Separation of G-CSF-mobilized PBSC transplants by counterflow centrifugal elutriation: modest enrichment of CD34+ cells but no loss of primitive haemopoietic progenitors

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Summary. The suitability of counterflow centrifugal elutriation (CCE) for reduction of the number of non-stem cells in autologous G-CSF-mobilized peripheral blood stem cell (PBSC) transplants was investigated. By cell size-monitored CCE, small cells could be rapidly separated from the haemopoietic progenitor cells present in leukapheresis product (LP) samples. The large cell fraction contained an average 86 ± 25% of the CD34+ cells and 76 ± 20% of the granulocyte-macrophage progenitors (CFU-GM) loaded into the separation chamber, and was depleted of 75 ± 18% of the lymphocytes, 89 ± 7% of the erythrocytes and 98 ± 2% of the platelets (n = 21). Due to the presence of high numbers of large immature myeloid cells, which co-elutriated with progenitor cells, enrichment of CD34+ cells in the large cell fraction was only modest (average 1.8 times). No indication of preferential co-elutriation of primitive stem cells with the small cells was obtained. There was no difference in expression of CD38 or Thy-1 on CD34+ cells between the two elutriation fractions. Frequencies of cobblestone-area-forming cells (CAFC) week 6, which are considered to represent cells with long-term repopulating ability, were reduced in the small cell fractions as compared to those in the unseparated samples and the large cell fractions. On average, 100% of CAFC week 6 were recovered in the large cell fractions (n = 5). In conclusion erythrocytes, platelets and 40–50% of leucocytes can be depleted from G-CSF-mobilized PBSC samples by CCE with an almost complete recovery of both clonogenic and primitive stem cells.

Keywords: PBSC, counterflow centrifugal elutriation, CD34+ cells, primitive stem cells, cobblestone-area-forming cells.

During the last few years autologous bone marrow has been largely replaced by peripheral blood stem cell (PBSC) leukapheresis products (LP) as a source of stem cells for transplantation after high-dose chemotherapy. PBSC transplantation has several advantages over bone marrow transplantation: (1) there is no need for general anaesthesia, (2) it is an option in case of bone marrow fibrosis due to previous treatments, (3) the degree of tumour cell contamination in PBSC transplants may in general be lower (Ross et al., 1993; Mariette et al., 1994; Passos-Coelho et al., 1995), and (4) it results in a more rapid haematological recovery (Sheridan et al., 1992; Bensinger et al., 1993; Chao et al., 1993). However, large numbers of nucleated cells have to be collected to obtain sufficient stem cells numbers, especially in patients in whom the mobilization of CD34+ cells is not very successful. This results in large-volume transplants which are cryopreserved with DMSO, and after treatment these transplants are usually rein infused without further manipulation. Availability of a rapid procedure for reduction of the cell number and volume of PBSC transplants would be beneficial to the patients. The DMSO-load associated with rein infusion would be diminished, and selection of CD34+ cells and/or purging of tumour cells would be possible on a smaller scale, with less cost, and probably greater efficiency.

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The number of cells in bone marrow grafts can be reduced by depletion of erythrocytes and granulocytes on density gradients (Noga, 1992; Davis et al. 1993; Glasser et al. 1994) or by differential centrifugation in automatic cell separators (Noga, 1992; Zingsem et al. 1992b; Davis et al. 1993; Hollingsworth et al. 1994). However, PBSC preparations collected by the most currently used leukapheresis machines consist mainly of mononuclear cells (Zingsem et al. 1992a; Rosenfeld et al. 1994; Mehta et al. 1995), which cannot be separated from progenitor cells by these techniques.

In the present study the suitability of counterflow centrifugal elutriation (CCE) for reduction of the number of non-stem cells in G-CSF-mobilized PBSC transplants was investigated. By CCE, cells are separated according to their differences in sedimentation characteristics, i.e. mainly on the basis of differences in size. This technique has been used by several groups for depletion of T cells from allogeneic bone marrow grafts (de Witte et al. 1986; Schattenberg et al. 1990; Wagner et al. 1988, 1990; Quinones et al. 1993). Most bone marrow clonogenic cells co-elutriate with the nucleated cells of larger size, like monocytes and myeloid cells, and can therefore be separated from lymphocytes, erythrocytes and platelets (de Witte et al. 1983; Noga et al. 1986a, b). In contrast, naturally circulating colony-forming cells have been found to co-elutriate with the larger lymphocytes (Hillyer et al. 1993; Herbein et al. 1994). Two recent studies indicate that G-CSF-mobilized progenitor cells are larger than progenitor cells in normal blood and could in principle be separated from lymphocytes by CCE (Grimm et al. 1995; Teofili et al. 1996). Both studies were, however, hampered by low recovery of clonogenic cells after the separation.

Although bone marrow clonogenic progenitors are apparently large cells, it has been postulated that primitive stem cells are small cells. Fractionation of murine bone marrow by CCE showed that only the fraction with the smallest cells was capable of long-term haemopoietic reconstitution upon transplantation in lethally irradiated mice, although it was devoid of CFU-GM (Jones et al. 1990). In another study (Yoder et al. 1993) a distinct subpopulation of mouse highly proliferative potential colony-forming cells (HPP-CFC), which required multiple haemopoietic growth factors for maximal proliferation, was isolated in elutriation fractions containing small cells. In separations of human bone marrow by CCE, the fraction with the smallest cells was found to contain progenitor cells capable of long-term haemopoietic activity in vitro, but no CFU-GM (Wagner et al. 1995). Moreover, although early engraftment in patients transplanted with bone marrow grafts from which T cells had been depleted by CCE was not impaired (de Witte et al. 1986; Wagner et al. 1988; Schattenberg et al. 1990), higher rates of late graft failure and mixed chimeraism, compared to patients receiving unseparated bone marrow, have been observed (Wagner et al. 1992; Schattenberg et al. 1989). Therefore, in the present study we carefully investigated the distribution of primitive stem cells from the PBSC transplants in the elutriation separations. For this purpose, CD34+ cells were immunophenotyped and frequencies of cobblestone-area-forming cells (CAFC) were quantified in long-term cultures with limiting dilution set-up.

MATERIALS AND METHODS

PBSC leukapheresis samples. PBSC LP samples were obtained from patients treated for breast cancer, germ cell cancer, or malignant lymphoma in the Antoni van Leeuwenhoek Hospital (Netherlands Cancer Institute) after informed consent according to the rules of the hospital. Haemopoietic progenitor cells were mobilized by chemotherapeutic treatment followed by daily subcutaneous injections of G-CSF (5 or 10 µg/kg/d) until completion of the leukapheresis procedures. As soon as the white blood cell count exceeded $3 \times 10^9/l$ and a consistent rise in CD34+ cell percentage was observed, leukapheresis was started. Leukaphereses were performed with a continuous-flow blood cell separator (Fenwall CS3000 Plus, Baxter, Utrecht, The Netherlands) and continued for 1–4 d, depending on the number of CD34+ cells that were collected. The LP were washed once in saline containing 0-1% (w/v) glucose, 0-38% (w/v) trisodium citrate (TSC), 2% (w/v) human serum albumin (HSA; CLB, Amsterdam) and resuspended in saline supplemented with 0-1% glucose, 0-38% TSC and 10% HSA. CCE experiments were performed within 2–18 h of LP collection.

Counterflow centrifugal elutriation. Aliquots of LP containing 300–700 × 106 leucocytes were sedimented by centrifugation and resuspended in 5–10 ml PBS supplemented with 2% HSA and 0-38% TSC. CCE was performed in a Curáme 3000 elutriation system (Dijkstra Veenegilde BV, Leystal, The Netherlands) equipped with four 5 ml disposable polycarbonate separation chambers (International Medical BV, Zutphen, The Netherlands) as described by Plas et al. (1988). The CCE medium consisted of phosphate-buffered saline (PBS) supplemented with 0-4% HSA and 0-38% TSC. In this study, only one chamber was used per experiment. Two air chambers were placed in shunt in the inlet tubing of this separation chamber. A medium counterflow was achieved by a roller pump. The medium flow to one of these chambers was clamped and the cell suspension was injected into it while the medium flow through the parallel chamber was continued. Subsequently the chamber containing the cells was opened, the parallel flow was stopped, and cells were introduced into the elutriation chamber at a fluid flow of 13.5 ml/min and a rotor speed of 3000 rpm. After introduction of the cells, the liquid flow was increased to 15 ml/min and a 100 ml fraction was collected. The separations were carried out at 6–10°C. The elutriation profile of cells in LP was determined by decreasing the rotation speed in steps of 100 rpm. Fractions of 50–100 ml were collected from the outlet at each rotation speed. Alternatively, LP were separated into two fractions: one containing small cells, and the other containing large cells. This was accomplished by determination of relative cell sizes in the effluent on a Coulter counter equipped with a Channelyzer. Rotation speed was first lowered to 2600 rpm, at least 100 ml effluent was collected, and subsequently rotation speed was decreased in steps of 50–100 rpm. After
each decrease in rotation speed the size of the cells in the output was determined. When only small cells were detected, 50 ml of effluent was collected, after which the rotation speed was decreased further. When large cells (monocytes and myeloid cells) were detected (which was seen as a second peak next to the lymphocyte peak in the monitor), the collection of a separate fraction of large cells was started by reducing the rotation speed rapidly to zero. The first fraction in which larger cells were detected was added to the large cell fraction, while the small cell fractions were pooled. Cells in the different fractions were sedimented by centrifugation and resuspended in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% heat-inactivated fetal calf serum (FCS) and 0-38% TSC.

Analysis of samples and flow cytometry. Leucocytes and erythrocytes were counted using a Coulter Counter (model ZF). Platelets were counted using a Cell-Dyn 100 thromboerythrocyte (Sequioa-Turner). Viability of cells was determined by the trypan blue dye exclusion test. Before immunophenotyping of leucocytes, erythrocytes were lysed by incubation in an isomolar NH4Cl/bicarbonate solution (pH 7.4) for 10 min at 4°C. Nucleated cells were labeled with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated mAb and analyzed in the presence of 1 μg/ml propidium iodide with a FACSscan flow cytometer (Becton Dickinson, San Jose, Calif.). The data files were analyzed with FACSscan or PC-lysis software. Dead (propidium-iodide-positive) cells and debris (events with very low forward and side scatter) were excluded from analysis. The following mAb were used for analysis of CCE fractions: Leu-4-PE (CD3) for T cells, Leu-M3-PE (CD14) for monocytes, Leu-16-PE (CD20) for B cells, anti-HPCA-2-PE (CD34) for progenitor cells, Leu-19-PE (CD56) for NK cells (all obtained from Becton Dickinson), CLB-gran/2-FITC (CD15; CLB, Amsterdam, The Netherlands) for myeloid cells.

CD34+ cells were immunophenotyped by double-labelling with anti-HPCA-2-FITC (CD34), and Leu-17-PE (CD18) or Leu-M9-PE (CD33) (from Becton Dickinson) or SE10-PE (Thy-1, DNH90) (Craig et al. 1993). For subtyping CD34+ cells a minimum of 1000 CD34+ was acquired for analysis by live-gating. Gates were set to exclude at least 99% of cells stained with a mouse IgG1-PE control antibody (Becton Dickinson). The percentage of CD34+ cells co-expressing a specific antigen was corrected for residual cells reactive with the control antibody.

Immunophenotype of cells forming clones beneath the stroma in the CAFC assay was determined by double-labelling with anti-HLe-1-FITC (CD45) (to differentiate the human cells from the mouse-derived FBMD-1 cells) and PE-conjugated Leu-5b (CD2), Leu-3a (CD4), Leu 2a (CD8) (from Becton Dickinson, CLB-gran/10 (CD66) or one of the above-mentioned PE-conjugated mAbs.

To isolate CD34+ cells from CCE fractions, cells were incubated with anti-HPCA-2-PE mAb, washed, resuspended in IMDM supplemented with 2% FCS and 0-38% TSC and sorted with a FACStar (Becton Dickinson, San Jose, Calif.) on fluorescence intensity versus side-angle scatter.

Morphological examination of the cell suspensions was done on May-Grünwald-Giemsa stained cytospin preparations.

RESULTS

Elutriation profile of LP cells

PBSC leukapheresis products, obtained after mobilization with chemotherapy and G-CSF and collected by the continuous flow cell separator CS3000, contained variable numbers of erythrocytes (11 ± 7 per leucocyte; n = 35) and platelets (18 ± 16 per leucocyte; n = 15). On average only 3% of the leucocytes were mature neutrophils, but considerable numbers of immature myeloid cells (myelocytes and metamyelocytes) were observed. Immunophenotyping showed that 26 ± 17% of the leucocytes were CD15-positive myeloid cells (n = 44). Only 45 ± 20% of leucocytes were lymphocytes.

To establish the CCE profile of the different cell types, PBSC samples were fractionated by lowering stepwise the centrifugation speed at constant counterflow rate. Fig 1 shows a representative example of the CCE profile of the nucleated cells from a PBSC preparation. At the higher rotation speeds (3000–2400 rpm) predominantly lymphocytes were leaving the separation chamber. By decreasing the rotation speed further, monocytes and myeloid cells were elutriated. Most CD34+ cells and all CFU-GM co-elutriated with these large cells.

Cell-size-monitored separation of LP samples

The co-separation of progenitor cells with monocytes and myeloid cells enabled the separation of small cells from progenitor cells by monitoring the size of cells in the eluent during the separation. The procedure was described in the Materials and Methods section. Briefly, to collect the small cells the rotation speed was lowered first to 2600 rpm and...
subsequently with steps of 50–100 rpm. After each change in rotation speed, the size of the cells in the effluent was examined. When a peak of large cells was observed next to the lymphocyte peak, it was decided to start collecting a separate fraction containing the large cells by rapidly lowering the rotation speed. Fig 2 summarizes the results of 21 cell-size-monitored separations of LP samples. Total recovery of leucocytes and erythrocytes after the procedure was about 85%. The viability of the leucocytes, as determined by the trypan blue dye exclusion test, was not compromised by elutriation. On average, 87% of the CD34\(^+\) cells and 99% of the CFU-GM which were recovered after the procedure were retained in the fraction containing the large cells. Of the total number of recovered lymphocytes (average 84%), 70% was separated into the small cell fraction. In addition to lymphocytes, about 85% of recovered erythrocytes and almost all platelets were elutriated into the small cell fraction. Together, \(36 \pm 1\%\) of the leucocytes recovered after the procedure were separated into the small cell fraction and \(1.8 \pm 0.6\) times enrichment of CD34\(^+\) cells in the large cell fraction was obtained.

**Immunophenotype of CD34\(^+\) cells in elutriation fractions**

Although in the separation experiments shown in Fig 2, only \(13 \pm 10\%\) of PBSC CD34\(^+\) cells were lost into the small cell fraction, almost no CFU-GM outgrowth was observed in this fraction. To assess whether the CD34\(^+\) cells lost in the small cell fraction were enriched for primitive stem cells, CD34\(^+\) cells were immunophenotyped with CD38 and Thy-1 (CDw90) mAb. Fig 3 shows that almost no CD34\(^+\)/CD38\(^-\) cells were observed in the LP. CD34\(^+\) cells could, however, clearly be divided into two subpopulations on basis of CD38 expression: one being CD38\(^{high}\)/CD34\(^{intermediate}\) and a smaller group being CD38\(^{low}\)/CD34\(^{high}\). The latter

<table>
<thead>
<tr>
<th>CD34(^+) cells with the indicated phenotype (%)</th>
<th>PBSC</th>
<th>Small cell fraction</th>
<th>Large cell fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thy-1(^+)</td>
<td>26 (\pm) 9</td>
<td>21 (\pm) 17</td>
<td>25 (\pm) 9</td>
</tr>
<tr>
<td>CD38(^-)</td>
<td>0.3 (\pm) 0.2</td>
<td>0.5 (\pm) 0.4</td>
<td>0.2 (\pm) 0.2</td>
</tr>
<tr>
<td>CD38(^{high})</td>
<td>15 (\pm) 6</td>
<td>11 (\pm) 9</td>
<td>14 (\pm) 4</td>
</tr>
<tr>
<td>CD33(^+)</td>
<td>97</td>
<td>92</td>
<td>97</td>
</tr>
</tbody>
</table>

subpopulation accounted for 9–26% of CD34+ cells in unseparated PBSC samples.

As shown in Table I, no significant enrichment of CD34+/CD38- or CD38lo cells in the fraction containing the small cells was observed. Also no difference in Thy-1 expression on CD34+ cells was noted in the CCE fractions as compared to the unseparated PBSC. Moreover, most CD34+ cells in both fractions expressed the myeloid marker CD33.

Distribution of stem cells in separations of PBSC by CCE as determined by CAFC frequency analysis

To determine frequencies of CAFC, limiting dilution type cultures on adherent FBMD-1 cell layers were initiated. Most of the clones underneath the stromal layer observed in cultures inoculated with the lymphocyte-enriched fractions were smaller than clones in cultures inoculated with the unseparated LP samples or with the large cell fractions. These small clones consisted exclusively of small cells with lymphocytic appearance. Although they did not proliferate further after week 2, they remained visible for the whole culture period. In parallel 6-week flask cultures the cells from the small cell fraction produced almost no non-adherent cells and CFU-GM, in contrast to cells from unseparated LP samples and from the progenitor-enriched CCE fractions.

Therefore we investigated whether these clones with small cells originated from haemopoietic stem cells by sorting CD34+ cells from the lymphocyte-enriched fractions. In cultures inoculated with these enriched CD34+ cells the clones with lymphocytic cells were not observed, whereas the frequency of clones with cells of normal appearance was strongly increased as compared to the lymphocyte-elutriation fraction (average 160 times for week 2 CAFC and 350 times for week 6 CAFC; n = 2). These observations indicate that the clones with lymphocytic appearance did not originate from CD34+ cells, whereas the clones with larger cells did. Therefore only the clones with larger cells were scored in subsequent experiments. Immunophenotyping of adherent cells (after 7 weeks of culture) showed that 20 ± 8% (n = 3) of human (CD45+) cells from the cultures initiated with cells from the lymphocyte-enriched CCE fractions expressed CD2, indicating that the cells with lymphocytic appearance probably were T cells. Part of these cells expressed CD4 and part CD8. No CD20+ cells were observed. In contrast, in cultures inoculated with cells from the large cell fractions adherent CD45+ cells did not express CD2 but the monocytic and myeloid markers CD14, CD33 and CD66.

As shown in Fig 4, CAFC frequencies in unseparated LP samples and in the CCE fractions containing the large cells remained the same between week 2 and week 6, whereas the frequencies of CAFC in cultures initiated with small cell CCE fractions gradually decreased in the course of culture time. In three of the five experiments frequencies of CAFC week 6 in the large cell fractions were higher than or equal to those in the unseparated LP samples. In one of the two other experiments (no. 4), the decreased CAFC outgrowth from the large cell fraction was probably due to aggregation of cells in this fraction. In contrast, in the three experiments in which cobblestone-areas of normal appearance were separately scored, frequencies of CAFC week 6 in the small cell fractions were considerably lower as in the unseparated LP samples.

CAFC recoveries were estimated by multiplication of CAFC frequencies with the numbers of leucocytes in the elutriation fractions. Mean recoveries (±SD) in the large cell-containing fractions were 55 ± 35%, 65 ± 50% and 100 ± 85% for weektype 2, weektype 4 and weektype 6 CAFC, respectively (n = 5). Recoveries of the CAFC weektypes in the lymphocyte-enriched fractions were 5 ± 3%, 1 ± 1% and 1 ± 0-5%, respectively (n = 3). The ability of the CAFC

in the large cell fractions to generate CFU-GM, as determined in parallel flask cultures, was comparable to those of CAFC in the unseparated LP samples. Mean productions of (CFU-GM per CAFC (±SD) in cultures initiated with the large cell fractions were 9 ± 6, 15 ± 18 and 46 ± 46 at week 2, 4 and 6, respectively, whereas 16 ± 18, 27 ± 12 and 33 ± 7 CFU-GM per CAFC weektype 2, 4 and 6 were produced in cultures inoculated with the unseparated LP samples (n = 4).

DISCUSSION

CCE has been applied extensively in a clinical setting for depletion of T cells from allogeneic bone marrow grafts (de Witte et al., 1986; Wagner et al., 1988, 1990; Schattenberg et al., 1990; Quinones et al., 1993). In this study we investigated whether this technique could be used for reduction of the number of non-stem cells in G-CSF-mobilized PBSC grafts. The elutriation pattern which we observed for the cells from LP samples was comparable to that of bone marrow cells (de Witte et al., 1983; Noga et al., 1986a, b). The co-separation of progenitor cells with the larger cells (i.e. monocytes and myeloid cells) enabled a rapid separation procedure, in which PBSC samples were separated into two fractions by monitoring the size of cells in the eluent.

Since the procedure was developed to process autologous transplants, we chose to optimize the yield of progenitor cells in the large cell fraction, rather than depleting T cells extensively. This was performed by starting the collection of the large cells fraction as soon as large cells became visible next to the small cells in the eluent. As a consequence of this, depletion of lymphocytes in our experiments was less efficient (70% of totally recovered lymphocytes) than the 97–99% depletion which has been realized in CCE separations of allogeneic bone marrow transplants (de Witte et al., 1986; Wagner et al., 1988, 1990). However, in our procedure only about 10% of CD34⁺ cells and almost no CFU-GM were lost in the lymphocyte-enriched fraction, which is considerably lower than reported losses of progenitor cells (10–40% of CFU-GM (de Witte et al., 1986; Wagner et al., 1988; Noga et al., 1986a, 1994) and 40% of CD34⁺ cells (Noga et al., 1994) upon rigorous T-cell depletion of bone marrow.

The extent of the reduction of nucleated cell numbers obtained by the procedure described is modest. This is partly due to the large proportion of immature myeloid cells which contaminate the samples after treatment with G-CSF and which co-elutriate with progenitor cells. It may be expected that higher leucocyte depletion from PBSC transplants could be achieved when alternative stem cell mobilization regimes, which do not induce such a prominent left shift in the myeloid lineage in blood, become available. We have recently developed a filtration technique by which monocytes and myeloid cells can be removed from LP samples. Combination of this technique with the elutriation procedure described here results in an average 7-fold enrichment of mobilized progenitor cells (unpublished observations).

Unfortunately, an average of 15% of the loaded cells (both leucocytes and erythrocytes) were lost during the elutriation procedures. This loss is largely due to the formation of a small cell aggregate in the separation chamber, which is probably caused by the high numbers of myeloid cells present in the PBSC grafts.

The CD34⁺ cells which co-separated with lymphocytes were virtually devoid of CFU-GM. However, in contrast to what has been found for human bone marrow cells (Wagner et al., 1995), these small CD34⁺ cells were not enriched with CD34⁺/CD38⁻ cells. Since nearly all CD34⁺ cells were CD38⁺ we also analysed the CD38⁺ population, which also contains many cells capable of long-term haemopoietic activity in vitro (Breems et al., 1996; Reems & Torok Storb, 1995), although these may be of a less immature state of differentiation (Hao et al., 1995; Prosper et al., 1996). Moreover, expression of Thy-1, which also has been shown

Fig. 4. CAFC frequencies in elutriation fractions of LP samples. Depicted are CAFC frequencies in the unseparated LP samples (A), the small cell CCE fractions (B) and the large cell CCE fractions (C). Data represent five experiments with different LP samples, except for the small cell CCE fractions (B) and the large cell CCE fractions (C). Depicted are CAFC frequencies in the unseparated LP samples (A), the small cell CCE (B) and the large cell CCE fractions (C).
to mark a subpopulation of CD34+ cells enriched for primitive stem cells in bone marrow (Craig et al. 1993), cord blood (Mayani & Lansdorf, 1994) and in PBSC (Murray et al. 1995; Humseau et al. 1996), was similar in both elutriation fractions.

To further analyse the possibility that stem cells were lost in the small cell fraction, CAF-C frequencies were determined. Considerable numbers of abnormal clones underneath the stroma, consisting of small cells with lymphocytic appearance, were observed in the cultures inoculated with cells from the lymphocyte-enriched elutriation fraction. These cells did not originate from CD34+ cells, produced almost no CFU-GM during the 6 weeks of culture, and were probably of T-cell origin (CD2+). For these reasons, these small clones were considered not to represent stem cells.

The frequencies of CAF-C clones of normal appearance observed in cultures inoculated with cells from lymphocyte-enriched CCE fractions decreased during the course of the cultures. Week type 6 CAF-C, which, as in the well-validated murine CAF-C assay, may be considered as indicators of stem cells capable of long-term haemopoietic reconstitution (Breems et al. 1994, 1996; Murray et al. 1995; Ploemacher et al. 1993; Van der Loo et al. 1994; Ploemacher, 1994), were nearly exclusively recovered in the elutriation fractions containing the large cells. These results indicate that primitive G-CSF mobilized stem cells were not separated from colony-forming units in the CCE procedure used in this study.

The difference between our findings on the distribution of primitive stem cells from PBSC grafts in CCE separations and those reported for murine and human bone marrow (Jones et al. 1990; Wagner et al. 1995) and normal human peripheral blood cells (Herbein et al. 1994) may result from an effect of the mobilization regime on stem cells. Elutriation profiles of murine HPP-CFC have been found to shift to fractions of larger cells when mice were pretreated with 5-fluorouracil and bone marrow was regenerating (Schwartz et al. 1986) and similar findings have been reported for elutriation profiles of human CD34+ cells mobilized with G-CSF as compared to CD34+ cells from normal blood (Grimm et al. 1995). However, it should be also realized that in the study of Wagner et al. (1995) with human bone marrow, progenitors capable of long-term haemopoietic activity in vitro were not exclusively recovered in the fractions with small cells but were also present in the fractions with large cells. Moreover, in other studies with murine bone marrow (Orlic & Bodine, 1992; Orlic et al. 1993) the observation of Jones et al. (1990) that only the elutriation fraction with the smallest cells was capable of long-term haemopoietic reconstitution upon transplantation, was not confirmed.

In conclusion, the CCE procedure described here, is a rapid means of concentrating PBSC samples directly after leukapheresis. No pre-separation step is needed, and the total procedure takes only about 2 h. The procedure results in removal of erythrocytes and of platelets, but only a modest reduction of the leucocyte number. No loss of primitive stem cells occurs. The depletion of the platelets (which may induce aggregation of cells) and of erythrocytes (which may interfere with immunolabelling and selection) may be advantageous for CD34-selection and antibody-based purging procedures. However, to enable separation of complete LP (which contain on the average 20×10^6 leucocytes) in one run, the capacity of the elutriation system has to be increased.

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