibited by depletion of $CD20^+$ cells before culture, and addition of IL-4 (250 U/ml) to the $CD10^+$ $sIgM^-$ $CD20^-$ cells failed to restore the appearance of $sIgM^+$ cells (Fig. 7). Addition of IL-4 (250 U/ml) to $CD10^+$ $sIgM^-$ BCP did not significantly affect the number of pre B ($c\mu^+$) cells recovered in culture (Fig. 6).

Finally, we consistently failed to detect the appearance of $CD21^+$ cells (Fig. 6) or $sIgD^+$ cells (not shown) in cultures of $CD10^+$ $sIgM^-$ cells performed either in the presence or absence of IL-4 (250 U/ml). Together, these data indicate that IL-4 does not signal the transition of normal BCP to mature Ig-bearing B cells.

Discussion

In the present report, we have studied the effects of IL-4 on normal and leukemic human BCP. We observed that freshly isolated BCP-ALL cells spontaneously undergo DNA replication in culture, in accordance with other studies (26–28). Of interest, IL-4 was found to significantly inhibit this spontaneous DNA replication in 8 of 14 Ph^1-negative B lineage ALL

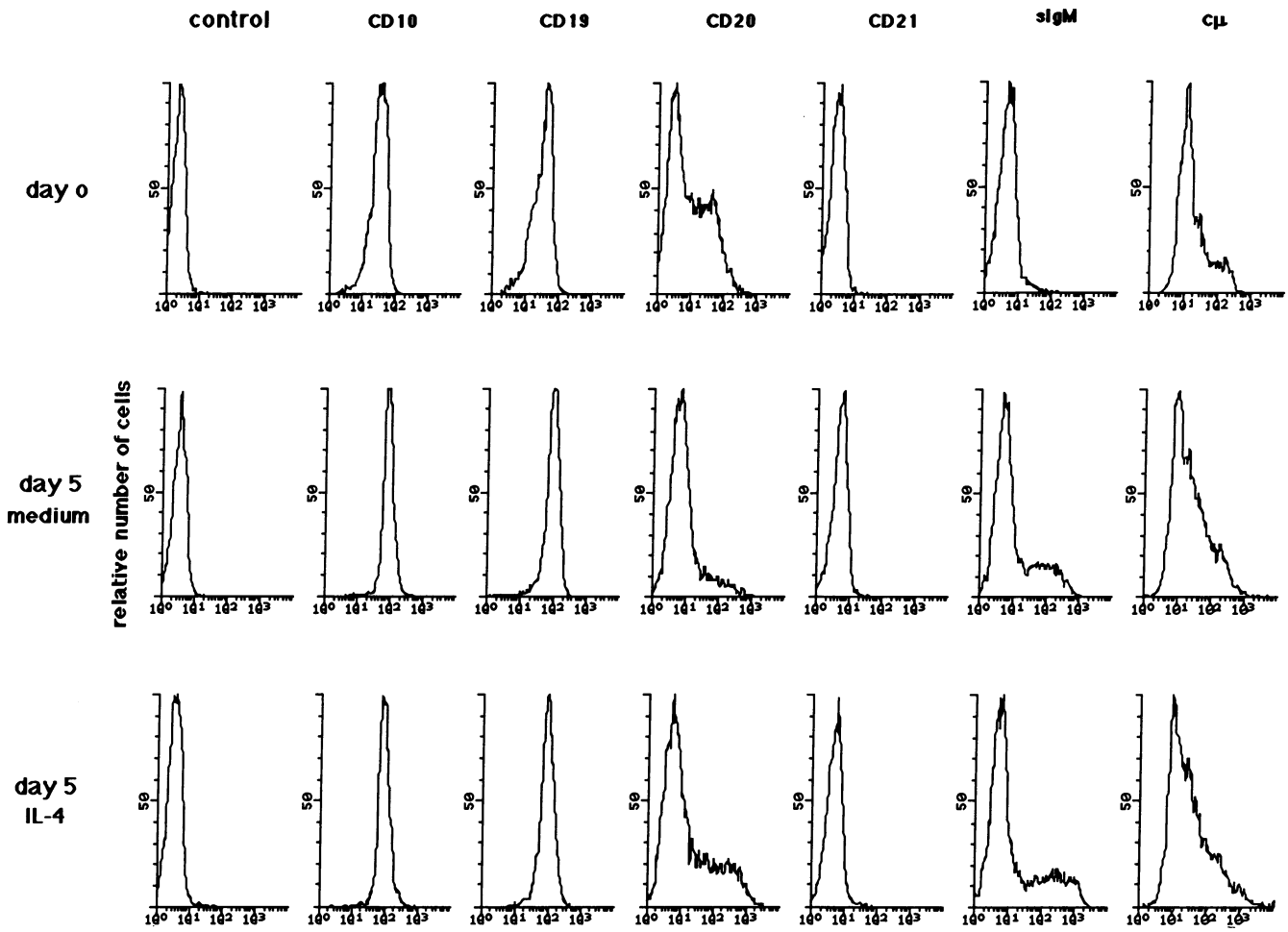


Figure 6. IL-4 does not favor the generation of $c\mu^+$ or $sIgM^+$ cells in cultures of fetal BCP ($CD10^+$ $sIgM^-$). (*A*) Phenotype of freshly-isolated $CD10^+$ $sIgM^-$ cells. (*B*) Phenotype of cells harvested after 5 d of culture of $CD10^+$ $sIgM^-$ cells in medium alone. (*C*) Phenotype of cells harvested after 5 d of culture of $CD10^+$ $sIgM^-$ cells in the presence of IL-4 (250 U/ml).

specimens tested. The important sample-to-sample variability observed with respect to this effect of IL-4 is likely to reflect the maturation heterogeneity inherent to ALL. Thus, the size and distribution of this IL-4–responsive population could be quite variable among cases of BCP-ALL. We observed no evident correlation between IL-4–dependent inhibition of [^{3}H]TdR incorporation and cell recovery. In this respect, some cases presented a more important IL-4–dependent decrease in cell numbers than expected to result from inhibition of proliferating cells, suggesting that IL-4 may also negatively affect the survival of cells which do not belong to the subset undergoing spontaneous DNA synthesis. Taken together, our data could imply the existence of different overlap patterns of the IL-4–responsive population and the cell subset undergoing spontaneous DNA replication.

We further observed that DNA replication was only inhibited by IL-4 in $CD20^-$ BCP-ALL. However, $CD20^-$ cells differed widely in their sensitivity to this effect, suggesting that lack of CD20 expression may be necessary but not sufficient for inhibition of DNA replication by IL-4. In this context, the $CD20^-$ BCP subset represents primitive cells ($CD34^+$ $c\mu^-$) in normal B cell ontogeny (29) but acquisition of CD20 antigen represents an asynchronous event in a number of BCP-ALL (30), as illustrated by case 3 in our present study ($CD20^-$ $c\mu^+$). Thus, the maturation heterogeneity of $CD20^-$ BCP-ALL cells could explain our observed variability in growth inhibition by IL-4.

Additional studies will be necessary to clarify the issue of BCP subsets and IL-4 responsiveness, particularly in relation to the expression of IL-4 receptors. In this respect, some murine BCP lines have been described among the rare cell types lacking detectable IL-4 receptors (31).

Our present results extend recent observations by Okabe et al. (17), who described IL-4–dependent inhibition of growth in the majority of Ph^1-positive BCP-ALL tested. However, in contrast to our study, these authors did not demonstrate a similar effect in Ph^1-negative ALL. This apparent discrepancy may be attributed to the fact that few examples of Ph^1-negative leukemias were evaluated in the study of Okabe et al. (only a single case of fresh BCP-ALL).

We recently established that highly purified $CD10^+$ $sIgM^-$ BCP from normal fetal bone marrow undergo DNA replication in response to IL-7 and IL-3, and that the proliferative response is enhanced by the combination of both factors (24). Here, we describe that, as on leukemic BCP, IL-4 has the potential to inhibit [^{3}H]TdR incorporation by normal human

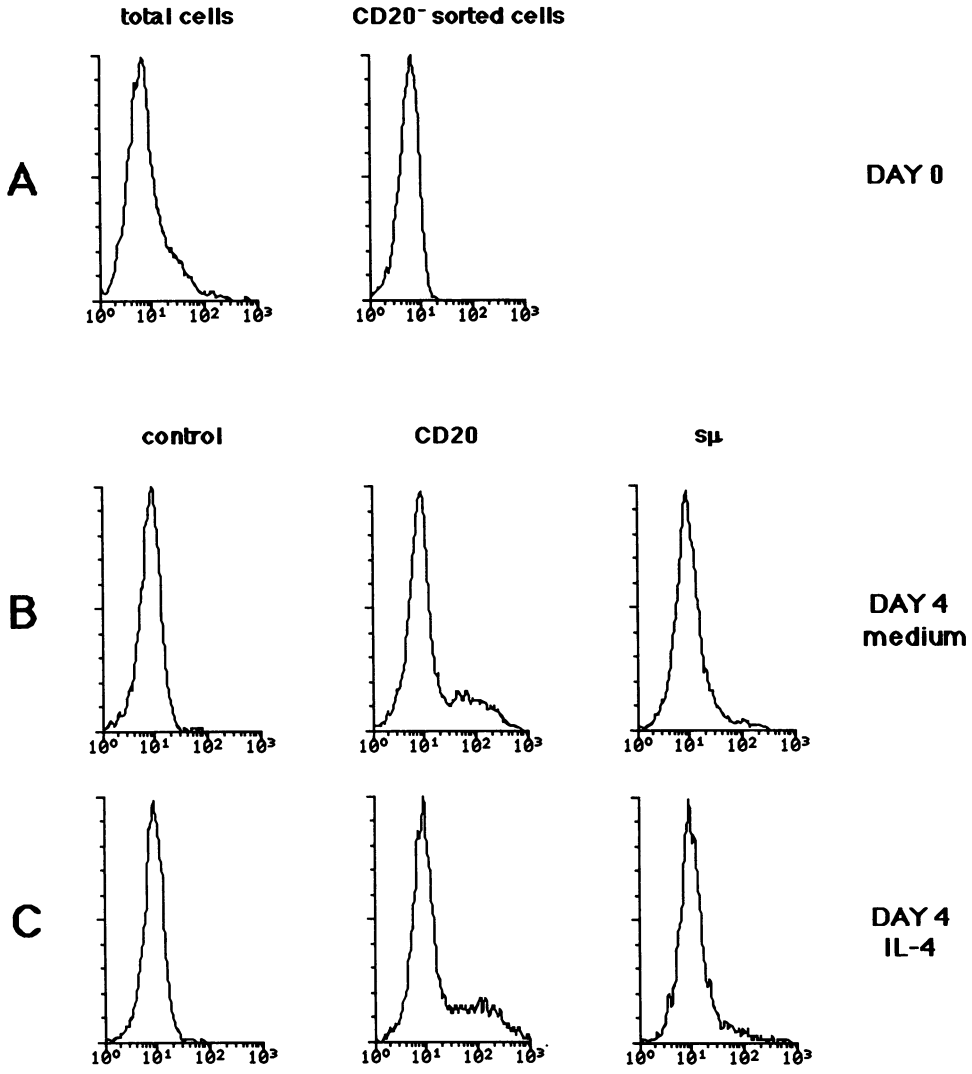


Figure 7. IL-4 does not promote the induction of CD20 antigen on normal CD20$^-$ fetal BCP. (*A*) CD20 expression by total CD10$^+$ sIgM$^-$ cells and by FACS-sorted CD20$^-$ cells. (*B*) Phenotype of CD20$^-$ cells cultured 4 d in medium. (*C*) Phenotype of CD20$^-$ cells cultured 4 d in the presence of IL-4 (250 U/ml).

BCP. Interestingly, IL-4 inhibited both spontaneous and growth factor (IL-3, IL-7)–induced DNA replication. This indicates that IL-4 acts as a general negative growth regulator in B cell development rather than as a specific antagonist of a given growth factor. This contrasts with findings on mature B cells where IL-4 acts as an antagonist of IL-2 action likely as a consequence of downregulation of IL-2R expression (32, 33). Our present data confirm and extend the results of Hofman et al. (15), who presented evidence for growth-inhibitory effects of IL-4 presumably borne by normal human BCP. It will be of interest to investigate the significance of our inability to detect a growth-stimulatory effect of IL-4 on normal human BCP, as IL-4 has been shown to promote the growth of mouse pro B cells (8, 9). These differences may reflect that IL-4–dependent proliferation of an early BCP subset would not be detected in our assay owing to growth inhibition of less primitive cells which are present at higher frequency.

Our data indicate that IL-4 promotes a selective survival of CD20$^+$ normal BCP which include the most mature precursors. This finding could be related to the lack of growth inhibition observed in CD20$^+$ BCP-ALL. It remains to be determined, however, if the normal CD20$^+$ subset (which includes spontaneously emerging sIgM$^+$ cells) contains cells that proliferate in response to IL-4.

In the present study, we further showed that IL-4 induces the appearance of CD20 antigen on leukemic BCP. We demonstrated that this effect represents de novo expression of CD20, because it could not be accounted for by the expansion of a marginal CD20$^+$ subset initially present within the leukemic population. Induction of CD20 expression in response to IL-4 was, however, not a general feature of all BCP-ALL cases tested. As discussed above, this is likely related to the notion that CD20$^-$ BCP-ALL cells cover a potentially broad maturation range. Our data suggest that lack of CD20 expression is only one of the parameters that determine the influence of IL-4 on BCP-ALL maturation. In this context, it appears of interest that spontaneous DNA replication was inhibited by IL-4 in all cases where CD20 was induced, suggesting that the populations responsive in these two assays overlap or are closely related.

In contrast to BCP-ALL cells, IL-4 failed to induce CD20 expression in normal BCP. These data would suggest that the CD20 gene may be regulated through different mechanisms in normal and leukemic cells. The CD20 surface molecule functions as a channel regulating the entry of Ca^{++} into the cell

(34). It remains to be determined how acquisition of this function is related to leukemic B cell development.

We further addressed whether the effect of IL-4 on CD20 expression was associated with an induction of BCP maturation as judged by expression of IgM. According to these criteria, our results demonstrate that IL-4 does not permit to override the maturation block in BCP-ALL, in that it failed to promote the emergence of $sIgM^+$ cells (and of $c\mu^+$ cells in the cases of $c\mu^-$ leukemia). In this context, little data is available on the effects of cytokines on the differentiation of leukemic BCP. Wörmann et al. (35) have described a human pre-B ALL cell line which spontaneously acquired sIgM through cloning selection, but not through exposure to a number of cytokines, including IL-4.

A number of studies have reported cytokine-mediated induction of Ig expression in normal murine and human BCP. In this context, we found that IL-4 did not enhance the spontaneous emergence of $sIgM^+$ cells from $CD10^+$ $sIgM^-$ cells, in accordance with Le Bien (16). Furthermore, IL-4 failed to affect the numbers of $c\mu^+$ (pre-B) cells present in our cultures.

In contrast to these data, Hofman et al. (15) have reported that IL-4 favors the transition to $c\mu^+$ and $sIgM^+$ cells in cultures of human $CD24^+$ fetal cells, in accordance with several studies on murine BCP (12–14). It has been suggested, however, that the effects of IL-4 on murine BCP are indirectly mediated through accessory cells (9), or reflect enhanced survival of $sIgM^+$ cells in the presence of IL-4 (36). In this respect, the $CD24^+$ cell population used in the study by Hofman et al. (15) initially contains a significant proportion of $sIgM^+$ cells, and thus cannot be directly compared to the $CD10^+$ $sIgM^-$ population presently analyzed. Although IL-4 failed to increase the numbers of $sIgM^+$ cells in our cultures of $CD10^+$ $sIgM^-$ fetal cells, it induced hyperexpression of sIgM. This effect is likely to be related to IL-4 induced hyperexpression of sIgM on $sIgM^+$ cells (37, 38).

Taken together, our results demonstrate that IL-4, by itself, does not drive the transition from BCP to fully mature B cells. Nevertheless, this conclusion does not rule out the possibility of an in vivo regulatory role for IL-4 in BCP maturation. In such a scheme, IL-4 derived from bone marrow stromal cells (13) could interact with accessory cells, and/or directly in association with another cytokine, to promote BCP maturation. Such a model would be compatible with our present demonstration that IL-4 can inhibit proliferation at some stages of BCP development. However, further studies will be required to address the precise role of IL-4 in human B cell ontogeny.

Acknowledgments

We are grateful to N. Courbière for editorial assistance and to Dr. P. Mannoni and Dr. J. A. Waitz for support and critical reading of the manuscript.

D. Pandrau is the recipient of a grant from the Fondation Mérieux (Lyon, France).

References

1. Kincade, P. W., G. Lee, C. E. Pietrangeli, S. I. Hayashi, and J. M. Gimble. 1989. Cells and molecules that regulate B lymphopoiesis in bone marrow. *Annu. Rev. Immunol.* 7:111–143.

2. Cooper, M. D. 1987. B lymphocytes: normal development and function. *N. Engl. J. Med.* 317:1452–1456.

3. Uckun, F. M. 1990. Regulation of human B-cell ontogeny. *Blood.* 76:1908–1923.

4. Rolink, A., A. Kudo, H. Karasuyama, Y. Kikuchi, and F. Melchers. 1991. Long-term proliferating early pre B cell lines and clones with the potential to develop to surface Ig-positive, mitogen reactive B cells in vitro and in vivo. *EMBO (Eur. Mol. Biol. Organ.)* 10:327–336.

5. Defrance, T., B. Vanbervliet, J. P. Aubry, Y. Takebe, N. Arai, A. Miyajima, T. Yokota, T. Lee, K. Arai, J. E. de Vries, and J. Banchereau. 1987. B cell growth-promoting activity of recombinant human interleukin-4. *J. Immunol.* 139:1135–1141.

6. Banchereau, J. 1991. Human interleukin-4 and its receptor. *In* Hematopoietic Growth Factors in Clinical Applications. R. Mertelsmann and F. Herrmann, editors. Marcel Dekker, Inc., New York. 433–469.

7. Rousset, F., E. Garcia, and J. Banchereau. 1991. Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen. *J. Exp. Med.* 173:705–710.

8. Palacios, R., H. Karasuyama, and A. Rolink. 1987. $Ly1^+$ PRO-B lymphocyte clones. Phenotype, growth requirements and differentiation in vitro and in vivo. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3687–3693.

9. Peschel, C., I. Green, and W. E. Paul. 1989. Preferential proliferation of immature B lineage cells in long-term stromal cell-dependent cultures with IL-4. *J. Immunol.* 142:1558–1568.

10. Rennick, D., G. Yang, C. Muller-Sieburg, C. Smith, N. Arai, Y. Takabe, and L. Gemmell. 1987. Interleukin 4 (B-cell stimulatory factor 1) can enhance or antagonize the factor-dependent growth of hemopoietic progenitor cells. *Proc. Natl. Acad. Sci. USA.* 84:6889–6893.

11. Billips, L., D. Petitte, and K. S. Landreth. 1990. Bone marrow stromal cell regulation of B lymphopoiesis: interleukin-1 (IL-1) and IL-4 regulate stromal cell support of pre-B cell production in vitro. *Blood.* 75:611–619.

12. Woodward, T. A., I. K. McNiece, P. L. Witte, P. Bender, R. Crittenden, D. S. Temeles, B. E. Robinson, G. B. Baber, D. H. Deacon, P. C. Isakson, et al. 1990. Further studies on growth factor production by the TC-1 stromal cell line: pre-B stimulating activity. *Blood.* 75:2130–2136.

13. King, A. G., D. Wierda, and K. S. Landreth. 1988. Bone marrow stromal cell regulation of B-lymphopoiesis. I. The role of macrophages, IL-1, and IL-4 in pre-B cell maturation. *J. Immunol.* 141:2016–2026.

14. Kinashi, T., K. Inaba, T. Tsubata, K. Tashiro, R. Palacios, and T. Honjo. 1988. Differentiation of an interleukin 3-dependent precursor B-cell clone into immunoglobulin-producing cells in vitro. *Proc. Natl. Acad. Sci. USA.* 85:4473–4477.

15. Hofman, F. M., M. Brock, C. R. Taylor, and B. Lyons. 1988. IL-4 regulates differentiation and proliferation of human precursor B cells. *J. Immunol.* 141:1185–1190.

16. LeBien, T. W. 1989. Growing human B-cell precursors in vitro: the continuing challenge. *Immunol. Today.* 10:296–298.

17. Okabe, M., Y. Kuni-eda, T. Sugiwura, M. Tanaka, T. Miyagishima, I. Saiki, T. Minagawa, M. Kurosawa, T. Itaya, and T. Miyazaki. 1991. Inhibitory effect of interleukin-4 on the in vitro growth of Ph^1-positive acute lymohoblastic leukemia cells. *Blood.* 78:1574–1580.

18. Saeland, S., C. Caux, C. Favre, J. P. Aubry, P. Mannoni, M. J. Pébusque, O. Gentilhomme, T. Otsuka, T. Yokota, N. Arai, et al. 1988. Effects of recombinant human interleukin-3 on CD34 enriched normal hematopoietic progenitors and on myeloblastic leukemia cells. *Blood.* 72:1580–1588.

19. Saeland, S., C. Caux, C. Favre, V. Duvert, M. J. Pébusque, P. Mannoni, and J. E. de Vries. 1989. Combined and sequential effects of human IL-3 and GM-CSF on the proliferation of $CD34^+$ hematopoietic cells from cord blood. *Blood.* 73:1195–1201.

20. LeBien, T. W., B. Wörmann, J. G. Villablanca, C. L. Law, L. M. Steinberg, V. O. Shah, and M. R. Loken. 1990. Multiparameter flow cytometric analysis of human fetal bone marrow B cells. *Leukemia.* 4:354–358.

21. Chrétien, I., A. Van Kimmenade, M. K. Pearce, J. Banchereau, and J. S. Abrams. 1989. Development of polyclonal and monoclonal antibodies for immunoassay and neutralization of human interleukin-4. *J. Immunol. Methods.* 117:67–81.

22. Caux, C., S. Saeland, C. Favre, V. Duvert, P. Mannoni, and J. Banchereau. 1990. Tumor necrosis factor-alpha strongly potentiates interleukin-3 and granulocyte-macrophage colony-stimulating factor-induced proliferation of human $CD34^+$ hematopoietic progenitor cells. *Blood.* 75:2292–2298.

23. Hunt, P., D. Robertson, D. Weiss, D. Rennick, F. Lee, and O. N. Witte. 1987. A single bone marrow-derived stromal cell type supports the in vitro growth of early lymphoid and myeloid cells. *Cell.* 48:997–1007.

24. Saeland, S., V. Duvert, D. Pandrau, C. Caux, I. Durand, N. Wrighton, J. Wideman, F. Lee, and J. Banchereau. 1991. Interleukin-7 induces the proliferation of normal human B-cell precursors. *Blood.* 78:2229–2238.

25. Villablanca, J. G., J. M. Anderson, M. Moseley, C. L. Law, R. L. Elstrom, and T. W. LeBien. 1990. Differentiation of normal human pre-B cells in vitro. *J. Exp. Med.* 172:325–334.

26. Findley, H. W. Jr., M. Zhou, R. Davis, Y. Abdul-Rahim, R. Hnath, and A. H. Ragab. 1990. Effects of low molecular weight B-cell growth factor on proliferation of leukemic cells from children with B-cell precursor-acute lymphoblastic leukemia. *Blood.* 75:951–957.

27. Touw, I., K. Pouwels, T. van Agthoven, R. van Gurp, L. Budel, H. Hoogerbrugge, R. Delwel, R. Goodwin, A. Namen, and B. Löwenberg. 1990. Interleukin-7 is a growth factor of precursor B and T acute lymphoblastic leukemia. *Blood.* 75:2097–2101.

28. Wörmann, B., S. R. Mehta, A. L. Maizel, and T. W. LeBien. 1987. Low molecular weight B cell growth factor induces proliferation of human B cell precursor acute lymphoblastic leukemias. *Blood.* 70:132–138.

29. Saeland, S., V. Duvert, C. Caux, D. Pandrau, C. Favre, A. Vallé, I. Durand, P. Charbord, J. E. de Vries, and J. Banchereau. 1992. Distribution of surface-membrane molecules on bone marrow and cord blood $CD34^+$ hematopoietic cells. *Exp. Hematol.* 20:24–33.

30. Hurwitz, C. A., M. R. Loken, M. L. Graham, J. E. Karp, M. J. Borowitz, D. J. Pullen, and C. I. Civin. 1988. Asynchronous antigen expression in B lineage acute lymphoblastic leukemia. *Blood.* 72:299–307.

31. Polla, B. S., J. Ohara, W. E. Paul, N. Nabavi, A. Myer, H.-C. Liou, F. W. Shen, S. Gillis, J. V. Bonventre, and L. H. Glimcher. 1988. Differential induction of class II gene expression in murine pre-B-cell lines by B-cell stimulatory factor-1 and by antibodies to B-cell surface antigens. *J. Mol. Immunol.* 3:363–373.

32. Defrance, T., B. Vanbervliet, J. P. Aubry, and J. Banchereau. 1988. Interleukin 4 inhibits the proliferation but not the differentiation of activated human B cells in response to interleukin 2. *J. Exp. Med.* 168:1321–1337.

33. Jelinek, D. F., and P. E. Lipsky. 1988. Inhibitory influence of IL-4 on human B cell responsiveness. *J. Immunol.* 141:164–173.

34. Bubien, J. K., P. D. Bell, R. A. Frizell, and T. F. Tedder. 1989. CD20 directly regulates transmembrane ion flux in B-lymphocytes. *In* Leucocyte Typing IV. W. Knapp, editor. Oxford University Press, Oxford. 51–54.

35. Wörmann, B., J. M. Anderson, J. A. Liberty, K. Gajl-Peczalska, R. D. Brunning, T. L. Silberman, D. C. Arthur, and T. W. LeBien. 1989. Establishment of a leukemic cell model for studying human pre-B to B cell differentiation. *J. Immunol.* 142:110–117.

36. Simons, A., and D. Zharhary. 1989. The role of IL-4 in the generation of B lymphocytes in the bone marrow. *J. Immunol.* 143:2540–2545.

37. Shields, J. G., R. J. Armitage, B. N. Jamieson, P. C. L. Beverley, and R. E. Callard. 1989. Increased expression of surface IgM but not IgD or IgG on human B cells in response to IL-4. *Immunology.* 66:224–227.

38. Rigley, K. P., S. M. Thurstan, and R. E. Callard. 1991. Independent regulation of interleukin 4 (IL-4)-induced expression of human B cell surface CD23 and IgM: functional evidence for two IL-4 receptors. *Int. Immunol.* 3:197–203.