IL-3 Increases Production of B Lymphoid Progenitors from Human CD34+CD38− Cells

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IL-3 Increases Production of B Lymphoid Progenitors from Human CD34<sup>+</sup>CD38<sup>-</sup> Cells<sup>1</sup>

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The effect of IL-3 on the B lymphoid potential of human hemopoietic stem cells is controversial. Murine studies suggest that B cell differentiation from uncommitted progenitors is positively prevented after short-term exposure to IL-3. We studied B lymphopoiesis after IL-3 stimulation of uncommitted human CD34<sup>+</sup>CD38<sup>-</sup> cells, using the stromal cell line S17 to assay the B lymphoid potential of stimulated cells. In contrast to the murine studies, production of CD19<sup>+</sup> B cells from human CD34<sup>+</sup>CD38<sup>-</sup> cells was significantly increased by a 3-day exposure to IL-3 (<i>p</i> < 0.001). IL-3, however, did not increase B lymphopoiesis from more mature progenitors (CD34<sup>+</sup>CD38<sup>-</sup> cells) or from committed CD34<sup>+</sup>CD19<sup>+</sup> B cells. B cell production was increased whether CD34<sup>+</sup>CD38<sup>-</sup> cells were stimulated with IL-3 during cocultivation on S17 stroma, on fibronectin, or in suspension. IL-3R<sup>a</sup> expression was studied in CD34<sup>+</sup> populations by RT-PCR and FACS. High IL-3R<sup>a</sup> protein expression was largely restricted to myeloid progenitors. CD34<sup>+</sup>CD38<sup>-</sup> cells had low or undetectable levels of IL-3R<sup>a</sup> by FACS. IL-3-responsive B lymphopoiesis was specifically found in CD34<sup>+</sup> cells with low or undetectable IL-3R<sup>a</sup> protein expression. IL-3 acted directly on progenitor cells; single cell analysis showed that short-term exposure of CD34<sup>+</sup>CD38<sup>-</sup> cells to IL-3 increased the subsequent cloning efficiency of B lymphoid and B lymphomyeloid progenitors. We conclude that short-term exposure to IL-3 significantly increases human B cell production by inducing proliferation and/or maintaining the survival of primitive human progenitors with B lymphoid potential. *The Journal of Immunology, 2000, 165: 2382–2389.*
that express very low levels of IL-3-Rα. We conclude that the incorporation of IL-3 into protocols using short-term ex vivo manipulation does not damage the B lymphoid potential of human hematopoietic stem cells.

Materials and Methods

Isolation of hematopoietic cells

Cord blood and bone marrow samples were obtained under guidelines of the Childrens Hospital Los Angeles’ Committee of Clinical Investigations and processed within 24 h of collection. Bone marrow samples were obtained from the filter screens of bone marrow harvests from the posterior iliac crests of two healthy donors (7 and 4 yr old). After Ficoll-Hypaque (Pharmacia, Piscataway, NJ) centrifugation, CD34+ cells were enriched from the mononuclear population with the MiniMACS device (Miltenyi Biotec, Auburn, CA) using manufacturer’s guidelines. CD34+–enriched cells were then incubated with CD34-FITC (HPCA2; Becton Dickinson, San Jose, CA) and CD34–PE (Leu-17; Becton Dickinson Immunocytometry Systems (BDIS), San Jose, CA) and CD38–PE (BDIS). Isotype controls were used to set positive and negative quadrants and CD34+CD38+ and CD34+CD38– cells were isolated by FACSvantage using an argon laser. Gates for cell sorting have been previously described (41); the gate for CD34+CD38– sorting is shown as R2 in Fig. 4C. Cell purity checked by reanalysis following isolation was 99.6%.

Initial stimulation conditions

Following isolation, cells were stimulated in 96-well plates (Becton Dickinson Labware, Franklin, NJ) for the first 3 days in B cell medium (RPMI 1640 (Irvine Scientific, Santa Ana, CA), 5% FCS (screened for B cell cultures), 50 μg/ml 2-ME (Sigma, St. Louis, MO), penicillin/streptomycin (Gemini Bio Products, Calabasas, CA), and glutamine (Gemini Bio Products) containing combinations of the following cytokines: IL-3 (10 ng/ml), FL (100 U/ml, a gift from Dr. Charles Hannum, DNAX, Palo Alto, CA), IL-7 (10 ng/ml). In most experiments, the 3-day stimulation took place during cocultivation on S17 stroma (a gift from Dr. Kenneth Dorshkind, UCLA). In certain experiments, cells were stimulated instead either on S17 stromal fibronectin fragment CH-296 (Takara Shuzo, Shiga, Japan) or in suspension (i.e., in tissue culture plates). Following stimulation, at day 3, approximately one-half the culture medium was changed to fresh B cell medium as below and, if not already present, S17 stroma was added to begin B cell culture.

Culture and analysis of human cells on S17 stroma

Following 3 days of stimulation with or without IL-3, cells were cultured in B cell conditions, i.e., RPMI, 5% FCS, 2-ME, penicillin/streptomycin, and glutamine with FL (100 U/ml) on S17 stroma (40). Half-medium changes were performed every 7 days. At 7–14-day intervals, cultures were harvested, and cells were stained with trypan blue and counted to determine the fold increase in total viable cells since day 0. Aliquots were then analyzed by FACS to determine the proportion of B lymphoid (using CD19-FITC; BDIS) and myeloid (using CD33-PE; BDIS) cells. In all experiments, cultured cells were used as negative controls (IgG-FITC and IgG-PE; BDIS) to set parameters for positive Ag expression (thus allowing for autofluorescence and nonspecific binding to Ab commonly seen when analyzing cultured cells). The remaining cells were replated to continue cultures. CD19+ cell numbers were compared across experiments by standardizing data to an input day 0 cell number of 1000. The relative number of CD19+ cells produced in culture was thus calculated using the following formula: (% CD19+ of the total population/100) multiplied by (fold increase in total viable cells from day 0 × 1000). Clonal analysis was performed as previously described (40). In brief, single cord blood CD34+CD38– cells were plated by the Automated Cell Deposition Unit on the FACSvantage onto S17 stroma in B cell medium in individual wells of 96-well plates and cultured for 3 days with or without IL-3. On day 3 and again on day 7, one-half the medium was removed and replaced with B cell medium containing FL only. Timing of appearance of each clone was recorded. Clones large enough for analysis (>1000 cells) were then analyzed by FACS for CD19 expression to measure the presence of B cells. Aliquots of each clone (approximately 50%) were also replated into methylcellulose culture to detect CFU and thus prove the presence of myeloid progenitors in which at least 2% of all cells were CD34+CD38– and which also produced CFU in methylcellulose culture were recorded as B lymphomyeloid. Clones in which at least 10% of cells were CD19+ but which did not produce CFU were recorded as B lymphoid.

IL-3-Rα expression

To analyze expression of IL-3-Rα by FACS analysis, CD34+–enriched cord blood cells were stained with combinations of CD34 (HPCA2)-FITC, CD38–APC (BDIS), CD19–FITC, and anti-human IL-3-Rα–PE (CDw123, clone 9E5, a nonblocking Ab; PharMingen, San Diego, CA). Anti-IL-3-Rα was used at 20 μl per 106 CD34+ cells (1/50 dilution). Cells were analyzed by FACS using a FACSVantage using argon and HeNe lasers, and populations of interest were FACS-sorted into tubes. Aliquots of each clone (approximately 50%) were also replated into methylcellulose culture to detect CFU and thus prove the presence of myeloid potential of human hematopoietic stem cells.

To determine whether short-term exposure of primitive human hematopoietic progenitors to IL-3 blocks their ability to subsequently differentiate into B cells, CD34+CD38– cells were initially cultured for 3 days with or without IL-3 on S17 stroma, and then assayed for subsequent B cell production during long-term culture on S17 stroma (in B cell medium without IL-3). A 3-day stimulation was chosen, as this is a commonly used period of ex vivo manipulation in many clinical hematopoietic gene transfer protocols. During the long-term B lymphoid culture, cell number was measured and cultures were serially analyzed by FACS for CD19 and CD33 expression. Early exposure to IL-3 did not inhibit, but instead increased B lymphoid production from both cord blood (n = 4) and bone marrow (n = 2) CD34+CD38– cells. Although the absolute level of B cell production varied from sample to sample, the effect of IL-3 was highly reproducible. The absolute number of CD19+ B cell progenitors was significantly increased in the S17 cultures initially exposed to IL-3 relative to those not exposed to IL-3 (n = 6, p < 0.001) (Fig. 1). The number of B lymphoid cells was higher after IL-3 exposure at all time points during subsequent long-term culture on S17 stroma. The proportion of CD33+ cells was not significantly changed by IL-3 stimulation. The increase in B cell numbers was accomplished by both a significant increase in total cell output (p < 0.001) and a significant increase in the purity (frequency) of CD19+ B cells in culture after IL-3 stimulation (p = 0.007). In cultures with cord blood, the number of CD19+ cells was 53.2 ± 26.5 (mean ± SEM)-fold greater with IL-3 stimulation than without IL-3, whereas total cell numbers in culture increased only 13.3 ± 2.7-fold with IL-3. Total cell and B cell proliferation, with or without IL-3 stimulation, was lower with bone marrow than with cord blood. Bone marrow total cell numbers were 4.9 ± 1.4-fold higher after short exposure to IL-3, and CD19+ cell numbers were 11.4 ± 4.3-fold higher after IL-3 exposure.

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IL-3 in combination with either FL or IL-7 further enhances CD19⁺ B cell output from CD34⁺CD38⁻ cells

We have previously noted that FL and IL-7 are able to increase B cell production from CD34⁺CD38⁻ cells cultured on S17 stroma (37; unpublished data). We therefore next studied whether early exposure to IL-3 would further enhance B cell production from CD34⁺CD38⁻ cells cultured in FL or IL-7. Cord blood cells were cultured on S17 stroma, either with FL or IL-7 each in the presence or absence of IL-3 during the first 3 days after isolation. As seen in Fig. 2, IL-3 increased CD19⁺ B cell production when added to either FL (p = 0.01, n = 4) or IL-7 (p = 0.004, n = 3). Adding IL-3 to FL or to IL-7 also slightly increased CD33⁺ myeloid cell production, but this effect did not reach statistical significance.

IL-3 stimulates uncommitted and primitive progenitors, and not more mature B lymphoid cells

The above experiments all studied the effect of a brief exposure to IL-3 early in culture. In contrast, B lymphoid cells were not produced from cord blood CD34⁺CD38⁻ cells cultured on S17 stroma during continuous exposure to IL-3 (data not shown). Similarly, when IL-3 was added late to established B lymphoid cultures (day 14), myeloid cells became predominant and B lymphoid cells rapidly disappeared. We thus reasoned that IL-3 may increase B cell production from early or uncommitted progenitors, but inhibit growth of more mature CD19⁺ B cells produced during S17 cocultivation.

To determine more specifically at which stage of lymphopoiesis IL-3 acts to increase B lymphoid production, we studied fresh cells isolated at different stages of B lymphoid differentiation. Early exposure of cord blood and bone marrow CD34⁺CD38⁻ cells (a mixture of committed lymphoid and myeloid progenitors) to IL-3 gave variable results with no consistent increase or decrease in B lymphoid production (n = 9); B cell production from these mature progenitors, however, was not prevented by IL-3. In contrast, myeloid cells (measured by % CD33 expression) were consistently increased in S17 stroma cultures of CD34⁺CD38⁻ cells after an initial 3-day stimulation with IL-3.

Freshly isolated cord blood CD34⁺CD19⁺ pro-B cells cultured on S17 stroma grew poorly relative to more primitive progenitors, whether or not they were stimulated by IL-3. However, early exposure to IL-3 slightly increased CD19⁺ cell numbers short-term; at day 16, the number of IL-3-stimulated CD19⁺ cells increased 1.94-fold compared with a decrease to 5% of the starting number of CD19⁺ cells without IL-3 stimulation. When more mature B cells (freshly isolated CD34⁺CD19⁺ cells) were exposed to IL-3, cultures rapidly died; by day 15, no cells were detectable in culture. Thus, IL-3 increased B cell production predominantly from primitive, immunophenotypically uncommitted progenitors. B cell production from committed progenitors was not blocked, but was barely if at all increased. Mature B cells were not maintained when exposed briefly to IL-3.

IL-3 effects in the absence of S17 stroma

As human IL-3 does not act on murine cells (44), it is unlikely that the results seen in the above experiments were due to an indirect

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**FIGURE 1.** IL-3 increases B cell production from CD34⁺CD38⁻ cells. Shown are two representative experiments of total four with cord blood, and one of total two experiments with bone marrow. IL-3 stimulation occurred only during the first 3 days of S17 cocultivation; shown is CD19⁺ cell production at two different time points during subsequent cocultivation on S17 stroma. Number of CD19⁺ cells is normalized for a starting (day 0) cell number of 1000 CD34⁺CD38⁻ cells (p < 0.001 for effect of IL-3 on CD19⁺ cell number by Student t test; n = 6 experiments).

**FIGURE 2.** IL-3 increases B cell production when added to other early acting cytokines. Shown are percentage of CD19⁺ cells (of total cultured cells) and number of CD19⁺ cells from S17 stromal assays established after initial 3-day (D) stimulation in FL ± IL-3 (p = 0.01 for increase in number of CD19⁺ cells with IL-3, n = 4) (A) and IL-7 ± IL-3 (p = 0.004, n = 3) (B). Individual representative experiments with cord blood are shown.
action of human IL-3 mediated through murine S17 stroma. To exclude this possibility, however, we studied the effect of IL-3 stimulation on B cell production in the absence of S17 stroma. Cord blood (n = 3) and bone marrow (n = 1) CD34⁺CD38⁻ cells were cultured for 3 days either in suspension or on fibronectin in the absence or presence of IL-3. Cells were then washed and replated onto S17 stroma to assay B lymphoid production. The presence of IL-3 during 3-day culture, either in suspension or on fibronectin, increased subsequent CD19⁺ B cell production (suspension, p = 0.06, n = 3; fibronectin, p = 0.02, n = 4) (Fig. 3). Thus, the effect of IL-3 on human B cell production was not mediated through the murine S17 stromal cells.

**IL-3R expression on progenitor cells**

To explore further the issue of the type of progenitor able to respond to IL-3, we analyzed the expression of IL-3Rα on the surface of freshly isolated cord blood populations. CD19⁺ cells did not express IL-3Rα (data not shown). Most CD34⁺ cells also had no IL-3Rα expression detectable by FACS (Fig. 4D). However, a small percentage of CD34⁺ cells (1.7%) expressed high levels of IL-3Rα (defined as CD34⁺ IL-3Rhigh) and approximately 12% of CD34⁺ expressed IL-3Rα levels near the threshold set by the isotype control (defined as CD34⁺ IL-3Rdim) (Figs. 4D and 5A). CD34⁺ cells with high IL-3Rα expression had a more mature progenitor immunophenotype, i.e., they were CD38 positive and had low CD34 expression (Fig. 4E). CD34⁺CD38⁻ cells were either IL-3Rdim or IL-3Rneg (Fig. 4F). RNA expression of IL-3Rα was detectable by RT-PCR in all CD34⁺ populations, including CD34⁺ IL-3Rneg cells (Fig. 5B).

**B lymphoid potential of progenitors isolated by IL-3Rα expression**

To determine whether IL-3 responsiveness of B cell progenitors was similar in all progenitors that express detectable levels of IL-3Rα on the cell surface, CD34⁺CD38⁺ IL-3R⁺ and CD34⁺CD38⁻ IL-3R⁻ cells were isolated by FACS, exposed to IL-3 for 3 days, and then studied in B lymphoid cultures. The IL-3R⁺ gate used for these studies included both IL-3Rhigh and IL-3Rdim cells. The CD34⁺CD38⁻ IL-3R⁻ cells produced a significantly higher purity (p = 0.045) and absolute number (p = 0.016) of CD19⁺ cells than did CD34⁺CD38⁻ IL-3R⁺ cells after stimulation in IL-3 (n = 2 experiments), demonstrating again that IL-3 acts on B lymphopoiesis at a primitive progenitor level (Fig. 6).

As IL-3Rα is expressed at lower levels in CD34⁺CD38⁻ than in CD34⁺CD38⁺ cells, we next determined whether the effect of IL-3 varies on populations expressing high, dim, and undetectable levels of IL-3Rα. CD34⁺ cells were isolated according to IL-3Rα expression, irrespective of CD38 expression, and cultured for 3 days in the presence or absence of IL-3. IL-3 exposure significantly increased the production of B lymphoid cells from both CD34⁺ IL-3Rdim (p = 0.001, n = 5) and CD34⁺ IL-3Rneg cells (p = 0.033, n = 3) (Fig. 7). There was no significant difference between the B lymphoid capacity of CD34⁺ IL-3Rdim and CD34⁺ IL-3Rneg cells either in the presence or absence of IL-3, CD34⁺ IL-3Rhigh cells, however, had little capacity for B lymphoid production, and IL-3 did not enhance B cell production. In the presence of IL-3, CD34⁺ IL-3Rhigh cells produced significantly lower numbers of CD19⁺ cells than did either CD34⁺ IL-3Rdim (p < 0.0001, n = 6) or CD34⁺ IL-3Rneg cells (p = 0.004, n = 3) (Fig. 7). Thus, B lymphoid cells are produced almost entirely from the most primitive CD34⁺ progenitors with low or undetectable expression of IL-3Rα by flow cytometry. Despite the low expression of IL-3, IL-3 significantly increases B lymphoid production from primitive human progenitors.

**IL-3 increases cloning efficiency of B lymphoid progenitors and B lymphomyeloid progenitors**

To determine whether IL-3 acts by increasing the number of B lymphoid progeny from each CD34⁺CD38⁻ cell or by increasing cloning efficiency of normally quiescent progenitors with B lymphoid potential, the clonal behavior of single CD34⁺CD38⁻ cells

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**FIGURE 3.** IL-3 increases B cell production from cord blood and bone marrow CD34⁺CD38⁻ cells stimulated on fibronectin (FN, n = 4 experiments; 3CB, 1BM; p = 0.02) or in suspension (n = 3 experiments; 2CB, 1BM; p = 0.06). Shown are mean and SEM of percentage of CD19⁺ cells and CD19⁺ cell number for all experiments.
IL-3-and B lymphoid cell production from CD34+CD38− cells

was studied. Individual CD34+CD38− cord blood cells were plated in each well of 96-well plates (containing S17 stroma) and cultured with or without IL-3 during the first 3 days. IL-3 exposure for 3 days increased the subsequent cloning efficiency of CD34+CD38− cells grown on S17 stroma (Fig. 8). It should be noted that the S17 culture system is not optimal to measure production of differentiated myeloid cells from progenitors (as demonstrated, for example, by CD33 expression). Coclure on S17 stroma does, however, allow preservation of myeloid progenitors in a relatively nondifferentiated state. The presence of myeloid stroma does, however, allow preservation of myeloid progenitors (e.g., methylcellulose medium with IL-3, IL-6, SF, and erythropoietin) (40). Thus, clones were analyzed by FACS (for CD19+ B cell production) and by secondary CFU plating (to prove myeloid potential). Exposure of single CD34+CD38− cells to IL-3 led to an increase in the frequency of total B lymphoid clones (i.e., CD19+ clones with or without myeloid potential) and of the subset of clones with both B lymphoid and myeloid cells (bipotent progenitors) (Fig. 8). IL-3, however, did not affect the proportion of CD19+ cells within the clones analyzed; IL-3-stimulated B cell clones contained 30.2 ± 4.1% (mean ± SEM) CD19+ cells, and clones arising without prior IL-3 stimulation contained 30.4 ± 4.1% CD19+ cells. These data support the contention that IL-3 either stimulates proliferation or improves survival of primitive progenitors, including pluripotent B lymphomyeloid progenitors. Furthermore, these single cell studies show that IL-3 acts directly on progenitors with B lymphoid and B lymphomyeloid potential rather than indirectly through accessory cells in the culture.

Discussion

These studies show that short-term exposure to IL-3 causes a consistent and marked increase in B lymphoid cell production from primitive human hemopoietic progenitors. IL-3 responsiveness was maximal in CD34+CD38− cells, the most immature progenitors studied. IL-3 increased the cloning efficiency of both B lymphoid and B lymphomyeloid progenitors, but did not increase the

FIGURE 7. B cell production from CD34+ cells isolated according to high, dim, and negative expression of IL-3Rα. Shown are mean and SEM (n = 6 independent experiments). Each experiment was analyzed at two to four different time points between days 7 and 45 of culture; the figure is a compilation of all analyses. *, Exposure to IL-3 significantly increased CD19+ cell numbers from both CD34+ IL-3Rdim (p = 0.001) and CD34+ IL-3Rnegative cells (p = 0.033), but not from CD34+ IL-3Rhigh cells. ψ, CD19+ cell production was significantly greater from CD34+ IL-3Rdim compared with CD34+ IL-3Rhigh cells (p < 0.0001) and from CD34+ IL-3Rnegative compared with CD34+ IL-3Rhigh cells (p = 0.004).

FIGURE 6. B lymphoid and myeloid growth from cord blood CD34+CD38− IL-3− and CD34+CD38− IL-3+ cells. Isolated cells were all exposed to IL-3 for 3 days (D) and then cultured in B cell conditions on S17 stroma. At time points shown, cells were counted and analyzed by FACS. A, Percentage of CD19+ of total cultured cells; B, percentage of CD33+ of total cells; C, total number of CD19+ cells (n = 2 experiments).
creased myeloid cell production from CD34
1
phomyeloid clones each as a percentage of total CD34
1
stroma. Data are presented as total B lymphoid clones arising and B lym-
phoid progenitors (45, 46). The proliferative effect of IL-3 was
limited to primitive progenitors. No proliferation or increased sur-
lymphoid progenitors (45, 46). The proliferative effect of IL-3 was
with B lymphoid or lymphomyeloid potential, rather than forcing
lymphoid production by either inducing proliferation and/or pro-
portion of CD19
1
cells in each clone. Thus, IL-3 increased B
lymphoid production by either inducing proliferation and/or pro-
moting survival of primitive and normally quiescent progenitors
with B lymphoid or lymphomyeloid potential, rather than forcing
B lymphoid differentiation. IL-3 was also synergistic with FL and
IL-7, growth factors known to act on stem cells and/or primitive B
lymphoid progenitors (45, 46). The proliferative effect of IL-3 was
limited to primitive progenitors. No proliferation or increased sur-
vival of CD34
1
B cells was seen. IL-3 consistently in-
creased myeloid cell production from CD34
1
CD38
2
cells, but results on B lymphopoiesis from this heterogeneous and lineage
committed population were inconsistent.

The results were unexpected in view of studies reporting the
effects of IL-3 on murine lymphohemopoietic progenitors using in
vitro clonal assays. Stimulation of murine stem cell populations
(e.g., murine Lin
- Sca-1
+ c-kit
+ cells) in IL-3 for more than
6 days has been reported to completely prevent the subsequent
production of B lymphoid cells (23–25, 47). Certain important
differences in the nature of the in vitro assays used for the murine
studies and our own should be noted. The murine studies used
semisolid medium during both the primary culture (IL-3 stimula-
tion) and the secondary culture (B cell assay). However, in vitro
identification of human B lymphopoiesis requires contact of pro-
genitors with an adherent stromal layer. In most cases, we used
S17 stroma layers during IL-3 stimulation and always in subse-
tent B cell assay. Nevertheless, IL-3 stimulation in the absence of
S17 stroma (on fibronectin or in suspension) also increased sub-
sequent B cell production. Thus, the actions of human IL-3 on B
lymphopoiesis were not mediated through S17 stroma.

IL-3 exposure during primary culture was slightly longer in the
murine studies than in our own (7–10 days vs 3 days). However,
in our own experience, after culturing CD34
+ CD38
- cells on pri-
mary human stroma continuously in IL-3 for at least 1 mo, B cells
can still be generated when cultures are switched to S17 stroma
without IL-3 (unpublished data). Thus, long-term exposure to IL-3
does not prevent subsequent B lymphopoiesis. It is possible, how-
ever, that a combination of the above differences in experimental
design could have produced the different results in the murine and
human studies. For example, the combination of the longer period
of IL-3 stimulation in the absence of stroma in the murine studies
may have led to apoptosis of cycling progenitors with B lymphoid
potential.

Other evidence supports our contention that IL-3 does not ab-
rogate human B lymphopoiesis. A number of investigators have
stimulated human CD34
+ or CD34
+ CD38
- cells in cytokine
combinations, which include IL-3 for up to 8 days and nevertheless
demonstrated robust human B lymphoid production in vivo after
transplantation into immunodeficient mice (30–34, 48). Previous
reports suggest that IL-3 may stimulate B lymphopoiesis from hu-
man pro-B cells and mature B cells (49–52). In one report, brief
initial exposure to IL-3 (in combination with other cytokines) ap-
ppeared to increase the frequency of B lymphoid clones arising
from single CD34
+ lin
- CD38
- cells during subsequent culture on
the murine stromal line AFT024 (53). In the same study, how-
ever, no increase in B lymphopoiesis was seen with IL-3 contain-
ing combinations using bulk cultures of CD34
+ lin
- Dr
- cells, possibly because overgrowth of other cell lineages affected B cell
growth (53). The effect of IL-3 used in isolation and the require-
ment of AFT024 stroma during IL-3 stimulation were not studied.

One explanation for the contrasting results is that intrinsic dif-
ferences in regulation of B lymphopoiesis may exist between the
species. The different requirements for IL-7 and for stromal con-
tact in postnatal in vitro human and murine B lymphopoiesis sup-
port this possibility (54, 55). The structures of murine and human
IL-3 and their receptors differ significantly. Only 29% amino acid
homology exists between murine and human IL-3 (44, 56). IL-3R
from both species are heterodimers consisting of α and β subunits
(42, 57). The α subunit confers low affinity, IL-3-specific binding;
the murine and human forms of IL-3Rα have the same low ho-
mology (30%), as do their ligands (58). The β subunit in both
species confers high affinity binding and mediates signal transduc-
tion. In the human, only one β subunit exists (βc), which is shared
with the receptors for GM-CSF and IL-5 (42, 57). The murine
IL-3R has two β subunits, βc and βIL-3, which have significant
sequence homology (91% at amino acid level). Either of the mu-
rine β subunits can combine with IL-3Rα to form high affinity
receptors (58), and some functional redundancy exists between murine βc and murine βIL-3. For example, either murine β sub-
unit can transduce the negative regulatory signals of IL-3; murine
B lymphopoiesis is abrogated when uncommitted progenitors from
knockout mice deficient in either βc or βIL-3 are exposed to IL-3
(25). The murine β subunits, however, are not completely inter-
changeable. For example, although murine βc is shared by the
receptors for murine GM-CSF and IL-5, βIL-3 is not. Thus, dif-
ferences both in ligand-receptor interactions and signal transduc-
tion pathways may result in critical differences in the responses of
murine and human progenitors to IL-3.

A recent report by Brown et al. (59) argues against the hypoth-
thesis that IL-3 responsiveness is restricted to human B lymphopoi-
esis in this study, IL-3 administration in vivo was found to par-
tially restore both T and B lymphopoiesis in Jak3-deficient mice,
suggesting that IL-3 also has a positive regulatory role in primitive
murine lymphopoiesis.

IL-3Rα expression on murine and human progenitors has been
previously reported (60–62). Of interest in the current studies was
the unexpected finding that IL-3 stimulation of B cell production
was most impressive in cells with low or undetectable cell surface
IL-3Rα expression. PCR revealed that message for IL-3Rα was
present even in cells with expression undetectable by flow cytom-
etry. IL-3Rα is the only subunit of the receptor that binds to IL-3;
it thus seems that a very low receptor number is sufficient for the
proliferative or survival effects on primitive progenitors with B
lymphoid potential. In contrast, high expression of IL-3Rα was
largely restricted to a mature, committed myeloid progenitor
population.
IL-3 AND B LYMPHOID CELL PRODUCTION FROM CD34⁺CD38⁻ CELLS

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