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

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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 [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 κ chain, nor the appearance of slgM+ 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 ofμ+ and slgM+ cells in cultures of CD24+ fetal bone marrow cells (15). However, the notion that IL-4 induces the emergence of slgM+ cells has been subsequently challenged (16). Finally, IL-4 has recently been described to inhibit the proliferation of Philadelphia (Ph1)-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 Ph1-negative B-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 slgM+ 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).
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 slgM, indicating that they represented a virtually pure normal BCP population. In some experiments, CD20+ cells were removed from the CD10+ slgM+ population by sorting with a FACstar+ flow cytometer (Becton, Dickinson & Co., Sunnyvale, CA).

Leukemic BCP. Blast-invaded (> 80%) bone marrow samples were obtained by iliæc crest aspiration from 14 pediatric patients at initial diagnosis or relapse of Ph1-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 (BLA, BL15, Institut Mérieux, Lyon, France), mature B cells (anti-λ and -κ 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 10^5 cells/100 µl. Cells were cultured at 37°C and 5% CO2 in complete medium alone or in the presence of cytokines (see below), and were subsequently pulsed with 1 µCi methyl-thymidine ([3H]Tdr) (Commissariat à l’Energie Atomique, Saclay, France, sp act 25 Ci/mmol) for the last 8 h of culture. [3H]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 × 10^3 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 10^3 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).

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

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>ID/R</td>
</tr>
<tr>
<td>1</td>
<td>ID</td>
</tr>
<tr>
<td>2</td>
<td>ID</td>
</tr>
<tr>
<td>3</td>
<td>ID</td>
</tr>
<tr>
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<td>R</td>
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</tr>
<tr>
<td>13</td>
<td>ID</td>
</tr>
<tr>
<td>14</td>
<td>ID</td>
</tr>
</tbody>
</table>

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, \( t = \frac{m_a - m_b}{s^2/(n_a + s^2/n_b)^{1/2}} \), where \( m_a \) and \( m_b \) are calculated means of compared values, \( n_a \) and \( n_b \) being the number of \([\text{H}]\text{TdR}\) incorporation determinations, and \( s^2 = \sum (x - m_a)^2 + \sum (x - m_b)^2/n_a + n_b - 2 \). 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 \([\text{H}]\text{TdR}\) 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. 1A) (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 \([\text{H}]\text{TdR}\) 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

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

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 \([\text{H}]\text{TdR}\) (1 \(\mu\)Ci) for the final 8 h of culture or counted by using a hemocytometer. \([\text{H}]\text{TdR}\) incorporation figures represent mean±SD of triplicate wells. Samples analyzed correspond to the cases described in Table I.

* Percent inhibition was calculated as: \(1 - \text{(value in IL-4/value in medium)} \times 100\).  
** NS, nonsignificant inhibition of \([\text{H}]\text{TdR}\) 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**

<table>
<thead>
<tr>
<th>([\text{H}]\text{TdR}) incorporation</th>
<th>(10\text{U/ml}) Mean + control serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{U/ml})</td>
<td>(\text{cpm} \times 10^{-3})</td>
</tr>
<tr>
<td>0</td>
<td>7.8±0.5</td>
</tr>
<tr>
<td>1</td>
<td>4.4±0.7</td>
</tr>
<tr>
<td>10</td>
<td>1.4±0.5</td>
</tr>
</tbody>
</table>

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 \([\text{H}]\text{TdR}\) (1 \(\mu\)Ci) for the final 8 h of culture. Results represent mean±SD of triplicate determinations.
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 [3H]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 [3H]TdR incorporation (stimulation index = 9.0). Fig. 2 further illustrates that IL-4 (250 U/ml) inhibited spontaneous [3H]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 [3H]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

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**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). (a) Cultures in the presence of IL-4 (250 U/ml); (•) cultures in the absence of IL-4. Data are expressed as [3H]TdR incorporation±SD of cells pulsed with 1 µCi for the final 8 h of culture.

---

**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 εμ+ or slgM+ 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 slgM in cases of pre B (εμ+) ALL (case 3, and εμ+ BCP-ALL lines) (data not shown). Finally, concomitant 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+ slgM- 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 × 10^3 CD20+ cells) were recovered when CD10+ slgM- cells were cultured for 5 days in the presence of IL-4 as compared to medium alone (28%) (1.9 × 10^3 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 × 10^3 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 a CD4-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+ slgM- population before culture. Data from such an experiment, illustrated in Fig. 7, demonstrate that, although CD20 spontaneously reappears on a subset of

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

<table>
<thead>
<tr>
<th>Case</th>
<th>Induction of CD20 expression*</th>
<th>Inhibition of [3H]Tdr incorporation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>71</td>
</tr>
<tr>
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<td>71</td>
</tr>
<tr>
<td>11</td>
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<td>25</td>
</tr>
<tr>
<td>12</td>
<td>26</td>
<td>60</td>
</tr>
</tbody>
</table>

* Numbers of CD20+ cells (×10^3) specifically appearing in cultures of 4 × 10^3 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.
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), slgM^+ cells emerged in culture of CD10^+ slgM^- cells in the absence of exogenous factors. Thus, in a representative experiment, after 5 d of culture of CD10^+ slgM^- cells in medium alone, we recovered 0.20 × 10^4 slgM^+ cells (Fig. 6). This figure did not differ significantly from the number of slgM^- cells recovered in the presence of IL-4 (250 U/ml) (0.21 × 10^4 cells), although a minor cell subset expressed higher levels of slgM in the IL-4-supplemented cultures (Fig. 6).

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

Finally, we consistently failed to detect the appearance of CD21^+ cells (Fig. 6) or slgD^+ cells (not shown) in cultures of CD10^+ slgM^- 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^'-negative B lineage ALL
represents an asynchronous event in a number of BCP-ALL (30), as illustrated by case 3 in our present study (CD20 + cμ +). 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 Ph1-positive BCP-ALL tested. However, in contrast to our study, these authors did not demonstrate a similar effect in Ph1-negative ALL. This apparent discrepancy may be attributed to the fact that few examples of Ph1-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 + slgM + 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 [3H]TdR incorporation by normal human

Figure 6. IL-4 does not favor the generation of cμ + or slgM + cells in cultures of fetal BCP (CD10 + slgM +). (A) Phenotype of freshly-isolated CD10 + slgM + cells. (B) Phenotype of cells harvested after 5 d of culture of CD10 + slgM + cells in medium alone. (C) Phenotype of cells harvested after 5 d of culture of CD10 + slgM + cells 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 Ca2+ into the cell.

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).
(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 matura-

tion as judged by expression of IgM. According to these crite-

ria, our results demonstrate that IL-4 does not permit to

overide the maturation block in BCP-ALL, in that it failed to

promote the emergence of slgM⁺ cells (and of cytotoxic B cells in

the cases of cytotoxic 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 slgM through cloning

selection, but not through exposure to a number of cytokines, including IL-4.

A number of studies have reported cytokine-mediated in-

duction of Ig expression in normal murine and human BCP. In this context, we found that IL-4 did not enhance the spontaneous emergence of slgM⁺ cells from CD10⁺ slgM⁻ cells, in accordance with LeBien (16). Furthermore, IL-4 failed to affect the numbers of cytotoxic (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 cytotoxic and slgM⁺ 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 slgM⁺ 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 slgM⁻ cells, and thus cannot be directly compared to the CD10⁺ slgM⁻ population presently analyzed. Although IL-4 failed to increase the numbers of slgM⁺ cells in our cultures of CD10⁺ slgM⁻ fetal cells, it induced hyperexpression of slgM. This effect is likely to be related to IL-4 induced hyperexpression of slgM on slgM⁻ 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.

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