Effects of Hematopoietin-1 and Interleukin 1 Activities on Early Hematopoietic Cells of the Bone Marrow

By K.M. Zsebo, J. Wyprych, V.N. Yuschenkoff, H. Lu, P. Hunt, P.P. Dukes, and K.E. Langley

Hematopoietin-1 (H-1) was purified from the human cell line 5637 and two amino acid sequences were observed in the preparation. One sequence was identical to that of interleukin 1α (IL 1α) and the other to that of IL 1β. The action of recombinant IL 1α and other hematopoietic growth factors was studied using (a) a proliferative potential colony-forming cell assay that uses primitive hematopoietic precursors from bone marrow, and (b) a spleen colony-forming unit assay. The results indicate that the IL 1α target cell population is different than the target cell populations of IL 3, granulocyte-macrophage colony-stimulating factor, and granulocyte colony-stimulating factor; that IL 1α in combination with mononuclear phagocyte colony-stimulating factor provides a proliferative stimulus; and that IL 1α has at least a survival-enhancing and possibly proliferation-inducing effect on primitive hematopoietic stem cells.

Personal communication, September, 1987. Moreover, based on serologic data and protein purification methods, the identity of H-1 and IL 1 by amino acid sequencing of H-1 purified from the human bladder carcinoma cell line 5637. In addition, we have further characterized the interaction of recombinant IL 1 (rIL 1) with early hematopoietic cells of the bone marrow.

MATERIALS AND METHODS

Sources of growth factors and antibodies. CSF-1 was prepared from MiaPaCa cell conditioned media. CSF-1 activity was determined by colony number in mouse bone marrow agar cultures as described. The amount giving half-maximal colony stimulation was assigned to be 50 U. Approximately 300 U/mL was used in the HPP-CFC assay. Human rIL 1α and rIL 1β were purchased from Genentech (Boston) or Genzyme (Boston). Murine rIL 3 and murine rGM-CSF were purchased from Genzyme. One unit is defined as the amount of test factor that produces a single colony (>50 cells) per 10° normal murine bone marrow cells in soft agar. Polyvalent anti-IL 1 antibodies made against rIL 1α or rIL 1β were purchased from Endogen. Human rG-CSF was purified from Escherichia coli. All recombinant factors were purified to homogeneity.

Conditioned medium from 5637 (1A6) cells. Supernatant fluids were prepared from the human bladder carcinoma cell line 5637 (subclone 1A6), essentially as described. In brief, cells were grown in Iscove’s medium (GIBCO, Santa Clara, CA) containing either 10% vol/vol fetal calf serum (FCS) (growth phase) or 0.2% (vol/vol) FCS (harvest phase) in roller bottles (Corning 900 cm²) containing Cytodex glass microcarriers (Pharmacia, Piscataway, NJ).

HPP-CFC assay (H-1 assay). H-1 was assayed by its ability to stimulate large high proliferative colony-forming cells (HPP-CFC) in combination with CSF-1. This assay differs from previous HPP-CFC assays primarily by the use of 2-day post 5-Fluorouracil (5-FU) bone marrow and by the enrichment for hematopoietic progenitor cells used in the assay. Previously described assays used bone marrow cells from mice injected 8 days prior with 5-FU, contained slightly different media components, and did not use partially purified progenitor cell populations. Female Balb/C mice (6 weeks of age, Simonsen, Gilroy, CA) were injected intravenously (IV) with 150 mg/kg 5-FU. Two days later, the femurs were removed and the bone marrow was harvested. Low density, nonadherent cells were obtained by Ficoll-Paque (Pharmacia) centrifugation and nonadherence to plastic plates. Bone marrow cells (1 × 10⁶ cells) were cultured in 1 mL 1× McCoy’s Complete as described containing 0.3% (wt/vol) Noble agar (Difco), 20% (vol/vol) FCS and combinations of hematopoietic growth factors in 35-mm Petri dishes. Dishes were incubated 14 days at 37°C in a humified atmosphere.
environment containing 5% CO2. Colonies 1 mm in diameter were scored under a 2x dissecting microscope. Due to the enrichment for hematopoietic progenitor cells, all colonies formed were 1 mm in diameter. One unit of H-1 activity is defined as the amount giving half-maximal stimulation of HPP-CFC in the presence of -100 U/mL CSF-1.

**Thymocyte comitogenicity assay for IL 1.** IL 1 was assayed essentially as described28 with minor modifications. In brief, samples were cultured with 1.2 x 106 murine thymocytes (female C3H/HeJ, 4 to 8 weeks of age; Jackson Labs, Bar Harbor, ME) in 200 mL of RPMI 1640 supplemented with 5 x 10-5 mol/L 2-mercaptoethanol, 200 mmol/L glutamine, 10% vol/vol FCS and 1.5 mmol/L phytohemagglutinin (PHA-L) (Sigma, St Louis). Cultures were incubated for 72 hours in 5% CO2 at 37°C and pulsed with 1.5 μCi 3H-thymidine (New England Nuclear) for the final 6 hours of culture. Cells were harvested onto filter paper disks by a PHD cell harvester (Cambridge Technology, Cambridge, MA). Disks were measured for radioactivity scintillation counting. Units of 5637-derived IL 1 were assigned by comparison to the activity of human rIL 1α where one unit is defined as the amount required to double the proliferative response of murine thymocytes stimulated with 1.0 μg/mL PHA-L alone.

**Measurements of growth factor effects on CFU-S in culture.** Female B6D2F1 mice, 12 to 16 weeks of age, were used (Jackson Labs). Marrow cells were flushed from femurs with α-medium into a sterile tube and dispersed to single cells by repeated pipetting. The cell number was determined in a hemocytometer, and resuspended in culture medium containing 30% (vol/vol) FCS with or without growth factor at 37°C in a humidified atmosphere of 5% CO2. After sample harvest by centrifugation, cells were harvested by centrifugation and resuspended in culture medium for assay of CFU-S.

The CFU-S assay was performed essentially as described27 as modified in our laboratory.Recipient mice were administered 900 rad whole body irradiation from a 60Co source at a dose rate of 80 rad/minutes. They were injected in the tail vein with 2.8 x 106 cells (based on the initial cell count) suspended in 0.3 mL medium. Eight to ten recipient mice were used for each experimental group. Irradiated mice were protected from infection by housing them in a laminar flow hood and by providing them with acidified water. Twelve days later, spleens were harvested, fixed in Bouin’s solution, and then transferred into 70% alcohol. Colonies were counted with a dissecting microscope at 8× magnification. Student’s t statistics were used to assess significance of the results.

**Purification of H-1/IL 1 activity.** The purification scheme used was based on the procedure reported by Jubinsky and Stanley.28 All operations were carried out at 4°C unless indicated otherwise.

Forty liters of 5637 conditioned medium was concentrated to 1.5 L by ultrafiltration using a Millipore Pellicon cassette system with two Millipore 10-μm cut-off polysulfone membrane cassettes. Following the concentration, the material was dialyzed against 8 L 50 mmol/L Tris/HCl, pH 8.3, and diluted with this buffer to a final volume of 2.85 L.

The dialyzed material was applied to diethylamino ethanol (DEAE)-cellulose (Whatman DE-52) anion-exchange column equilibrated with 50 mmol/L Tris/HCl, pH 8.3. After sample application, the column was washed with 2 L Tris buffer and a linear 10-L gradient of 0 to 300 mmol/L NaCl in the Tris buffer was then applied for elution. Two separated pools with H-1/IL 1 activity were obtained: pool a of 680 mL eluted at 80 to 105 mmol/L NaCl, and pool b of 570 mL eluted at 115 to 135 mmol/L NaCl. The two pools were treated separately through subsequent purification steps, as indicated by designations a and b below.

Pools a and b from ion exchange were each applied to an Ultrogel ACA34 (LKB) gel filtration column. Pools of active material from the gel filtration columns (cases a and b, handled separately) were subjected to chromatofocusing. Two separate active pools (corresponding to pH ~4.8 and pH ~5.3) were obtained from chromatofocusing for both case a and case b. The pH 4.8 pools from the two cases were combined; similarly, the pH 5.3 pools from the two cases were combined.

The pH 4.8 pool and the pH 5.3 pool from chromatofocusing (handled separately) were chromatographed on phenyl-Sepharose CL-4B columns. The pool of active dialyzed fractions from phenyl-Sepharose (pH 4.8 case) was applied to a sodium dodecyl sulfate (SDS)-polyacrylamide gel.29 Sample preparation was accomplished by heating at 55°C for 50 minutes in sample treatment buffer containing 0.7 mol/L 2-mercaptoethanol; thioglycolic acid (0.1 mmol/L) was included in the cathode reservoir buffer. After electrophoresis, the section in which H-1 activity migrates (described in the Results section) was cut out of the fixed gel and subjected to electroelution29; 700 μL of electroeluted material was obtained.

**N-Terminal amino acid sequence analysis.**Automated Edman degradation was performed with a gas phase sequencer (Applied Biosystems, Foster City, CA) according to previously reported procedures.28,29 Approximately 100 pmol electroeluted sample was subject to sequence analysis. The released phenylthiohydantoin (PTH)-amino acids were identified by reverse-phase narrow-bore high-pressure liquid chromatography (HPLC). 30

**Analytical methods.** Protein was determined using bovine serum albumin (BSA) as standard.24 Silver staining of SDS-polyacrylamide gels was performed according to Morrissey.31

**RESULTS**

**Purification of H-1/IL 1.** The purification of H-1/IL 1 is based on previously reported procedures,28 and results are summarized in Table 1. The H-1 and IL 1 activities copurified throughout the procedure. Most column eluates (ion exchange, chromatofocusing, phenyl-Sepharose) were assayed on a fraction-by-fraction basis for both activities, and coelution was observed in each case.

Two well-separated peaks of activity eluted from the ion-exchange column (step 2), and these peaks were taken separately through subsequent purification steps. In chromatofocusing (step 4), both pools arriving through gel filtration showed two peaks of activity, eluting at pH ~5.3 and pH ~4.8. This suggests that the difference in the two peaks of activity separated by ion exchange (step 2) reflects molecular heterogeneity other than difference in charge alone. The combined major chromatofocusing peaks at pH 4.8 and the combined minor peaks at pH 5.3 were applied to phenyl-Sepharose, but only the pH 4.8 material was then further subjected to preparative SDS-polyacrylamide gel electrophoresis (PAGE). In general, the behavior of the H-1 material upon chromatofocusing, gel filtration, and phenyl-Sepharose chromatography was very similar to that described by Jubinsky and Stanley.28 Using the method described by these authors, we confirmed their result that H-1 activity was present in a gel region corresponding to ~20 kDa in mol wt; activity was not evident in any other gel regions. This information was used to cut out and electroelute a gel region covering mol-wt range 17.5 kDa to 20.5 kDa after electrophoresis of the pH 4.8 material. Purity of material before and after this preparative electrophoresis step is shown in Fig 1. Two closely migrating bands are evident after the electroelution (apparent mol wt 19.6 kDa and 18.5 kDa from www.bloodjournal.org by guest on August 30, 2017. For personal use only.
### Table 1. Purification of H-1/IL-1 from 5637 Conditioned Medium

<table>
<thead>
<tr>
<th>Purification Step</th>
<th>Origin of Applied Sample</th>
<th>Total Protein (mg)</th>
<th>Total H-1 Activity (U)</th>
<th>Total IL-1 Activity (U)</th>
<th>Specific Activity (U/mg)</th>
<th>Purification Fold, H-1</th>
<th>Overall Yield, H-1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5637 Conditioned Medium</td>
<td>—</td>
<td>7,720</td>
<td>2.4 x 10^6</td>
<td>8.3 x 10^4</td>
<td>311</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>1. Ultrafiltration</td>
<td>—</td>
<td>7,980</td>
<td>2.3 x 10^6</td>
<td>288</td>
<td>0.93</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>2. Anion exchange Pool a</td>
<td>Step 1</td>
<td>3,244</td>
<td>8.2 x 10^4</td>
<td>7 x 10^4</td>
<td>252</td>
<td>0.81</td>
<td>54</td>
</tr>
<tr>
<td>2. Anion exchange Pool b</td>
<td>—</td>
<td>1,470</td>
<td>3.4 x 10^4</td>
<td>1.2 x 10^4</td>
<td>233</td>
<td>0.75</td>
<td>54</td>
</tr>
<tr>
<td>3. Gel filtration a. Anion exchange pool a</td>
<td>—</td>
<td>4.1</td>
<td>2.0 x 10^4</td>
<td>4 x 10^3</td>
<td>48,780</td>
<td>157</td>
<td>26</td>
</tr>
<tr>
<td>3. Gel filtration b. Anion exchange pool b</td>
<td>—</td>
<td>2.6</td>
<td>3.2 x 10^4</td>
<td>2.8 x 10^3</td>
<td>123,077</td>
<td>396</td>
<td>26</td>
</tr>
<tr>
<td>4. Chromatofocusing a. Step 3a</td>
<td>—</td>
<td>1.34</td>
<td>2.52 x 10^4</td>
<td>8 x 10^3</td>
<td>188,059</td>
<td>605</td>
<td>11</td>
</tr>
<tr>
<td>4. Chromatofocusing b. Step 3b</td>
<td>—</td>
<td>0.344</td>
<td>2 x 10^4</td>
<td>3.6 x 10^3</td>
<td>581,395</td>
<td>1,869</td>
<td>9</td>
</tr>
<tr>
<td>5. Phenyl-Sepharose from steps 4a + 4b</td>
<td>0.344</td>
<td>2 x 10^4</td>
<td>3.6 x 10^3</td>
<td>581,395</td>
<td>1,869</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>5. Phenyl-Sepharose pH 5.3 pool from steps 4a + 4b</td>
<td>ND</td>
<td>1.7 x 10^4</td>
<td>3 x 10^4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6. Preparative electrophoresis Step 5 (pH 4.8 case)</td>
<td>0.005</td>
<td>(4.5 x 10^3)t</td>
<td>(1.2 x 10^3)t</td>
<td>900,000</td>
<td>4.5%t</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Starting with 40 L culture medium; as described in Materials and Methods.

*Estimate, based on intensity of silver-stained gel band after SDS-PAGE.

†Recovery of H-1/IL-1 polypeptide at this step was ~50% relative to the amount present at step 5 (see overall yield column); however, recoveries of activities were very low, probably because of the harsh nature of this step. The overall purification, based on recovery of polypeptide, would be ~64,000-fold.

‡Estimate based on intensity of silver-stained gel band after SDS-PAGE; recovery values for steps 1 through 5 are based on H-1 activity.

Starting with 40 L culture medium; as described in Materials and Methods.

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‡Estimate based on intensity of silver-stained gel band after SDS-PAGE; recovery values for steps 1 through 5 are based on H-1 activity.

![SDS-PAGE (reducing conditions) with silver staining.](image)

Fig 1. SDS-PAGE (reducing conditions) with silver staining. Lane 1, pool after phenyl-Sepharose, step 5a; lane 2, material after preparative electrophoresis/electroelution, step 6; lane 3, mol-wt markers [BioRad; the six major bands, from top to bottom in the gel lane, represent phosphorylase b (92.5 kDa), BSA (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and lysozyme (14.4 kDa)].

![N-Terminal sequence of material purified as in Table 1 (H-1)].](image)

Fig 2. N-Terminal sequence of material purified as in Table 1 (H-1). Two sequences were identified. Both IL 1α and IL 1β sequences from cDNA38 are shown for comparison; X denotes cycles in which no residues could be identified.

relative to the mol-wt markers used). The preparation containing both species was subjected to N-terminal amino acid sequencing (below). The material was active in biologic assays for both H-1 and IL 1 (Table 1), although there was a significant loss of biologic activity as a result of the electrophoresis step.

**N-Terminal amino acid sequence.** Purified material after SDS-PAGE/electroelution (Table 1, step 6; Fig 1) was subjected to sequence analysis. Two major N-terminal sequences could be identified, one through 12 cycles and the other through 19 cycles (Fig 2). The two sequences match those described for human IL 1α and IL 1β38 except that the Ser residue after Leu7 in the reported IL 1α sequence was not detected. The ratio of IL 1α to IL 1β was ~60:40 as judged by the yield of PTH-amino acids.

**Effect of IL 1 on early hematopoietic progenitors.** To investigate the effects of H-1/IL 1 on the growth of the earliest hematopoietic cells, the assay system utilized mouse bone marrow cells derived from animals 2 days after 5-FU treatment. Other researchers37 have shown that 5-FU selec-
tively depletes precursor cells responsive to CSF-1. In addition, as shown in Table 2, the combination of 5-FU treatment and enrichment of early progenitors by density-gradient fractionation and adherence depletion removes the target cells responsive to rIL 3, rGM-CSF, and rG-CSF. H-1/IL 1 does not stimulate colony formation by itself, but CSF-1 in combination with H-1/IL 1 or rIL 3 induces the formation of large colonies of HPP-CFC. Both rIL 1α and rIL 1β are active in combination with CSF-1 in the HPP-CFC assay (Table 2).

The N-terminal sequence analysis of the H-1/IL 1 material purified from 5637 conditioned medium suggested that IL 1α and IL 1β were present in roughly equal amounts. Both HPP-CFC colony generation (H-1 assay) and thymocyte comitogenicity (IL-1 assay), however, could be completely neutralized by an anti-IL 1α but not by an anti-rIL 1β antibody (Tables 2 and 3). Thus, the IL 1β in the H-1 preparation was apparently not active.

We sought to determine whether the target cells for H-1/IL 1 plus CSF-1 and IL 3 plus CSF-1 are the same. Combinations of these factors in the presence of CSF-1 were tested for the capacity to form colonies of HPP-CFC. If the factors act on the same progenitor population, combining them at dosages that generate maximal colony formation by each alone should not increase the total number of colonies formed. The dose responses in the HPP-CFC assay for H-1, IL 1α, and IL 3 are shown in Fig 3. These assays are performed in the presence of CSF-1 since the generation of HPP-CFC has an absolute requirement for CSF-1. The combination of IL 1 plus IL 3, GM-CSF, and G-CSF does not lead to HPP-CFC colony formation in the absence of CSF-1. Similar results were obtained in two separate experiments. Both H-1 and rIL 1α generated similar numbers of HPP-CFC colonies, but IL 3 consistently generated fewer colonies than did H-1 or IL 1α. Combining maximal concentrations of H-1 and rIL 1α did not increase the total number of colonies formed, suggesting that the two molecules act on the same target population. In contrast, the effects of rIL 1α and rIL 3 on colony numbers were additive; this observation supports previous reports that the targets of H-1 and IL 3 that synergize with CSF-1 are different.

Table 2. Effect of Various Growth Factors in the HPP-CFC Assay

<table>
<thead>
<tr>
<th>Growth Factor Added</th>
<th>No. of Colonies/10⁶ Cells*</th>
<th>- CSF-1†</th>
<th>+ CSF-1§</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-1† (2 U)</td>
<td>0</td>
<td>19 ± 3</td>
<td></td>
</tr>
<tr>
<td>Human rIL 1β (100 U)</td>
<td>0</td>
<td>20 ± 2</td>
<td></td>
</tr>
<tr>
<td>Human rIL 1α (50 U)</td>
<td>0</td>
<td>19 ± 2</td>
<td></td>
</tr>
<tr>
<td>Murine rIL 3 (100 U)</td>
<td>0</td>
<td>10 ± 1</td>
<td></td>
</tr>
<tr>
<td>Murine GM-CSF (250 U)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Human G-CSF (250 U)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>H-1† (4 U) + anti-rIL 1α antibody†</td>
<td>-</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>H-1† (4 U) anti-rIL 1β antibody†</td>
<td>-</td>
<td>18 ± 3</td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>-</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*One SD, n = 3.
†H-1 from step 5 (Table 1) was used.
‡No colonies present of any size.
§All colonies ≥ 1 mm in diameter.
Polyvalent anti-rIL 1 antibodies 40 µg/mL.

Does IL 1 induce the synthesis of other factors that enhance the proliferation of HPP-CFC? Although early hematopoietic progenitor cells were enriched by 5-FU treatment, density separation, and adherence depletion, it remained possible that H-1/IL 1 induced the synthesis of other hematopoietic growth factors from accessory cells that actually led to the generation of HPP-CFC colonies. For example, IL 1 is known to stimulate release of GM-CSF and G-CSF. To investigate this possibility, conditioned medium was generated from 5-FU–treated bone marrow stimulated with 50 U/mL rIL 1α for 6 days. This conditioned medium was then tested in the HPP-CFC assay. As shown in Table 4, there was significant activity; however, it could be completely neutralized by an antibody directed against rIL 1α. This antibody is specific for rIL 1α since it does not inhibit HPP-CFC colony formation by murine rIL 3 (Table 4) or IL 1β activity in the thymocyte proliferation assay.

Table 3. Effect of Various Growth Factors in IL 1 Thymocyte Comitogenicity Assay

<table>
<thead>
<tr>
<th>Growth Factor Added*</th>
<th>Antibody†</th>
<th>cpm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>940 ± 97</td>
</tr>
<tr>
<td>rIL 1α</td>
<td>—</td>
<td>36,871 ± 10,260</td>
</tr>
<tr>
<td>rIL 1α anti-rIL 1α</td>
<td>—</td>
<td>1,041 ± 316</td>
</tr>
<tr>
<td>rIL 1α anti-rIL 1β</td>
<td>—</td>
<td>38,090 ± 6,313</td>
</tr>
<tr>
<td>rIL 1β</td>
<td>—</td>
<td>16,584 ± 501</td>
</tr>
<tr>
<td>rIL 1β anti-rIL 1α</td>
<td>—</td>
<td>14,704 ± 3,039</td>
</tr>
<tr>
<td>rIL 1β anti-rIL 1β</td>
<td>—</td>
<td>1,491 ± 231</td>
</tr>
<tr>
<td>H-1</td>
<td>—</td>
<td>16,914 ± 5,156</td>
</tr>
<tr>
<td>H-1 anti-rIL 1α</td>
<td>—</td>
<td>902 ± 23</td>
</tr>
<tr>
<td>H-1 anti-rIL 1β</td>
<td>—</td>
<td>13,262 ± 2,122</td>
</tr>
</tbody>
</table>

*Human rIL 1α was 15 U/mL; human rIL 1β was 12.5 U/mL; H-1 from step 4 (Table 1) was used at a concentration equivalent to 10 U/mL rIL 1α.
†10 µg/mL.

Fig 3. Combinations of growth factors in the HPP-CFC assay. Various amounts of growth factors were added to the HPP-CFC assay as described in Materials and Methods in the presence of CSF-1. Error bars indicate 1 SD of triplicate cultures. The IL 1α was human rIL 1α; the H-1 was from step 5 (Table 1); the IL 3 was murine rIL 3.
Three experiments were performed with similar results; suspensions. The effect of growth factor was dose dependent. The CFU-S assay serves as an in vivo test for the presence of hematopoietic progenitor cells injected into irradiated recipient mice. Recently, two subsets of CFU-S have been described: a subset that leads to colonies apparent in the spleen on day 7 after injection (day 7 CFU-S); a proportion of these colonies disappear shortly thereafter and are believed to have little self-replicating capacity), and a subset that leads to colonies on day 12 (day 12 CFU-S). The latter is believed to have a high self-replicating ability and to be more representative of early hematopoietic progenitors.

Because the effect of rIL 1α on early hematopoietic progenitors in the HPP-CFC assay depends on the expression of CSF-I receptors on primitive, early hematopoietic progenitor cells. The combination of rIL 1α, rIL 3, and CSF-I leads to a greater number of colonies than saturating levels of either rIL 1α + CSF-I or rIL 3 + CSF-I (Fig 3), evidently there is more than one class of early hematopoietic progenitors.

In this article, we have demonstrated that rIL 1α acts on early hematopoietic progenitor cells. The combination of IL 1 or IL 3 with CSF-I generates HPP-CFC colonies from 5-FU-treated bone marrow. H-1/IL 1 has been shown to induce the expression of CSF-1 receptors on primitive, hematopoietic progenitors. Because the combination of rIL 1α, rIL 3, and CSF-1 leads to a greater number of colonies than saturating levels of either rIL 1α + CSF-1 or rIL 3 + CSF-1 (Fig 3), evidently there is more than one class of early hematopoietic progenitors.
hematopoietic cell giving rise to HPP-CFC colonies. These classes could correspond to those observed in HPP-CFC replating experiments; the results were suggestive of one class of cell responding to IL 3 plus CSF-1 directly and another class responding to IL 3 plus CSF-1 only after incubation with H-1/IL 1. Because H-1/IL 1 induces the expression of IL 2 receptors and CSF-1 receptors, a plausible mechanism of action of H-1/IL 1 on early hematopoietic progenitors is that it induces the expression of receptors for a number of growth factors normally expressed only on more mature cell types. The data in this article suggest that the action of IL 1 on primitive hematopoietic cells is not mediated through accessory cells since there are no factors produced that are active in the HPP-CFC assay that cannot be neutralized by an anti-IL 1e antibody.

The hematopoietic factors erythropoietin and IL 3 have previously been reported to increase the number of CFU-S present in bone marrow cultures. In the case of erythropoietin it was demonstrated (examining colonies on day 7) that a particular erythroid committed subset of CFU-S with no self-replicating capacity in the recipient spleen was affected. The day 12 CFU-S appear to have a high self-replicating ability and appear to be more primitive than day 7 CFU-S, although there may be as much as 50% overlap between the two subsets.

We report that the addition of only IL 1 (or H-1) to bone marrow cultures increases the number of day 12 CFU-S that can subsequently be recovered. This indicates that, in addition to synergizing with CSF-1 to promote proliferation and differentiating cells making them responsive to IL 3, IL 1 also has a maintenance or survival-enhancing effect on primitive hematopoietic stem cells. That this effect also could take place in an adherent cell depleted system is suggestive of a direct action of IL 1 on day 12 CFU-S.

ACKNOWLEDGMENT

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REFERENCES

2. Oppenheim JJ, Kovacs EO, Marusshka K, Durum SK: There is more than one interleukin 1. Immunobiol Today 7:45, 1986


Effects of hematopoietin-1 and interleukin 1 activities on early hematopoietic cells of the bone marrow

KM Zsebo, J Wypych, VN Yuschenkoff, H Lu, P Hunt, PP Dukes and KE Langley