Human Interleukin-4 Enhances Stromal Cell–Dependent Hematopoiesis: Costimulation With Stem Cell Factor

By Ulrich Keller, M. Javad Aman, Günther Derigs, Christoph Huber, and Christian Peschel

Interleukin-4 (IL-4) has distinct hematopoietic activities, primarily as a costimulant with other cytokines to enhance colony formation of hematopoietic progenitors. We investigated the influence of IL-4 on stromal cell–supported long-term cultures (LTCs) of normal human bone marrow. Addition of IL-4 to LTCs of unseparated bone marrow or highly enriched CD34+ cells resulted in a significant increase of myeloid progenitors in the nonadherent, as well as in the stromal cell–adherent cell populations. In contrast, the total cell number was not influenced by IL-4, suggesting a selective effect on primitive progenitor cells. Cord blood cells or CD34+ bone marrow cells were incubated with stem cell factor (SCF) and/or IL-4 in stromal cell–free cultures. In these experiments, a twofold to fivefold increase of myeloid progenitor cells was observed in the presence of SCF and IL-4 as compared with SCF alone. Preincubation of the stromal cell cultures with IL-4 resulted in an enhanced adherence of CD34+ cells to the stromal layer. Secretion of hematopoietic growth factors produced by the stromal cells, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and IL-1, was inhibited by IL-4. Thus, the increase of hematopoietic progenitors in LTCs, as observed in the presence of IL-4, can be at least partially explained by a costimulation of SCF and IL-4 on primitive progenitor cells and by an enhancement of hematopoietic cells to stroma. The downregulation of CSFs by IL-4 might prevent the expansion of the mature hematopoietic cell compartment.

INTERLEUKIN-4 (IL-4) is a pleiotropic cytokine that is produced by T cells in the process of immune recognition and by a nonlymphocytic cell population predominantly consisting of mast cells and basophils. In addition to multiple biological functions exerted on B and T lymphocytes, IL-4 also significantly influences the proliferation of hematopoietic progenitor cells. In normal murine and human hematopoiesis, IL-4 acts as a costimulant with other hematopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), and IL-6, inducing the proliferation of colony-forming unit–granulocyte, macrophage (CFU-GM), burst-forming unit–erythrocyte (BFU-e), and colony-forming unit–granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM). A direct stimulatory effect of IL-4 has also been described in primitive murine mast cell–forming cells. In recent reports investigating the effects of IL-4 in leukemic cell populations, suppressive effects of IL-4 have been detected by some investigators. Inhibitory activities of IL-4 have also been described for normal monocyte/macrophage lineage cells.

Long-term hematopoiesis in vitro is strictly dependent on direct contact of hematopoietic progenitor cells with mesenchymal cells of nonhematopoietic origin, collectively referred to as stromal cells. In addition to direct cellular mechanisms, stromal cells mediate their hematopoietic effects by producing cytokines with stimulatory or inhibitory actions, which can be locally presented to hematopoietic progenitors by binding to matrix proteins or by membrane-bound forms as reported for stem cell factor (SCF). Exogenously added cytokines can influence stromal cell–dependent hematopoiesis either by direct action on progenitor cells or by regulation of the production of stromal cell factors.

IL-4 receptors were detected on nonhematopoietic fibroblasts and stromal cell lines, which suggests a role of IL-4 in the regulation of the bone marrow microenvironment. In the murine system, IL-4 has been described to mediate an inhibitory activity for hematopoietic and pre-B lineage cells, whereas preincubation of another stromal cell line with IL-4 caused an enhancement of colony formation when bone marrow cells were physically separated from the stromal cells.

In the present report, we evaluated the effect of IL-4 on human stromal cell–dependent hematopoiesis. We found a profound hematopoietic effect of IL-4, which appears to be restricted to primitive hematopoietic precursors. Costimulation with SCF appears to be involved in the stimulatory action of IL-4 on early hematopoiesis.

MATERIALS AND METHODS

Growth factors. The following cytokines were used: recombinant human IL-4 was generously provided by Schering-Plough (Kenilworth, NJ), recombinant human G-CSF was obtained from Amgen (Munich, Germany), recombinant human IL-3 was obtained from Sandzö (Nürnberg, Germany), and recombinant human EPO was obtained from Cilag (Sulzbach, Germany). Recombinant human SCF was a generous gift from Amgen (Thousand Oaks, CA).

Cell separation. Normal bone marrow was obtained from patients harvested for autologous bone marrow transplantation or from patients undergoing diagnostic bone marrow biopsy after informed consent had been given. Umbilical cord blood samples were obtained from normal, full-term newborns. Cell samples were anticoagulated with preservative-free heparin 10 U/mL and gently layered over a Ficoll/Paque layer of density 1.077 g/mL (Seromed, Berlin, Ger-

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many). Light-density mononuclear cells (MNC) were separated from the interface after centrifugation for 30 minutes at 400 g at room temperature. When indicated, adherent cells were removed by passing the MNC over a Sephadex G10 column (Pharmacia, Freiburg, Germany). Bone marrow cells were enriched for hematopoietic precursors by positive selection for cells expressing the CD34 antigen using immunomagnetic beads coated with CD34 (Dynal, Hamburg, Germany). Detachment of immunomagnetic beads from the CD34-expressing cell population was obtained using DetachA Bead (Dynal) according to the manufacturer's instructions. The efficiency of the selection was controlled by flow cytometry. The separation procedure resulted in an enrichment of 85% to 95% CD34-expressing cells. More than 90% of these cells expressed CD33 and major histocompatibility complex (MHC) class II.

Stromal cell cultures. Stromal cell layers of Dexter type,14 which support hematopoietic long-term proliferation, were initiated as previously described.23 Briefly, bone marrow MNC separated by centrifugation over Ficoll-Hypaque were incubated at a cell density of 2 × 10^6/mL in a total volume of 5 mL in 25-cm² tissue flasks at 33°C in culture medium consisting of RPMI 1640 medium (Seromed) supplemented with 1 mmol/L sodium pyruvate, modified Eagle's medium (MEM) nonessential (1:100) and essential (1:50) amino acids, 2 mmol/L L-glutamine, 16 μg/mL L-asparagine, 10 mmol/L HEPES buffer, penicillin-streptomycin, and 5 × 10^-5 mol/L 2-mercaptoethanol, 10% fetal calf serum, 10% horse serum, and 1 × 10^-4 mol/L hydrocortisone (Sigma Chemicals, Deisenhofen, Germany). In weekly intervals, cultures were fed by replacing 75% of culture medium. When cultures were confluent, the primary cultures were treated with trypsin-EDTA (Bioschorn, Berlin, Germany), detached stromal cells were pooled and expanded in six-well culture plates or 25-cm² culture flasks at a ratio of 1:5. The adherent cells were incubated under the same culture conditions until the cultures became confluent again. Nonadherent bone marrow cells, cord blood cells, or CD34⁺ bone marrow cells were added to identical stromal cultures, which were derived from the same pool of primary cultures. Unselected cells were seeded at a concentration of 3 × 10⁵ cells per 25-cm² culture flask. CD34⁺ cells were incubated in six-well plates at a density of 2 × 10⁵ cells per well. IL-4 was added to the cultures at a concentration of 500 U/mL, and expansion of hematopoietic cells was compared with cultures containing culture medium alone. In some experiments, stromal cell cultures were supplemented with IL-3 (50 ng/mL) or SCF (10 ng/mL). Cultures were fed weekly by replacing 50% of the medium with the same growth factors as added initially. The number of CFU-GM present in the nonadherent cell population was determined weekly. Hematopoietic progenitors residing in the adherent cell layer were evaluated after a culture period of 14 to 28 days. In some experiments, hematopoietic cells were physically separated from the stromal cell layer using culture inserts with semipermeable membranes (Millipore, Eschborn, Germany).

Alternatively unselected or CD34⁺ cells were incubated in liquid suspension culture without stromal cell support. These cultures were supplemented with SCF and IL-4 or IL-3 alone or in combinations of IL-4 or IL-3 and SCF. The total cell number and CFU-GM numbers were determined after 14 to 21 days.

Hematopoietic progenitor cell assay. The assays for detecting CFU-GM and BFU-e were performed as previously described elsewhere.8 Cells obtained from the nonadherent population of long-term cultures were plated in agar without further separation procedures. Hematopoietic cells residing in the adherent cell layer were counted in a hemocytometer and then depleted from stromal cells by passing over a Sephadex G10 column before seeding in agar. Cells from unselected long-term cultures (LTCs) were plated at a cell density of 0.5 × 10⁶ cells per well. CD34⁺ cells were used at a cell density of 2 × 10⁵ cells per well after in vitro culture. These cell numbers were suspended in culture medium containing 0.3% agar in a volume of 0.25 mL and plated in multiwell tissue culture plates (Nunc, Wiesbaden, Germany). After solidification of the agar layer, a liquid overlay of culture medium was added to the culture plates containing the hematopoietic growth factors. The final concentration of growth factors was as follows: G-CSF, 200 ng/mL; IL-3, 50 ng/mL; and EPO, 5 U/mL. At the end of the incubation period, the agar cultures were fixed with glutaraldehyde 2.5%, washed with distilled water for 4 hours, transferred onto microscopic slides, dried at 40°C, stained with May-Grünwald-Giemsa, and permanently mounted. Colony number was evaluated in such slides by light microscopy.

Cytokine enzyme-linked immunosorbent assay. Enzyme-linked immunosorbent assay (ELISA) kits from commercial sources were used according to the manufacturers' instructions. Protein levels of IL-1β, GM-CSF, G-CSF, and IL-6 were determined in supernatants of stromal cell cultures. First-passage stromal cell cultures were incubated with culture medium without hydrocortisone. To obtain readily detectable protein levels by the immunoassays, cytokine production was stimulated by the addition of IL-1-α (50 U/mL). IL-4 was added in combination with IL-1-α at a concentration of 500 U/mL. Parallel cultures with IL-1 alone were used as controls. The culture supernatants were collected 48 hours after addition of the cytokines. For detection of cell-bound IL-1β, stromal cells stimulated with IL-4 or control cultures were overlayed with 1 mL fresh culture medium and detached using a cell scraper. Stromal cells from three cultures were pooled and homogenized by a ultrasound sonifier. Cell-bound IL-1 was measured after pelleting the cell particles. The level of cell-bound IL-1 was expressed as picograms per culture medium.

Adhesion of hematopoietic cells to bone marrow stroma. Adhesion of CD34⁺ cells to stromal layers was determined by a ⁵¹Cr-labeling assay as previously described.28 Briefly, bone marrow cells positively selected for expression of CD34 antigen were labeled with ⁵¹Cr (DuPont, Bad Homburg, Germany). Labeled cells were incubated with stromal cells in six-well culture plates, which were prestimulated with IL-4 for 24 hours or which contained culture medium alone. All experiments were performed in triplicate. After 1 to 2 hours, nonadherent cells were removed by gently washing three times with 2 mL phosphate-buffered saline (PBS). Then the adherent layer was trypsinized, and radioactivity in both the adherent and nonadherent cell populations was measured in a gamma-counter. The percentage of CD34⁺ cells adherent to the stromal layer was calculated based on the amount of radioactivity recovered from both cell fractions.

RESULTS

Influence of IL-4 on stromal cell–dependent hematopoiesis. Long-term bone marrow cultures (LTBMCs) initiated in the presence of IL-4 failed to form confluent stromal cell layers as observed in conventional LTBMC with culture medium alone. IL-4 appears to prevent the formation of cellular stroma components, such as fibroblasts and adipocytes, and promotes the development of foci consisting primarily of macrophage-like cells (data not shown). However, IL-4 dramatically enhances stromal cell–dependent hematopoiesis if added to preformed stromal layers. In six individual experiments, nonadherent bone marrow cells or cord blood cells were added to stromal layers in the presence or absence of IL-4 (500 U/mL) and the number of CFU-GM was determined after 2 and 3 weeks of incubation (Fig 1). Addition of IL-4 to LTBMCs caused a twofold to threefold increase...
of myeloid progenitor cells in the nonadherent cell population. The number of erythroid progenitor cells in these cultures, which was always low, was not influenced by IL-4 (data not shown).

The effect of IL-4 in LTBMCS was compared with IL-3 as a growth factor with well-defined activity on early hematopoietic progenitors. One representative experiment is shown in Fig 2. In weekly intervals, the total cell number and the number of CFU-GM were determined in the nonadherent cell population. Both IL-3 and IL-4 modulated stromal cell–dependent hematopoiesis during a culture period of up to 5 weeks. IL-4 caused a substantial increase of the concentration of myeloid progenitors without affecting the total number of mature cells per culture. In contrast, IL-3 initiated an expansion of the total cell number, whereas the relative amount of progenitor cells was unchanged. Thus, mediated by different mechanisms, both factors significantly increased the absolute numbers of progenitor cells in LTBMCS as compared with controls without additional growth factor. In separate experiments, we examined the number of CFU-GM residing in the adherent layer, which are considered to represent the more immature progenitor cell population, in LTBMCS with and without IL-4 after a longer incubation period. In a representative experiment, we found, after 28 days of incubation, 41 CFU-GM per 5 × 10⁴ adherent cells in conventional stromal cultures, whereas in the presence of IL-4, the number of CFU-GM was 123 per 5 × 10⁴ cells adherent to the stromal layer.

The effect of IL-4 on stromal cell–dependent hematopoiesis was further investigated using highly enriched CD34⁺ bone marrow cells to minimize an influence of accessory cells. In four experiments, the total cell number and the number of myeloid progenitors was determined in the nonadherent cell compartment and in the hematopoietic cells adhering to the stromal layer. Furthermore, in two of these experiments, the CD34⁺ cells were physically separated from the stromal cells by culture inserts (Fig 3). In cultures stimulated with IL-4, an increase of the myeloid progenitor
number of CFU-GM per culture induced by IL-4 as shown in Fig 3C was caused by a selective expansion of early hematopoietic cells. When direct cell-cell interaction was prevented by placing the CD34+ cells in culture inserts, IL-4 caused only a marginal increase of the progenitor cell number. To obtain an estimate of the capacity to expand the hematopoietic progenitor compartment in vitro, the number of CFU-GM added to the cultures initially was compared with the number of CFU-GM after an incubation period of 2 weeks. In control cultures, the mean number of CFU-GM was 155% (range, 59% to 235%) of the number of myeloid progenitors at culture initiation, whereas in IL-4-stimulated stromal cultures, the number of CFU-GM increased to 307% (range, 103% to 455%).

Costimulation of IL-4 with SCF in liquid culture. The stimulatory effect of IL-4 on stromal cell–dependent hematopoiesis can be caused by several mechanisms. One possible explanation could be a costimulatory activity with a constitutively expressed stromal factor, such as SCF. Therefore, we incubated nonadherent cord blood cells with SCF, IL-4, and combinations of SCF and IL-4 or IL-3 and evaluated the number of myeloid progenitors after 2 weeks of liquid culture. In three independent experiments, an increase of CFU-GM was observed in the presence of SCF and IL-4 alone as compared with control cultures without growth factor (Table 1). The combination of SCF and IL-4 led to a significant increase of the progenitor cell number, which suggests a synergism between those cytokines on early progenitor cells. In contrast, the total cell number was enhanced by the combination of SCF and IL-4 in one experiment only. Control experiments with IL-3 and SCF again showed that the total cell population was increased without affecting the propor-

Table 1. Effect of IL-4 in Costimulation With SCF on the Expansion of CFU-GM in Cord Blood MNC

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Experiment No.</th>
<th>CFU-GM /10^5</th>
<th>Cell No. /10^5</th>
<th>CFU-GM /Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>1</td>
<td>106</td>
<td>175</td>
<td>369</td>
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<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>23</td>
<td>310</td>
<td>143</td>
</tr>
<tr>
<td>IL-4</td>
<td>1</td>
<td>381</td>
<td>360</td>
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<tr>
<td></td>
<td>2</td>
<td>36</td>
<td>250</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>41</td>
<td>700</td>
<td>574</td>
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</tr>
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<td></td>
<td>2</td>
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<td>411</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>69</td>
<td>270</td>
<td>373</td>
</tr>
<tr>
<td>SCF + IL-4</td>
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<td>1,874</td>
<td>410</td>
<td>15,367</td>
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<tr>
<td></td>
<td>2</td>
<td>450</td>
<td>150</td>
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<td></td>
<td>3</td>
<td>114</td>
<td>840</td>
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</tr>
<tr>
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<td>165</td>
<td>1,120</td>
<td>4,356</td>
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<td>75</td>
<td>670</td>
<td>1,006</td>
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<tr>
<td></td>
<td>3</td>
<td>158</td>
<td>430</td>
<td>1,359</td>
</tr>
</tbody>
</table>

Nonadherent cord blood cells (3 x 10^6/culture at a concentration of 1 x 10^6/mL) were incubated in liquid culture in the presence of various combinations of cytokines. After 2 weeks of incubation, the total cell number and the number of CFU-GM were determined. Results of three individual cultures are expressed as the mean of triplicate values. Values of CFU-GM are presented as the relative number and absolute number per culture.
Costimulation of IL-4 and SCF on CD34+ bone marrow cells. CD34+ cells (2 x 10^5/culture) were incubated in culture medium or in the presence of IL-4 (500 U/ml), SCF (10 ng/ml), or IL-4 + SCF. Cell number and CFU-GM number were determined after 14 days of incubation. Values represent the mean ± SEM of four individual experiments.

Effect of IL-4 on adhesion to stromal cells. A stimulatory effect on hematopoiesis in LTCs might also be transmitted by an improvement of the direct cellular interaction between stroma and hematopoietic precursors, as suggested by the recent finding that IL-4 upregulates the expression of adhesion molecules in stromal cells. To evaluate the influence of IL-4 on cell-cell interactions, we measured the proportion of ^51Cr-labeled CD34+ cells adhering to stromal layers after preincubation with IL-4. As shown in Table 2, preincubation of enriched hematopoietic progenitors with IL-4 resulted in a small, but significant increase of the cell fraction that tightly bound to the stromal cells.

Effect of IL-4 on cytokine production by stromal cells. The influence of IL-4 on production of hematopoietic growth factors by stromal cells was investigated. GM-CSF, G-CSF, and IL-6 levels were evaluated in stromal cell supernatants after stimulation with IL-1 or. IL-1β was measured in stromal cell lysates, since this protein could never be detected in supernatants, even after exogenous stimulation with IL-1 or LPS. In a representative experiment, shown in Fig 5, the
secretion of IL-1–stimulated GM-CSF, G-CSF, and IL-1β by stromal cells was markedly reduced in the presence of IL-4. The influence on IL-6 production was inconsistent. Stimulation of IL-6 secretion was observed in some experiments (Fig 5), whereas IL-6 secretion was not affected by IL-4 in other experiments.

**DISCUSSION**

Hematopoiesis in stromal cell cultures can be influenced by the exogenous addition of cytokines. Stimulation of long-term hematopoiesis in vitro has been described by the substitution of stromal cell cultures with hematopoietic growth factors or by the addition of IL-1, which caused an enhanced expression of GM-CSF in stromal cells. We demonstrate in this report that in the presence of IL-4 the number of myeloid progenitor cells is markedly increased in LTCs, whereas IL-4 has little influence on the expansion of mature myeloid cells, resulting in a shift in the proportion of immature cells.

The increase of CFU-GM numbers after several weeks of in vitro cultures suggests that the primary target cell of this novel activity by IL-4 is a progenitor cell at the pre-CFU level. An effect of IL-4 on an early progenitor cell population has been demonstrated previously by its direct stimulation of murine blast cell colony formation. Whether IL-4 primarily induces the differentiation of primitive progenitor cells, rendering them susceptible to the action of colony-stimulating factors as described for IL-1, or whether IL-4 promotes also self-renewal of primitive progenitors remains to be determined. In stromal cell cultures propagated over several weeks, no evidence was found that the increase of CFU-GM by IL-4 observed was followed by an emptying of the progenitor cell pool at a later stage of the incubation period, which suggests that the self-renewal capacity of primitive progenitors was not reduced by IL-4.

Several modes of action can explain the stimulation of hematopoiesis in stromal cell cultures. An exogenous cytokine such as IL-4 can directly stimulate hematopoietic precursors or it can costimulate with a constitutively secreted stromal cell product. Alternatively, IL-4 might also stimulate hematopoiesis by an indirect mechanism: it might modulate the balance of cytokine expression in stromal cells or in the hematopoietic accessory cells, either by induction of a stimulatory factor or by suppression of a hematopoietic inhibitor. Finally, the direct cellular interaction between hematopoietic progenitors and stromal cells might be improved by IL-4.

We attempted to elucidate the underlying mechanisms of action by IL-4 using several approaches. The separate examination of CFU-GM residing in the adherent layer and those in the nonadherent cell population confirmed that in fact IL-4 expanded the total progenitor cell number rather than caused a shift of CFU-GM from the adherent layer to the supernatant. In cultures of CD34-enriched bone marrow cells at a low cell density, the stimulatory effect of IL-4 on hematopoietic progenitors was still evident, which suggests that modulation of accessory bone marrow cells is of minor importance for the enhancing effect of IL-4 in stromal cell–dependent hematopoiesis.

A direct effect of IL-4 on early hematopoiesis has been demonstrated in costimulation with SCF, which could partially replace the role of stromal cells. In the past, interaction of IL-4 with hematopoietic growth factors has predominantly been detected in clonal assays for myeloid and erythroid progenitor cells. However, a synergistic interaction has recently been described for IL-4 and IL-11 in bone marrow from fluorouracil-treated mice, which was used as a source of primitive hematopoietic progenitors. These, as well as our results, indicate a novel role of IL-4 as a regulator of early hematopoiesis in conjunction with cytokines produced by stromal cells.

Long-term hematopoiesis in vitro appears not to be entirely dependent on direct contact with stromal cells, but can also be mediated by soluble stromal cell products. However, the enhancing effect of IL-4 in stromal cell cultures was abolished when the hematopoietic cells were physically separated from the stromal layer, which suggests that a protein located at the membrane of stromal cells is the main costimulant with IL-4. The improved adherence of CD34+ cells to stromal cells on stimulation with IL-4 as observed in this study might be related to the effect of IL-4 to enhance the expression of VCAM-1 in stromal cells. An improved binding to stromal cells mediated by adhesion molecules, such as VCAM-1, might enhance the exposure of progenitor cells to membrane-bound SCF and/or other surface proteins and, therefore, might further optimize the costimulatory effect of IL-4 with stromal cell products.

Despite its enhancing effect on long-term hematopoiesis, IL-4 inhibits the expression of several cytokines with stimulatory activity in hematopoietic cells, including IL-1, G-CSF, and GM-CSF. Such regulatory actions of IL-4 on cytokine expression are well defined in monocytic cells. Macrophages represented only a minority of the adherent cells after passing the stromal cell cultures in the presence of hydrocortisone. Therefore, the hematopoietic factors measured in our experiments appear to be derived predominantly from the stromal cells, which suggests a regulatory role of IL-4 in this cell population. It is difficult to assess the implication of cytokine regulation by IL-4 on stromal cell–dependent hematopoiesis. It can be assumed that comparable effects, as described in Fig 5 in exogenously stimulated stromal cells, are mediated by IL-4 in the constitutive secretion of stromal cell factors as well. Thus, based on current experimental evidence, IL-4 might interact with stromal cell–dependent hematopoiesis in vitro according to the following model: IL-4 costimulates with stromal cell–derived cytokines acting on early progenitors such as SCF, IL-11, and probably IL-6. On the other hand, the reduction by IL-4 of differentiating factors, such as GM-CSF and G-CSF, might counteract an increased production of mature myeloid cells derived from the expanded progenitor cell pool. Overall, the stimulation of early hematopoiesis might predominate the effects of differentiating cytokines, resulting in a more immature composition of hematopoietic cells in LTCs in the presence of IL-4.
In the past several years, hematopoietic actions of IL-4 in vitro have been described in numerous reports. However, the importance of IL-4 in the regulation of hematopoiesis in vivo remains to be determined. Helper T lymphocytes are induced to produce cytokines by presentation of antigens, in vivo remains to be determined. Helper T lymphocytes in vitro have been described in numerous reports. However, IL-4 and long-term hematopoiesis.

**REFERENCES**


34. Le Beau MM, Lemons RS, Espinola R III, Larson RA, Arai N, Rowley JD: Interleukin-4 and interleukin-5 map to human chromosome 5 in a region encoding growth factors and receptors and are deleted in myeloid leukemias with a del (5q). Blood 73:647, 1989
Human interleukin-4 enhances stromal cell-dependent hematopoiesis: costimulation with stem cell factor

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