Interleukin-8 Induces Rapid Mobilization of Hematopoietic Stem Cells With Radioprotective Capacity and Long-Term Myelolymphoid Repopulating Ability

By L. Laterveer, I.J.D. Lindley, M.S. Hamilton, R. Willemze, and W.E. Fibbe

Interleukin-8 (IL-8) belongs to a family of chemotractant cytokines involved in chemotaxis and activation of neutrophils. As in vivo administration of IL-8 induces granulocytosis and the release of immature white blood cells into the circulation, we assessed a possible mobilizing effect of IL-8 on myeloid progenitor cells. IL-8 was administered at intraperitoneal doses ranging from 0.1 to 100 μg per mouse to female Balb/C mice (aged 8 to 12 weeks; weight, 20 to 25 g). Animals were killed at time intervals ranging from 1 to 240 minutes after IL-8 administration, and blood, bone marrow, and spleen cells were harvested. Injection of 30 μg IL-8 resulted in an increment from 25 ± 9 to 418 ± 299 granulocyte-macrophage colony-forming units (CFU-GM) per milliliter blood at 15 minutes after a single intraperitoneal injection. Sixty minutes after the injection of IL-8, the numbers of circulating CFU-GM per milliliter blood had almost returned to pretreatment values (82 ± 39 CFU-GM per milliliter). A dose of 100 μg IL-8 per animal did not result in a further increment in the numbers of circulating CFU-GM. Transplantation of 5 × 10⁶ blood-derived mononuclear cells (MNC) obtained at 30 minutes after IL-8 injection (30 μg) resulted in 69% survival of lethally irradiated (8.5 Gy) recipient male and female recipients of MNC derived from IL-8-treated donors resulted in 100% survival. Six months after transplantation, female recipients of MNC derived from IL-8–treated male donors were killed, and chimerism was determined in bone marrow, spleen, and thymus using a Y chromosome-specific probe and fluorescent in situ hybridization (FISH). The majority of bone marrow, spleen, and thymus cells (83% ± 25%, 89% ± 5%, and 64 ± 28%, respectively) consisted of Y chromosome-positive cells, showing that the IL-8–mobilized cells had myelolymphoid repopulating ability. We conclude that IL-8 is a cytokine that induces rapid mobilization of progenitor cells and pluripotent stem cells that are able to rescue lethally irradiated mice and that are able to completely and permanently repopulate host hematopoietic tissues.

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parts in posterior-anterior and anterior-posterior position at a dose rate of 4 Gy/min. Male, blood-derived mononuclear cells (MNC) were injected in the tail vein of lethally irradiated female recipients.

IL-8. Recombinant human IL-8 was purified from Escherichia coli expressing a synthetic gene. IL-8 had no colony-stimulating activity; as reported previously and as confirmed in our laboratory (unpublished observations, March and May 1991). The concentration of endotoxin was less than 0.05 endotoxin units (EU/mL) as tested in the Limulus amoebocyte lysate assay. For in vivo experiments, IL-8 was diluted to the desired concentration in endotoxin-free phosphate-buffered saline (PBS) and administered as a single intraperitoneal injection. Polyclonal anti-IL-8 antibody, raised in goats, was able to neutralize an amount of 0.1 µg/mL IL-8, as tested in an elastase release assay.

Preparation of cell suspensions. Mice were killed by CO2 asphyxia at various time intervals after IL-8 injection. Peripheral blood was drawn by cardiac puncture, and white blood cell counts were performed on a Sysmex F800 (Toa Medical Electronics Co LTD, Kobe, Japan). Manual neutrophil counts were performed after May-Grünwald-Giemsa staining. Blood-derived MNC suspensions were obtained by Ficoll separation as described earlier. Bone marrow cells were harvested by flushing the femur under sterile conditions with RPMI 1640 containing 500 µg/mL penicillin, 250 µg/mL streptomycin, and 2% fetal bovine serum (FBS; Gibco, Grand Island, NY). Single-cell suspensions of spleen and thymus cells were prepared by mashing the organs and washing once in RPMI 1640 with 5% FBS. In transplantation experiments, a fixed number of 5 × 10^6 blood-derived MNC from IL-8- or PBS-treated donors was suspended in PBS containing 1% syngeneic mouse serum.

GM colony-forming unit (CFU-GM) cultures. Cells were cultured as described previously. Briefly, bone marrow cells were cultured in microtiter plates containing 10^5 cells per well in semisolid medium in the presence of murine GM-CSF (1.25 ng/mL). Peripheral blood MNC and spleen cells were cultured in 3.5-cm dishes containing 5 × 10^6 cells per milliliter and 10^6 cells per milliliter, respectively. After 6 days of culture, the number of CFU-GM (defined as aggregates of more than 20 cells) were scored using an inverted microscope.

In situ hybridization. In some experiments, long-term surviving (greater than 6 months) animals were killed, bone marrow, spleen, and thymus were harvested, and single-cell suspensions were prepared. Fluorescent in situ hybridization (FISH) with a Y chromosome-specific probe was performed on single-cell suspensions as described. Amplification of the hybridization signal was performed according to Pinkel et al. Two hundred interphase nuclei were analyzed on each slide.

Statistical analysis. Differences were evaluated using the Student's t test. In survival analysis, differences were evaluated using the Mantel-Haenszel test for linear association. P values of less than .05 were considered statistically significant.

RESULTS

Mobilization of myeloid progenitor cells. IL-8 was administered as a single intraperitoneal injection at doses ranging from 0.1 to 100 µg per mouse. Doses of 0.1, 1, and 10 µg IL-8 per mouse all induced neutropenia followed by granulocytosis (Fig 1A) but did not mobilize hematopoietic progenitor cells into the circulation (Fig 1B). Dose levels of 30 or 100 µg IL-8 per mouse induced granulocytosis that reached maximum levels at 4 hours after injection (mean ± SD neutrophil counts: 2.80 × 10^6 ± 1.0 × 10^6/mL and 3.20 × 10^6 ± 0.61 × 10^6/mL for 30 and 100 µg, respectively, v 1.35 × 10^6 ± 0.54 × 10^6/mL for saline-treated controls; n = 3 to 20 per dose per time interval; Fig 1A). At 15 minutes after injection of a dose of 30 or 100 µg IL-8, a significant (P < .001) increase in the number of circulating CFU-GM was observed from 25 ± 9/mL blood at t = 0 to 418 ± 299/mL blood (30 µg) and 290 ± 43/mL blood (100 µg) (mean ± SD; seven and three experiments, respectively; 3 to 20 animals per time interval; Fig 1B). This effect was observed between 5 and 15 minutes after administration of IL-8 and preceded the granulocytosis that developed between 1 and 4 hours after injection of IL-8. At 1 hour after injection of IL-8, the number of circulating CFU-GM returned to almost pretreatment levels (82 ± 39/mL blood; mean ± SD, n = 5). Subcutaneous injection of IL-8 (30 µg) resulted in a similar effect on the number of progenitor cells mobilized (397 ± 178 CFU-GM/mL at t = 30 minutes; mean ± SD, two

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Therefore, we used splenectomized mice in some experiments. Intravenous administration of 0.5 mg anti-IL-8 antibody at 1 hour before an intraperitoneal injection of 30 µg IL-8 prevented the IL-8-induced neutrophilia and mobilization of hematopoietic progenitor cells (Fig 2), showing the IL-8 specificity of the effect. No significant changes in the numbers of progenitor cells in spleen or bone marrow were observed after injection of IL-8 (Table 1).

Effect of splenectomy on mobilization of CFU-GM. Accumulation of progenitor cells in the spleen may decrease their numbers in the circulation. In splenectomized mice, the effect on mobilization of progenitor cells by G-CSF was threefold higher than similar treatment of intact mice. Therefore, we used splenectomized mice in some experiments in an attempt to increase the numbers of circulating progenitor cells after injection of IL-8. The numbers of circulating CFU-GM after a single intraperitoneal injection of IL-8 were not significantly higher in splenectomized mice. Peak numbers of circulating CFU-GM were 470 ± 263/mL blood (mean ± SD, n = 6) in splenectomized mice versus 418 ± 299/mL blood (mean ± SD, n = 20) in intact control mice at 15 minutes after IL-8 injection.

Radioprotective capacity of hematopoietic progenitor cells mobilized by IL-8. To study the radioprotective capacity of the circulating progenitor cells, recipient female mice were lethally irradiated (8.5 Gy) and transplanted with 5 × 10⁶ blood-derived MNC obtained from male donors at 30 minutes after an intraperitoneal injection of saline or 30 µg of IL-8. Control recipient mice were lethally irradiated and received 1 × 10⁶ bone marrow cells or no cells at all (irradiation controls). At 60 days after transplantation, 69% of recipients that received peripheral blood MNC (PBMC) obtained from IL-8-treated donor animals were alive versus 22% of recipients transplanted with an equal number of PBMC obtained from saline-treated control donor mice, showing the presence of a higher number of circulating cells with radioprotective capacity in IL-8-treated mice (P < .05; n = 42 and n = 41, respectively; six experiments; Fig 3). In three of six experiments, no short-term survival was observed after transplantation of PBMC derived from salinetreated controls. In two experiments, groups of 10 to 20

<table>
<thead>
<tr>
<th>Table 1. Hematopoietic Progenitor Cells in Bone Marrow and Spleen After Injection of IL-8</th>
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<tr>
<td><strong>Time (min)</strong></td>
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<tr>
<td></td>
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<tr>
<td>0</td>
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<td>15</td>
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<tr>
<td>45</td>
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<tr>
<td>60</td>
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Balb/C mice were injected intraperitoneally with 30 or 100 µg IL-8 per mouse. Controls were injected with saline (time 0). Mice were killed at 15 minutes up to 60 minutes after injection, and unseparated nucleated cells (1 × 10⁶/mL for bone marrow and 1 × 10³/mL for splenic cells) were plated in a CFU-GM assay. Colonies were scored after 6 days, and numbers of CFU-GM per organ were calculated. Differences in numbers of CFU-GM were not significant.
Lethally irradiated mice were transplanted with increasing numbers (0.15 \times 10^9 up to 50 \times 10^9 cells per mouse) of PBMCN derived from IL-8–treated donors or from untreated control animals. Transplantation of 5 \times 10^9 IL-8–mobilized MNC protected 70% of the lethally irradiated recipients, whereas transplantation of 1.5 \times 10^9 or 5 \times 10^9 MNC resulted in 100% radioprotection. In these experiments, none of the mice transplanted with MNC (up to 5 \times 10^9) of non-treated animals survived lethal irradiation (Fig 4).

**Long-term repopulating ability of IL-8–mobilized peripheral blood cells.** Six months after transplantation of IL-8–mobilized MNC, mice were killed, and chimerism of bone marrow, spleen, and thymus was assessed using a Y chromosome-specific probe and FISH. Bone marrow, spleen, and thymus contained 83% \pm 25%, 89% \pm 5%, and 64% \pm 28% Y chromosome-positive donor-derived nucleated cells, respectively (Table 2). Cell suspensions of the thymus of control mice contain 98% T lymphocytes as assessed by immunofluorescence using a combination of monoclonal antibodies for CD4 and CD8, whereas spleen cell suspensions contain 50% B220-positive B lymphocytes. Thus, although the T cells, B cells, and myeloid cells were not separated before analysis, it is justified to state that the myeloid and both lymphoid lineages originate from transplanted donor cells due to the high percentage of donor-derived cells in these hematopoietic organs. Chimerism at 6 months after transplantation could not be assessed in recipients of PBMCN derived from saline-treated donors, as all mice died between 3 and 4 months after transplantation. Thus, these cells had no long-term repopulating ability to the extent of supporting survival.

**DISCUSSION**

In this study we report the stem cell-mobilizing properties of IL-8. Mice treated with a single injection of 30 or 100 \( \mu \)g IL-8 show a rapid increase of CFU-GM in the peripheral blood starting within 5 minutes after injection and preceding granulocytosis. The effect was specific for IL-8, as the mobilizing and neutrophilia-inducing effect was completely blocked by treatment of the animals with a polyclonal antı–IL-8 antibody before IL-8 injection. The initial neutropenia after injection of IL-8, also observed by others,\(^{27, 38} \) is likely caused by pulmonary sequestration\(^{32, 33} \) due to decreased deformability of the cytoskeleton.\(^{43} \) IL-8 may induce shedding of L-selectin and detachment of polymorphonuclear cells (PMN).\(^{23, 25, 32, 46, 47} \) An initial binding of PMN via L-selectin seems essential for a secondary and more potent binding with CD11b/CD18 \( \beta 2 \)-integrins.\(^{47} \) Consequently, PMN may not attach to endothelium despite upregulation of CD11b/ CD18 \( \beta 2 \)-integrins\(^{24} \) at the cell surface, which could contribute to the neutrophilic phase observed. The effect on mobilization of leukocytes restricted to neutrophils indicates a direct effect.\(^{27, 38} \) In contrast, the release of progenitor cells and stem cells into the circulation after IL-8 injection has no lineage specificity. In addition, the dissimilar kinetics and dose-response relationship of progenitor cell and neutrophil mobilization also implicate involvement of distinct mechanisms. Various integrins (ie, very late antigen 4 [VLA\(_4\)], lymphocyte function-associated antigen 1 [LFA-1], and L-selectin) are expressed on hematopoietic progenitor cells,\(^{39, 50} \) and interference with adhesion molecules by treatment with anti-VLA\(_4\) antibody may induce release of progenitor cells in monkeys.\(^{50} \) Similarly, the mobilizing effect may be

**Table 2. Long-Term Repopulating Ability of IL-8–Mobilized Hematopoietic Precursor Cells**

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>WBCs (x 10(^6)/mL)</th>
<th>PMNs (x 10(^6)/mL)</th>
<th>PLTs (x 10(^9)/mL)</th>
<th>ERYs (x 10(^12)/mL)</th>
<th>% Donor-Derived Cells in Recipient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.5</td>
<td>2.38</td>
<td>419</td>
<td>6.49</td>
<td>96</td>
</tr>
<tr>
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<td>10.3</td>
<td>1.44</td>
<td>336</td>
<td>4.88</td>
<td>96</td>
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<td>3</td>
<td>7.5</td>
<td>2.25</td>
<td>376</td>
<td>6.06</td>
<td>98</td>
</tr>
<tr>
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<td>9.8</td>
<td>2.25</td>
<td>558</td>
<td>7.13</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>10.6</td>
<td>3.29</td>
<td>743</td>
<td>10.9</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>11.7</td>
<td>3.63</td>
<td>400</td>
<td>10.7</td>
<td>87</td>
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</tbody>
</table>

Lethally irradiated (8.5 Gy) female recipients received 5 \times 10^8 IL-8–mobilized MNC obtained from male donors. At 6 months after transplantation, four mice were killed, and chimerism was determined using a Y chromosome-specific probe and FISH.

Abbreviations: WBCs, white blood cells; PLTs, platelets; ERYs, erythrocytes.
related to an IL-8–induced effect on these adhesion molecules. This hypothesis would suggest the expression of IL-8 receptors on hematopoietic progenitor cells, which is at present unknown.

Another mechanism for rapid mobilization of progenitor cells would be an indirect effect involving interference with binding to matrix molecules in the marrow microenvironment. IL-8 induces mobilization as well as activation of neutrophils with the subsequent release of proteases, including gelatinase-B, elastase, and β-glucuronidase—all involved in degradation of the extracellular matrix. In vitro studies indicate that primitive progenitor cells are preferentially bound to the extracellular matrix molecules. Thus, neutrophils in the bone marrow may release gelatinase-B within 5 to 10 minutes after IL-8 injection, resulting in nonspecific release of progenitor cells into the circulation. To investigate the involvement of gelatinase-B release in IL-8–induced stem cell mobilization, studies in rhesus monkeys are presently ongoing in our laboratory.

In the transplantation experiments, the radioprotective property of peripheral mononuclear cells mobilized by IL-8 was shown by the sharp reduced death rate between the 10th and 20th day after transplantation of 5 × 10^5 cells into lethally irradiated mice, as compared with animals transplanted with MNC after saline treatment (69% survival vs 22% response). Even 100% radioprotection of lethally irradiated mice was obtained after transplantation of 1.5 × 10^5 MNC derived from IL-8–treated donors. In addition to their radioprotective capacity, the MNC mobilized by IL-8 showed also long-term repopulating ability (LTRA). At 6 months after transplantation, the vast majority of the nucleated cells in the spleen, thymus, and bone marrow were of donor origin. Although (sub)sets of blood cells were not separated before analysis, the high percentages of donor-derived cells indicate that stem cells were transplanted and capable of differentiating in the myeloid as well as in the B- and T-lymphoid lineages.

The dose of IL-8 required for stem cell mobilization is higher than that necessary for neutrophil mobilization. It should be noted that IL-8 is relatively species-specific, and, therefore, lower doses may be required in humans to obtain similar results. Experiments in rhesus monkeys are in progress, and, indeed, preliminary results show similar levels of circulating progenitor cells at 10-fold lower doses than used in mice (Laterveer et al, manuscript in preparation).

These results suggest that IL-8 could be a useful cytokine in the setting of peripheral stem cell transplantation. Application of IL-8 in this setting would allow elective mobilization of progenitor cells in a reproducible fashion without requiring monitoring of hematopoietic cells. This contrasts with factors like G-CSF and GM-CSF that are already used clinically for recruitment of progenitor cells but that require treatment for several days. Pretreatment with G-CSF or stem cell factor (SCF) may expand the pool of progenitor cells susceptible for IL-8 mobilization and may further increase the mobilizing effect of IL-8. A similar effect has been reported for the combination of G-CSF and SCF. Studies are ongoing in an attempt to further increase the IL-8–induced mobilization of progenitor cells after a short pretreatment with hematopoietic cytokines (ie, GM-CSF, G-CSF, IL-3, and SCF).

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