Stroma-Contact Prevents Loss of Hematopoietic Stem Cell Quality During Ex Vivo Expansion of CD34+ Mobilized Peripheral Blood Stem Cells


Mobilized peripheral blood stem cells (PBSC) are increasingly used to restore the formation of blood cells after high-dose chemotherapy for solid tumors and hematological malignancies.1-3 More recently, PBSC from cancer patients are cultured ex vivo4-6 because expanded hematopoietic stem cells (HSC) may possibly reduce the time to hematopoietic recovery after their transplantation. Furthermore, the use of smaller transplants may lead to a reduction of contaminating tumor cells. Although clinical ex vivo expansion trials have already started, many questions are still unanswered. Firstly, although many investigators have shown that the total number of CD34+ cells, progenitors, and primitive stem cells in PBSC can be expanded in vitro,4-10 there has been no report of improved hematopoietic recovery using such in vitro propagated HSC.4-6

Secondly, because of the nonmyeloablatative conditioning regimen and/or cotransplantation of unmanipulated HSC in these studies, it is also not apparent if primitive stem cells are still capable of long-term engraftment after ex vivo culture. Ex vivo propagation studies in mice have shown both loss of in vivo engraftment11 and an increased ability of cultured cells to repopulate irradiated hosts.12 Diminished engraftment in vivo may result from a reduced ability of stem cells to home to the bone marrow (BM). Indeed, we have recently shown that short incubations of murine stem cells with several cytokine combinations diminish their lodgement in hematopoietic organs and hence their ability to repopulate the hematopoietic system of irradiated recipients.13 In addition, loss of primitive stem cell quality may also lead to a reduced in vivo repopulating ability. Previously, we have studied 47 mobilized PBSC harvests of 21 autologous transplantation cancer patients and shown that poorly mobilized PBSC harvests contain a low number of primitive HSC (cobblestone area forming cell [CAFC] week 6), and also produce less progenitors per primitive stem cell in stroma-supported long-term cultures (LTC-CFC).14 This poor primitive stem cell quality was related with the number of cytokoreductive pretreatment rounds administered to the patients. In addition, we have observed low CAFC week 6 numbers and low primitive stem cell quality in the original autologous transplant of patients that failed to engraft within 6 months after transplantation.15

It has been shown that primary stromal feeder layers and stromal cell lines support the culture of HSC.16-19 In 2- or 5-week cultures without exogenous cytokines, the Verfaillie group has shown that primitive stem cell (LTC-initiating cell; LTC-IC) recovery and colony-forming cell (CFC) production in LTC was improved when normal BM-derived HSC were propagated in stroma-noncontact cultures as compared to stroma-contact.20,21 This improvement was explained by proliferation inhibition of CFC and LTC-IC during direct stroma-contact, possibly via adhesion of the fibronectin receptor to stroma.22,23 The stroma-noncontact cultures were further improved by the addition of interleukin-3 (IL-3) and macrophage inflammatory protein-1α (MIP-1α) to the medium and simplified by using stroma-conditioned medium (SCM) instead of stroma-noncontact transwell inserts.24 However, Koller et al25,26 have shown that cytokine-driven LTC-IC expansion can only be achieved with the use of a stromal feeder layer.

In contrast to studies on normal BM-derived HSC, only a limited number of studies are dedicated to ex vivo expansion of LTC-IC or CAFC week 6 from clinically relevant mobilized PB from cancer patients. In 7- to 12-day static liquid cultures of CD34-selected mobilized PBSC, a maintenance of LTC-IC or CAFC week 6 has been reported.7,9 Two- to 20-fold expansion of LTC-IC or CAFC week 6 from mobilized PBSC was...
only observed in cultures containing stromal factors and/or accessory cells and in perfusion bioreactors. However, those studies did not provide information about the HSC quality.

In the present study we have focused on the effect of ex vivo propagation on both the number and quality of HSC because, in our view, numerical expansion of HSC can only be effective if their quality is not reduced at the same time. We used CD34-selected mobilized PBSC from myeloma and lymphoma patients in our experiments because this HSC source is also used in most clinical ex vivo expansion studies. In 7-day serum-free cultures supplemented with IL-3, stem cell factor (SCF), and IL-6 with or without FLT3-ligand (FL) and thrombopoietin (TPO), the effect of SCM addition from various stromal cell lines was tested in comparison with direct contact with a murine stromal cell layer and stroma-noncontact cultures on the recovery of progenitors cell and primitive stem cell numbers and their quality. The assessment of different HSC subsets was performed in 35-mm bacterial dishes (Greiner, Alphen a/d Rijn, The Netherlands) to prevent strong adherence of the hematopoietic cells to the plastic surface. The serum-free Iscove’s modified Dulbecco’s medium with Glatmax-1 (IMDM; Gibco, Breda, The Netherlands) contained 1% bovine serum albumin (A9418; Sigma, St Louis, MO), penicillin (100 U/mL; Gibco), streptomycin (100 µg/mL; Gibco), 10⁻⁴ mol/L β-mercaptoethanol (Merck, Darmstadt, Germany), bovine insulin (10 µg/mL; Gibco), 15 µM/L cholesterol (Sigma), 15 µM/L linolic acid (Merck), iron-saturated human transferrin (0.62 g/L; Intergen, Purchase, NY), cytidine (1 µg/mL; Sigma), adenosine (1 µg/mL; Sigma), uridine (1 µg/mL; Sigma), guanosine (1 µg/mL; Sigma), thymidine (1 µg/mL; Sigma), 2'-deoxyctydine (1 µg/mL; Sigma), 2'-deoxyadenosine (1 µg/mL; Sigma), 2'-deoxyguanosine (1 µg/mL; Sigma). Forty thousand CD34⁺ PBSC in 2 mL serum-free medium supplemented with IL-3 (25 ng/mL), human SCF (100 ng/mL), IL-6 (100 ng/mL), and anti-human-transforming growth factor-β1 (sTGFβ1; 1.0 µg/mL; R&D Systems, Abingdon, UK) with or without FL (100 ng/mL) and TPO (10 ng/mL) were cultured at 37°C and 10% CO₂. After 7 days of culture, the cells were collected from the dishes after scraping with a cell scraper (Greiner) and rinsing with IMDM. After washing, the cells were resuspended in IMDM and plated in CFC, CAFC, and flask-LTC assays.

**Stroma-conditioned media.** Confluent layers were grown of the stromal cell lines FBMD-1, L87/4, and L88/5.18,27 The cells were cultured in IMDM supplemented with 10% fetal calf serum (FCS; Summit, Fort Collins, CO), penicillin (100 U/mL), streptomycin (100 µg/mL), and 10⁻⁴ M β-mercaptoethanol. The FBMD-1 cells were maintained at 33°C and 10% CO₂ and the L87/4 and L88/5 cells at 37°C and 10% CO₂. When the layers were confluent, the medium was removed and rinsed twice with IMDM. Serum-free medium was added to the confluent stromal layers and conditioned for 7 days. The SCM were harvested, the nonadherent (NA) cells were removed by centrifugation, and the media were stored at −20°C until use. Control medium was prepared by parallel incubations without the stromal cell lines. In the cultures, 50% SCM or control medium was used.

**Stroma-contact cultures.** In 35-mm tissue culture dishes (Falcon, Franklin Lakes, NJ) confluent layers were grown of the stromal cell line FBMD-1. When the layers were confluent, 40,000 CD34⁺ PBSC were cultured on these stromal feeders in the same medium and under the same conditions as used for the serum-free liquid cultures. After 7 days of culture, the NA cells were collected from the dish and after two rinses with IMDM replaced by 1 mL of 0.1% trypsin (Gibco) for 5 minutes. The digestion was stopped by adding 1 mL of ice-cold FCS and the dish was scraped with a cell scraper to include all adherent cells. The NA and adherent cells were pooled and after washing the cells were resuspended in IMDM and plated in CFC, CAFC, and flask-LTC assays.

**Table 1. Mean Frequency of Different Progenitor and CAFC Subsets in Mobilized PBSC Before Ex Vivo Culture**

<table>
<thead>
<tr>
<th>Progenitor or CAFC Subset</th>
<th>Frequency per 100 Cells (± 1 SEM)</th>
<th>No. of Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34⁺</td>
<td>84 (± 3)</td>
<td>9</td>
</tr>
<tr>
<td>CFC</td>
<td>22 (± 3)</td>
<td>9</td>
</tr>
<tr>
<td>CAFC wk 2</td>
<td>3.0 (± 0.7)</td>
<td>9</td>
</tr>
<tr>
<td>CAFC wk 4</td>
<td>4.6 (± 1.0)</td>
<td>9</td>
</tr>
<tr>
<td>CAFC wk 6</td>
<td>2.7 (± 1.0)</td>
<td>9</td>
</tr>
</tbody>
</table>

**Materials and Methods**

**Mobilized PB.** Nine leukapheresis products from four patients with non-Hodgkin’s lymphoma, four with multiple myeloma, and one with Burkitt’s lymphoma in remission were used in this study. Before leukapheresis the HSC were mobilized to the blood after several courses of chemotherapy using granulocyte colony-stimulating factor (Filgrastim, recombinant-methionyl human G-CSF; Roche, Midjrecht, The Netherlands) as described before. After cell collection, an excess of erythrocytes was removed using buffy coat centrifugation. Fresh or frozen and thawed leukaphereses were subjected to CD34-selection to enrich for HSC. For CD34-selection the following methods were used according to the guidelines of the suppliers: Ceprate SC column (CellPro, Bothell, WA), Dynal CD34 progenitor cell selection system (Dynal, Oslo, Norway), and MACS CD34 isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany). Before CD34-selection using the Dynal system and the MACS kit a density gradient was performed (1.077 g/mL, Lymphoprep; Nycomed, Oslo, Norway). After selection the percentage CD34⁺ cells was determined as described before. Table 1 shows the frequency of the different stem cell subsets in the CD34⁺ selected PBSC before ex vivo culturing as determined using flow cytometry, CFC, and CAFC assays.

**Cytokines.** The following purified recombinant cytokines were kindly provided: human granulocyte-macrophage-CSF (GM-CSF), human IL-6, and murine SCF from Genetics Institute (Cambridge, MA); human FL, human G-CSF, and human SCF from Amgen (Thousand Oaks, CA); human IL-3 from Gist Brocades (Delft, The Netherlands); and human TPO from Genentech (South San Francisco, CA).

**Serum-free liquid culture.** Serum-free liquid culture experiments were performed in 35-mm bacterial dishes (Greiner, Alphen/a/d Rijn, The Netherlands) to prevent strong adherence of the hematopoietic cells to the plastic surface. The serum-free Iscove’s modified Dulbecco’s medium with Glatmax-1 (IMDM; Gibco, Breda, The Netherlands) contained 1% bovine serum albumin (A9418; Sigma, St Louis, MO), penicillin (100 U/mL; Gibco), streptomycin (100 µg/mL; Gibco), 10⁻⁴ mol/L β-mercaptoethanol (Merck, Darmstadt, Germany), bovine insulin (10 µg/mL; Gibco), 15 µM/L cholesterol (Sigma), 15 µM/L linolic acid (Merck), iron-saturated human transferrin (0.62 g/L; Intergen, Purchase, NY), cytidine (1 µg/mL; Sigma), adenosine (1 µg/mL; Sigma), uridine (1 µg/mL; Sigma), guanosine (1 µg/mL; Sigma), thymidine (1 µg/mL; Sigma), 2'-deoxyctydine (1 µg/mL; Sigma), 2'-deoxyadenosine (1 µg/mL; Sigma), 2'-deoxyguanosine (1 µg/mL; Sigma). Forty thousand CD34⁺ PBSC in 2 mL serum-free medium supplemented with IL-3 (25 ng/mL), human SCF (100 ng/mL), IL-6 (100 ng/mL), and anti-human-transforming growth factor-β1 (sTGFβ1; 1.0 µg/mL; R&D Systems, Abingdon, UK) with or without FL (100 ng/mL) and TPO (10 ng/mL) were cultured at 37°C and 10% CO₂. After 7 days of culture, the cells were collected from the dishes after scraping with a cell scraper (Greiner) and rinsing with IMDM. After washing, the cells were resuspended in IMDM and plated in CFC, CAFC, and flask-LTC assays.

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** Colony-forming cell assay.** Quantification of the number of colony-forming units granulocyte/macrophage (CFU-GM) and burst-forming units erythroid (BFU-E) was performed using a semisolid CFC assay containing erythropoietin (Boehringer, Mannheim, Germany), G-CSF, GM-CSF, IL-3, and murine SCF as described before. CFU-GM and BFU-E were counted on day 14 of culture in the same dish.
**RESULTS**

Expansion of progenitor and primitive stem cells in liquid cultures. In 7-day serum-free liquid cultures containing IL-3, SCF, IL-6, and αTGFβ1 (3/5/6/αT), we were able to expand progenitor cells (CFC and CAFC week 2 to 4) 2.6-fold and to maintain primitive stem cells (CFC and CAFC weeks 2 to 6) and (B) the ability of stem cells to produce NA CFC in parallel flask long-term cultures (LTC) for 6 weeks. At week 6 the stroma-adoherent (SA) CFC content was also determined. Comparison between no SCM and with SCM: *P < .05, No SCM (n = 7 to 9); FBMD-1 SCM (n = 7 to 9); L87/4 SCM (n = 3 to 5); L88.5 SCM (n = 3 to 5).

**Table 2. Total Long-Term Culture Colony-Forming Cell Production of Mobilized PBSC Before Ex Vivo Expansion**

<table>
<thead>
<tr>
<th></th>
<th>Total LTC-CFC Production per 100 Input Cells (± 1 SEM)</th>
<th>No. of Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA-CFC at wk 2</td>
<td>36 (±12)</td>
<td>7</td>
</tr>
<tr>
<td>NA-CFC at wk 4</td>
<td>30 (±8)</td>
<td>7</td>
</tr>
<tr>
<td>NA-CFC at wk 6</td>
<td>21 (±6)</td>
<td>8</td>
</tr>
<tr>
<td>SA-CFC at wk 6</td>
<td>12 (±5)</td>
<td>8</td>
</tr>
<tr>
<td>NA + SA-CFC at wk 6</td>
<td>33 (±8)</td>
<td>8</td>
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</tbody>
</table>

Loss of graft quality of expanded CD34+ mobilized PBSC after liquid culture. Using the same setup for liquid cultures as described above, the in vitro graft quality of CD34+ PBSC was determined after a 7-day serum-free liquid culture. In contrast to the expansion of HSC numbers (Fig 1A), the ability of stem cells to produce LTC-CFC was diminished as compared to the input cells (Fig 1B). CD34+ PBSC that had been cultured in the presence of 3/5/6/αT produced 80% NA-CFC at week 2 as compared with unexpanded CD34+ PBSC. At later weeks there was a further reduction of NA-CFC production (week 4, 31%; week 6, 13%). Also, at week 6 the SA-CFC content was only a fraction of the control CD34+ PBSC (13%). The addition of FBMD-1 and L88.5 SCM showed only a modest improvement of the graft quality (NA-CFC week 2, 100% and 88%; NA-CFC week 4, 51% and 42%; NA-CFC week 6, 16% and 14%; SA-CFC week 6, 27% and 34%, respectively, as compared with unexpanded cells). L87/4 SCM did not influence the graft quality.
Expansion of progenitor and primitive stem cells in stroma-contact and stroma-noncontact cultures. To investigate whether direct contact with stroma cells would similarly improve the recovery and quality of primitive stem cells as did soluble stromal factors, we studied the fate of HSC when cultured for 7 days in serum-free medium containing 3/S/6/αT in FBMD-1 SCM, in direct contact with a FBMD-1 stromal layer and in FBMD-1 stroma-noncontact (Fig 2A). SCM and stroma-contact showed no significant differences in their effect on the numerical expansion of progenitors and primitive stem cells, and both significantly improved generation of progenitor cells (CFC and CAFC weeks 2 to 4) as compared with stroma-noncontact cultures.

**Stroma-contact prevents loss of primitive stem cell quality in expansion cultures.** CD34+ PBSC cultured in FBMD-1 stroma-contact showed a significantly improved graft quality as compared with FBMD-1 SCM and stroma-noncontact cultures (Fig 2B). At weeks 2 and 4 the stroma-contact expanded CD34+ PBSC produced 143% and 91% NA-CFC, respectively, as compared with the input CD34+ PBSC. The NA-CFC and SA-CFC at week 6 of stroma-contact expanded cells were still 60% and 73%, respectively, as compared with unexpanded cells.

In Table 3 the primitive stem cell quality in week 6 LTC is summarized. In 7-day serum-free liquid cultures containing 3/S/6/αT there was a dramatic loss of graft quality (13% of input) as expressed in NA + SA LTC-CFC at week 6 (Table 3, second column). The addition of FBMD-1 SCM could only slightly prevent this quality loss (21% v 13%). In FBMD-1 stroma-noncontact cultures quality of primitive stem cells was more preserved (38% v 13%), while FBMD-1 stroma-contact cultures proved to be the best in preventing the loss of graft quality (66% v 13%). By calculating the mean number of LTC-CFC produced in week 6 flask-LTC per CAFC week 6, we were able to estimate the average individual primitive stem cell quality of expanded CD34+ PBSC (Table 3, third column). Although the CD34+ PBSC that had been propagated in FBMD-1 stroma-contact had also the best average LTC-CFC per CAFC at week 6, in all culture conditions there was extensive loss (6- to 13-fold) of individual primitive stem cell quality as compared with unexpanded CD34+ PBSC.

**TGFα** and TPO further improve the numerical expansion and quality maintenance of both progenitor and primitive stem cells. To test whether the effects of stroma and stroma-elaborated activities was due to the recently cloned cytokines FL and TPO, we performed experiments in which FL and TPO were added to 7-day cultures in the presence of 3/S/6/αT with or without FBMD-1 SCM or FBMD-1 stroma-contact. The combination of IL-3, SCF, IL-6, αTGFβ1, FL, and TPO with FBMD-1 stroma-contact led to a 21.1- and 4.9-fold expansion of CAFC weeks 2 and 6, respectively (Table 4). Addition of FL/TPO to 7-day FBMD-1 stroma-contact cultures also further enhanced the nonadherent CFC production in LTC leading to a complete maintenance of LTC-CFC quality (Table 5). As a result, inclusion of FL/TPO improved the recovery of all progenitor and stem cell subsets tested, and their ability to generate CFC. Remarkably, the inclusion of FBMD-1 SCM or FBMD-1 stroma-contact still gave further improvement of these parameters, indicating that the stroma-related effects described here were not mediated by FL and/or TPO.

![Fig 2. The effect of FBMD-1 SCM, FBMD-1 stroma-contact, and FBMD-1 stroma-noncontact on (A) the expansion of different stem cell subsets (CFC and CAFC week 2 to 6) and (B) the ability of stem cells to produce NA CFC in parallel flask LTC for 6 weeks in 7-day serum-free liquid cultures containing IL-3, SCF, IL-6, and αTGFβ1. At week 6 the stroma-adherent (SA) CFC content also was determined. Comparison between FBMD-1 SCM and FBMD-1 stroma-contact or stroma-noncontact: †, P < .05. Comparison between FBMD-1 stroma-noncontact and FBMD-1 stroma-contact: ‡, P < .05. (■), SCM (n = 5 to 6); (□), stroma-contact (n = 5 to 6); (○), stroma-noncontact (n = 3).](image-url)
in long-term stroma-supported cultures, or alternatively, that not at the expense of the ability of primitive HSC to generate CFC. The proliferation of progenitors and primitive stem cells may have occurred at later weeks. This showed that an absolute numerical expansion of progenitors was only maintained in week 2 flask-LTC, which is consistent with previous reports. As reported before, the grafts’ ability to generate CFC in LTC was very low. In addition, we have reported that only few CAFC week 6 are mobilized in patients that have received intense cytotoxic chemotherapy, and that their quality is reduced.

In the second part of this study we have investigated factors that could improve the expansion of primitive stem cells (CAFC week 6) and maintain their quality (LTC-CFC at week 6). We have found that both soluble stromal factors and stroma-contact increase the expansion of CAFC week 6 in the presence of cytokines (IL-3/SCF/IL-6) to both stroma-contact and stroma-noncontact cultures supplemented with cytokines (IL-3/SCF/IL-6). The improved generation of CAFC week 6 is probably the combined result of their proliferation and conservation. The Verfaillie group has shown that the maintenance or expansion of LTC-IC in 2-week stroma-noncontact cultures with cytokines (IL-3/SCF/IL-6) and αTGFβ1. The improved generation of CAFC week 6 could have further abrogated the proliferation inhibition of stroma-contact on CFC and LTC-IC proliferation as compared with stroma-noncontact cultures. In our study we do not observe inhibitory effects of stroma-contact on CAFC expansion in the presence of cytokines.

In the present study we investigated the recovery of both progenitors and primitive stem cells in CD34+/CD38− PBSC after ex vivo propagation in more detail. We showed an expansion of both progenitor and primitive stem cell numbers, which was consistent with previous reports. Although we found an expansion of CFC and various CAFC subsets in these cultures, the grafts’ ability to generate progenitors was only maintained in week 2 flask-LTC, while the cultured cells produced dramatically less progenitors at later weeks. This showed that an absolute numerical expansion of progenitors and primitive stem cells may have occurred at the expense of the ability of primitive HSC to generate CFC in long-term stroma-supported cultures, or alternatively, that not all primitive HSC survived. This situation could explain the inability of expanded grafts to improve hematologic recovery in conditioned recipients.

Although the clinical relevance of a reduced ability of expanded primitive stem cells to produce CFC at later weeks is not fully clear, we believe that this observation is more than just “an in vitro artifact.” Our group has recently observed that autologous grafts that were unable to lead to significant hematopoietic repopulation of patients at 3 months posttransplantation contained low CAFC week 6 numbers, while their ability to generate CFC in LTC was very low. In addition, we have reported that only few CAFC week 6 are mobilized in patients that have received intense cytotoxic chemotherapy, and that their quality is reduced.

In the second part of this study we have investigated factors that could improve the expansion of primitive stem cells (CAFC week 6) and maintain their quality (LTC-CFC at week 6). We have found that both soluble stromal factors and stroma-contact increase the expansion of CAFC week 6 in the presence of cytokines (IL-3/SCF/IL-6) to both stroma-contact and stroma-noncontact cultures supplemented with cytokines (IL-3/SCF/IL-6) and αTGFβ1. The improved generation of CAFC week 6 could have further abrogated the proliferation inhibition of stroma-contact on CFC and LTC-IC proliferation as compared with stroma-noncontact cultures. In our study we do not observe inhibitory effects of stroma-contact on CAFC week 6 expansion. This can be explained by the addition of cytokines (IL-3/SCF/IL-6) to both stroma-contact and stroma-noncontact cultures in our experiments, which may have overruled this stroma-contact mediated proliferation block. Furthermore, the addition of neutralizing antibodies directed against TGFβ1 could have further abrogated the proliferation inhibition of stroma-contact, because TGFβ1 is an important inhibitor of primitive stem cell proliferation and is produced by the FBMD-1 stromal cell line. In addition to the favorable effect of stroma-contact for numerical CAFC expansion, direct stroma-contact appears to be required for conservation of total graft quality (LTC-CFC week 6). Because soluble stromal factors only partly protect against quality loss of HSC, it may be argued that the concentration of SCM in our cultures may have been too low; however, stroma-noncontact cultures gave comparable results as SCM-containing cultures. As a result, our data support the observations of Koller et al in that stromal cells exert a favorable effect on BM-derived LTC-IC expansion in 2-week cultures containing IL-3, SCF, GM-CSF, and erythropoietin. In the light of the recent observations from other investigators that in vitro expanded progenitor and primitive stem cell grafts do not improve time to hematologic recovery, it seems indeed pertinent to include stromal elements in bioreactors for cytokine-driven ex vivo expansion of HSC contained in mobilized PB.

A recent study of Petzer et al reports that in 10-day liquid cultures IL-3, SCF, FL, and TPO are the most important cytokines for the expansion of LTC-IC from CD34+/CD38− BM cells. In addition, it has been shown that in these cultures

### Table 4. CFC and CAFC Week-Type Expansion of Mobilized PBSC After Ex Vivo Expansion With or Without the Addition of FL and TPO

<table>
<thead>
<tr>
<th>7-Day Culture Condition</th>
<th>CFC</th>
<th>CAFC Week 2</th>
<th>CAFC Week 4</th>
<th>CAFC Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-3/SCF/FL-6αT-GFβ1</td>
<td>0.9</td>
<td>0.7</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>+ FL/TPO</td>
<td>4.4</td>
<td>5.5</td>
<td>2.7</td>
<td>2.1</td>
</tr>
<tr>
<td>3/5/6αT/FBMD-1 SCM</td>
<td>2.4</td>
<td>1.6</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>+ FL/TPO</td>
<td>10.2</td>
<td>13.7</td>
<td>4.1</td>
<td>3.4</td>
</tr>
<tr>
<td>3/5/6αT/FBMD-1 Contact</td>
<td>4.6</td>
<td>2.1</td>
<td>2.1</td>
<td>1.7</td>
</tr>
<tr>
<td>+ FL/TPO</td>
<td>15.9</td>
<td>21.1</td>
<td>6.7</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**Abbreviation:** 3/5/6αT, IL-3/SCF/IL-6αT-GFβ1.

### Table 5. Nonadherent LTC-CFC Production of Mobilized PBSC After Ex Vivo Expansion With or Without the Addition of FL and TPO

<table>
<thead>
<tr>
<th>7-Day Culture Condition</th>
<th>NA LTC-CFC Production (% of day 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 2</td>
</tr>
<tr>
<td>IL-3/SCF/FL-6αT-GFβ1</td>
<td>9</td>
</tr>
<tr>
<td>+ FL/TPO</td>
<td>34</td>
</tr>
<tr>
<td>3/5/6αT/FBMD-1 SCM</td>
<td>7</td>
</tr>
<tr>
<td>+ FL/TPO</td>
<td>24</td>
</tr>
<tr>
<td>3/5/6αT/FBMD-1 Contact</td>
<td>19</td>
</tr>
<tr>
<td>+ FL/TPO</td>
<td>39</td>
</tr>
</tbody>
</table>

**Abbreviation:** 3/5/6αT, IL-3/SCF/IL-6αT-GFβ1.
there is no loss of CFC-producing ability. Therefore, we performed additional experiments in which FL and TPO were added to the expansion cultures. Indeed, the addition of FL/TPO to expansion cultures improved the recovery of progenitors and primitive stem cells and that this FL/TPO effect was still present in the presence of soluble stromal factors and direct stromal contact. In addition, FL/TPO together with stromal factors further improved the LTC-CFC production at week 6 resulting in a complete maintenance of primitive stem cell quality. These observations strongly support the addition of FL and TPO to stroma-dependent expansion strategies.

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