Rapid Mobilization of Hematopoietic Progenitor Cells in Rhesus Monkeys by a Single Intravenous Injection of Interleukin-8


Interleukin-8 (IL-8) is a chemoattractant cytokine involved in chemotaxis and activation of neutrophils. Because in vivo administration of IL-8 induces mobilization of hematopoietic stem cells in mice, we assessed the mobilizing properties of IL-8 in rhesus monkeys. Recombinant human IL-8 was administered as a single intravenous injection at doses of 10, 30, and 100 µg/kg to rhesus monkeys (age, 2 to 3 years; weight, 2.5 to 4.5 kg). Venous blood samples were obtained at time intervals ranging from 1 to 480 minutes after IL-8 administration. Cell counts, colony-forming unit-mix assays, and fluorescence-activated cell sorter analysis were performed. Plasma was harvested to assess IL-8 levels.

Interleukin-8 (IL-8) belongs to a family of proinflammatory molecules called chemokines.1-2 It was previously known as neutrophil activating protein (NAP-1)3-7 and is a member of the CXC chemokine subfamily that includes several related ligands involved in the activation and chemotraction of neutrophils.8 The genes encoding these peptides are clustered on the human chromosome 4q13-q21.9 IL-8 is produced by a variety of cells, ie, monocytes, neutrophils, fibroblasts, endothelial cells, lung epithelial cells, mast cells, and keratinocytes,3-15 in response to stimulation with lipopolysaccharide (LPS), tumor necrosis factor α (TNFα), granulocytemacrophage colony-stimulating factor (GM-CSF), IL-1, IL-2, or IL-3.16-19 The in vitro bioactivities of IL-8 include chemotaxis,1-2 neutrophil activation, induction of release of metalloproteinases (ie, elastase, gelatinase-B, and β-glucuronidase), production of toxic metabolites in neutrophils,4,13-21 stimulation of histamine release by basophilis,22,23 inhibition of neutrophil-endothelial interaction,10,23-25 shedding of L-selectin,23-26 upregulation of CD11b/CD18,23,24 and transendothelial migration of neutrophils.25

In vivo, local administration of IL-8 induces a typical inflammatory response characterized by neutrophil margination and infiltration, plasma exudation, and angiogenesis.20-33 Systemic treatment with IL-8 in monkeys, rabbits, and mice induces an initial neutropenia that is rapidly followed by granulocytosis.20,34-36

Previously, we reported the rapid mobilization of hematopoietic stem cells after a single injection of IL-8 in mice.36 These stem cells had radioprotective capacity and long-term lymphomyeloid repopulating ability. Because IL-8 is relatively species-specific37 and in view of its potential application in humans, we wished to study these effects of IL-8 in nonhuman primates. Up to now, no reports have been published on the mobilization of hematopoietic progenitor cells in primates by IL-8.

In this report, we show that a single intravenous injection of IL-8 in rhesus monkeys causes an immediate neutropenia due to sequestration of neutrophils in the lungs. This neutropenia is followed by a mean 30-fold increase in the numbers of circulating progenitor cells until, at several hours after injection, an absolute neutrophilia occurs. These progenitor cell mobilizing properties of IL-8 indicate its potential use in the setting of peripheral blood stem cell transplantation.
The concentration of endotoxin was less than 0.05 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) with 0.1% bovine serum albumin (BSA) and administered as a time-controlled (30 seconds) intravenous bolus injection.

Preparation of cell suspensions. Blood samples were taken by venous puncture at various intervals and total blood cell counts were performed on a Sysmex F-1000 (Toa Medical Electronics Co LTD, Kobe, Japan). Manual differential counts were performed on May-Grunwald-Giemsa-stained blood films. Plasma was separated from the blood cells by centrifugation and the cell pellet was resuspended with an equal volume of RPMI 1640 containing 8 U/mL heparin and 2% [vol/vol] FBS). Erythrocytes were lysed by incubation with NH₄Cl-buffer for 10 minutes at 0°C. Leukocytes were washed two times with washing buffer (RPMI 1640 supplemented with 500 μg/mL penicillin, 250 μg/mL streptomycin, 8 U/mL heparin, and 2% [vol/vol] FBS). Cell lysate assay. For in vivo experiments, IL-8 was diluted to the desired concentration in endotoxin-free phosphate-buffered saline (PBS)

Table 1. Peak Plasma Levels of Circulating IL-8 After a Single Bolus Injection of IL-8

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Bodyweight (kg)</th>
<th>Dose of IL-8 (μg/kg)</th>
<th>Plasma Peak Level (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>89</td>
<td>2.90</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>9141</td>
<td>3.00</td>
<td>10</td>
<td>307</td>
</tr>
<tr>
<td>C89</td>
<td>2.90</td>
<td>10</td>
<td>153</td>
</tr>
<tr>
<td>9201</td>
<td>2.50</td>
<td>30</td>
<td>482</td>
</tr>
<tr>
<td>9123</td>
<td>3.20</td>
<td>30</td>
<td>396</td>
</tr>
<tr>
<td>9142</td>
<td>3.90</td>
<td>30</td>
<td>717</td>
</tr>
<tr>
<td>9142</td>
<td>3.75</td>
<td>100</td>
<td>610</td>
</tr>
<tr>
<td>9137</td>
<td>3.00</td>
<td>100</td>
<td>2,314</td>
</tr>
<tr>
<td>9201</td>
<td>2.50</td>
<td>100</td>
<td>8,766</td>
</tr>
<tr>
<td>9141</td>
<td>2.90</td>
<td>100</td>
<td>2,510</td>
</tr>
</tbody>
</table>

Peak levels of circulating IL-8 at 1 minute after injection of rhuIL-8. Monkey identification numbers are presented in the first column.

![Figure 1: Effect of IL-8 on the levels of circulating IL-8. Saline or IL-8 (10, 30, and 100 μg/kg) was injected intravenously at t = 0. Results are expressed as the means of two (10 μg/kg), three (30 μg/kg), or four (100 μg/kg) experiments. The saline experiment was performed once. The inserted figure shows the plasma levels of IL-8 up to 480 minutes after injection.](image-url)
analysis, using the Biosoft (Cambridge, UK) scientific parameter fit software program P.fit, version 5.1 (Fig.P Software Corp, Durham, NC).

Monoclonal antibody labeling for fluorescence-activated cell sorting (FACS) analysis. Commercially available fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-conjugated monoclonal antibodies CD2, CD4, CD8, CD14, CD34, CD20, and CD56 (Becton Dickinson, Mountain View, CA) were used. FN18, an antibody for rhesus CD3 (obtained from Dr M. Jonker, TNO) was indirectly labeled with FITC conjugate goat-antimouse IgG. Data were obtained by sorting at least 10,000 cells using a FACScan flow cytometer (FACScan; Becton Dickinson) with a single argon (488 nm) laser. FITC intensity was measured using a combined 510/515 long-pass and 540 short-pass filter set. PE intensity was measured using a 580/590 long-pass filter.

Experimental protocol. Monkeys were placed in a chair-like restraining device and blood was drawn from the right calf vein (t = 0 sample). Using the same route, saline or IL-8 was injected as a time-controlled bolus injection. Venous blood samples were always obtained from the left calf vein at several time intervals after injection.

Statistical analysis. Differences were evaluated using the Student’s t-test. P values of <.05 were considered to be statistically significant.

RESULTS

Pharmacokinetics of IL-8. To study the pharmacokinetics of IL-8, plasma levels of IL-8 were determined at various time intervals after injection. At 1 minute after injection of IL-8, maximum levels of IL-8 were reached ranging from 230 ± 109 ng/mL for animals treated with a dose of 10 µg/kg (mean ± SD, n = 2) up to 3,550 ± 3,580 for animals treated with a dose of 100 µg/kg (mean ± SD, n = 4; Fig 1). No circulating IL-8 could be detected after 120 minutes. Interindividual peak values varied considerably between 610 ng/mL up to 8,766 ng/mL for a dose of 100 µg IL-8 per kilogram of body weight (Table 1). The half-life time for circulating IL-8 calculated from these data was 9.9 ± 2.2 minutes (mean ± SD, n = 12, elimination phase). Within the detection limits, the levels of circulating IL-8 decreased in a monophasic fashion.

Effect of IL-8 on peripheral blood leukocyte counts. All animals injected with IL-8, regardless of the dose, showed an instant neutropenia occurring between 1 and 5 minutes after injection (Fig 2A). The numbers of circulating lymphocytes and monocytes decreased only slightly (Fig 2C). This neutropenia was followed by neutrophilia. At lower doses, maximum levels of circulating neutrophils were reached at earlier time intervals after IL-8 injection (t = 30 for 10 µg/kg, t = 45 for t = 30 µg/kg, and t = 120 for 100 µg/kg; Fig 2A).

The neutrophil fraction consisted of mature and immature cells (Fig 2B). Not only were bands seen, but, in several experiments, myelocytes, metamyelocytes, and erythroblasts were also seen (data not shown). Upon FACS analysis using monoclonal antibodies against CD2, CD3, CD4, CD8, CD20, and CD56, no significant changes between lymphocyte subsets were observed (data not shown).

Distribution of labeled leukocytes after injection of IL-8 as measured by scintigraphy. In 1 of 18 experiments, a
rhesus-monkey became dyspnoeic 1 minute after the injection of IL-8. Dyspnoea resolved spontaneously after 5 minutes. Because this period coincided with the neutropenic phase, we tested the hypothesis that the IL-8–induced neutropenia was caused by sequestration of neutrophils in the lung. A rhesus monkey therefore received an injection of autologous leukocytes labeled with 99mTc. At 22 minutes after injection, equal distribution of labeled leukocytes over the body had occurred and IL-8 (100 µg/kg) was injected. Immediately after injection and coinciding with the neutropenia, an increment of radioactivity was observed over the lungs concurring with a decrease of activity over the aorta and the brains. No change in radioactivity was observed over the liver or the spleen (Fig 3).

Mobilization of myeloid progenitor cells. To study a possible mobilizing effect, IL-8 was injected intravenously at dose levels of 10, 30, or 100 µg per kilogram of body weight. This resulted in a dose-dependent increase in the numbers of circulating progenitor cells starting at 5 minutes after injection and reaching maximum levels at \( t = 30 \) minutes of \( 1,382 \pm 599 \) CFU/mL for 100 µg IL-8 per kilogram (mean ± SD, \( n = 8 \)), whereas no increase was observed in control monkeys treated with the vehicle alone (9 ± 7 CFU/mL for saline, mean ± SD, \( n = 2, t = 30 \) minutes; Fig 4). The increment in the absolute numbers of progenitor cells ranged from 10 to 100 fold at a dose of 100 µg IL-8 per kilogram. The various types of mobilized progenitor cells were not different between blood derived from IL-8–treated animals and controls (Fig 5).

Effect of a second bolus injection of IL-8. To study the possibility of tachyphylaxis, a second bolus injection of IL-8 was administered. An interval of 72 hours was chosen to ensure full restoration of the mobilizing capacity. A second injection resulted in similar levels and kinetics of circulating numbers obtained after the first injection (Fig 6).

DISCUSSION

In this study, we report the stem cell mobilizing properties of IL-8 in rhesus monkeys. Rhesus monkeys treated with a single intravenous bolus injection of IL-8 showed an instant
IL-8 MOBILIZES PROGENITOR CELLS IN MONKEYS

Fig 5. Distribution of progenitor subtypes as determined in a CFU-Mix assay as a percentage (left) of the total numbers of IL-8 (100 pg/kg) mobilized progenitor cells (right). Results are expressed as the means of four experiments. Cells were plated in triplets for each experiment.

A high level of circulating IL-8 that rapidly declined. The t of free plasma IL-8 was calculated to be 9.9 ± 2.2 minutes, which is in accordance with the t described by van Zee et al (7.5 ± 1.6 minutes).

IL-8 injection had a profound effect on the numbers of hematopoietic progenitor cells (HPCs) in the peripheral blood. Within 5 minutes after injection, the numbers of circulating HPCs increased rapidly. Maximum levels of circulating progenitor cells were reached at 30 minutes after injection, regardless of the dose. In contrast, the maximum numbers of mobilized progenitor cells were clearly dose-dependent, although considerable interindividual differences were observed, ranging from a 10- to 100-fold increment (mean, 28-fold; n = 8) in circulating HPCs for a dose of 100 µg IL-8 per kilogram of body weight. These interindividual differences could not be explained by differences in levels of circulating IL-8. We previously reported that the mobilization of progenitor cells in mice was a specific effect of IL-8, because the mobilizing and neutrophilia-inducing effect was completely blocked by treatment of the animals with a polyclonal anti-IL-8 antibody before IL-8 injection. In contrast to mature circulating leukocytes, the progenitor cells appearing in the circulation after injection of IL-8 were not lineage-specific. All subtypes of colony-forming cells were observed in the circulation of IL-8-treated animals. Based on the numbers that are commonly used in blood stem cell transplantation in humans (20 × 10⁴ CFU-GM per kilogram of body weight), a leukapheresis procedure processing 300 mL of blood would be sufficient for autologous stem cell transplantation. These data illustrate the potential application of IL-8 in blood stem cell transplantation.

Rapid release of progenitor cells within 30 minutes is, to our knowledge, a unique property of chemoattractant agents. It was previously reported for complement 5a, another chemoattractant. However, the mechanism of stem cell mobilization is unclear. Papayannopoulou and Nakamoto have shown that involvement of adhesion molecules plays a role in mobilization of progenitor cells by treating nonhuman primates with injections of anti-VLA₄ antibody. They reported increments in the numbers of HPCs with similar interindividual variety and almost similar levels as reported here. However, mobilization was induced at 24 hours after the first injection of anti-VLA₄ antibody, implicating that another mechanism was involved in IL-8-induced mobilization. Other adhesion molecules such as LFA-1 and L-Selectin are also expressed on hematopoietic progenitor cells. IL-8 induces shedding of L-selectin on neutrophils; similarly, IL-8 may induce instant shedding of L-selectin of the surface of hematopoietic progenitor cells, contributing to the rapid
mobilization of progenitor cells. However, this hypothesis supposes the expression of IL-8 receptors on HPCs, which is at present unknown.

Another mechanism for rapid mobilization of progenitor cells would be an indirect interference with adhesion of progenitor cells to matrix molecules in the bone marrow microenvironment. Activation of neutrophils by IL-8 induces within a few minutes release of several metalloproteases involved in the degradation of the extracellular matrix.\textsuperscript{45,50,22} Primitive progenitor cells are preferentially bound to the extracellular matrix molecules.\textsuperscript{45} Neutrophils in the bone marrow may release these proteases after activation by IL-8,\textsuperscript{46} resulting in nonspecific release of progenitor cells into the circulation. Experiments in primates are in progress to test this hypothesis.

Systemic administration of IL-8 induces an immediate neutropenia also described by others.\textsuperscript{29,34-36} The neutropenic period in our experiments lasted for at least 5 minutes. In this study, we showed that \textsuperscript{99m}Tc-labeled leukocytes accumulated in the lungs instantly after injection of IL-8, coinciding with a decrement of activity over the aorta. Leukocytes did not accumulate in the liver, spleen, or brain. Because only the numbers of circulating neutrophils decreased significantly, it was likely that the accumulation of radioactivity in the lungs was due to neutrophil sequestration. This may also explain the short and self-resolving period of dyspnea after injection of IL-8 observed in one of the monkeys. Rot\textsuperscript{37} and Hechtman et al.\textsuperscript{38} observed a similar accumulation of neutrophils in the lungs of rabbits. Considering the very rapid induction of neutropenia, an instantaneously induced effect must play a principal role in this phenomenon. Instant cell stiffening is described on activation of neutrophils.\textsuperscript{48} The decreased deformability may then result in accumulation of neutrophils in the microvessels of the lungs.\textsuperscript{35,48} Upregulation of adhesion molecules such as CD11b/CD18 \( \beta2 \)-integrins may also participate in the accumulation of neutrophils, but does not explain the very rapid neutropenia and the preference for accumulation in the lungs.\textsuperscript{27,29,35,47}

At 5 minutes after injection of IL-8, neutrophil counts started to increase. Maximum numbers were reached 30 to 120 minutes after injection, depending on the dose of IL-8 administered. This phenomenon is less well explained. IL-8 promotes neutrophils to adhere to endothelium followed by migration into tissues.\textsuperscript{36,49,50} As shown by Hechtman et al.,\textsuperscript{38} intravascular IL-8 inhibits the migration of neutrophils into tissues. This may be due to the shedding of L-selectin by neutrophils.\textsuperscript{25,26} These selectins are necessary for the initial contact of neutrophils before a more potent adhesion via \( \beta2 \)-integrins and transendothelial migration.\textsuperscript{50} In this way, shedding of L-selectin may also induce circulation of marginating neutrophils. IL-8 is also a chemotactic agent for neutrophils and may recruit neutrophils from the bone marrow reservoir.\textsuperscript{5,57,37} As shown here, the increment in numbers of neutrophils comprises mature as well as immature cells derived from the bone marrow. This phenomenon was also observed by Hechtman et al.\textsuperscript{38} Therefore, recruitment of neutrophils from the marginal pool as well as the bone marrow reservoir may be responsible for the observed neutrophilia.

These two IL-8–induced mechanisms of neutrophil mobilization may explain the dose-dependent time interval between injection of IL-8 and maximal numbers of circulating neutrophils. The maximal numbers of circulating immature neutrophils (bone marrow compartment) are identical for all dose levels at 30 minutes. However, neutrophils initially trapped in the lungs are released sooner after injection of lower doses of IL-8 due to a shorter effective plasma level of IL-8. Therefore, desequstration of neutrophils from the lungs would account for the earlier peaking.

In summary, our studies show that a single injection of IL-8 induces instant mobilization of hematopoietic progenitor cells in nonhuman primates in a reproducible fashion. Because IL-8 is a rapid inducer of mobilization of hematopoietic progenitor cells, mobilization may be performed electrochemically. Side effects may be very limited because IL-8 is a cytokine at the end of the acute-phase cascade and has been shown not to induce release of other acute-phase proteins in vivo.\textsuperscript{27} Therefore, IL-8 may be potentially applicable for stem cell mobilization in humans.

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IL-8 MOBILIZES PROGENITOR CELLS IN MONKEYS


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