Production of Hematopoietic Stem Cell–Chemotactic Factor by Bone Marrow Stromal Cells

Cherry, Ryoji Yasumizu, Junko Toki, Hiroaki Asou, Tomoyoshi Nishino, Yasuhiro Komatsu, and Susumu Ikehara

Bone marrow stromal cells play a crucial role in hematopoiesis. Developmentally, the formation of stromal cells precedes the settlement of hematopoietic cells. Similarly, reconstitution of the bone marrow in adult mice after bone marrow transplantation is characterized by the initial establishment of the marrow microenvironment. When hematopoietic stem cells (HSCs) are injected into irradiated mice, they migrate into the spleen and bone marrow, where they form colonies. However, it is still unknown why HSCs home in on the bone marrow and spleen.

In in vitro culture systems, HSCs can proliferate and differentiate into different lineages with the support of adherent stromal cell layers. Various stromal cell lines with the capacity to support the growth and differentiation of hematopoietic cells have been established. However, the interaction between stromal cells and HSCs is still poorly understood. Direct contact between these two compartments is documented as being necessary for stroma-dependent hematopoiesis. On the other hand, biologically active substances produced by stromal cells have also been reported to be the regulatory elements for hematopoiesis. Thus, the supporting mechanisms appear to be involved in both direct cell-to-cell interaction and the secretion of humoral factors.

According to our observation using time-lapse video microscopy, HSCs first move toward a stromal layer and, after coming into contact with the stromal cells, they burrow under the stromal layer and start to proliferate. This unidirectional movement of HSCs toward stromal cells prompted us to examine a possible guidance by chemotactic factors produced by the stromal cells.

We herein show a chemotactic factor prepared from the culture supernatants of stromal cell lines with the potential to support long-term hematopoiesis. The factor has a molecular weight of more than 200 kDa and attracts low-density bone marrow cells with Thy1+, WGA+, and H-2Kb phenotypes.

MATERIALS AND METHODS

Mice. Female C3H/Hej mice (8 to 12 weeks old) were purchased from Japan Clea Inc (Osaka, Japan) and maintained in our animal facility.

Cell preparation. Mice were killed by decapitation, and the bone marrow cells (BMCs), which were collected from the femurs, tibias and humeruses, were then passed through a Sephadex G-10 (Pharmacia Fine Chemicals, Uppsala, Sweden) column to deplete adherent cells. The nonadherent BMCs were fractionated on Percoll (Pharmacia) discontinuous density gradients.

Cells in the low-density fraction, which ranged between 1.063 and 1.075 (40% and 60% Percoll solutions), were collected and used for the chemotactic assay. This fraction consisted mainly of the cells in the blast window and lymphoid window, which showed high colony-forming units assay. This fraction consisted mainly of the cells in the blast window and lymphoid window, which showed high colony-forming units. The high-density fraction, which ranged between 1.075 and 1.090 (60% and 70% Percoll solutions), were collected a week later and stored at -20°C until use.

Culture supernatants. The stromal cell lines (10T1/2, MS-5, MC3T3-G2/Pa6, RGB 3T3-1, and ST-2) were cultured in RPMI 1640 with 10% fetal calf serum (FCS) or 10% horse serum. When the growth became confluent in a 75-cm² flask, the culture medium was replaced with a fresh medium. The supernatants were collected a week later and stored at -20°C until use. As a negative control, we used RPMI 1640 with 10% FCS or horse serum.

Biochemical characteristics. The supernatants of 10T1/2 were heated at 56°C for 30 minutes, at 70°C for 10 minutes, 20 minutes, and 30 minutes, and at 100°C for 10 minutes. Activity was then examined. For trypsin digestion, 10 µg/mL of trypsin was added to the 10T1/2-conditioned medium and incubated at 37°C for 30 minutes. Trypsin inhibitor (40 µg/mL) was then added. To examine sensitivity to a reducing agent, 2-mercaptoethanol (2ME; 50 mM/mL) was added to the medium, which was then incubated at room temperature for 2 hours. The 10T1/2 medium was also mixed well with either ether or chloroform for 5 minutes, then recovered by centrifugation and dialysed overnight before use.

© 1994 by The American Society of Hematology.
HEMATOPOIETIC STEM CELL-CHEMOTACTIC FACTOR

Fig 1. Chemotactic activities in the culture supernatants produced by stromal cell lines. The chemotactic activities in the conditioned media of stromal cell lines (10T1/2, MS-5, MC3T3-G2/PA6, RGB3T3-1, and ST-2) and bone marrow adherent cells were analyzed using Boyden’s chambers, as described in Materials and Methods. Low-density BMCs (2 \times 10^7) were placed in the upper chambers. Each column represents the mean ± SD of triplicate cultures.

molecular weight of the factor, the 10T1/2-conditioned medium was fractionated using ultra-filters UK 200, UK 50, and UK 10 (Toyoh Roshi, Tokyo, Japan). Fractions (Fr) I, II, and III were recovered by washing the respective filter. The last filtrate was collected as Fr IV; relative molecular weights were greater than 200 kD (Fr I), 200 to 50 kD (Fr II), 50 to 10 kD (Fr III), and less than 10 kD (Fr IV). Each fraction was lyophilized and resuspended in the original volume. All test solutions were dialysed in RPMI 1640 medium before use.

Chemotactic assay. The micro pore filter assay was performed in accordance with Wilkinson’s method. Briefly, cellulose nitrate filters of 5 \mu m porosity (GM64, Sartorius, Germany) were placed in blind-well Boyden’s chambers (Neuro Probe, Cabin John, MD) to separate into upper and lower chambers. The lower chambers contained 200 \mu L of test media. Test cell suspensions (200 \mu L) at an appropriate concentration were added to the upper chambers. The Boyden’s chambers were incubated for 3 hours at 37°C in 5% CO2 air. After incubation, the filters were removed, fixed, and stained with hematoxylin. The cells passing through the membranes were counted under microscope by focusing 120 \mu m away from the upper surface.

For a kinetic study of chemotaxis and cytological study, polycarbonate filters of 5 \mu m porosity (Nucleopore Inc, Costar, MA) were used instead of cellulose nitrate filters to facilitate the passage of cells. The attracted cells were recovered from the lower chambers.

Assays for hematopoietic progenitors. To evaluate hematopoietic activity, CFU-S and CFU-granulocyte macrophage (GM) assays were performed, as previously described. CFU-S activity was assessed on day 8 and on day 12 after the injection of BMCs. The control mice without BMC injection had no colonies in the spleens on day 8 or on day 12. Each group consisted of five mice.

Fluorescence-activated cell sorter (FACS) analyses. Cells (10^6) were suspended in 25 \mu L of phosphate-buffered saline (PBS) with 2% FCS and mixed with 25 \mu L of the following staining reagents at the appropriate concentrations: fluorescein isothiocyanate (FITC) wheat germ agglutin (WGA) (Polyscience, Warrington, PA), FITC-anti-Thyl.2 monoclonal antibody (MoAb Becton Dickinson Immunocytometry Systems [B-D], San Jose, CA), FITC-anti-Ly1 MoAb (B-D), FITC-anti-L3T4 MoAb (B-D), FITC-anti-Ly2 MoAb (B-D), FITC-anti-Ly5 MoAb (Caltag, S San Francisco, CA), and FITC-goat-anti-murine Ig (Cappel, West Chester, PA). After incubation for 30 minutes on ice, the cells were washed and analyzed using a FACScan (B-D).

Recombinant hematopoietic factors. Purified recombinant human granulocyte colony-stimulating factor (G-CSF) was kindly provided by Toyoy Brewery Company (Shizuoka, Japan). Purified recombinant human macrophage (M) CSF was donated by Kirin Brewery Company (Tokyo, Japan). Purified recombinant murine GM-CSF and murine IL-6 were purchased from Biogen International (Camarillo, CA). Purified recombinant murine stem cell fac-

Fig 2. Chemotactic activities of MS-5 and 10T1/2. One million unfractionated BMCs, 1 \times 10^6 high-density BMCs, or 2 \times 10^5 low-density BMCs were placed in the upper chambers. The lower chambers contained the conditioned media of 10T1/2 or MS-5. Migrated cells from the upper surface were counted after incubation for 3 hours. Each column represents the mean ± SD of 5 or 6 cultures. (□) MS-5; (□) 10T1/2; (m) control.
Table 1. Discrimination Between Chemotactic and Chemokinetic Activities

<table>
<thead>
<tr>
<th>Condition</th>
<th>Upper Chamber</th>
<th>Lower Chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control medium</td>
<td>85 ± 17</td>
<td>45 ± 12</td>
</tr>
<tr>
<td>Conditioned medium</td>
<td>289 ± 13</td>
<td>44 ± 23</td>
</tr>
</tbody>
</table>

The experiments were performed using Boyden's chambers. Low-density bone marrow cells (2 × 10⁶) were introduced to upper chambers. The representative data are shown here as the mean ± SD of quadruplicate culture.

* RPMI 1640 with 10% FCS was used as a control medium.
† 10T1/2-conditioned medium was prepared, as described in Materials and Methods.
‡ P < .0001, compared with the cell counts (44 ± 23).

Table 2. Biochemical Nature of Chemotactic Factor in 10T1/2 Medium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Chemotaxis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity to heat</td>
<td></td>
</tr>
<tr>
<td>56°C 30 min</td>
<td>80</td>
</tr>
<tr>
<td>70°C 10 min</td>
<td>73</td>
</tr>
<tr>
<td>20 min</td>
<td>12</td>
</tr>
<tr>
<td>30 min</td>
<td>17</td>
</tr>
<tr>
<td>100°C 10 min</td>
<td>2</td>
</tr>
<tr>
<td>Sensitivity to protease enzyme†</td>
<td></td>
</tr>
<tr>
<td>trypsin</td>
<td>-42</td>
</tr>
<tr>
<td>Sensitivity to reducing agent†</td>
<td></td>
</tr>
<tr>
<td>2 mercaptoethanol</td>
<td>110</td>
</tr>
</tbody>
</table>

Table 3. Chemotactic Activity in Fractionated 10T1/2 Medium

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Fractions of Supernatants*</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I  II III IV</td>
<td>Unfraction RPMI + 10% serum</td>
</tr>
<tr>
<td>1</td>
<td>52 11 8 0 ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>34 5 4 0 ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>67 ND 13 ND</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>55 ND 25 ND</td>
<td>65</td>
</tr>
</tbody>
</table>

BMCs (HSC-enriched population) but also high-density BMCs (granulocyte-enriched population), whereas 10T1/2-conditioned medium attracted only low-density BMCs (HSC-enriched population) (Fig 2). May-giemsa staining showed that MS-5-conditioned medium attracts both blasts and neutrophils, whereas 10T1/2-conditioned medium attracts only blasts (data not shown).

The next step was to examine whether 10T1/2-conditioned medium attracts "true HSCs." We used the HSCs in the Go phase (5-FU treated Lin- WGA+ blast window sorted BMCs), as previously described. The 10T1/2-conditioned medium attracted the HSCs in the Go phase (data not shown). Therefore, we used the 10T1/2-conditioned medium for further characterization of the HSC-chemotactic factor.

To confirm that this reflects chemotactic activity and not...
HEMATOPOIETIC STEM CELL-CHEMOTACTIC FACTOR

Original Experiment Control

Fig 4. Light scatter profile of BMCs recovered from lower chambers. The cells were collected from the lower chambers, which contained 10T1/2-(B) conditioned medium or (C) control medium. The chemoattracted cells by the 10T1/2-conditioned medium mostly consist of (B) blast window and lymphocyte window cells, whereas (C) nonspecifically migrating cells consist dominantly of granulocyte window cells. (A) The original unfractionated bone marrow cells are shown. The clusters of granulocytes, blast cells, and lymphocytes are shown as a, b, and c, respectively.

a simple enhanced movement of BMCs caused by activation (chemokinesis), 10T1/2-conditioned medium was introduced into the upper chambers with low-density BMCs. In this condition, there was less migration of BMCs. Significant migration is observed only when conditioned medium is introduced into the lower chambers (Table 1).

Kinetics of chemotaxis. To study the kinetics of chemotaxis, cells were recovered from the lower chambers and counted at the intervals. A significant number of cells were collected from the lower chambers, even in the control group, because of nonspecific movement of BMCs (Fig 3). When the lower chambers were filled with the 10T1/2-conditioned media, the migration of cells into the lower chambers increased rapidly. Within 1 hour, the cell counts in the experimental group exceeded those in the control group. After 2 hours, the cell counts in the experimental

Fig 5. Thy1-expression on chemoattracted low-density BMCs. Cells were collected as shown in Fig 4, then stained with FITC-anti-Thy1.2 MoAb and analyzed using a FACSscan. A distinguishable cluster of Thy1<sup>+</sup> cells is clearly identified in (A) the experimental group, but not in (B) the control group. (C) The histogram shows fluorescence intensity of blast window cells in the experimental group (--- - -), control group (- - -), thymocytes (-- - -), and unstained BMCs (- - -).

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
Fig 6. Expression of WGA and H-2 in chemotactically attracted BMCs. Cells were collected from the lower chambers, as shown in Fig 4, then stained with (A) FITC-WGA and (B) FITC-H-2K MoAb and analyzed using a FACScan. The merge graphs show the fluorescence intensity of lower chamber cells of the experimental group (-- - -), control group (-- ---), low density BMCs (.- . .), and unstained BMCs ( - - -).

Characterization of chemotactic factors secreted by stromal cell lines. To understand the nature of the chemotactic factor, the serum-free 10T1/2-conditioned medium was heated at 56°C for 30 minutes, at 70°C for 10 minutes, 20 minutes, and 30 minutes, and at 100°C for 10 minutes. Activity after heating is shown in Table 2. The factor was sensitive to heat: the activity was lost after the treatment of 70°C for 20 minutes, although activity was not lost after the treatment at 56°C for 30 minutes. The medium was also treated with trypsin and the reducing agent, 2ME. Activity deteriorated as a result of trypsin digestion, but not after 2ME-treatment. The factor was not extractable in organic solvents such as ether or chloroform (data not shown).

The 10T1/2-conditioned medium was ultrafiltrated into four fractions. As shown in Table 3, chemotactic activity was detected only in Fr 1 (the fraction with a molecular weight of more than 200 kD). When Fr 1 was serially diluted and assayed, a clear dose-dependent response was observed (data not shown). The similar results were found in MS-5-conditioned medium (data not shown).

FACS analyses of migrating BMCs. The low-density BMCs were placed into the upper chambers and cultured for 1.5 hours. The cells recovered from the lower chambers were stained and analyzed using a FACScan. Figure 4 shows a typical light scatter profile. The cells attracted by the 10T1/2-conditioned medium consisted of more blast window and lymphoid window cells and less granulocytes than the control.

Blastic window cells were further characterized. A clear independent cluster in Thy-1 staining was found in the experimental group (Fig 5A), the level of expression being significantly lower than that of thymocytes. The population of Thy-1~ cells in the lower chambers of the experimental group was approximately 40%, whereas that of the control and original low-density BMCs was 11% and 12%, respectively (6% in the unfractonated BMCs). These Thy-1~ cells were confined to the mid-upper region of the blast window and were brightly stained with FITC-WGA and anti-H-2K MoAb in double staining analyses (Fig 6), whereas Ly1, L3T4, Ly2, and Ly5 antigens as well as surface Ig were negative (data not shown).

Regarding lymphoid window cells, the surface markers were Thy1~ , WGA~ , Ly1~ , Ly5~ , L3T4~ , and H-2K~ ; approximately 70% of the cells expressed Ly5 and 20% expressed sIg (data not shown). Thus, the 10T1/2-conditioned medium attracts not only blastic window cells but also lymphoid window cells.

The specific clusters of Thy-1~ cells were sorted using a FACStar, and cytospin specimens were prepared. The majority of sorted Thy-1~ cells were round in shape and had scanty cytoplasm—the characteristics of blastic cells. Some cells showed deep basophilic cytoplasm compatible with the transitional cells, as previously documented as stem cells.16,17

Hematopoietic activity of attracted BMCs. The day 8 CFU-S, day 12 CFU-S, and CFU-GM activities of attracted low-density BMCs are summarized in Table 4. Both CFU-S counts on day 8 and on day 12 were approximately three times higher in the cells attracted by the 10T1/2-conditioned medium than in those migrated in the control experiments. CFU-GM counts (day 7) showed a slight increase in the experimental group: 182/10^5 cells in the experimental group against 121/10^5 cells in the control group and 159/10^5 cells in the low-density BMCs.

Chemotactic activity of putative hematopoietic factors. It has been reported that bone marrow stromal cells produce several factors that contribute to hematopoiesis. Some have chemotactic activity specific for certain types of cells; for example, IL-3 and SCF attract mast cells.8,9 We analyzed
Table 4. Hematopoietic Clonogenic Activities of Chemotactically Acted BMCs

<table>
<thead>
<tr>
<th>Cells</th>
<th>CFU-S (10^4)</th>
<th>CFU-GM (10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMcs</td>
<td>13.5 ± 0.9</td>
<td>13.9 ± 2.5</td>
</tr>
<tr>
<td>Low density BMcs</td>
<td>30.9 ± 2.9 (2.3)</td>
<td>28.6 ± 3.8 (2.1)</td>
</tr>
<tr>
<td>Experiment</td>
<td>34.7 ± 4.9 (2.6)*</td>
<td>35.1 ± 4.8 (2.5)*</td>
</tr>
<tr>
<td>Control</td>
<td>11.2 ± 3.7 (0.8)</td>
<td>11.5 ± 3.0 (0.8)</td>
</tr>
</tbody>
</table>

Attracted BMcs were collected from lower chambers and processed for CFU-S and CFU-GM assays. Data are represented as the mean ± SD of five counts. Enrichment factors are shown in parenthesis.

* P < .0001, compared with each control group.
† P < .001, compared with the control group (120.9 ± 12.6).

M-CSF, G-CSF, GM-CSF, SCF, IL-6, and IL-3 for chemotactic activity. The 10T1/2-conditioned medium was used as a positive control, and RPMI 1640 medium with 10% FCS was used as a negative control. The results are represented as percent chemotaxis according to the formula shown in Table 2. None of the cytokines tested here showed HSC-chemotactic activity. We also examined the activity using the combinations of these cytokines. Some combinations (GM-CSF plus IL-3, GM-CSF plus SCF) showed chemokinetic activity, but not HSC-chemotactic activity (data not shown). This strongly suggests that the 10T1/2-conditioned medium contains a newly identified chemotactic factor that is different from putative growth factors and cytokines.

DISCUSSION

In this report, we analysed the chemotactic activities of cultured supernatants to obtain some insight into the interaction between stromal cells and hematopoietic cells using stromal cell lines instead of a heterogeneous stromal population. We have found that MS-5 and 10T1/2 cell lines secrete chemotactic factors; MS-5 secretes two kinds of factors (one attracts low-density BMcs and the other, high-density BMcs), whereas 10T1/2 secretes only one factor that attracts low-density BMcs. These findings strongly suggest that the migration of hematopoietic cells and their contact with stromal cells are guided by chemotactic factors produced by stromal cells.

The molecular mechanisms involved in the chemotaxis and homing of lymphocytes and phagocytes are well documented as receptor-mediated events that control and amplify specific cellular activities. A degraded fragment of C5a (C5a5), which is an anaphylatoxin and chemotactic factor, usually binds to its receptors, which are different from those for adhesion molecules. The binding of C5a to the receptors leads to upregulation and downregulation of the specific adhesion molecule’s receptors; it is supposed that this phenomenon determines the homing tissue for the cells. On the other hand, the high endothelial cells of postcapillary venules express the adhesion molecule, which is thought to guide lymphocytes from the circulation into the lymph nodes. In addition, the same high endothelial cells are found to secrete a factor that stimulates lymphocyte mobility. Taken together, it appears that circulating cells are influenced by chemotactic factors, followed by the modulation of their receptors for adhesion molecules, and that they then migrate and adhere to the relevant stromal cells to start a direct cell-to-cell interaction.

After intravenous injection, BMcs, particularly hematopoietic progenitors, migrate to the bone marrow and spleen, as evaluated by the marrow-repopulating ability and CFU-S assay. Here we would like to propose that the homing and traffic of BMcs in vivo may occur in a manner similar to the circulating leukocytes mentioned above. However, it is uncertain whether chemotactic factors modulate the expression of adhesion molecules in BMcs. A series of reports from Aizawa and Tavassoli showed the interaction between the putative lectins on BMcs and galactosyl and mannosyl moieties on the hematopoietic stromal cells involved in the adhesion mechanism. Knope et al. reported that cellulose membranes coated with BMc-derivived extracellular matrix induced bone formation, followed by hematopoiesis on the membrane, indicating that the extracellular matrix plays a pivotal role in the generation of hematopoietic tissue. The well-organized process during the regeneration of the bone marrow occurs in the sequence of several types of cell interaction: matrix-stromal cells, stromal cells-stromal cells, and stromal cells-hematopoietic progenitors. Within the context of these observations, the chemotactic factors described in this report might be involved in the early stages of homing of hematopoietic progenitor cells from the circulation to the bone marrow. Further investigation, including molecular analyses of this factor and its receptors, will elucidate the interaction between stromal and hematopoietic progenitor cells.

In this report, we focused on the target BMcs that were attracted by the chemotactic factors produced by the stromal cell lines. Target cells consisted of two kinds of cells; one is the blasts and the other is the lymphoid cell, as shown in Fig. 4. First, we would like to discuss the blasts cell. The chemotactically low-density BMcs contained a clearly separated cluster of Thy1 BMcs (Fig 4) that resided in the mid-upper area of the blast window, and expressed WGA46 and H-2K (Fig 6), but lacked Ly1, Ly2, L3T4, and Ly5 (data not shown). Visser et al. have shown that hematopoietic progenitor cells are localized in the blast window in a light scatter profile as shown on FACS analyses. Visser et al. have also found that the progenitor cells have a high affinity to WGA and express H-2K antigen at a high level. Similarly, Lord and Spooner reported a high enrichment of the progenitor cells by sorting WGA "blast window" cells. Weissman's group showed that Thy1 BMcs, which lack lineage markers such as Ly5, CD4, CD8, Gr1, and Mac1, have hematopoietic progenitor activities. Recently, Hirabayashi et al. have shown that the counts of CFU-S on day 24 are enriched in Thy1 BMcs, WGA+, Lin-, e-kit BMcs, and that Thy1 expression precedes e-kit expression. The early emergence of Thy1 antigen on hematopoietic progenitors was also observed in ontogeny. In the present study, we have confirmed that low-density Thy-1 BMcs are localized in the blasts window.

Regarding lymphoid cells (second-type cells) attracted by the 10T1/2-conditioned medium, it has been reported that...
primitive progenitor cells with a long-term repopulating ability have lymphoid characteristics in morphology. Although the chemoattracted BMCs showed a significant enrichment in lymphoid cluster (Fig 4), the lymphoid cells were Thy-1+, and the majority expressed Ly5, suggesting that they belong to B-lineage lymphocytes; some expressed surface Ig. In our assay system, the chemoattracted BMCs consisted of both blastic and lymphoid cells. It is uncertain whether there are one or more responsible factor(s) in the 10T1/2-conditioned medium. We are in the process of clarifying this.

To examine whether the chemotaxis described here is specific for hematopoietic progenitor cells, clonogenic assays in vivo and in vitro were performed on the chemoattracted BMCs. Enrichment factors of CFU-S day 8, CFU-S day 12, respectively); although there are statistically significant differences between the experimental and control groups (Table 3). However, there are two possible explanations. One is that attracted cells are contaminated by nonspecifically moving cells which were shown in the control group (Fig 3), because it is difficult to separate chemotactically migrated cells from chemokinetically moved cells. The other possibility is that the 10T1/2-conditioned medium may contain two or more factors, and that one factor attracts blast cells while the other attracts lymphoid cells; indeed, the primitive progenitor cells with lymphoid characteristics with a long-term repopulating ability were found to show neither CFU-S activity nor radioprotective activity. Until the concept of HSCs has been clearly defined and an appropriate assay system has been established, we cannot draw any conclusions about this issue.

In conclusion, the HSC-chemotactic factor appears to have a molecular weight of more than 200 kDa (Table 3), and is sensitive to heat (70°C for 20 minutes) and trypsin digestion (Table 2). Various putative hematopoietic factors (G-CSF, M-CSF, GM-CSF, SCF, IL-6, and IL-3) and their combinations were found to have no HSC-chemotactic activity (Table 5). Therefore, we believe that the present factor is a newly identified factor, and we are in the process of purifying it.

ACKNOWLEDGMENT
We thank K. Higuchi, Y. Umeda, and Y. Shinno for their expert technical assistance, and K. Ando for manuscript preparation.

REFERENCES
9. Ogawa M, Nishikawa S, Ikuta K, Yamamura F, Naito M, Takahashi K, Nishikawa SI: B cell ontogeny in murine embryo studied by a culture system with the monolayer of a stromal cell clone, ST2; B cell progenitor develops in the embryonal body rather than in the yolk sac. EMBO J 7:1337, 1988

<p>| Table 5. HSC-Chemotactic Activity of Growth Factors and Cytokines |
|-------------------|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Concentration (U/mL)</th>
<th>G-CSF</th>
<th>M-CSF</th>
<th>GM-CSF</th>
<th>SCF</th>
<th>IL-6</th>
<th>IL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.6</td>
<td>2.5</td>
<td>ND</td>
</tr>
<tr>
<td>10^3</td>
<td>ND</td>
<td>ND</td>
<td>7.0</td>
<td>10.4</td>
<td>4.1</td>
<td>ND</td>
</tr>
<tr>
<td>10^4</td>
<td>6.1</td>
<td>-1.8</td>
<td>13.3</td>
<td>ND</td>
<td>3.3</td>
<td>ND</td>
</tr>
<tr>
<td>10^5</td>
<td>4.2</td>
<td>-6.9</td>
<td>6.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10^6</td>
<td>8.7</td>
<td>-7.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10^7</td>
<td>12.7</td>
<td>-7.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Percent Chemotaxis* 7.7

G-10 nonadherent low-density BMCs (2 x 10^6) were introduced into the upper chambers, as described in Materials and Methods. Purified recombiant cytokines were diluted in media with 10% serum and introduced into the lower chambers.

Abbreviation: ND, not determined.

* The percentage of chemotaxis was calculated as shown in Table 2.

† WEHI-3 supernatant was used without dilution as a source of IL-3.

For personal use only.on October 3, 2017. by guest

From www.bloodjournal.org by guest on October 3, 2017. For personal use only.
HEMATOPOIETIC STEM CELL-CHEMOTACTIC FACTOR

29. Ploemacher RE, Brons NHC: Cells with marrow and spleen repopulating ability and forming spleen colonies on day 16, 12, and 8 are sequentially ordered on the basis of increasing Rhodamine 123 retention. J Cell Physiol 136:531, 1988
46. Ploemacher RE, Brons NHC: Isolation of hematopoietic stem cell subsets from murine bone marrow: I. Radioprotective ability of purified cell suspensions differing in the proportion of day-7 and day-12 CFU-S. Exp Hematol 16:21, 1988
Production of hematopoietic stem cell-chemotactic factor by bone marrow stromal cells

Cherry, R Yasumizu, J Toki, H Asou, T Nishino, Y Komatsu and S Ikehara