Expression of Interleukin-4 Receptors on Early Human B-Lineage Cells

By Che-Leung Law, Richard J. Armitage, Judith G. Villablanca, and Tucker W. LeBien

Interleukin-4 (IL-4) regulates multiple stages of the antigen-dependent phase of B-cell development. However, its precise role in regulating B lymphopoiesis in bone marrow is not as well defined. We examined whether surface IgM-normal and leukemic human B-cell precursors (BCP) expressed IL-4 receptors using biotinylated IL-4. Constitutive expression of IL-4 receptors was detected on both normal and leukemic BCP. A higher percentage of normal BCP (82% ± 15%) expressed IL-4 receptors compared with leukemic BCP (44% ± 8%). Using mean fluorescent intensity as an indicator of receptor level on the IL-4 receptor positive cells, normal (91 ± 41) and leukemic (44 ± 37) BCP expressed comparable numbers of receptors. IL-4 induced the expression of CD23 on 30% of the leukemic BCP cases examined. IL-4 induced CD23 on surface IgMmant fetal bone marrow lymphoid cells but not on the surface IgMnormal BCP, despite the presence of detectable receptors on the surface IgM cells. IL-4 did not stimulate proliferation of normal BCP, nor could it enhance the effect of recombinant IL-7 or low molecular weight B-cell growth factor. However, IL-4 increased the expression of surface IgM and surface Igk on in vitro differentiated pre-B cells. Our collective results identify no role for IL-4 in the proliferation of normal or leukemic BCP, but identify a role in the enhancement of surface Ig expression during pre-B to B-cell differentiation.

MATERIALS AND METHODS

Cells. Newly diagnosed fresh BCP-acute lymphoblastic leukemia (ALL) bone marrow aspirates expressing at least two of the three B-cell-associated antigens CD10, CD19, and CD24 were obtained from the Cell Marker Laboratory, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis. These specimens consisted of ≥ 80% (and frequently > 90%) leukemic blasts. Normal fetal bone marrow lymphoid cells were obtained by flushing fetal femoral bones with 10 mL of RPMI-1640, 5% fetal calf serum (FCS). Mononuclear cells were isolated by centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) and washed thrice with RPMI-1640, 5% FCS. The preparations were adherent cell-depleted by incubation on plastic at 37°C. 5% CO2 for at least 3 hours. The resulting cells were greater than 95% viable by trypan blue exclusion. The use of all human tissue had the approval of the University of Minnesota Committee on the Use of Human Subjects in Research. BCP-ALL cell lines NALM-6, HPB-NULL, and BLIN-1, the pre-B lymphoblastic lymphoma cell line JM-1,13 and the Burkitt’s lymphoma cell line RAJI were routinely maintained in RPMI-1640, 10% FCS, supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin.

Antibodies. 13B3 (IgG2a)/anti-CD2 was produced and characterized in this laboratory. EBVCS2 (IgG1)/anti-CD23 was a gift from Dr Bill Sugden (University of Wisconsin, Madison). Polyclonal rabbit antihuman IL-4 antibody was a gift from murine pre-B cells to induce expression of surface IgG. Hofman et al10 reported a similar differentiation-inducing function of human IL-4 on human fetal bone marrow lymphoid cells. They showed that IL-4 increased the percentage of cytoplasmic Ig and surface Ig cells in a population initially enriched for expression of the B-cell-associated antigen CD24. However, using CD10+/surface IgM+ B-cell precursors (BCP) as a starting population, we observed that in vitro acquisition of surface Ig was independent of any exogenous growth factors, including IL-4.11 Similar growth factor-independent in vitro differentiation of pre-B cells has also been described in the mouse.12

To critically assess the effect of IL-4 on the proliferation and/or differentiation of human BCP, we investigated the expression of IL-4 receptors on normal and leukemic human BCP. In this report, we show that IL-4 receptors are constitutively expressed on both normal and leukemic BCP. IL-4 did not stimulate proliferation in either population. Nevertheless, IL-4 induced the expression of CD23 on 30% of the leukemic BCP cases examined. IL-4 did not induce cell surface CD23 expression on normal BCP, but enhanced the expression of surface Ig on in vitro differentiated pre-B cells.
were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). J5 (IgG2a/anti-CD10-phycocerythrin (PE) and IgG2a-PE control myeloma protein were purchased from Coulter Immunology. IgG1 (MOPC) control myeloma proteins were purchased from Cappel (Durham, NC). FITC-goat antimouse IgG1 (FITC-GAM IgG1) was purchased from Southern Biotechnology Associates, Inc. (Birmingham, AL). FITC conjugation and biotinylation of HB57, HB61, and IgG1 myeloma protein was accomplished using the methods described by Goding. Flow cytometry. Two-color analysis was used to examine the expression of CD23 and CD10 on BCP-ALL. Cells, 1 x 10^6, were stained with saturating amounts of J5 (IgG2a/anti-CD10-PE and EBVCS2 (IgG1/anti-CD23 in 50 μL of fluorescence buffer (1x phosphate buffered saline [PBS], 2.5% newborn calf serum) for 30 minutes at 4°C. Cell-bound anti-CD23 was then detected by FITC-GAM IgG1 at a final dilution of 1:40. Negative controls consisted of cells incubated with identical concentrations of IgG2a-PE and IgG1 myeloma protein, followed by counterstaining with FITC-GAM IgG1. A similar method was used to examine the expression of CD23 and surface IgM on fetal bone marrow lymphoid cells. Cells, 1 x 10^6, were stained with a saturating amount of EBVCS2 (IgG1/anti-CD23, and cell-bound anti-CD23 was detected by FITC-GAM IgG1. Unbound sites on FITC-GAM IgG1 were blocked by incubating cells with IgG1 myeloma protein at 40 μg/mL for 30 minutes at 4°C. Surface IgM was then detected using a saturating amount of biotinylated HB57 (IgG1/anti-IgM, followed by counterstaining with streptavidin-PE (SA-PE) (Caltag, San Francisco, CA). For negative controls, EBVCS2 and biotinylated HB57 were substituted with IgG1 myeloma protein and biotinylated IgG1 myeloma protein, respectively. Single-color analysis using FITC-conjugated HB57 and HB61 was used to detect surface IgM and surface IgG, respectively. Stained cells were washed thrice with fluorescence buffer and fixed with 0.5 mL of 1% paraformaldehyde in PBS before analysis on a FACScan (Becton Dickinson, Mountain View, CA).

IL-4 receptor binding assay. Human recombinant IL-4 was biotinylated as previously described. Five hundred nanograms of biotinylated IL-4 in 25 μL of staining buffer consisting of PBS, 0.5% bovine serum albumin (BSA) was incubated with 5 x 10^5 cells at 4°C for 30 minutes. Cells were washed once in staining buffer, counterstained with SA-PE (dilution of 1:40) for 30 minutes, and then analyzed on a FACScan. Competition assays were conducted by preincubating cells with log concentrations of unlabeled IL-4 before staining with biotinylated IL-4. Induction of CD23. Fresh BCP-ALL or fetal bone marrow low-density leukocytes were incubated in RPMI-1640, 10% FCS, 2 mmol/L L-glutamine, 50 μmol/L 2-mercaptoethanol with or without 1 to 800 U/mL of IL-4 (Genzyme). Expression of surface proteins was analyzed by flow cytometry after 24 to 48 hours of incubation. Magnetic bead depletion. Fetal bone marrow nonadherent low-density leukocytes were suspended to 15 x 10^6 cells/mL in RPMI-1640, 2% FCS containing a cocktail of monoclonal antibodies (MoAbs) at saturating concentrations against myeloid/monocytoid cells (My 8, CD11b, CD33), erythroid precursors (glycophorin A), T cells (CD2), and mature B cells (surface IgM). Cells were incubated at 4°C for 45 minutes, and washed twice in RPMI-1640, 2% FCS. They were then incubated with goat antimouse IgG-coated Dynabeads M-450 (Robbins Scientific, Mountain View, CA) at a concentration of 5 to 10 beads/contaminating cell with constant rotation at 4°C for 60 to 90 minutes. The suspension was diluted twofold and placed next to a flat plate magnet for 5 to 10 minutes. Cells not bound to the Dynabeads were subsequently aspirated and used for various experiments. These cells were ~95% CD10^+ and less than 2% surface Ig^+.

RESULTS

Expression of IL-4 receptors on normal and leukemic human BCP. Successful application of biotinylated IL-4 in the evaluation of receptor expression using flow cytometry has been reported using both mouse and human B cells. To facilitate the comparison of relative levels of receptor expression based on the percentage of positive cells and mean fluorescent intensity (MFI), a single batch of biotinylated, recombinant human IL-4 was used throughout this study. Figure 1 illustrates the binding of biotinylated IL-4 to the pre-B lymphoblastic lymphoma cell line JM-1. Binding was effectively blocked by the presence of a 200-fold excess of unlabeled IL-4, confirming the specificity of biotinylated IL-4. A survey of IL-4 receptor expression on BCP-ALL cell lines, a Burkitt's lymphoma cell line, and fresh BCP-ALL is presented in Fig 2. Clearly positive signals were obtained using BCP-ALL cell lines (NALM-6, HPB-NULL, and BL-1) and the Burkitt's lymphoma cell line RAJI (Fig 2A). IL-4 receptor positive cells were arbitrarily defined by subtracting the fluorescence signal obtained in the absence of biotinylated IL-4 from the fluorescence signal obtained in the presence of biotinylated IL-4. Using this definition, greater than 90% of the cells were IL-4 receptor positive. As determined by MFI, the level of receptors was in the order of BL-1 > RAJI > NALM-6 > HPB-NULL. Using the aforementioned definition for receptor positivity, only 44% ± 8% of the cells from any given fresh BCP-ALL expressed IL-4 receptors (Fig 2B), compared with 95% ± 1% of the cells from any given leukemic cell line (Fig 2A).
IL-4 receptor expression was then examined on normal BCP. CD10+/surface IgM- fetal bone marrow cells were isolated by magnetic bead depletion. Results obtained from two fetal bone marrow donors (FBM-1, FBM-2) are presented in Fig 3. Approximately 85% and 95% of the CD10+/surface IgM- fetal bone marrow lymphoid cells stained with biotinylated IL-4. Specificity of IL-4 binding was evaluated by cold competition using cells obtained from an additional donor (FBM-3, Fig 3). CD10+/surface IgM- cells were incubated with biotinylated IL-4 plus unlabeled IL-4 at ratios of 1:200 (FBM-3, left histogram) and 1:1,000 (FBM-3, right histogram). At a ratio of 1:200, unlabeled IL-4 reduced the percentage of positive cells from 64 to 41, and the MFI from 41 to 31. At a ratio of 1:1,000, unlabeled IL-4 reduced the percentage of positive cells from 58 to 6, and the MFI from 21 to background.

IL-4-induced CD23 expression occurs on some BCP-ALL but not on normal BCP. The major outcome of IL-4/IL-4 receptor interaction on mature B cells is the induction of cell surface CD23. Hence, the ability of IL-4 to upregulate CD23 on BCP-ALL was assessed to determine the functional status of the IL-4 receptors on these cells. Ten of the 34 cases (30%) of BCP-ALL tested responded to IL-4 stimulation by expressing CD23. Figure 4 shows the response of one representative case. Greater than 90% of the BCP-ALL cells were CD10+. Few CD23+ cells were detectable in the fresh cells or cells incubated in medium alone for 24 or 48 hours. Addition of IL-4 induced expression of CD23 by 24 hours with a more striking induction by 48 hours, and virtually all the CD23+ cells were CD10+. This...
result provided direct evidence that CD10⁺ leukemic BCP expressed CD23 in response to IL-4. The specificity of IL-4 was confirmed using a rabbit anti–IL-4 neutralizing antibody. When IL-4 was incubated with anti–IL-4 for 30 minutes before the addition of BCP-ALL cells, all the CD23-inducing activity was negated (Fig 4). Moreover, substituting IL-4 with IL-1β, -2, -3, -6, -7, and L-BCGF did not result in CD23 upregulation on leukemic BCP (data not shown). A major polypeptide of 46 Kd was immunopurified from an IL-4–treated BCP-ALL using the EBVCS2/anti-CD23 MoAb, confirming that the IL-4–induced antigen detected by flow cytometry had the expected molecular weight of CD23 (data not shown). Of the five cases of BCP-ALL assessed for IL-4 receptor expression in Fig 2, only two (patients LC and MD) responded to IL-4 by expressing CD23.

Adherent cell-depleted fetal bone marrow low-density leukocytes were then examined for their response to IL-4 stimulation. After 48 hours in culture with 800 U/mL of IL-4, 20% of the surface IgM⁺ cells became CD23⁺ (Fig 5A). In contrast, surface IgM⁺ fetal bone marrow lymphoid cells did not show an increase in CD23 expression (Fig 5A). Figure 5B shows the IL-4 dose-dependent induction of CD23 on fetal bone marrow lymphoid cells. Consistent with the data in Fig 5A, CD23 was only induced on surface IgM⁺ cells. IL-4 also did not induce CD23 on purified CD10⁻/surface IgM⁻ fetal bone marrow BCP (data not shown). This result ruled out the possibility that cells of other hematopoietic lineages present in fetal bone marrow low-density leukocytes interfered with the response of BCP to IL-4 stimulation.

Function of the IL-4 receptor on normal BCP. The functional status of IL-4 receptors on normal BCP was further investigated using proliferation and differentiation assays. CD10⁺/surface IgM⁺ fetal bone marrow cells were incubated with 1 to 100 U/mL of IL-4. These concentrations were effective in inducing CD23 in BCP-ALL (Fig 4) and surface IgM⁺ B cells (Fig 5). The ability of IL-4 to stimulate [³H]-TdR incorporation alone, or in combination with other growth factors (L-BCGF or IL-7), is shown in Table 1. IL-4 alone did not deliver any significant, dose-dependent, reproducible stimulation of normal BCP in three experiments. CD40 is expressed on approximately 2/3 of CD10⁺/surface IgM⁺ normal BCP. Because IL-4 plus anti-CD40 is a potent growth stimulus for mature B cells, the efficacy of this combination in stimulating proliferation of normal BCP was also tested. Consistent with our other studies, anti-CD40 did not enhance [³H]-TdR incorporation, nor did anti-CD40 plus 1 to 100 U/mL of IL-4. In contrast, L-BCGF (5% vol/vol) and IL-7 (1 or 10 ng/mL) significantly stimulated [³H]-TdR incorporation in CD10⁺/surface IgM⁺ BCP, with stimulation indices ranging from 1.4 to 6.6. IL-4 did not significantly enhance the effects of L-BCGF or IL-7.

Because IL-4 has been reported to play a role in the differentiation of pre-B to B cells in both humans and mice, the effect of IL-4 on the expression of surface IgM during in vitro differentiation of normal pre-B cells was examined. Using our recently described short-term culture for studying differentiation of normal human pre-B cells, IL-4 did not exert any appreciable effect on the acquisition of surface IgM⁺ cells, compared with medium alone (Table 2). However, incubation with IL-4 resulted in B cells
expressing a higher level of surface IgM and surface Igk. This effect was IL-4 specific, because it was totally abolished by rabbit anti–IL-4 antibody (Table 2). IL-4 also increased the level of cell surface Igk (data not shown).

**DISCUSSION**

Biotinylated IL-4 and flow cytometric analysis were used to assess the expression of IL-4 receptors on normal and leukemic BCP in the current study. Although data on binding affinity and quantitation of receptor number per cell cannot be derived using biotinylated ligands, they can provide rapid evaluation of receptor expression on a cell-per-cell basis. Accordingly, this approach is particularly well suited for studying receptor expression on small numbers of cells. The validity of the biotinylated IL-4 binding assay used in this study is predicated on the ability of unlabeled IL-4 to effectively block the binding of biotinylated IL-4 to the pre-B lymphoblastic lymphoma cell line JM-1 (Fig 1), and normal BCP (Fig 3). Analysis of BCP-ALL cell lines (NALM-6, HPB-NULL, and BLIN-1), the Burkitt's lymphoma cell line RAJI, and CD10+/surface IgM- normal BCP showed clear positive staining for IL-4 receptors on nearly all cells (Figs 2A and 3). On the other hand, only 44%±8% of the leukemic cells from any given fresh BCP-ALL were IL-4 receptor positive according to the definition used in this study. Nevertheless, when MFI was used to compare the level of IL-4 receptors expressed on the receptor positive cells, the number of receptors expressed on different BCP populations was comparable, ie, 58±28 for BCP-ALL cell lines, 91±44 for CD10+/surface IgM- normal BCP, and 44±37 for fresh BCP-ALL.

The human IL-4 receptor is a member of a newly described receptor superfamily characterized by four conserved cysteine residues and a double tryptophan-serine motif.

**Table 1. Effect of IL-4 on [3H]-Tdr Incorporation by CD10+/Surface IgM- Fetal Bone Marrow Cells Isolated by Magnetic Bead Depletion**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>CD10+/Surface IgM-</th>
<th>CD10+/Surface IgM-</th>
<th>CD10+/Surface IgM-</th>
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<tbody>
<tr>
<td>Medium</td>
<td>5,338 ± 35,381</td>
<td>537 ± 321</td>
<td>5,300 ± 3,304</td>
</tr>
<tr>
<td>1 U/mL</td>
<td>5,738 ± 14,576</td>
<td>4,810 ± 2,021</td>
<td>5,658 ± 5,304</td>
</tr>
<tr>
<td>10 U/mL</td>
<td>6,741 ± 13,648</td>
<td>6,269 ± 4,193</td>
<td>6,148 ± 4,566</td>
</tr>
<tr>
<td>100 U/mL</td>
<td>7,254 ± 22,892</td>
<td>6,161 ± 3,671</td>
<td>6,178 ± 4,366</td>
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</table>

CD10+/surface IgM- fetal BM cells (>95% CD10+, <2% surface IgM+) isolated by magnetic bead depletion were seeded in triplicate at 5 x 10⁴ cells/well in 96-well round-bottom plates and were cultured for 80 hours. Wells were pulsed with 1 µCi/well of [3H]-Tdr for 16 hours before harvesting.

*IL-BCGF was used at 5% vol/vol.
†IL-7 was used at 1 ng/mL.
§Anti-CD40 MoAb 5 was used at 4 µg/mL.
$Significantly greater than the control value (medium), P < .05.
motif located proximal to the transmembrane region. Binding assays using 125I-labeled IL-4 have detected 2,280 ± 210 IL-4 receptors/RAJI cell and 115 ± 15 IL-4 receptors/ NALM-6 cell. Analysis of NALM-6 cells showed an easily detectable signal using biotinylated IL-4, comparable with that obtained from RAJI cells (Fig 2A). This may reflect a higher level of IL-4 receptors expressed on the NALM-6 cells used in this study. Leukemic cells from patient LC (Fig 2B) gave a biotinylated IL-4 fluorescence signal less than NALM-6 and RAJI (Fig 2A), and underwent a functional response to IL-4 by expressing CD23. This result argues that the low fluorescence signal is a true reflection of IL-4 receptor expression on cells from patient LC, and biotinylated IL-4 can detect IL-4 receptors ≤ 200/cell.

The results in this study constitute the first report of the ability of IL-4 to induce expression of CD23 on some BCP-ALL. This is an intriguing observation in light of the recent reports describing an autoproteolytic action of CD23, and the reported ability of soluble CD23 to stimulate proliferation of cells from different lineages including T cells, B cells, and myeloid precursors. Soluble CD23 can also promote the functional maturation of prothymocytes. The potential of CD23 induced on BCP-ALL to be shed from cell surface and the response of BCP-ALL to soluble CD23 remains to be determined. Several mechanisms can be proposed to explain the variable response of BCP-ALL to IL-4, despite the constitutive expression of IL-4 receptors on all cases examined. Human IL-4 mediates transient polyphosphoinositol hydrolysis, Ca2+ mobilization, and cAMP generation in tonsillar B cells, all of which are required for CD23 induction. Hence, it is conceivable that in those cases of BCP-ALL that did not respond to IL-4 by expressing CD23, this full complement of signals was not generated. Alternatively, transcription or translation of CD23 may be specifically altered in some leukemic cells, whereas changes in the expression of other (undefined) molecules may be intact. The difference in response to IL-4 may also reflect a structural difference in the IL-4 receptor itself.

A major distinction between normal and leukemic BCP is the inability of normal BCP to express CD23 (Fig 5), despite the expression of IL-4 receptors (Fig 3). The capacity of normal fetal bone marrow lymphoid cells to express CD23 is correlated with the expression of surface IgM (Fig 5). The acquisition of surface IgM may be necessary but not sufficient for B cells to upregulate CD23 in response to IL-4 stimulation because not all fetal bone marrow surface IgM+ B cells could express CD23 (Fig 5). When CD10+/surface IgM+ BCP were cultured with or without IL-4 some of them differentiated into surface IgM+ cells within 24 hours (Table 2), but CD23 could not be detected on these differentiated surface IgM+ cells (data not shown). This finding suggests that a full complement of CD23-inducing signals is only generated in some surface IgM+ fetal bone marrow B cells, and appears to be restricted to mature B cells expressing higher levels of surface IgM. It is also conceivable that an extremely minor subpopulation of normal BCP may be capable of expressing CD23 in response to IL-4, but were not detected by flow cytometry. The BCP-ALL capable of expressing CD23 may then represent leukemic transformation and clonal expansion of such rare BCP. On the other hand, asynchronous programming of maturation in leukemic cells may underlie the difference between normal and leukemic BCP.

The absence of an agonistic effect of IL-4 on the proliferation of leukemic BCP agrees with previously published data. Touw et al observed no proliferative effect of IL-4 on BCP-ALL. Cell cycle analysis of the IL-4 responsive BCP-ALL in this study showed no increase in cells in S/G2 + M phases of the cell cycle compared with cells cultured in medium alone (data not shown). The ability of IL-4 to induce proliferation in CD10+/surface IgM normal BCP was evaluated in three fetal bone marrow donors (Table 1). IL-4 delivered no significant stimulation and did not enhance the agonistic action of IL-7 or L-BCGF. IL-4 plus anti-CD40 delivers a potent stimulus to mature B cells, but this combination did not stimulate normal BCP (Table 1). IL-4 acts at multiple stages of murine B-cell ontogeny by stimulating proliferation in early precursors and differentiation in late precursors. Thus, it maintains long-term cultures of pro-B cell clones, and supports self-renewal in the early precursor population during which the cells retain their immature phenotype. Although purified according to our definition of CD10 expression and lack of surface IgM, the normal BCP used in this study constitute a heterogeneous population encompassing cytoplasmic mu+ pro-B cells, cytoplasmic mu+ pre-B cells, and potentially other minor subpopulations. Therefore, it is conceivable that IL-4 may have limited proliferation enhancing actions on a very minor population of human BCP.

Table 2. Upregulation of Surface Ig Expression on CD10+/Surface IgM+ Fetal Bone Marrow Lymphoid Cells by IL-4

<table>
<thead>
<tr>
<th>Surface IgM Expression</th>
<th>Surface K Light Chain Expression</th>
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<tr>
<td></td>
<td>FBM-1</td>
</tr>
<tr>
<td>Medium</td>
<td>43/128</td>
</tr>
<tr>
<td>1 U/mL IL-4</td>
<td>52/190</td>
</tr>
<tr>
<td>100 U/mL IL-4</td>
<td>53/199</td>
</tr>
<tr>
<td>100 U/mL IL-4 + anti-IL-4†</td>
<td>45/117</td>
</tr>
<tr>
<td>Anti-IL-4</td>
<td>46/116</td>
</tr>
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CD10+/surface IgM+ fetal bone marrow lymphoid cells (> 95% CD10+, <10% surface IgM) isolated by fluorescence-activated cell sorting were seeded at 5 x 10^6 cells/vial in 96-well round-bottom plates, and were analyzed for expression of surface IgM and K light chains after 48 hours. *Percentage of positive cells/mean fluorescent intensity. †Anti-IL-4 was used at 4 μg/mL.
examined. However, cells cultured in IL-4 had levels of surface IgM and surface IgK on the in vitro differentiated cells that were consistently higher than cells maintained in medium alone. IL-4 may act on surface IgM- cells so that on successful differentiation cells will express higher levels of surface IgM. In this circumstance IL-4 may transduce a message to surface IgM- BCP. Alternatively, the expression of surface IgM may render cells responsive to IL-4, leading to an increase in surface Ig and, potentially, an increased sensitivity to antigenic stimulation.

In conclusion, we have described the constitutive expression of IL-4 receptors on normal and leukemic BCP. Our collective data indicate that IL-4 does not directly promote proliferation of human BCP. Progress has recently been made in our laboratory in modifying the murine Whitlock-Witte culture to support self-renewal of human BCP under serum-free conditions (Wolf ML, Buckley JA, Goldfarb A, Law C-L, LeBien TW: submitted for publication). Using this culture system, experiments are currently in progress to more critically assess the role of IL-4 on in vitro B lymphopoiesis. Likewise, the potential application of this system to study the role of IL-4 and CD23 in the self-renewal of leukemic BCP is also under investigation.

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