Whole-blood leukodepletion filters as a source of CD34+ progenitors potentially usable in cell therapy

Zoran Ivanovic, Pascale Duchez, Doris A. Morgan, Francis Hermitte, Xavier Lafarge, Jean Chevaleyre, Vincent Praloran, Bernard Dazey, Gérard Vezon, and Jean-Michel Boiron

BACKGROUND: Used leukodepletion filters (LDFs), containing billions of white blood cells (WBCs), are discarded. Because the steady-state blood contains low quantities of stem and progenitor cells that are retained in LDFs, the viability and the functional properties of mononuclear cells (MNCs) and CD34+ cells recovered from LDFs were investigated.

STUDY DESIGN AND METHODS: WBCs were recovered from LDFs by use of a closed system. MNCs and CD34+ cells were isolated from freshly LDF-recovered WBCs or after their overnight incubation. The CD34+ cells were enumerated, as well as the number of colony-forming unit (CFU)–granulocyte-macrophage, burst-forming unit–erythroid, and CFU-Mixed. The expansion in clinical-scale volume cultures (serum-free medium plus stem cell factor, granulocyte–colony-stimulating factor, and megakaryocyte growth and development factor) was performed starting from MNCs, freshly isolated CD34+ cells, and CD34+ cells isolated after overnight incubation of WBCs. The erythroid, megakaryocytic, eosinophilic, and monocyte-myelocytic lineage differentiation of LDF-recovered CD34+ cells was challenged in liquid cultures by adding relevant cytokines.

RESULTS: Nearly 450×10^3 viable CD34+ cells were recovered per LDF. These cells exhibit unimpaired colony-forming ability. It is possible to expand these cells ex vivo, but their response to cytokines is different compared to mobilized peripheral blood and cord blood CD34+ cells. Thus, further work is necessary to optimize their ex vivo expansion. These cells give rise to the mature cells and precursors of erythroid, megakaryocytic, eosinophilic, and monomyelocytic lineage in liquid cultures.

CONCLUSION: MNCs and CD34+ cells recovered from the LDFs exhibit unimpaired functional capacities. Recent development of ex vivo technologies for expansion, retrodifferentiation, and differentiation reinforces the value in cell therapy of these LDG-recovered peripheral blood progenitor cells that are routinely discarded.

The increase in the number of committed progenitors in peripheral blood after chemotherapy or/and cytokine mobilization attracted the attention of researchers and clinicians resulting in a shift in clinical practice to the transplantation of mobilized peripheral blood cells. Conversely, the steady-state progenitors and stem cells were not intensively studied and are not used in clinical cell therapy today.

Steady-state blood contains low quantities of stem and progenitor cells. The capacity of circulating stem cells to reconstitute hematopoiesis was documented in rats[1] even before the first publication on the existence of a pluripotent hematopoietic progenitor in syngeneic mice transplantation model.[2] Later, these findings were confirmed in baboons[3] and humans.[4] Apart from the stem cells with the capacity of hematopoietic reconstitution in vivo, steady-state peripheral blood contains the committed[5-7] and pluripotent[8-10] progenitors.

Leukodepletion of blood products is mandatory in France since 1998. Used leukodepletion filters (LDFs), containing billions of white blood cells (WBC), are dis-

ABBREVIATIONS: CFC(s) = colony-forming cell(s); LDF(s) = leukodepletion filter(s); MGDF = megakaryocyte growth and development factor.

From the French Blood Establishment Aquitaine-Limousin, Bordeaux, France; Drexel University, Philadelphia, Pennsylvania; and CNRS UMR 5164, Université de Bordeaux 2, Bordeaux, France.

Address reprint requests to: Zoran Ivanovic MD, PhD, Etablissement Français du Sang Aquitaine-Limousin, 5 Place Amélie Raba Léon BP 24, 33035 Bordeaux Cedex, France; e-mail: zoran.ivanovic@efs.sante.fr.

This work was supported by Grant 2004.11 of Scientific Council of French Blood Establishment (Etablissement Français du Sang).

Received for publication March 2, 2005; revision received May 17, 2005, and accepted May 19, 2005.

doi: 10.1111/j.1537-2995.2006.00677.x

TRANSFUSION 2006;46:118-125.

carded. Thus, even if the concentration of CD34+ cells is low in steady-state peripheral blood (approx. 0.15% of mononuclear cells [MNCs]),[11] an enormous quantity of these cells retained at the LDFs are destroyed on a daily basis in blood centers. For example, Bordeaux Blood Center (Etablissement Français du Sang Aquitaine-Limousin, Bordeaux) alone prepares approximately 160,000 leukodepleted red blood cell (RBC) units per year.

The problem of relatively low quantity of CD34+ cells per filter could be resolved by collecting and storing frozen cells recovered from several LDFs obtained from the same donors for a period of several years (in France a regular male donor volunteer gives blood five times per year). In addition, the development of ex vivo expansion procedures,[12-15] as well as the techniques of ex vivo precursors and mature cell production,[16-21] make the idea of clinical utility of the stem and progenitor cells recovered from LDFs even more attractive. In this article, we demonstrate preserved functional capacities of CD34+ cells recovered from the LDFs.

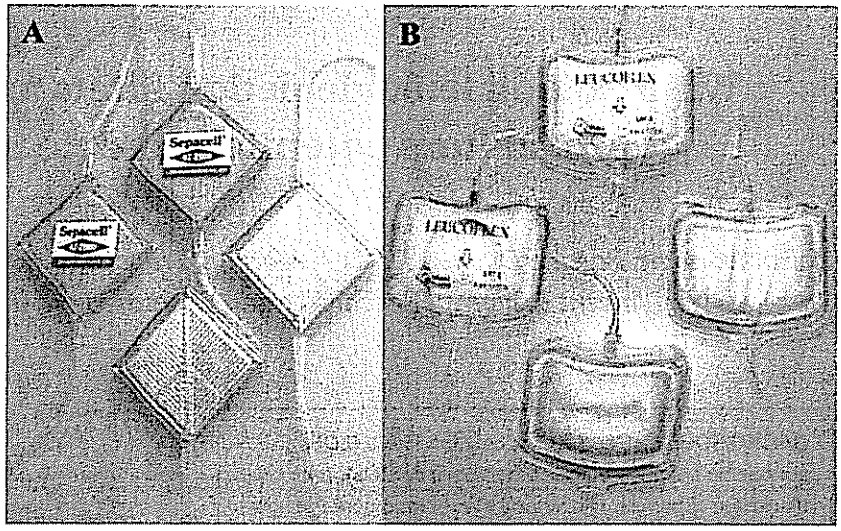

Fig. 1. LDFs used in this article. (A) Sepacell RZ-2000, filters incorporated in a rigid plastic shell; (B) Leukoflex, filters welded in nonrigid polyvinylchloride bags.

MATERIALS AND METHODS

Recovery of WBCs and MNCs from the LDF

Two standard kits for the leukodepletion of RBCs were used (Sepacell RZ-2000, Baxter, Maurepas, France; and Leucoflex, Macopharma, Tourcoing, France). Although the design of filters in these two kits was different (Fig. 1), we did not see any evidence of any difference in WBC recovery nor in MNC and CD34+ viabilities. LDFs were used within 22 hours after blood collection at which time they were removed from the leukodepleted RBC bags that were still in connection with the original whole-blood collection (emptied) bag. A sterile connection (connector TSCD SC 201 A, Terumo, Guyancourt, France) was established between the distal part (exit tube) of the filter and the tube of another bag filled with phosphate-buffered saline (PBS; B/Braun Medical S.A., Boulogne, France). The cells were flushed from filters by a gravitation-induced reversal stream of PBS and collected in the original blood collection bag. By use of the same thermal sterile connection device, the filter was simultaneously removed and a new "transfer bag" (600-mL transfer bag, Macopharma) tube connected to the tube of the original blood sampling bag. The cell suspension was then transferred to the transfer bag; the original blood collection bag was removed by a thermal cutter that also welded the tube of the transfer bag. The cells were then centrifuged in these transfer bags at the low speed (10 min at 20°C, 370 × g; Heraeus Multifuge S3, Yutz, France) to remove platelets (PLTs) from the buffy coat. The PLT-rich supernatants were then removed by use of a manual press, and the buffy coats with the residual RBCs were aspirated by a syringe and directly used for further experimentation or layered onto Ficoll (Bicoll separation solution, Biochrom AG, Berlin, Germany) and the MNC fraction was removed after 20 minutes of centrifugation at 290 × *g* (Heraeus Multifuge S3). MNCs were then washed and counted, their viability was assessed (trypan blue test), and the MNCs were resuspended in buffer for the CD34+ isolation.

Isolation of CD34+ cells

CD34+ cells were isolated by use of the Miltenyi (Miltenyi Biotec, Paris, France) "indirect" immunomagnetic technique (human CD34+ progenitor cell isolation kit "Macs"):[22] the first passage used the "midi" column and the second passage used the "minicolumn" (Vario Macs Device). This approach enabled, in spite of a very low starting concentration of CD34+ cells, relatively good CD34+ purity (80%-98%).

Flow cytometry CD34+ cell detection

The purity and frequency of CD34+ cells in MNCs after Ficoll separation, selected CD34+ cells, or cultured cell suspensions were detected following the recommendation of the International Society of Hematotherapy and Graft Engeneering[23] currently in use at our facility.[24] Briefly, three-color fluorescence was used to detect viable (7-aminoactinomycin–negative) CD45+/CD34+ cells that

were counted directly by use of tricount microbeads (Becton Dickinson, San Jose, CA) by means of a flow cytometer (FACSCalibur, Becton Dickinson).

Detection of clonogenic progenitors

Colony-forming unit–granulocyte-macrophage (CFU-GM) assay, a standard quality control test in cell therapy, was performed by use of a commercial ready-to-use kit (IG, Stem Alpha, Saint Clement les Places, France) to determine the functional preservation of progenitor cells in MNCs recovered from all LDFs examined. In some experiments, the progenitor activity of LDF-recovered purified CD34+ cells (fresh or cultured) was assayed for three types of progenitors (CFU-GM, burst-forming unit–erythroid [BFU-E], and CFU-Mixed; ready-to-use kit ID, Stem Alpha). In any case, the freshly isolated CD34+ cells or cultured cells were plated in methylcellulose cytokine-supplemented kits and cultured for 14 days in 35-mm petri dishes (NUNC, Roskilde, Denmark). The colonies (>50 cells) were then counted.[22]

Evaluation of ex vivo expansion capacity of LDF-recovered MNCs and CD34+ cells

The MNCs were cultured with a starting concentration of 1×10^6 to 3×10^6 cells per mL (the mean Day 0 CD34+ concentration in these cultures was 4×10^3/mL). CD34+ cells isolated from fresh MNCs as well as from MNCs after an overnight incubation in Iscove's modified Dulbecco's medium with 2 percent human serum albumin at 37°C and 5 percent CO_2 were cultured for 7 days as follows: Cells were seeded in bags (Lifecell OptiCyte cell culture container 180 cm^2, Nexell Therapeutics Inc., Irvine, CA) at a concentration of 20×10^3 cells per mL of serum-free medium (Macopharma HP01) supplemented with stem cell factor (SCF), granulocyte–colony-stimulating factor (G-CSF), and megakaryocyte growth and development factor (MGDF; 100 ng/mL each).[12,22]

Ex vivo differentiation potential of CD34+ cells freshly isolated from LDFs

CD34+ cells were seeded in 48-well plates at a density of 5×10^4 to 10×10^4 per mL of RPMI containing 10 percent human serum supplemented with 1) SCF (50 ng/mL) plus erythropoietin (EPO; 4 U/mL) for the erythroid lineage; 2) SCF (50 ng/mL) plus GM-CSF and G-CSF (5 ng/mL) or, alternatively, SCF (50 ng/mL) plus interleukin (IL)-3 (5 ng/mL) for myeloid lineage; and 3) SCF (50 ng/mL) plus thrombopoietin (20 ng/mL) for megakaryocytic lineage (all cytokines from R&D Research, Minneapolis, MN). After 12 days, the cells were harvested and deposited on to slides by cytocentrifugation. Morphology was examined after Wright-Giemsa staining, and heme was detected in erythroid-directed cultures by the benzidine reaction.

Statistical analysis

An unpaired one-sided t test was used to test the significance of differences between the groups. The correlation factor was calculated by use of a computer program (StatView, SAS, Cary, NC) and the significance of correlation was calculated by a t test.

RESULTS

In this work, we tested a series of LDFs recovered after the leukodepletion of normal voluntary donors with an equal distribution of sexes and with age distribution from 18 to 60 years (36.4 ± 13.8). The mean CD34+ cell frequency in MNC fraction was 0.16 ± 0.14 percent (not shown). The means ± SD of blood volume; number and viability of total MNCs, CD34+ cells, and CFU-GM recovered; and the CFU-GM frequency are presented in Table 1. Thus, applying the technique described above, it was possible to recover

TABLE 1. Donor age, blood volume, and quantity of recovered cells per LDF

Variable	Mean ± SD
Donors' age (years) (n = 48)	36.4 ± 13.8
Blood volume (mL) (n = 48)	419 ± 36.3
Total MNC ($\times 10^6$) (n = 48)	358.3 ± 121.8
MNC viability (%) (n = 60)	96.6 ± 1.85
Total CD34+ ($\times 10^3$) (n = 48)	447.2 ± 337.8
CD34+ cell viability (%) (n = 60)	89.0 ± 6.85
Total CFU-GM ($\times 10^3$) (n = 37)	157.9 ± 106.4
CFU-GM/10^5 MNCs (n = 37)	45.5 ± 28.5

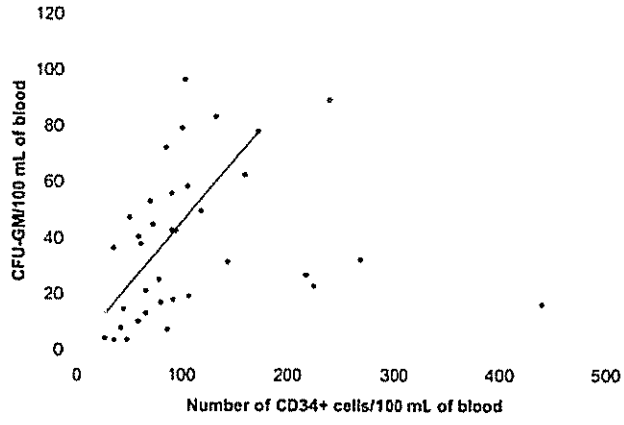

Fig. 2. Correlation between CD34+ cells and CFU-GM in MNC fractions recovered from the filters (n = 38). The values are normalized to the same volume (100 mL) of filtered blood. (◇) Samples showing a nonproportionally high number of CD34+ cells in MNC cell suspension.

approximately 360×10^6 MNCs and 450×10^3 CD34+ cells per LDF.

We further examined the correlation between the CD34+ cells and CFU-GM detected in MNC fractions normalized to the same blood volume (100 mL) before leukodepletion (Fig. 2). In spite the fact that they were the most abundant in CD34+ cells (open symbols), several samples (10%) exhibited a relatively lower clonogenic activity. The majority of samples (90%) showed a very good correlation between the number of CD34+ cells and the CFU-GM numbers ($R^2 = 0.3734$; $y = 0.4457X + 1.1188$; $p = 0.0002$).

Whereas the number of MNCs recovered per 100 mL of blood was stable regardless the age of donors (Fig. 3A), the number of CD34+ cells recovered per same blood volume appeared to be stable only for donors between 18 and 30 years of age. Approximately 30 percent of donors over the age of 30 had two- to threefold more CD34+ cells ($>200 \times 10^3$/100 mL of blood) than those aged 18 to 30 years (Fig. 3B). The number of CFU-GM per 100 mL of blood was also very different in individual filters and was not correlated with age (Fig. 3C).

The more complete clonogenic potential (CFU-GM, BFU-E, CFU-Mixed) of freshly isolated MNCs and CD34+ cells either freshly isolated or after an overnight incubation is shown in Table 2. Owing to the action of accessory cells, the number as well as the respective proportions of CFU-GM, BFU-E, and CFU-Mixed detected varied in function of purity of CD34+ cells plated in semisolid cultures. The plating efficiency of 1000 CD34+ cells contained in the MNC fraction was higher than that of 1000 purified CD34+ cells, but the relative proportion of CFU-Mixed with respect to CFU-GM and BFU-E was much lower. After 7 days of expansion of cultures initiated with MNCs, the incidence of CFU-GM and BFU-E per 1000 CD34+ cells did not increase with respect to Day 0 values, whereas those of CFU-Mixed even decreased. When CD34+ cells were plated in semisolid cultures immediately after purification

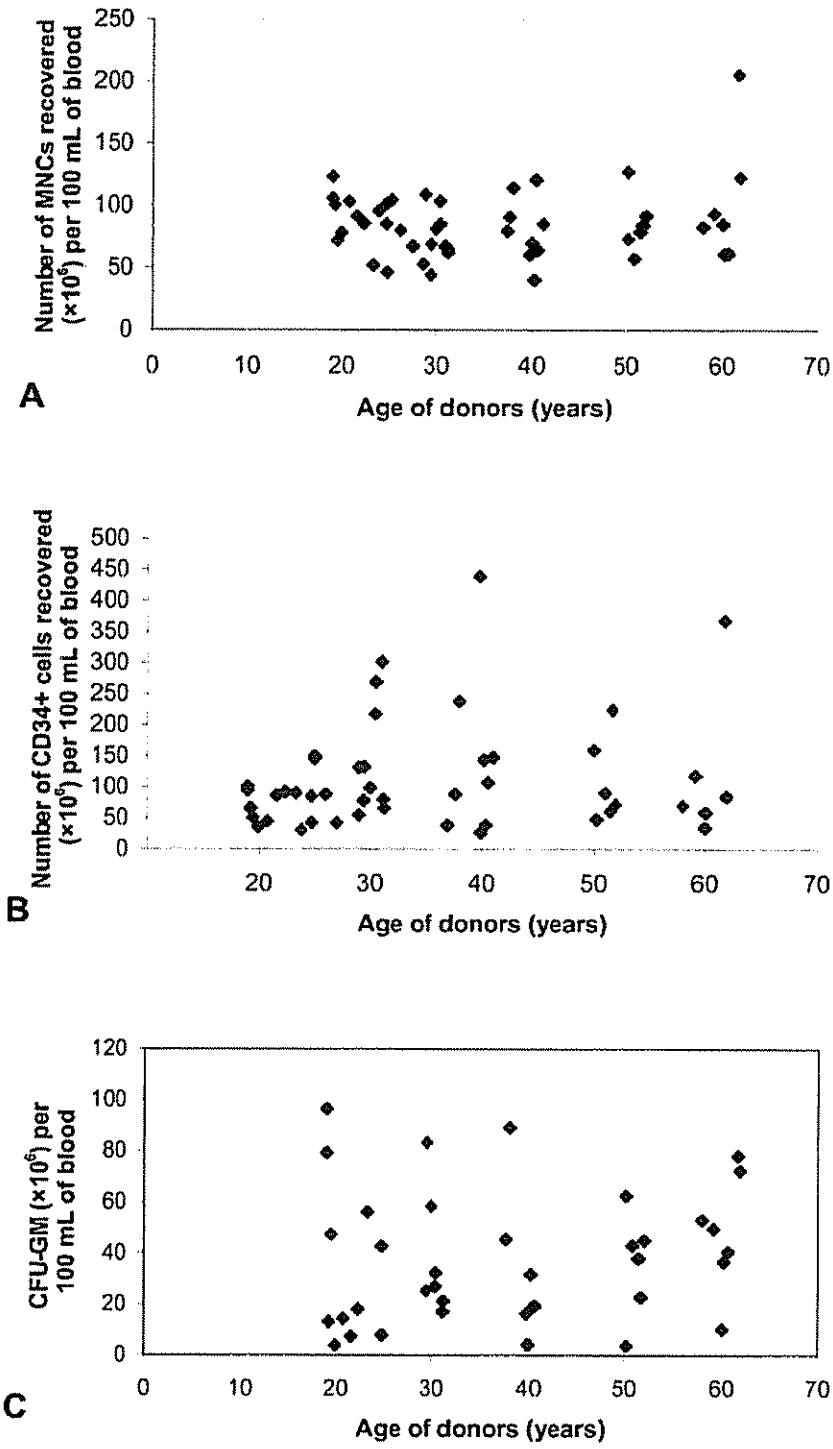

Fig. 3. Relation age-yield of (A) MNCs (n = 49), (B) CD34+ cells (n = 49), and (C) CFU-GM (n = 38) per 100 mL of filtrated blood. No significant correlations. Note that the samples with extremely elevated CD34+ yield are detected only in donors aged more than 30 years.

TABLE 2. Incidence of committed progenitors in cell populations obtained from LDFs, differing in CD34+ cell concentration, way of isolation (Day 0), and their amplification capacities ex vivo*

Starting cell population	Progenitor type	Day 0 (progenitors per 1000 CD34+ cells)	Day 7 (progenitors expanded from 1000 Day 0 CD34+ cells)
Freshly isolated MNCs (n = 6)	CFU-GM	189.3 ± 57.3	424.0 ± 400.0
	BFU-E	279.0 ± 154.0	351.0 ± 160.0
	CFU-Mix	25.1 ± 21.5	1.7 ± 1.6
Freshly isolated CD34+ cells (n = 6)	CFU-GM	59.0 ± 33.5	2893.4 ± 1842.4
	BFU-E	73.7 ± 20.9	1578.4 ± 1269.4
	CFU-Mixed	47.1 ± 31.2	168.6 ± 151.0
CD34+ cells isolated after overnight incubation of total cells (n = 4)	CFU-GM	69.7 ± 41.7	1326.7 ± 997.0
	BFU-E	111.7 ± 42.2	914.1 ± 409.9
	CFU-Mixed	22.5 ± 9.7	158.9 ± 68.6

* MNCs were plated in expansion cultures (see Materials and methods) at a concentration of 3×10^6 per mL (mean real CD34+ cell concentration, 4×10^3/mL), and CD34+ cells were plated at a concentration of 20×10^3 per mL. To compare these different conditions, the number of progenitors is expressed per 1×10^3 Day 0 CD34+ cells. Consequently the expansion product of the same number of CD34+ cells (1×10^3) is shown.

or after being incubated overnight before purification, the plating efficiency per 1000 cells for three classes of progenitors was comparable for two conditions and equilibrated between each progenitor class. After 7 days of expansion their incidence was dramatically increased, but important dispersion between individual experiments was evident. The relative increases for each progenitor class with respect to Day 0 values were not significantly different for CD34+ cells isolated immediately or after an overnight incubation of total cells.

We further investigated the capacity of ex vivo expansion of peripheral blood MNCs and CD34+ cells with the cytokine cocktail used in our institution for clinical expansion of CD34+ cells mobilized in peripheral blood by G-CSF (SCF, G-CSF, and MGDF, 100 ng/mL each).[12] The only difference with respect to this clinical assay was the new serum-free medium (Macopharma HP01), which supported more efficiently the amplification of colony-forming cells (CFCs) and maintenance of SCID-repopulating cells than the medium used for clinical assay (Irvine Scientific, Santa Ana, CA).[25] Thus, if we started with MNCs recovered from the LDF, the amplification of total cells, CD34+ cells, and CFCs (CFU-GM plus BFU-E plus CFU-Mixed) at the end of 7-day cultures was very modest (less than twofold; Fig. 4). Under these conditions, the CFU-Mixed progenitors almost completely disappeared (Table 2). The selection of CD34+ cells (freshly or after an overnight incubation of total cells) and their plating in expansion cultures enabled a more efficient amplification of total and CD34+ cells. If the cells recovered from LDFs were incubated overnight, and the CD34+ selection then purified, however, the amplification fold of CFCs in Day 7 cultures was at least two times lower than those of freshly isolated CD34+ cells.

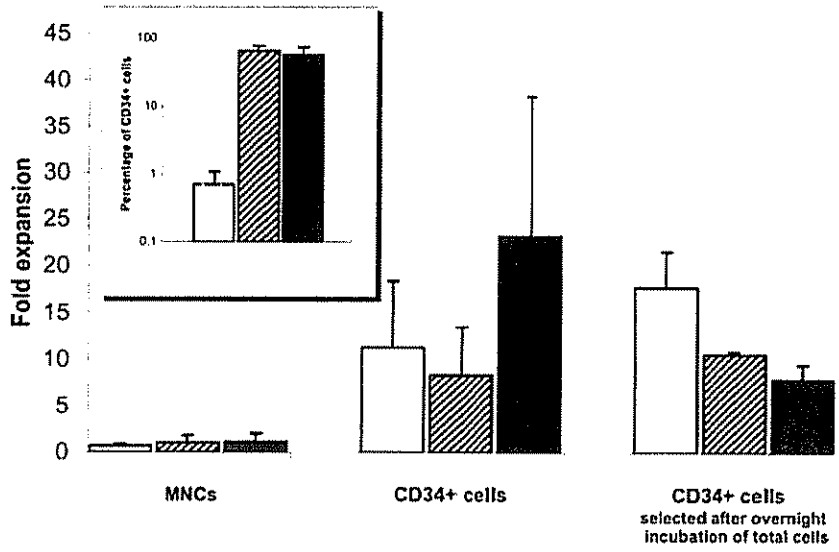

Fig. 4. Fold expansion in 7-day serum-free cultures supplemented by SCF, G-CSF, and MGDF (100 ng/mL each) of MNCs (□; n = 6), CD34+ cells (▨; n = 6), and CFU-C (CFU-GM plus BFU-E plus CFU-Mixed; ■; n = 4) starting with LDF-recovered MNCs, freshly isolated CD34+ cells, and CD34+ cells isolated after overnight incubation of total WBCs. (S) Percentage of CD34+ cells on Day 7 of cultures initiated with MNCs (□), freshly isolated CD34+ cells (▨), and CD34+ cells isolated after an overnight incubation of LDF-recovered WBCs (■). Means ± SD.

The percentage of CD34+ cells after 7 days of expansion was slightly higher than in Day 0 in cultures of MNCs (0.7%) and persisted in cultures of fresh CD34+ cells (67% vs. approx. 80% at Day 0) and in cultures of CD34+ cells isolated after an overnight incubation of total cells (58% vs. approx. 87% on Day 0; Fig. 3, inset). These percentages

of CD34+ cells persisting in Day 7 cultures were much higher than in similar expansion cultures of cord blood and mobilized blood CD34+ cells.[12,22,25]

Cytokine-directed lineage commitment and terminal differentiation of LDF-recovered CD34+ cells (>95% pure after selection for this series of experiments) were achieved in all tested conditions. Owing to the fact that the decline of cultures occurred after approximately 2 weeks, we evaluated cultures on Day 12 for the presence of differentiated cells. Cultures supplemented by SCF and EPO gave rise to hemoglobinized erythroid cells (benzidine-reactive brown cells) that were dominant (Fig. 5A and 5B). A minor population of cells remained immature. It is obvious that these cultures were not synchronized because of heterogeneous progression of progenitors through terminal differentiation and maturation (Fig. 5A). Figure 5B shows a higher resolution of the small compact erythroid cell. Cultures receiving SCF plus IL-3 (Fig. 5C) directed the CD34+ cells into predominantly the eosinophilic lineage, whereas GM-CSF or G-CSF induced these cells into the monomyelocytic lineage (Fig. 5D). SCF plus thrombopoietin resulted in megakaryocytes seen in Fig. 5E.

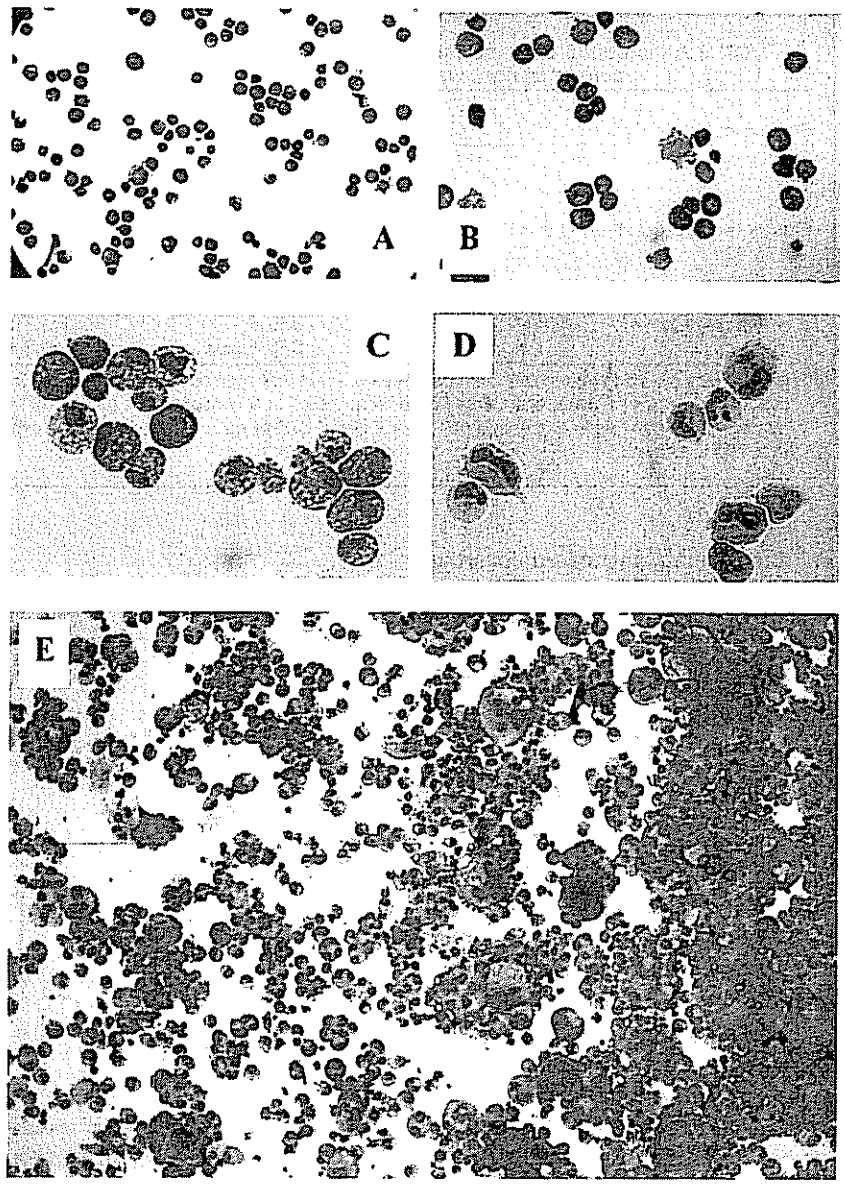

Fig. 5. Differentiation ex vivo of LDF-recovered CD34+ cells. (A and B) Hemoglobinized erythroid cells (benzidine-reactive brown cells); (C) eosinophilic lineage cells; (D) monomyelocytic lineage cells; (E) megakaryocytes.

DISCUSSION

We present here a technique permitting the recovery of MNCs from the LDFs discarded after the leukodepletion of whole blood. An effort was made to develop a technique compatible with the clinical practice. The procedure described in this report is a "closed system" with the exception of MNC separation by Ficoll. This step could also be performed in a closed system[26] meeting the requirements of good manufacturing practice–compatible procedures.

There were significant variations in total number of MNCs, CD34+ cells, and CFU-GM obtained per same volume of blood of different individuals as well as per individual filters. This is expected because relatively large individual variations in number of progenitors in peripheral blood of adults in physiologic conditions were reported in humans[6,27] and large animals.[28,29] Nevertheless, the results of amelioration of several technical points of our technique as well as selection of more "fresh" filters are promising in terms of better yield of these cells and a significant reduction of the individual variations (ongoing work). For a potential use in cell therapy, these individual variations are of relative importance because it may be necessary to pool cells recovered from several LDFs of the same donor.

The clonogenic activity of CD34+ cells and the capacity of ex vivo expansion in cytokine-supplemented serum-free liquid cultures (as already employed in our center for clinical trials) were evaluated to estimate their functional state. On the basis of these two systems, we

conclude that the functionality of LDF-recovered CD34+ cells was well preserved. It is evident that these cells exhibit an important capacity of ex vivo expansion, but with some unique properties compared to mobilized peripheral blood and cord blood. The fact that an overnight incubation of total cells influenced cytokine responsiveness of LDF-recovered progenitors implies that additional studies will be required for optimization of their ex vivo expansion.

We also demonstrated that LDF-derived CD34+ cells could differentiate into hemoglobinized erythroid cells as well as cells of megakaryocytic, eosinophilic, and monocyte-myelocytic lineages, depending on the presence of certain cytokines in liquid cultures. This point suggests that LDF-recovered CD34+ cells could be used for the development of clinical-grade procedures for the production of mature cells ex vivo, as it has been shown for cord blood and mobilized and steady-state peripheral blood CD34+ cells.[16-18,21]

It is known that the steady-state peripheral blood CD34+ cells do not engraft the NOD/SCID mice, making the detection of primitive stem cells very difficult. An improvement of the NOD/SCID model, however, by treatment with an antiasialo-GM1 antibody immediately before the cell transplantation (increasing the sensibility of the technique approx. five times)[30] recently permitted the detection of the SCID-repopulating cells in steady-state peripheral blood suspension.[31] We are currently working on this problem to evaluate the engraftment capacity of steady-state LDF-recovered CD34+ cells and their maintenance (amplification) in the course of ex vivo expansion, as well as the impact on this capacity of low oxygen concentration.[22]

Recently, intriguing results were published that demonstrated a retrodifferentiation of steady-state peripheral blood MNC ex vivo (i.e., their transformation in CD34+ progenitors and primitive stem cells) and a possibility of their further differentiation in several nonhematopoietic cell lineages.[32,33] If these results are confirmed, the MNCs recovered from the LDFs, which are actually a medical waste, may become a principal source of adult cells for cell therapy. This possibility is made more feasible by the results published recently that described the in vitro growth of endothelial cells from MNCs recovered from LDFs.[34]

ACKNOWLEDGMENTS

We thank J.P. Maurel, PhD, and C. Hamsany, PhD, for providing us the LDFs.

REFERENCES

1. Brecher G, Cronkite EP. Post-irradiation parabiosis and survival in rats. Proc Soc Exp Biol Med 1951;77:292-4.
2. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961;6:133-48.
3. Storb R, Graham TC, Epstein RB, et al. Demonstration of hemopoietic stem cells in the peripheral blood of baboons by cross circulation. Blood 1977;50:537-42.
4. Kessinger A, Armitage JO, Landmark JD, Weisenburger DD. Reconstitution of human hematopoietic function with autologous cryopreserved circulating stem cells. Exp Hematol 1986;14:192-6.
5. McCredie KB, Hersh EM, Freireich EJ. Cells capable of colony formation in the peripheral blood of man. Science 1971;171:293-4.
6. Chervenick PA, Boggs DR. In vitro growth of granulocytic and mononuclear cell colonies from blood of normal individuals. Blood 1971;37:131-5.
7. Barr RD, Whang-Peng J, Perry S. Hemopoietic stem cells in human peripheral blood. Science 1975;190:284-5.
8. Udomsakdi C, Landsdorp PM, Hogge DE, et al. Characterisation of primitive hematopoietic cells in normal human peripheral blood. Blood 1992;80:2513-21.
9. Ivanovic Z, Petakov M, Jovcic G, et al. Pluripotent and committed haemopoietic progenitor cells in rat peripheral blood. Comp Haematol Int 1997;7:1-6.
10. Ivanovic Z, Milenkovic P. Pluripotent haemopoietic progenitor cells (CFU-Sd8) in peripheral blood of hereditarily anaemic Belgrade (b/b) rats. Lab Anim 1999;33:77-82.
11. Herbein G, Sovalat H, Wunder E, et al. Isolation and identification of two CD34+ cell subpopulations from normal human peripheral blood. Stem Cells 1994;12:187-97.
12. Reiffers J, Cailliot C, Dazey B, et al. Abrogation of post-myeloablative chemotherapy neutropenia by ex-vivo expanded autologous CD34-positive cells. Lancet 1999;354:1092-3.
13. McNiece I, Jones R, Bearman SI, et al. Ex vivo expanded blood progenitor cells provide rapid neutrophil recovery after high-dose chemotherapy in patients with breast cancer. Blood 2000;96:3001-7.
14. Jaroscak J, Goltry K, Smith A, et al. Augmentation of umbilical cord blood (UCB) transplantation with ex-vivo-expanded UCB cells: results of a phase 1 trial using the AastromReplicell system. Blood 2003;101:5061-7.
15. Oki M, Ando K, Nakajima H, et al. Cord blood transplantation supported with ex vivo expanded fraction for a patient with myelodysplastic syndrome and metastatic breast cancer. Rinsho Ketsueki 2004;45:1048-52.
16. Neildez-Nguyen A, Wajcman H, Mardan MC, et al. Human erythroid cells produced ex vivo at large scale differentiate into red blood cells in vivo. Nat Biotechnol 2002;20:467-72.
17. Giarratana MC, Kobari L, Lapillonne H, et al. Ex vivo generation of fully mature human red blood cells from hematopoietic stem cells. Nat Biotechnol 2005;23:69-74.

18. Lathers DM, Achille N, Young MR. Dendritic cell development from mobilized peripheral blood CD34+ cells. Methods Mol Biol 2003;215:409-15.
19. Proulx C, Dupuis N, St-Amour I, et al. Increased megakaryopoiesis in cultures of CD34-enriched cord blood cells maintained at 39 degrees C. Biotechnol Bioeng 2004;88:675-80.
20. Decaudin D, Vantelon JM, Bourhis JH, et al. Ex vivo expansion of megakaryocyte precursor cells in autologous stem cell transplantation for relapsed malignant lymphoma. Bone Marrow Transplant 2004;34:1089-93.
21. Lefebvre P, Cohen I. Large-scale ex vivo expansion of human megakaryocytes for clinical use. Methods Mol Biol 2003; 215:375-85.
22. Ivanovic Z, Hermitte F, Brunet de la Grange P, et al. Simultaneous maintenance of human cord blood SCID-repopulating cells and expansion of committed progenitors at low O2 concentration (3%). Stem Cells 2004;22:716-24.
23. Sutherland DR, Anderson L, Keeney M, et al. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother 1996;5:213-26.
24. Jeanne M, Bouzgarrou R, Lafarge X, et al. Comparison of CD34+ cell collection on the CS-3000+ and Amicus blood cell separators. Transfusion 2003;43:1423-7.
25. Ivanovic Z, Duchez P, Dazey B, et al. A clinical-scale expansion of mobilized CD34+ hematopoietic stem and progenitor cells by using a new, serum-free medium. Transfusion 2006; in press.
26. Celluzzi CM, Welbon C. Dendritic cell culture: a simple closed culture system using ficoll, monocytes, and a table-top centrifuge. J Hematother Stem Cell Res 2003;12:575-85.
27. To LB, Haylock DN, Dowse T, et al. A comparative study of the phenotype and proliferative capacity of peripheral blood (PB) CD34+ cells mobilized by four different protocols and those of steady-phase PB and bone marrow CD34+ cells. Blood 1994;84:2930-9.
28. Kovacevic M, Bozic T, Ivanovic Z. Erythroid progenitor cells from pig bone marrow and peripheral blood. Vet J 1999; 158:196-203.
29. Kovacevic M, Bozic T, Jovcic G, et al. Pig bone marrow and peripheral blood granulocyte-macrophage progenitor cells. Acta Vet 2001;51:15-26.
30. Yoshino H, Ueda T, Kawahata M, et al. Natural killer cell depletion by anti-asialo GM1 antiserum treatment enhances human hematopoietic stem cell engraftment in NOD/Shi-scid mice. Bone Marrow Transplant 2000;11: 1211-6.
31. Hirayama F, Yamaguchi M, Yano M, et al. Spontaneous and rapid reexpression of functional CXCR4 by human steady-state peripheral blood CD34+ cells. Int J Hematol 2003; 78:48-55.
32. Abuljadayel IS. Induction of stem cell-like plasticity in mononuclear cells derived from unmobilised adult human peripheral blood. Curr Med Res Opin 2003;19:355-75.
33. Abuljayadel IS, Afghan RK, McCaffrey TA, et al. SCID repopulating cells derived from unmobilised adult human peripheral blood. Curr Med Res Opin 2004;20:87-100.
34. Teleron AA, Carlson B, Young PP. Blood donor white blood cell reduction filters as a source of human peripheral blood-derived endothelial progenitor cells. Transfusion 2005;45: 21-5.