Interleukin-1 (IL-1) Directly and Indirectly Promotes Hematopoietic Cell Growth Through Type I IL-1 Receptor

By Kjetil Hestdal, Francis W. Ruscetti, Richard Chizzonite, Mariaestela Ortiz, John M. Gooya, Dan L. Longo, and Jonathan R. Keller

Interleukin-1 (IL-1) has been shown to stimulate hematopoietic progenitor cell growth both in vitro and in vivo. Although IL-1 alone lacks the ability to promote hematopoietic progenitor growth in vitro, it is a potent synergistic factor in combination with other colony-stimulating factors (CSFs). Because it was unknown whether type I (p80), type II (p68), or other IL-1-binding proteins mediated the synergistic effects of IL-1 on purified progenitor cells, we used the difference in immunoreactivity between type I and type II IL-1 receptor (IL-1R) to better assess the role of these receptors in hematopoietic progenitor growth. Therefore, the synergistic effects of IL-1α on IL-3-, CSF-1-, and granulocyte macrophage (GM)-CSF-induced progenitor growth, both in CFU-c and single-cell assays, were determined in the presence of monoclonal antibodies (MoAbs) 35F5 and 4E2 that block the binding of IL-1α to type I and type II IL-1R, respectively. The synergistic effect of IL-1α on IL-3 responsive Lin- and Lin+ progenitors was indirectly mediated and could be inhibited by MoAb 35F5. In contrast, IL-1α directly synergized with CSF-1 and GM-CSF to promote progenitor cell growth. The direct synergistic effect of IL-1α on CSF-1-induced progenitor growth was observed in all progenitor populations examined (Lin-, Lin-Thy-1+, and Lin-Thy-1) and was inhibited by MoAb 35F5. However, the direct synergistic effect of IL-1α on GM-CSF-responsive progenitors, Lin- and Lin-Thy-1+, was partially inhibited by MoAb 35F5. In contrast, the MoAb antitype II IL-1R (MoAb 4E2) could not inhibit the direct synergistic effects of IL-1α on CSF-1- or GM-CSF-induced progenitor growth. Thus, IL-1α directly and indirectly stimulates the growth and differentiation of purified progenitors through the type IIL-1R but not the type II IL-1R.

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MATERIALS AND METHODS

Growth factors and anti–IL-1R antibodies. Purified recombinant human IL-1α, IL-1β, and the MoAbs against type I and type IL-1α-induced production of CSFs from hematopoietic accessory cells in vitro and the production of CSFs in vivo, and block the radioprotective effect of IL-1 in vivo.39-42 In addition, we have shown that MoAb 35F5 inhibits the IL-1α-mediated upmodulation of both IL-1α and CSF receptors on purified BMCs in vivo.30,31 Taken together, the indirect effects of IL-1α are most likely mediated through the type I IL-1R.

Therefore, to determine the type of IL-1Rs that mediates the direct proliferative effect of IL-1 on hematopoietic progenitors in vitro, we examined the synergistic effects of IL-1α on BMCs in colony-formation assays and in single-cell assays in the presence or absence of the MoAbs 35F5 and 4E2.

From the Laboratory of Molecular Immunoregulation, Biological Response Modifiers Program, and Biological Carcinogenesis and Development Program, Program Resources, Inc/DynCorp, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD; and the Department of Immunopharmacology and Molecular Genetics, Hoffmann-LaRoche, Nutley, NJ.

Address reprint requests to Jonathan R. Keller, PhD, Biological Carcinogenesis and Development Program, PRI/DynCorp, PO Box B, Frederick, MD 21702-1201.

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Blood, Vol 84, No 1 (July 1), 1994: op 125-132
II IL-1Rs (35F5 and 4E2); both monoclonal rat IgG) were prepared at Hoffmann-LaRoche (Nutley, NJ). The activity of IL-1α and IL-1β was 3 × 10^6 U/mg determined by thymocyte proliferation assay. Purified recombinant murine granulocyte macrophage (GM)-CSF was kindly provided by Dr Ian K. McNiece (Amen Corp, Thousand Oaks, CA) and recombinant murine IL-3 was purchased from R&D Systems (Minneapolis, MN). Recombinant human CSF-1 was a generous gift from Dr Michael Geier (Cetus Corp, Emeryville, CA).

**BMCs.** Normal murine BMCs were obtained by aspirating femurs of BALB/c mice with RPMI and then washed before resuspension in Iscove’s modified Dulbecco’s Medium (IMDM) supplemented with 10% fetal calf serum (FCS; Inovar, Gaithersburg, MD), 1% L-glutamine, and antibiotics (complete IMDM). The Lin^- BMCs were purified by immunomagnetic bead separation according to a previously described protocol.15 Briefly, light-density BMCs were obtained by separating BMCs on lymphocyte separation medium (Organon Teknika Corp, Durham, NC). The light-density BMCs were incubated in complete IMDM at 4°C for 30 minutes with a cocktail of antibodies: B220 (CD45; Pharmingen, San Diego, CA), RB6-8C5 (Gr-1; gift from Dr R.L. Coffman, DNAXCorp, Palo Alto, CA), MAC-1 (Boeringer Mannheim, Indianapolis, IN), Lyt-2 (CD8), and L3T4 (CD4) (both purchased from Becton Dickinson & Co, Sunnyvale, CA). The cells were washed and resuspended in complete IMDM before incubation with immunomagnetic beads (Dynal, Great Neck, NY) at a ratio 40:1 (beads/cell) for 30 minutes at 4°C. The Lin^- cells were removed by magnetic particle concentrator (Dynal). Lin- cells were labeled with biotinylated anti-Thy-1.2 or isotype-matched control IgG (Pharmingen) and washed before streptavidin-fluorescein isothiocyanate were added. Lin-Thy-1^+ and Lin-Thy-1^- cells were then sorted using FACSstar (Becton Dickinson).

**Thymocyte proliferation assay.** Thymus cells were obtained from 4- to 6-week-old normal C3H/HeN mice. The cells were washed in RPMI before being resuspended in complete IMDM. The cells, 1 × 10^6 cells/well, were seeded in 96-well flat microtiter plates (Costar, Cambridge, MA) and incubated with phytohemagglutinin (1 μg/mL) and IL-1α (1 μg/mL) in the presence or absence of MoAb 35F5 or control IgG for 72 hours at 37°C, 5% CO₂. The cells were pulsed with 1 μCi of ^3H-thymidine (6.7 Ci/mmol, New England Nuclear, Boston, MA) for the last 12 hours. Cell cultures were harvested with a multiple-sample automated cell harvester on glass fiber filters, individual filters were placed in scintillation fluid, and radioactivity was assessed by liquid scintillation counting.

**Colony growth.** Lin-, Lin-Thy-1^-, or Lin-Thy-1^+ BMCs were plated in 35-mm Lux Petri dishes (Miles Laboratories, Inc, Naperville, IL) in 1 mL of complete IMDM and 0.3% Seaplaque agarose (FMC Bioproducts, Rockland, ME) and supplemented with predetermined optimal concentrations of indicated HGFs. Dishes were incubated at 37°C in 5% CO₂ for 6 to 8 days before scoring for colony growth (>50 cells). Single-cell proliferation assays were performed in Terasaki plates (Nunc, Kamstrup, Denmark) at a concentration of 1 cell per well in 20 μL of complete IMDM and indicated growth factors. For each determination a total of 300 cells were examined. The frequency number represents the number of responding progenitors divided by the number of single cells plated (300 cells). Wells were scored for proliferation (>10 cells/well) after 6 days of incubation at 37°C and 5% CO₂. MoAbs against IL-1^- binding proteins or control IgG were preincubated with target cells for 45 minutes at 37°C before HGFs were added.

**Radioiodinated cytokines and binding assay.** Radioiodination of GM-CSF was performed by a modification of the chloramine-T method as described previously for IL-3.16 The specific radioactivity of ^125I-GM-CSF ranged from 4.0 to 7.0 × 10^6 cpm/μL. There was no significant loss in biologic activity of cytokines after the iodination procedure. The binding assays were performed by a previously described phtolate oil separation method.17 Lin^- cells were washed in RPMI 1640 and resuspended in 50 mmol/L glycine-hydrochloric acid for 1 minute to release bound ligands. The cells were washed in binding medium (RPMI 1640 containing 2% bovine serum albumin, 20 mmol/L HEPES, and 0.1% sodium azide) before radioiodinated GM-CSF was added to 2 × 10^6 cells. After 75 minutes of incubation at 20°C, cell-bound radioactivity was separated from unbound radioiodinated ligands by centrifugation of samples through a mixture of dibutyl phthalate and bis(2-ethylhexyl) phthalate oil (ratio 1:5:1) (Eastman Kodak, Rochester, NY). Specific binding was measured as the difference between counts in the presence and absence of 50-fold excess of cold competitor.

**RESULTS**

**Role of type 1 IL-1R in the synergistic effects of IL-1α on BMC growth in soft-agar colony assays.** Although the synergistic effects of IL-1 on hematopoietic progenitor growth have been proposed to be indirect, possibly through the induction of HGF production from hematopoietic accessory cells, we have also shown that IL-1α in combination with HGFs can directly promote the growth of purified progenitor cells in single-cell assay. In particular, the growth of Lin^- progenitors responsive to GM-CSF was enhanced by IL-1α in single-cell assays. However, using ^125I-IL-1α we have been unable to detect and define the type of IL-1R(s) constitutively expressed on purified progenitors. Therefore, using MoAb 35F5 we examined whether the synergistic effect of IL-1α in colony assays was mediated through IL-1R. First, Lin^- BMCs were plated in soft-agar assays with IL-1α (10 ng/mL) in combination with either IL-3 (30 ng/mL), GM-CSF (30 ng/mL), or CSF-1 (50 ng/mL). As previously shown, IL-1α enhanced the CSF-induced colony formation of all three CSFs examined (Fig 1). The highest synergy was observed between IL-1α and GM-CSF (140%), whereas IL-1α enhanced the CSF-1^- and IL-3^- induced colony formation by 90% and 60%, respectively (Fig 1). The ability of MoAb 35F5 (20 μg/mL) to block IL-1α^-enhanced CSF^-induced colony growth varied between the CSFs examined (Fig 1). Specifically, MoAb 35F5 inhibited 90% to 100% of the synergistic effect of IL-1α on IL-3^-induced colony formation, whereas the synergistic effect of IL-1α on CSF-1^- and GM-CSF^-induced progenitor growth was reduced 55% to 60% and 40% to 50%, respectively (Fig 1). Finally, 35F5-mediated inhibition of the synergistic effects of IL-1α was dose dependent, with maximal inhibition observed at 20 μg/mL of antibody (data not shown).

To further control for the amount of antibody required, we examined the concentration of MoAb 35F5 needed to recognize the type 1 IL-1R and inhibit IL-1α^-induced T-cell proliferation. The proliferation of thymocytes (1 × 10^6 cells/well) was determined by ^3H-thymidine incorporation after 72 hours of treatment with phytohemagglutinin (1 μg/mL) in combination with IL-1α (1 ng/mL) (Fig 2). When compared with the effect of MoAb 35F5 on IL-1α^-induced progenitor cell growth, MoAb 35F5 inhibited the IL-1α^-induced T-cell proliferation in a dose-dependent way with maximal inhibition (100%) obtained at 2.0 μg/mL (200-fold...
higher concentration compared with IL-1α; molar basis) of MoAb 35FSF (Fig 2).

Role of type I IL-1R in the direct synergistic effects of IL-1α. Because MoAb 35FSF could partially inhibit the synergistic effects of IL-1α on GM-CSF– and CSF-1–induced colony growth, we examined the ability of MoAb 35FSF to inhibit the direct synergistic effect of IL-1α on the growth of isolated Lin- cells in Terasaki plates. The frequency of Lin- cells that respond to IL-3 was 1:12 and, as previously reported and shown here for comparison, IL-1α did not enhance the frequency of Lin- progenitors responsive to IL-3 in single-cell assays (Table 1). Therefore, the synergistic effect of IL-1α on IL-3–induced Lin- progenitor growth was indirect and mediated through the type I IL-1R.

In contrast to the results observed with IL-3, the frequency of single Lin- cells responsive to GM-CSF and CSF-1 was increased by IL-1α; from 1:17 to 1:10 and from 1:50 to 1:27, respectively (Table 1). Similar to the results in colony assays, the synergistic effect of IL-1α on CSF-1–responsive single
Table 2. Effect of MoAb 35F5 on the Direct Synergistic Response of IL-1α on Progenitor Proliferation

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>MoAb 35FS</th>
<th>Freq.</th>
<th>% Stimulation</th>
<th>Freq.</th>
<th>% Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-3</td>
<td>−</td>
<td>6</td>
<td>−</td>
<td>12</td>
<td>−</td>
</tr>
<tr>
<td>IL-3 + IL-1α</td>
<td>−</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>−</td>
<td>75</td>
<td>−</td>
<td>10</td>
<td>−</td>
</tr>
<tr>
<td>GM-CSF + IL-1α</td>
<td>−</td>
<td>18</td>
<td>316</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>GM-CSF + IL-1α</td>
<td>+</td>
<td>20</td>
<td>261</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CSF-1</td>
<td>−</td>
<td>300</td>
<td>−</td>
<td>50</td>
<td>−</td>
</tr>
<tr>
<td>CSF-1 + IL-1α</td>
<td>+</td>
<td>27</td>
<td>713</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td>CSF-1 + IL-1α</td>
<td>+</td>
<td>42</td>
<td>638</td>
<td>43</td>
<td>13</td>
</tr>
</tbody>
</table>

Lin-Thy-1' and Lin-Thy-1- cells were separated according to Materials and Methods and seeded as single cells in Terasaki plates as described. The single-cell cultures were incubated at 37°C, 5% CO₂ in IL-3 (30 ng/mL), IL-1α (10 ng/mL), GM-CSF (30 ng/mL), or CSF-1 (50 ng/mL) in the absence (−) or presence (+) of MoAb 35FS (20 μg/mL). The frequency of responding progenitors (>10 cells/well) was determined after 6 days of incubation. The frequency of responding progenitors was obtained as described in Materials and Methods and Table 1. The results are representative of at least four separate experiments. The percent stimulation presented is the mean of at least four separate experiments, with at least 1,200 wells scored per group.

Abbreviation: ND, not determined.

progenitors was reduced 75% to 80% by MoAb 35FS (Table 1), and the synergistic effect on GM-CSF-induced single progenitor growth was only partially reduced (20%) by MoAb 35FS (Table 1). Thus, the synergistic effect of IL-1α on CSF-1-induced growth was direct and mainly mediated through the type I IL-1R, and the synergistic effect of IL-1α on GM-CSF-responsive Lin- progenitors was direct and only partially mediated through the type I IL-1R.

Because the Lin- cells represent a partially purified population of progenitors, this might account for the differential effects of MoAb 35FS on the synergistic effect of IL-1α. Lin+ cells expressing the Thy-1 antigen have previously been shown to be enriched for multipotential hematopoietic stem cells. The direct proliferative effect of IL-1α (10 ng/mL) on CSF-1- (50 ng/mL), GM-CSF- (30 ng/mL), and IL-3-induced (30 ng/mL) growth was examined on Lin-Thy-1' (Thy-1+) and Lin-Thy-1- (Thy-1-) cells in Terasaki plates. The frequency of progenitors responsive to IL-3 in single-cell assays was higher in Thy-1+ cells compared with Thy-1- cells, 1:6 versus 1:2 (Table 2), and similar to the results with Lin- cells no direct synergy between IL-1α and IL-3 was observed in either population (Table 2).

The proliferation of isolated progenitors responsive to CSF-1 was enhanced by IL-1α; 85% to 95% for Thy-1+ cells and approximately 700% for Thy-1- (Table 2). Although the direct synergistic effect of IL-1α on CSF-1-induced growth of Thy-1+ cells was inhibited 85% to 100% by MoAb 35FS (Table 2), the direct synergistic effect of IL-1α on the proliferation of CSF-1-induced by Thy-1+ single cells was marginally reduced (10% to 15%) in the presence of MoAb 35FS (Table 2). GM-CSF-induced proliferation of isolated Thy-1+ cells was enhanced (300%) by IL-1α, but IL-1α did not affect GM-CSF-induced progenitor growth of single Thy-1+ progenitors (Table 2). Similar to the results obtained with CSF-1 on the Thy-1+ population, the frequency of single Thy-1+ cells responsive to the combination of GM-CSF and IL-1α was partially reduced (15% to 25%) by the MoAb against the type I IL-1R (Table 2).

Thus, IL-1α and CSF-1 directly synergized through the type I IL-1R on Thy-1+ progenitors, whereas the direct synergistic response of IL-1α on both CSF-1 and GM-CSF-responsive Thy-1+ progenitors was only marginally inhibited by MoAb 35FS.

Role of type II IL-1R in the growth of Lin-Thy-1+ cells. These results suggested that the direct synergistic effect of IL-1α on Lin-Thy-1+ progenitors could be mediated through the type II IL-1R. Therefore, the effect of MoAb 4E2 on IL-1α-induced progenitor growth was examined on Thy-1+ cells in single-cell assays. The MoAb 4E2 has been shown to block the binding of radioiodinated IL-1α to the type II IL-1R but not the type I IL-1R; however, the affinity of this MoAb for the type II IL-1R is lower than the affinity of MoAb 35FS for the type I IL-1R (R.C., unpublished observation, October 1992). Preliminary experiments showed that the binding of radioiodinated IL-1α on both unseparated BMCs and NFS-60 cells (murine myeloid progenitor cell line with high numbers of IL-1R) was completely inhibited by MoAb 4E2 (200-fold higher concentration compared with IL-1α in a molar basis) (data not shown). Because MoAb 4E2 has a reduced affinity for type II IL-1R, we also reduced the concentration of IL-1α in the single-cell assays; therefore, we used IL-1α at 0.5 ng/mL and MoAbs 4E2 and 35FS at 20 μg/mL (4,000-fold higher concentration of MoAb compared with IL-1α; molar basis). As a result of using 0.5 ng/mL IL-1α, the direct synergistic effect of IL-1α on CSF-1 and GM-CSF-induced proliferation of Thy-1+ cells was reduced 50% to 60% compared with the optimal dose of IL-1α (10 ng/mL). Similar to the results obtained with IL-1α at 10 ng/mL, the addition of the MoAb 35FS reduced the frequency of Thy-1+ single progenitor responding to GM-CSF plus IL-1α (0.5 ng/mL) by approximately 20% (Fig 3). However, in some experiments we could detect up to 60% to 70% inhibition. The synergistic effect of IL-1α (0.5 ng/mL) on CSF-1-induced Thy-1+ progenitor growth in Terasaki plates was inhibited 100% by MoAb 35FS (20 μg/mL) (Fig 3). In comparison, the MoAb 4E2 at 4,000-fold higher concentration than IL-1α did not decrease the direct synergistic effect of IL-1α on GM-CSF responsive Thy-1+ progenitors or significantly reduce the frequency of single Thy-1+ cells responsive to IL-1α (0.5 ng/mL) and CSF-1 (Fig 3).

Taken together, the direct synergistic effect of IL-1α on CSF-1-responsive progenitors, both Thy-1+ and Thy-1- occurred through the type I IL-1R and not the type II IL-1R. GM-CSF and IL-1α directly synergize to promote the proliferation of Thy-1+ but not Thy-1- progenitors, partially through the type II IL-1R but not the type II IL-1R.

To show that we could completely inhibit the direct synergistic response of IL-1 on GM-CSF-responsive progenitors
CELL GROWTH THROUGH TYPE I IL-1 RECEPTOR

In our assay system, we examined the effect of MoAb 4C5 on IL-1α- and IL-1β-induced proliferation of Thy-1+ progenitors in single assays. MoAb 4C5 recognizes a 96-kD cell membrane protein expressed by all cells with IL-1Rs (R. Chizzonite, P. Nunes, T. Truit; manuscript in preparation, October 1993). The cDNA for this protein has been recently cloned and shows homology with the type I IL-1R (approximately 65%) but is distinct from both type I and type II IL-1Rs. The 4C5 antibody binds to recombinant 4C5 protein expressed in COS cells. MoAb 4C5 has been shown to specifically inhibit the proliferative effect of IL-1β on lymphocytes but had no effect on the proliferative response of IL-1α (R.C., unpublished observation, October 1992). Although IL-1α–induced proliferation was not affected by MoAb 4C5, the direct synergistic effect of IL-1β on GM-CSF–responsive progenitors was completely (100%) inhibited by MoAb 4C5 (Table 3).

Finally, because MoAb 4E2 has been shown to have an agonistic effect on the growth of lymphocytes (R.C., unpublished observation, January 1991), we examined the effect of MoAb 4E2 on the growth of isolated Thy-1+ cells in the presence of CSF-1 or GM-CSF. The MoAb 4E2 (25 μg/mL) did not enhance GM-CSF– or CSF-1–induced progenitor cell growth in single-cell assays (Table 4).

IL-1α indirectly enhanced the IL-3–induced progenitor growth of Lin-Thy-1+ cells. Although IL-1α did not directly synergize with IL-3 to promote the growth of Lin+, Lin-Thy-1+, or Lin-Thy-1+ cells in Terasaki plates, it did synergize with IL-3 to promote the growth of Lin+ cells in colony assays (Fig 1). Therefore, we examined whether IL-1α could also indirectly promote the growth of FACS-separated Thy-1+ or Thy-1+Lin+ progenitors in colony assays. Lin-Thy-1+ and Lin-Thy-1+ cells were plated in soft-agar assays with IL-3 (30 ng/mL) alone or in the combination with IL-1α (10 ng/mL). Interestingly, although IL-1α (10 ng/mL) did not enhance IL-3–induced colony growth of Thy-1+ cells, it did enhance the growth of IL-3–responsive Thy-1+ cells (2.5 × 105 cells/well) by 60% (Fig 4). The synergistic effect of IL-1α on IL-3–responsive Thy-1+ progenitors in colony assay was completely inhibited by MoAb 35F5 (20 mg/mL) (Fig 4).

**Table 3. Effect of MoAb 4C5 on IL-1α– and IL-1β–Induced Progenitor Growth**

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>Single-Cell Growth (N/300)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>5</td>
</tr>
<tr>
<td>GM-CSF + IL-1α</td>
<td>10</td>
</tr>
<tr>
<td>GM-CSF + IL-1α + MoAb 4C5</td>
<td>13</td>
</tr>
<tr>
<td>GM-CSF + IL-1β</td>
<td>13</td>
</tr>
<tr>
<td>GM-CSF + IL-1β + MoAb 4C5</td>
<td>3</td>
</tr>
</tbody>
</table>

Lin-Thy-1+ cells were separated according to Materials and Methods and seeded as single cells in Terasaki plates as described. The single-cell cultures were incubated at 37°C, 5% CO₂ in IL-1α (0.5 ng/mL), IL-1β (50 ng/mL), GM-CSF (30 ng/mL), or MoAb 4C5 (20 μg/mL) as indicated. The growth of single cells was presented as the number (N) of responding progenitors per 300 cells (1 cell/well) and after 6 days of incubation scoring positive wells (>10 cells/well). The results are one representative of two separate experiments.

**Table 4. Agonistic Effect of MoAb 4E2 on Progenitor Growth**

<table>
<thead>
<tr>
<th>Stimulator</th>
<th>Single-Cell Growth (Thy-1+) Freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>33</td>
</tr>
<tr>
<td>GM-CSF + IL-1α</td>
<td>15</td>
</tr>
<tr>
<td>GM-CSF + MoAb 4E2</td>
<td>37</td>
</tr>
<tr>
<td>GM-CSF + control IgG</td>
<td>37</td>
</tr>
<tr>
<td>CSF-1</td>
<td>100</td>
</tr>
<tr>
<td>CSF-1 + IL-1α</td>
<td>21</td>
</tr>
<tr>
<td>CSF-1 + MoAb 4E2</td>
<td>100</td>
</tr>
<tr>
<td>CSF-1 + control IgG</td>
<td>100</td>
</tr>
</tbody>
</table>

Lin-Thy-1+ cells were separated according to Materials and Methods and seeded as single cells in Terasaki plates as described. The single-cell cultures were incubated at 37°C, 5% CO₂ in IL-1α (0.5 ng/mL), GM-CSF (30 ng/mL), CSF-1 (50 ng/mL), MoAb 4E2 (25 μg/mL), or control IgG (25 μg/mL) as indicated. The frequency of responding progenitors (>10 cells/well) was determined after 6 days of incubation. The frequency of responding progenitors was obtained as described in Materials and Methods and Table 1. The results are one representative of two separate experiments.
was scored after 7 to 10 days respectively. The cultures were incubated at 37°C, 5% CO₂ in IL-1α and plated in soft-agar assay at 2,500 cells/plate.

Lin-Thy-1' cells were separated according to Materials and Methods of HEStDAL ET AL as the mean (10 ng/mL) and 11-3 (30 ng/mL) in the presence of IL-1α and IL-3 was observed in colony assays using both Lin and Lin 'Thy-1' cells at a low-plating density (2,500 Lin 'Thy-1' cells/plate). Thus, the use of purified progenitors plated at low-cell density in colony assays does not exclude the possibility of indirect effects.

IL-1α has been shown to induce the production of CSFs both in vitro and in vivo; however, the synergistic effect of IL-1α on IL-3–responsive progenitors could be indirectly mediated through HGF(s) production by Lin cells. We were able to detect any synergistic effect of conditioned medium obtained from IL-1α–stimulated Lin cells (IL-1–conditioned medium) on IL-3–responsive progenitor growth (K.H. et al, unpublished observation, May 1993). Furthermore, IL-1–conditioned medium did not stimulate the proliferation of cell lines responsive to IL-3, GM-CSF, IL-6, G-...
GM-CSF receptor upmodulation but only partially reduces effect of combined HGFs, or membrane-bound forms of HGFs cannot be ruled out to explain the observed indirect effects.

We and others have shown that MoAb 35F5 inhibits the biologic effects of IL-1α on cells expressing type I IL-1R both in vitro and in vivo.\(^7,8,13^{-15}\) Because IL-1R I is expressed on hematopoietic accessory cells such as T cells and stromal cells but not detectable on progenitor cells, the effects of IL-1α on hematopoiesis were proposed to be indirectly mediated through type I IL-1R. However, in this study we show that the effect of IL-1α on the proliferation of CSF-1-responsive Thy-1+ and Thy-1- progenitors is direct and blocked by antibody to the type I receptor.

In contrast to the direct effect of IL-1α on CSF-1-responsive progenitors, the direct synergistic effect of IL-1α on GM-CSF-induced progenitor growth was only partially inhibited by MoAb 35F5. However, in some experiments at lower doses of IL-1α we could inhibit as much as 60% to 70% of the direct synergistic effects of IL-1α and GM-CSF. In this regard, we have recently shown that GM-CSF can induce IL-1R expression on hematopoietic progenitor cells in vitro;\(^12\) therefore, upmodulation of IL-1Rs by GM-CSF may explain the incomplete inhibition of the direct synergistic effect of IL-1α on GM-CSF-responsive progenitors observed by both MoAbs 35F5 and 4E2. Finally, IL-1α–binding protein(s) other than type I and type II IL-1R, as previously described,\(^9,12,15\) may be important in the direct synergistic effect of IL-1α in hematopoietic progenitor growth.

Despite the inability of anti–IL-1R II and anti–IL-1R I to completely inhibit the direct synergistic effects of IL-1α on GM-CSF–induced progenitor growth, we showed that MoAb 4C5 completely inhibited the synergistic response of IL-1/β in combination with GM-CSF on these progenitors, whereas the synergistic effect of IL-1α was not inhibited. This is comparable with the observation using MoAb 4C5 on IL-1–induced B-cell growth (R.C., unpublished observation, January 1992). This indicates that blocking IL-1 binding and signal transduction inhibits the direct synergistic effects of IL-1α on GM-CSF–responsive progenitors.

We also show that IL-1α enhanced GM-CSF–induced colony formation of Lin– cells by 140% (Fig 1) and that this was reduced to 80% in the presence of MoAb 35F5 compared with GM-CSF alone (Fig 1). In comparison, IL-1α enhanced GM-CSF–induced growth of isolated Lin– cells by 70% in single-cell assays that were partially inhibited by MoAb 35F5 (Table 1). This indicates that a portion of the synergistic response between IL-1α and GM-CSF in colony assay is indirectly mediated through the type I IL-1R whereas the remainder of the synergistic response is direct and partially mediated through the type I IL-1R.

We have proposed that one mechanism for the synergistic effect of IL-1α on GM-CSF–induced progenitor growth could be IL-1α–induced upmodulation of GM-CSF receptors on Lin– progenitor cells.\(^21\) We show here that pretreatment of Lin– cells with MoAb 35F5 inhibits IL-1α–induced GM-CSF receptor upmodulation but only partially reduces IL-1α–induced progenitor growth. Thus, the marginal inhibition of the direct synergistic effect of IL-1α on GM-CSF–induced progenitor growth by MoAb 35F5, together with the observed inhibition of GM-CSF receptor upmodulation by anti–IL-1R I, indicates that IL-1α may directly synergize with GM-CSF on progenitor cells by mechanism(s) other than receptor modulation.

IL-1 is important in the regulation of hematopoietic growth and differentiation. In particular, it has proven to be essential in the stimulation of very immature progenitor cells, such as high proliferative potential colony-forming cells.\(^3,4\) We have shown that IL-1α can enhance progenitor growth both indirectly and directly through the type I IL-1R. Because IL-1R II has been shown to be expressed on BMCs the role of this receptor in the regulation of hematopoietic development remains to be determined.

**REFERENCES**

Interleukin-1 (IL-1) directly and indirectly promotes hematopoietic cell growth through type I IL-1 receptor

K Hestdal, FW Ruscetti, R Chizzonite, M Ortiz, JM Gooya, DL Longo and JR Keller