Marrow- and Spleen-Seeding Efficiencies of All Murine Hematopoietic Stem Cell Subsets Are Decreased by Preincubation With Hematopoietic Growth Factors

By Johannes C.M. van der Loo and Rob E. Ploemacher

The cobblestone-area forming cell (CAFC) assay permits a direct measurement of the seeding of primitive and more mature murine hematopoietic stem cell subsets by comparing the number of CAFC in the original transplant with the number of CAFC retrieved from bone marrow (BM) and spleen after transplantation. We found no differences in seeding efficiency between the more mature and primitive CAFC subsets, nor between seeding efficiencies of stem cells from low-density (LD) fractions of normal and day-6 post-5-fluorouracil BM. The data show that 18% to 20% of all intravenously transplanted stem cell subsets seed to the BM, whereas 8% to 10% seed to the spleen. In addition, similar seeding efficiencies were found for day-12 spleen colony-forming unit (CFU-S-12) as was determined by re-transplantation. Previously, it has been reported that a 2- to 3-hour preincubation of BM with interleukin-3 (IL-3) enhances the in vivo repopulating ability of a graft. To test whether hematopoietic growth factors affected this increased engraftment by enhancing the seeding of the transplanted marrow, we assessed the 16- to 18-hour seeding efficiency of short- and long-term in vivo repopulating stem cell subsets to BM and spleen using the CAFC assay, after preincubation with or without hematopoietic growth factors. A 2- to 3-hour preincubation with IL-3, or a combination of IL-3, IL-12, and steel factor, at 37°C, led to a substantial decrease in seeding compared with control (which was kept on ice) of all hematopoietic subsets measured, both in spleen and BM. In concert with these data, the long-term in vivo repopulating ability of growth-factor incubated BM was also decreased when compared with control. In conclusion, we have been unable to observe a beneficial effect of growth factor preincubation on the repopulating ability of a graft.

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The homing of hematopoietic stem cells and progenitors and binding to the bone marrow (BM) stroma is mediated by a complex of interactions, involving stromal cells as well as components of the extracellular matrix. These components include the different types of collagen and several glycoproteins such as fibronectin, hemnectin, thrombospondin, the intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), and the family of proteoglycans with their different glycosaminoglycan side chains. Human and murine hematopoietic stem cells and progenitors have been shown to interact with the stromal microenvironment using several pathways simultaneously. The β1 integrins very late antigen 4 (VLA-4; α4β1) and VLA-5 (α5β1) mediate the binding to stromal fibronectin by interacting with its heparin- and cell-binding domains, respectively. In addition, VLA-4 also interacts with VCAM-1 and the family of proteoglycans with their different glycosaminoglycan side chains. Human and murine hematopoietic stem cells can bind chondroitin-sulfate produced by the progenitor cell. Other pathways involve the interaction of a β2 integrin with ICAM-1 and CD44/Pgp-1 with hyaluronic acid. Ample evidence exists on the role of a membrane lectin in the specific homing of intravenously transplanted stem cells to the marrow. This lectin receptor, which has been purified from the cloned progenitor cell lines FDCP-1 and B6SUT, is a 110-kD glycoprotein heterodimer with specificity for galactosyl and mannosyl residues of a membrane-associated glycoconjugate on marrow stromal cells. It has been shown that the expression of homing receptors on FDCP-1 and FDCP-mix cells can be upregulated by a brief incubation with IL-3 or granulocyte-macrophage colony-stimulating factor (GM-CSF). In addition, it has been reported that an incubation of mouse BM cells or sheep or monkey fetal liver cells with hematopoietic growth factors (either IL-3, GM-CSF, or phytohemagglutinin-stimulated lymphocyte-conditioned medium) before transplantation significantly enhances the engraftment when compared with preincubation without these factors. It has been suggested that this enhancement could be attributed to an increased seeding efficiency of the transplanted stem cells as a result of an upregulation of homing protein expression.

In the present study, we investigated the BM and spleen-seeding efficiency of day-12 spleen colony-forming units (CFU-S-12) and of short-term and long-term in vivo repopulating stem cell subsets as assessed by the cobblestone area forming cell (CAFC). In addition, we determined the effect of a brief 2- to 3-hour preincubation of BM cells with rIL-3, or with a combination of IL-3, IL-12, and steel factor (SF), on the CAFC-seeding efficiency and long-term repopulating ability of the graft in vivo. The results show that all hematopoietic stem cell subsets tested, in normal and day-6 post-5FU LD BM, have similar seeding efficiencies. In addition, we show that a brief 2- to 3-hour preincubation of BM cells at 37°C with hematopoietic growth factors has a negative effect on the seeding of hematopoietic stem cells and progenitors to BM and spleen. Furthermore, we show that this preincubation did not enhance the long-term in vivo engraftment of the BM when compared with BM that was not incubated, but kept on ice during the whole procedure.

MATERIALS AND METHODS

Animals. Male and female inbred (CBA × C57BL) F1 mice (BCBA), 14 to 35 weeks old, were bred and maintained under specific pathogen-free conditions at the Central Animal Department of the Erasmus University, and received acidified water (pH 2.8) and...
food pellets ad libitum. In specific experiments, male mice were injected intravenously with 150 mg 5-fluorouracil (5FU; Sigma, St Louis, MO) in phosphate-buffered saline (PBS) per kg body weight 6 days before harvest of the BM.

Preparation of BM cells and density centrifugation. BM from untreated or 5FU-treated animals was harvested from tibiae and femurs and separated by buoyant density centrifugation using a discontinuous Ficoll–400 (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient as previously described. Cells with a density of 1.069 to 1.078 g/mL (10% to 5% of total BM cells, 5% to 6% of post-5FU BM cells) were collected from the interphases, washed in cold Dutton’s balanced salt solution (Dutton; Gibco, Breda, The Netherlands) containing 5% fetal calf serum (FCS), and maintained on ice.

Incubation with hematopoietic growth factors. To determine the effect of growth factor preincubation on spleen and BM seeding, normal low-density (LD) BM cells were incubated for 2 to 3 hours at 37°C at a density of 5 × 10^6 cells/mL in Dutton containing 5% FCS, with or without specific growth factors. The control group (1) was maintained on ice. Other groups contained either 100 U/mL murine IL-3 (group 2); 100 U/mL IL-3, 10 ng/mL murine IL-12, and 100 ng/mL murine steel factor (SF) (group 3); 10 ng/mL IL-12 and 100 ng/mL SF (group 4); or no additional growth factors (group 5). After incubation the cells were washed, counted, and used immediately for transplantation. Part of the cells were kept on ice until transfer into the different in vitro assays (within 2 hours). All groups were tested for in vitro clonable myeloid progenitors (CFU-GM) and viability of the cells was tested using propidium iodide staining and FACScan analysis. Groups 1 to 3 were also tested for cobbles- stone area formation in the CAFC assay, for CFU-S-12 and for long- term in vivo repopulating ability (LTRA). In addition, the cells in groups 1 to 3 were tested for their spleen- and BM-seeding efficiency (next section). The recombinant factors used were kindly provided by Dr S. Neben of the Genetics Institute (Cambridge, MA).

Determination of the seeding is femur and spleen. The seeding of normal LD BM, after preincubation with or without growth factors, and post-5FU LD-BM, was determined at 16 to 18 hours after transplantation, as is schematically shown in Fig 1. Male recipients (four to five mice per group, 15 weeks old) received 11.1 Gy total body irradiation (gamma) at a dose rate of 0.98 Gy/min, 7 to 9 hours before transplantation. The dose was chosen to maximally reduce residual hematopoietic activity allowing the detection of low numbers of seeded stem cells. Mice were transplanted with 4 × 10^6 post-5FU or 1.3 × 10^7 normal LD-BM cells, respectively, by injection of 0.5 mL of single cell suspensions into a lateral tail vein. Part of the cells were used to determine the input number of transplanted CFU-S-12 and CAFC subsets (CAFC-7 through CAFC-28) in the CFU-S and CAFC assay, respectively. Spleens and femurs were removed under sterile conditions at 16 to 18 hours after transplantation, which is 24 to 26 hours after irradiation. Cells from spleens and femurs were collected quantitatively and were pooled per organ per group. Single cell suspensions were prepared by gently sieving the cells through 100-μm and 30-μm sterile nylon gauze. Spleen and BM cells were subsequently tested for CFU-S-12 and CAFC. The BM- and spleen-seeding efficiencies were then calculated on the basis of the number of input CAFC and CFU-S-12, and number of output CFU-S-12 and CAFC per spleen or femur. The transplanted
established in flat-bottomed 96-well microtiter plates for limiting dilution analysis of CAFC as previously described.\textsuperscript{41,43,44} Briefly, an adherent stromal layer from BM of BCBA mice (5 to $10 \times 10^{5}$ cells/well) was grown in 10 to 14 days at $33^\circ C$, 10% CO$_2$ in an 100%-humidified incubator. At confluency, the cultures were irradiated with 20 Gy$^{37}$Cs gamma. For limiting dilution analysis, the stroma was overlaid with 12 dilutions of a BM or spleen cell suspension, twofold apart, using 15 wells per concentration. The stroma could not be overlaid with more than 1/20 femur or 1/40 spleen per well, ie 3 to $4 \times 10^{5}$ cells per well, as higher numbers of cells disrupted the stromal layer. Wells were inspected 1 to 2 times per week, from 1 to 5 weeks after inoculation, using a phase-contrast inverted microscope, and were scored positively when at least one hematopoietic clone (cobblestone area [CA], a cluster containing five or more phase-dark cells) was observed. The frequency of CAFC was calculated from the proportion of negative wells on the basis of Poisson statistics.\textsuperscript{45}

**Colony assay.** The number of CAFC-GM was determined in 1 mL semisolid cultures consisting of 1.2% (wt/vol) methylcellulose (Methocel MC, Fluka Chemie, Buchs, Switzerland) in a-modified Dulbecco’s modified Eagle’s medium (GIBCO, Grand Island, NY) at an osmolarity of 280 mOsmol/kg. The medium was supplemented with 20% horse serum (GIBCO), 1% bovine serum albumin (BSA), 80 U/mL penicillin, 80 µg/mL streptomycin, 3.2 mmol/L L-glutamine (Merck, Darmstadt, Germany), $8 \times 10^{-4}$ mol/L sodium selenite (Merck) and $8 \times 10^{-4}$ mol/L $\beta$-mercaptoethanol (final concentrations). Cultures (four replicate dishes per group) were stimulated by 10% (vol/vol) pokeweed mitogen-stimulated mouse spleen conditioned medium, and were kept at 37°C, 5% CO$_2$, and 100% humidity. Colonies consisting of 50 cells or more were counted at day 8 using an inverted microscope.

**LTRA.** The LTRA was determined by injection of different numbers of male BM cells into sublethally irradiated syngeneic female recipients (10 mice per group). We used four cell doses per experimental group, twofold apart. Female recipients (15 to 25 weeks old) received 7.8 Gy total body irradiation 1 day before transplantation. The dose was chosen to enable survival of all animals, independent of the BM dose injected. Mice, 15 to 35 weeks old, were randomly divided over the groups, each containing 10 to 15 mice. The primary transplant mice were injected with $5 \times 10^{5}$ to $1 \times 10^{6}$ LD cells. Secondary recipients were infused with either 1/60 to 1/200 spleen (equaling 8 $\times 10^{4}$ to 3 $\times 10^{5}$ cells), or with 1/10 to 1/30 femur (equaling 1 $\times 10^{5}$ to 6 $\times 10^{5}$ cells), per mouse. A control group of 10 to 15 mice that had not received any cells was included in all experiments. Mice were kept in laminar air flow cabinets during the experiments and were killed at day 12 after injection. Their spleens were excised and fixed in Tellyesniczky’s solution. Macroscopically visible colonies were counted.

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**CAFC assay.** Dexter-type murine long-term BM cultures were performed using primary BM cultures established in flat-bottomed 96-well microtiter plates for limiting dilution analysis of CAFC as previously described.\textsuperscript{41,43,44} Briefly, an adherent stromal layer from BM of BCBA mice (5 to $10 \times 10^{5}$ cells/well) was grown in 10 to 14 days at $33^\circ C$, 10% CO$_2$ in an 100%-humidified incubator. At confluency, the cultures were irradiated with 20 Gy$^{37}$Cs gamma. For limiting dilution analysis, the stroma was overlaid with 12 dilutions of a BM or spleen cell suspension, twofold apart, using 15 wells per concentration. The stroma could not be overlaid with more than 1/20 femur or 1/40 spleen per well, ie 3 to $4 \times 10^{5}$ cells per well, as higher numbers of cells disrupted the stromal layer. Wells were inspected 1 to 2 times per week, from 1 to 5 weeks after inoculation, using a phase-contrast inverted microscope, and were scored positively when at least one hematopoietic clone (cobblestone area [CA], a cluster containing five or more phase-dark cells) was observed. The frequency of CAFC was calculated from the proportion of negative wells on the basis of Poisson statistics.\textsuperscript{45}

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IN VIVO SEEDING AND GROWTH OF STEM CELLS

Seeding of CAFC in normal and post-5FU LD BM. To determine the seeding of more mature and primitive hematopoietic stem cell subsets to BM and spleen, LD BM cells from normal and 5FU-treated mice were transplanted into 11.1 Gy-irradiated recipients (four to five mice/group). Part of the cells were tested in the CAFC assay to calculate the number of CAFC subsets (CAFC-7 through CAFC-28/35) that were transplanted. After 16 to 18 hours, the mice were killed and cells from their spleens and femurs were collected and tested for their CAFC content (Figs 2A and 3A). Significant differences between the seeding efficiencies of early (day 7 through 14) and late (day 28 through 35) CAFC derived from normal BM (Fig 2B), representing the more mature and primitive hematopoietic stem cell subsets, respectively,41,43 were not observed. However, compared with the late CAFC, the seeding of the more mature progenitor cells (CAFC-7) in post-5FU BM was slightly lower (Fig 3B). This may reflect an effect of the 5FU-treatment as it was not observed in normal BM. On average, 9.3% ± 0.5% (mean ± SEM) and 9.5% ± 0.5% of the CAFC-10 through CAFC-28/35, in normal and post-5FU BM, respectively, seeded to the spleen, whereas 1.1% ± 0.1% and 1.2% ± 0.1% of the CAFC, respectively, were recovered per femur (Figs 2B and 3B). The seeding efficiencies of normal and post-5FU CAFC were not significantly different (P > .1).

Previously, extensive correlation studies have shown that day-10 CAFC (CAFC-10) enumerate CFU-S-12 numbers, whereas CAFC-28 through CAFC-35 are related to the long-term repopulating ability of a graft.41,43,48-51 Therefore, the present results show that the different murine hematopoietic stem cell subsets, ranging from the most primitive long-term repopulating stem cells up to CFU-S-12, in normal as well as in day-6 post-5FU BM, have equal seeding efficiencies.

Seeding of CAFC subsets after growth factor incubation. To test whether a brief preincubation with hematopoietic growth factors would influence the marrow- and spleen-seeding efficiency of hematopoietic stem and progenitor cells, LD BM was incubated for 2 to 3 hours at 37°C with 100 U/mL IL-3, or with a combination of growth factors, and was
transplanted into 11.1 Gy–irradiated recipients (four to five mice/group) as indicated in Fig 1. The combination of IL-3, IL-12, and SF was included as it has been shown to be more powerful than IL-3 alone in preserving hematopoietic stem cell function in 7-day liquid cultures. The control suspension remained on ice in medium (Dutton with 5% FCS) without additional growth factors. FACScan analysis with propidium iodide showed a viability of 97% in the control, and 87% to 93% in all other groups (data not shown). Compared with the control (group 1), incubation with IL-3 (group 2) or IL-3 plus IL-12 plus SF (group 3) did not change the number of day-8 CFU-GM and CFU-S-12, as was shown in the primary assays (Fig 4). However, after preincubation in IL-12 plus SF (group 4), or in medium without extra growth factors (group 5), the number of CFU-GM was slightly decreased, indicating that IL-3 seems necessary for the survival of progenitors at 37°C. This was roughly reflected by the total cellular viability (>95%, data not shown).

The average of three independent experiments shows that the apparent frequency of primary CAFC was slightly enhanced by preincubation with IL-3 or IL-3 plus IL-12 plus SF (Fig 5A). Despite this, the CAFC subsets that could be recovered from spleen and marrow after growth factor preincubation, 16 to 18 hours after transplantation, were at 50% to 70% of control values (Fig 5B and 5C). The overall seeding efficiency of all hematopoietic stem cell subsets tested, calculated from the CAFC frequencies of the graft before transplantation and from BM and spleen suspensions after transplantation, was significantly decreased by growth factor preincubation (Fig 6, A and B). A difference between preincubation with IL-3 or IL-3 plus IL-12 plus SF was not observed.

**Seeding of CFU-S-12 after growth factor incubation.** The seeding efficiency of CFU-S-12 was also determined on the basis of their number before transplantation and the CFU-S-12 content of femurs and spleen 16 to 18 hours after transplantation (Table 1). On average, growth factor preincubation also decreased the seeding of CFU-S-12 to the spleen, from 11.4% (group 1) to 7.3% (group 2) or 7.9% (group 3). The lack of statistical difference between group 1 and groups 2 and 3 in seeding to the femur may be caused by the large variation inherent to the spleen-colony assay and retransplantation technique. Spleens and femurs of animals that had been irradiated with 11.1 Gy, but had received no cells, contained no detectable CFU-S-12 nor CAFC in any of the assays performed (not shown).

**Long-term in vivo repopulating ability.** To determine the effect of hematopoietic growth factor preincubation on the stable engraftment ability of stem cells in vivo, LD BM cells from male mice were incubated with and without growth factors (groups 1 to 3, Fig 1) and transplanted in limiting dilution into sublethally irradiated syngeneic female recipients (10 mice per group). At 4 months after transplantation, donor-type repopulation was assessed by fluorescent in situ hybridization on blood smears of the sex-mismatched chimeraic mice using a murine Y-chromosome–specific probe (Fig 7). The data show that chimeraism was lower in the groups preincubated with either IL-3 or IL-3 plus IL-12 plus SF, when compared with control marrow that was kept on ice throughout the whole incubation.

**DISCUSSION**

The present study shows that the seeding efficiency of different hematopoietic stem cell subsets, ranging from CFU-S-12 (assessed by the frequency of CAFC-10) to the long-term repopulating stem cells (as assessed by the frequency of CAFC-28/35), can be determined directly without the need for retransplantation studies. The data show that all hematopoietic stem cell subsets tested, in normal as well as in post-5FU LD BM, have similar seeding efficiencies. They
Table 1. Seeding Efficiency of Low-Density BM Cells in Spleen and Femur After Preincubation With or Without Hematopoietic Growth Factors Using Primary and Secondary CFU-S-12 Assays

<table>
<thead>
<tr>
<th>Group and Exp No.</th>
<th>CFU-S-12* per 10^6 Cells</th>
<th>CFU-S-12† Infused per Mouse</th>
<th>CFU-S-12† Infused per Mouse</th>
<th>CFU-S-12‡ Retrieved per Spleen</th>
<th>CFU-S-12‡ Retrieved per Femur</th>
<th>% Seeding† Spleen</th>
<th>% Seeding† Femur</th>
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<tbody>
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<tr>
<td>a</td>
<td>43.33 ± 3.60</td>
<td>7.50 x 10^5</td>
<td>3,249.8 ± 270.0</td>
<td>282.9 ± 18.6</td>
<td>40.61 ± 3.7</td>
<td>8.7 ± 0.9</td>
<td>1.25 ± 0.15</td>
</tr>
<tr>
<td>b</td>
<td>73.27 ± 8.49</td>
<td>1.30 x 10^7</td>
<td>9,525.3 ± 1,103.1</td>
<td>1,020.0 ± 91.4</td>
<td>114.29 ± 14.2</td>
<td>10.7 ± 1.6</td>
<td>1.20 ± 0.20</td>
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<tr>
<td>c</td>
<td>81.63 ± 5.56</td>
<td>1.90 x 10^7</td>
<td>10,611.7 ± 722.4</td>
<td>1,577.8 ± 164.6</td>
<td>154.29 ± 13.5</td>
<td>14.9 ± 1.8</td>
<td>1.45 ± 0.16</td>
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<td>11.4 ± 1.4</td>
<td>1.30 ± 0.17</td>
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<td>a</td>
<td>34.29 ± 3.74</td>
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<td>245.0 ± 33.5</td>
<td>44.00 ± 5.16</td>
<td>9.5 ± 1.7</td>
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<tr>
<td>b</td>
<td>75.47 ± 9.04</td>
<td>1.14 x 10^7</td>
<td>8,620.7 ± 1,030.9</td>
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<td>152.37 ± 15.5</td>
<td>4.9 ± 1.5</td>
<td>1.77 ± 0.28</td>
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<td>c</td>
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<td>1.35 x 10^7</td>
<td>12,381.6 ± 794.6</td>
<td>900.0 ± 63.2</td>
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<td>7.3 ± 1.4</td>
<td>1.45 ± 0.24</td>
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<td>a</td>
<td>40.50 ± 4.59</td>
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<td>b</td>
<td>62.61 ± 6.94</td>
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<td>Averages</td>
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<td>7.9 ± 1.2</td>
<td>1.15 ± 0.18</td>
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LD BM cells were either kept on ice (group 1) or were incubated for 2 to 3 hours at 37°C in Dutton, 5% FCS containing 100 U/mL IL-3 (group 2), or 100 U/mL IL-3, 10 ng/mL IL-12, and 100 ng/mL SF (group 3) before transplantation (see Fig 1). All data are corrected for the number of endogenous colonies in the control-irradiated group (average 0.2 colony/spleen). Survival in all groups averaged between 90% and 95%. Letters a, b, and c indicate individual experiments.

* Mice (10 to 15 per group) were irradiated with 8.6 Gy 4 to 6 hours before transplantation, and received 5 x 10⁴ to 1 x 10⁵ LD BM cells, giving rise to 3 to 7 colonies per spleen. Data represent mean ± SEM.

† Number of cells and CFU-S-12 transplanted per recipient (four to five mice per group). Recipient mice received 11.1 Gy total body irradiation 7 to 9 hours before transplantation.

‡ Mice received 1/60 to 1/200 spleen (from primary recipients) by intravenous injection, equalling 8 x 10⁴ to 3 x 10⁵ cells per mouse (10 to 15 mice per group). CFU-S-12 colonies ranged from 3 to 9 per spleen.

§ Mice received 1/10 to 1/30 femur (from primary recipients) by intravenous injection, equalling 1.2 x 10⁴ to 6.2 x 10⁵ cells per mouse (10 to 15 mice per group). CFU-S-12 colonies ranged from 3 to 7 per spleen.

‖ Seeding efficiencies in spleen and femur, 16 to 18 hours after transplantation.

also show that 1.2% of the CAFC could be recovered from a femur, whereas 9.5% seeded to the spleen, which is in agreement with previous estimates using the CFU-S assay.53-56 Because one femur contains about 6% of all BM cells,37 approximately 30% of the intravenously injected hematopoietic stem cells seeded to the two major hematopoietic organs. In addition, we show that a brief 2- to 3-hour preincubation of BM cells at 37°C in medium containing IL-3, or a combination of IL-3, IL-12, and SF, did not improve but even significantly impaired the seeding of hematopoietic stem cells to both spleen and BM. The in vivo engraftment, determined at four months after transplantation, was similarly compromised, indicating that the net effect of preincubation of BM cells with hematopoietic growth factors at 37°C is not beneficial for BM transplantation.

In contrast with our findings, it has been suggested that the engraftment of hematopoietic stem cells could be improved by a brief incubation with IL-3 or GM-CSF before transplantation.37,39,44 Fabian et al and Tavassoli et al37,39 studied the effect of growth factor addition on the engraftment of murine BM cells that were preincubated at 37°C. Unlike in our experiments, these investigators did not include a group that was either nonincubated, or kept on ice. Their data show that numbers of primary CFU-S and CFU-GM were slightly decreased in the 37°C control group compared with the 37°C group that had received IL-3 or GM-CSF.37,39 In line with their data, we observed a loss of CFU-GM after incubation in medium without IL-3. In addition, it has previously been shown that cloned progenitors deprived of growth factors not only undergo apoptosis, but also gradually lose their ability to adhere to a stromal layer.58,59 Consequently, incubation of hematopoietic cells at 37°C without growth factors might influence their capacity to home to hematopoietic organs after intravenous transfer in vivo. Therefore, the previously observed beneficial effect of growth factor preincubation may only bear on the survival of stem cells at 37°C rather than showing an increased engraftment ability after preincubation as compared with no incubation. Hence, the difference between their findings and ours may be caused by the use of different controls.

In an allogeneic transplantation setting, using T-cell-depleted, major histocompatibility complex-disparate donor BM, the effects of growth factor preincubation seem even more complex. A brief ex vivo preincubation with GM-CSF facilitated engraftment across extensive histocompatibility barriers.60 In contrast, using the same transplantation model, these investigators found a lower engraftment after preincubation with IL-3.61 In both studies, seeding efficiencies were not determined. Because these effects might have been related to graft-versus-host disease the results are difficult to interpret.
The effect of ex vivo growth factor preincubation has reportedly also been tested using sheep fetal liver cells that were incubated for 2 to 3 hours at 37°C with (W) 11-3, or rism in the recipients of multiple growth factor-incubated BM cells term repopulating ability compared with freshly transplanted control hematopoietic stem cells home exclusively to the fetal liver and not to the BM. Hematopoietic progenitor cell membrane with the RGD domain of hyaluronic acid expressed by human fetal liver and BM stromal cells. As described in the present study, and the in utero transplantation of fetal liver cells, as described previously, are difficult to compare because the cells not only have a different sensitivity for hematopoietic growth factors, but also home to different organs with qualitatively different microenvironments. Consequently, the receptors involved in the homing, and the modulation of their expression, may differ between the two models. This is supported by the observation that in the adult, homing receptor protein expression is organ specific.

In summary, the present study shows that all subsets of CAFC have equal BM- and spleen-seeding efficiencies. These subsets include the CFU-S-12 and the short and long-term in vivo repopulating stem cells as has been extensively shown by correlation studies. In addition, the present study shows that a 2- to 3-hour incubation of mouse BM cells with IL-3 or IL-3 plus IL-12 plus SF before transplantation significantly impairs the seeding efficiency of CFU-S-12 as well as more primitive hematopoietic stem cell subsets. This was accompanied by a loss of long-term in vivo engrafting ability. The lack of a beneficial effect of preincubation on the long-term in vivo engraftment of mouse BM stem cells questions a general application of preincubation for BM transplantation.

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