Characterization of mononuclear cells remaining in the leukoreduction system chambers of apheresis instruments after routine platelet collection: a new source of viable human blood cells

Sonia Néron, Louis Thibault, Nathalie Dussault, Geneviève Côté, Éric Ducas, Nicolas Pineault, and Annie Roy

BACKGROUND: The yield of white blood cells (WBCs) extracted from whole-blood leukoreduction filters can be affected by the storage conditions and delay before filtration. Platelets (PLTs) collected with apheresis instruments (Trima Accel, Gambro BCT) are leukoreduced during the procedure on a fluidized particle bed in a leukoreduction chamber (LRS chamber). In this report, the residual cell content of these LRS chambers was characterized to determine whether it would be a valuable source of viable human blood cells.

STUDY DESIGN AND METHODS: The content of LRS chambers was eluted by gravity, and peripheral blood mononuclear cells (PBMNCs) were purified on a Ficoll-Paque gradient. Analyses were performed before and after freezing. Proportions of CD3+, CD14+, CD16+, CD19+, CD34+, and CD45+ cells were determined by flow cytometry. The frequency of T cells expressing CD4, CD8, and CD27 and of B cells expressing immunoglobulin G (IgG), IgM, and CD27 was also determined.

RESULTS: LRS chambers held approximately 10^9 CD45+ cells representing the normal proportions of CD3+, CD14+, CD16+, and CD19+ cell populations of PBMNCs. A small fraction of these CD45+ cells were CD34+CD38+ cells (0.3 ± 0.2%). The viability of these cells, measured before and after freezing, was more than 95 percent.

CONCLUSION: The residual cell content of Trima Accel LRS chambers recovered after PLT collection is a good source of viable monocytes and lymphocytes. These PBMNCs, containing CD3+, CD14+, CD16+, CD19+, and CD34+ cells can be frozen to prepare cell banks, which opens new avenues for utilization in several physiologic studies or even in cellular therapy applications.

Clinical and experimental investigations on human blood cells usually require blood samples from healthy volunteers as experimental controls, and it is often difficult for investigators to obtain access to large numbers of these cells due to the complexity of volunteer recruitment, blood collection, and blood testing. To solve these issues, one may consider volunteers from the laboratory staff, which then raises ethical concerns. We and others have previously reported that cryopreservation of lymphocytes prepared from whole-blood (WB) leukoreduction filters, combining safety and convenience, was an asset for investigations of human white blood cells (WBCs).[1] Several groups have also shown that leukoreduction filters could be a good source of normal human cells,[2-7] and the growing interest in cellular therapy applications also added importance to identify new sources of normal human cells. Toward this, two groups recently reported the isolation of 0.45×10^6 to 1.1×10^6 CD34+ cells from WB leukoreduction filters[7,8] as cells, which represent normally despite their naturally low abundance in the circulation of approximately 0.06 percent. In addition, dendritic cells

ABBREVIATIONS: 7-AAD = 7-aminoactinomycin D; LRS chamber(s) = leukoreduction chamber(s); NK cell(s) = natural killer cell(s); WB = whole blood.

From Héma-Québec, Research and Development, Québec, Quebec; Department of Biochemistry and Microbiology, Laval University, Québec, Quebec, Canada.

Address reprint requests to: Sonia Néron, PhD, Héma-Québec, Recherche et développement, 1009, route du Vallon, Québec, Québec, Canada G1V 5C3; e-mail: sonia.neron@hema-quebec.qc.ca.

Received for publication October 17, 2006; revision received December 6, 2006, and accepted December 8, 2006.

doi: 10.1111/j.1537-2995.2007.01233.x

TRANSFUSION 2007;47:1042-1049

that can be used in vaccination therapy[9] have also been generated from CD14+ precursors contained in WB leukoreduction filters.[3] Delays to obtain these filters, storage conditions, filter types, and processing steps can, however, affect the yield of WBCs extracted from these filters.[1,7] Blood components can be leukoreduced by filtration or during apheresis collection without subsequent processing. Approximately 70 percent of Héma-Québec's leukoreduced platelet (PLT) concentrates are produced by apheresis with apheresis instruments (Trima Accel, Gambro BCT, Inc., Lakeview, CO). During these procedures, the leukoreduction of blood components is done through a saturated fluidized particle bed in a small leukoreduction chamber (LRS chamber) which allows the separation of WBC and PLTs. After PLTs collection the majority of WBCs are returned to the donor while a small fraction remains in the LRS chamber. Based on manufacturer's information, these residual cells are thought to be mostly mononuclear cells; however, there is currently no published data characterizing their detailed phenotype distribution and quantity, though a study showing their efficient response to mitogenic activation has just recently been published.[10] On the other hand, the PLT content of LRS chambers was also examined as a tool for quality control in apheresis procedures.[11] In this study, we have investigated the value and nature of the peripheral blood mononuclear cells (PBMNCs) trapped in the LRS chambers to prepare frozen cell banks. The cell content, viability, and phenotype distribution of the cells isolated from LRS chambers used for single and double PLTs collection procedures is described in detail. Taken together, these results demonstrate that LRS chambers can be used as a valuable new source rich in viable human cells.

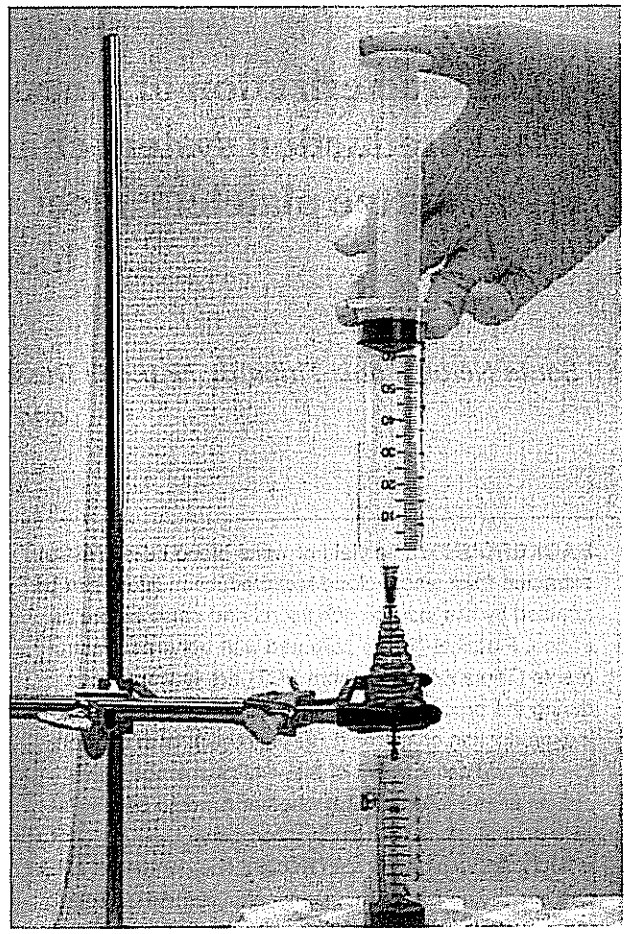

Fig. 1. Cell recovering from LRS chambers. LRS chambers disconnected from Trima Accel collection systems were held on a support, as illustrated, and the cells were recovered aseptically in a laminar hood as described under Materials and Methods.

MATERIALS AND METHODS

PLT apheresis blood donors and collection of LRS chambers

Regular blood donors were recruited during the predonation interview by nurses. Those who agreed to participate in this study have signed an informed consent. Automated PLTs collections by apheresis were done at our permanent blood donor centers with an apheresis collection system (Trima Accel, Gambro BCT, Lakewood, CO). The LRS chambers were recovered by nurses at the conclusion of the apheresis procedures. Tubing of LRS chambers was sealed before removing the collection set from the Trima Accel. After disconnection, LRS chambers were kept at room temperature until WBC recovery (within 3 hr), and the remaining collection sets were discarded.

Cell recovering from LRS chambers

Cell recovering from LRS chambers was done aseptically in a laminar flow hood as illustrated in Fig. 1. Briefly, the LRS chambers and tubing were cleaned with 70 percent ethanol and held upright to dry. The downstream tubing was cut and then the upstream tubing, to allow the chamber content to flow in a sterile 50-mL tube. The chamber was next rinsed with approximately 45 mL phosphate-buffered saline (PBS; 10 mmol/L potassium/sodium phosphate buffer with 136 mmol/L NaCl, pH 7.4, Dulbecco's PBS [Invitrogen, Grand Island, NY]) supplemented with 10 percent ACD (Baxter Healthcare Corp., Deerfield, IL) with a 60-mL syringe with 22-gauge needle. Complete blood counts were done with a hematologic analyzer (Cell-Dyn 3200, Abbott Laboratories, Abbott Park, IL). WBC counts were also determined with a hemocytometer after RBC lysis with 3 percent acetic acid.

Preparation of PBMNC banks

The LRS chamber eluates, volume ranging from 50 to 55 mL, were used directly to isolate PBMNCs by centrifu-

gation on Ficoll-Paque following manufacturer's instructions (Amersham/Pharmacia Biotech, Baie D'Urfé, Quebec, Canada). After centrifugation, PBMNCs were extracted from the Ficoll-Paque tubes and washed with PBS supplemented with 10 mmol per L glucose. PBMNCs were finally frozen and thawed as previously described.[1] The viability of PBMNCs, before and after thawing, was determined with Trypan blue exclusion counting.

Flow cytometry analysis

Peridin chlorophyll protein-cyanin 5.5–conjugated anti-CD19; allophycocyanin-conjugated anti-CD14, anti-CD27, or anti-CD45; phycoerythrin (PE)-conjugated anti-CD27, anti-CD45, anti-immunoglobulin G (IgG), or anti-IgD; fluorescein isothiocyanate (FITC)-conjugated anti-CD3, anti-CD27, or anti-CD38; Alexa 488-conjugated anti-CD16; and Alexa 488-allophycocyanin-, PE-, and FITC-conjugated isotype controls were used in quadruple or triple staining procedures. All these antibodies were IgG1 mouse monoclonal antibodies (MoAbs), except anti-CD14 which was IgG2a, and obtained from BD Biosciences (Mountain View, CA). PE-conjugated anti-CD34 was IgG1 mouse MoAbs obtained from Beckman Coulter (Burlington, ON, Canada) and FITC–anti-IgM were polyvalent goat IgG (The Jackson Laboratory, Mississauga, Ontario, Canada). PBMNCs were stained with 1 µg of antibody by 10^6 cells at 4°C and then fixed with 2 percent paraformaldehyde as described previously.[1] In all analyses, antibody isotype controls were run in parallel to ensure the specificity of the cell staining (more than 95% of cells were double-negative). Samples were analyzed on a flow cytometer and its software (FACSCalibur and CellQuest Pro, BD Biosciences). The instrument was adjusted to delineate dead cells with propidium iodine (PI) or 7-aminoactinomycin D (7-AAD) staining following the manufacturer's instructions (BD Biosciences). Acquisition of data was performed by gating more than 80,000 cells for PBMNCs or more than 5,000 for purified B cells, with the flow cytometer. Data were subsequently analyzed with computer software (FCS express II, De Novo Software, Thornhill, Ontario, Canada). Mean fluorescence intensity (MFI) was used, when indicated, to compare a marker expression level between two populations.

RESULTS

The LRS chambers contain a large amount of viable human blood cells

The total number of cells isolated from LRS chambers was evaluated before (WBCs) and after (PBMNCs) Ficoll-Paque separation (Fig. 2). The total amount of WBCs isolated from LRS chambers after a single or a double plateletpheresis were $0.94 \times 10^9 \pm 0.49 \times 10^9$ and $1.4 \times 10^9 \pm 0.56 \times 10^9$ cells, respectively. The recovery of PBMNCs after elimination of the PLTs, RBCs, and granulocytes by centrifugation over Ficoll-Paque was good with approximately 70 percent of all the WBCs eluted. The final yields in cells were $0.7 \times 10^9 \pm 0.4 \times 10^9$ and $0.9 \times 10^9 \pm 0.5 \times 10^9$ PBMNCs per LRS chamber following single or double PLT apheresis procedures, respectively. The viability of PBMNCs was greater than 95 percent, with a mean

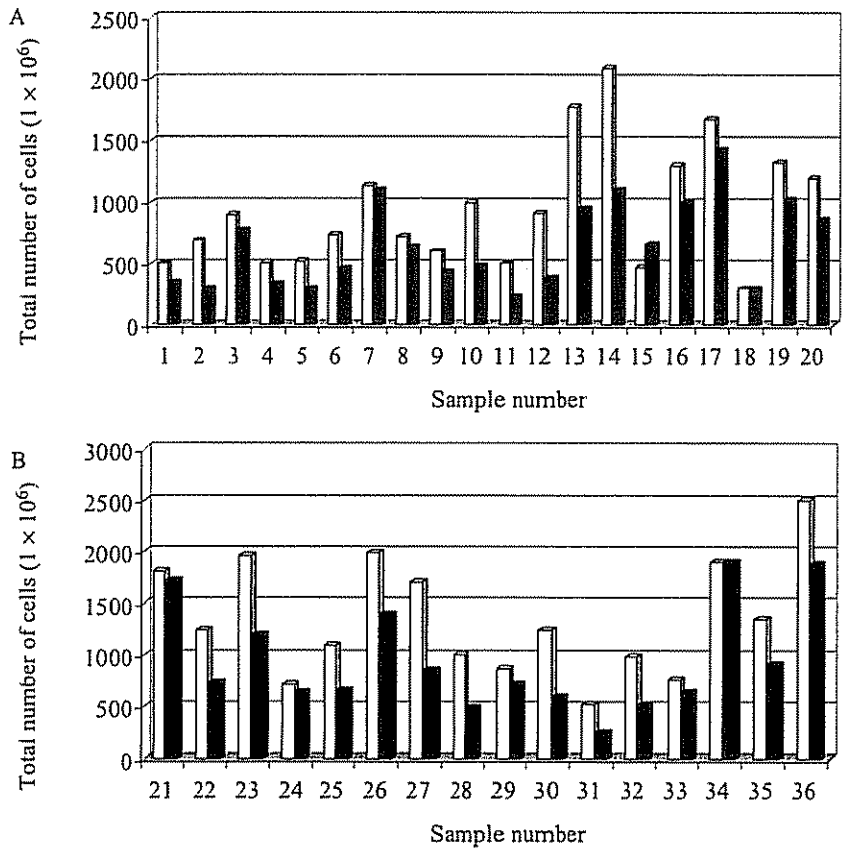

Fig. 2. WBCs (□) and PBMNCs (■) recovered from LRS chambers are viable. The total number of WBCs recovered directly from the LRS chambers and PBMNCs isolated after centrifugation over Ficoll-Paque were determined by cell count with Trypan blue exclusion for 36 independent samples issued from (A) single- and (B) double-unit PLT concentrates. WBCs concentrations were corroborated by complete blood counts with a Cell-Dyn 3200 hematologic counter.

recovery yield of 77 ± 24 percent, after one freezing-thawing cycle (n = 17; data not shown). The total cell content was evaluated on 20 samples taken immediately after the elution of LRS chamber (Table 1). Lymphocytes and monocytes represented, respectively, 68 ± 9 and 25 ± 9 percent of WBCs and similar proportions were recovered in PBMNCs after one freezing-thawing cycle, namely, 66 ± 12 and 21 ± 7 percent, respectively. Neutrophils, eosinophils, and basophils were present in very low frequency representing less than 5 percent of total cell count. RBCs and PLTs were also present at concentrations $1.15 \times 10^{12} \pm 0.18 \times 10^{12}$ and $2.63 \times 10^{11} \pm 1.10 \times 10^{11}$ per L, respectively. The presence of RBCs and PLTs, in addition to minor granulocyte populations, justified the need for a Ficoll-Paque separation step to MNCs. Overall, lymphocytes and monocytes proportions in LRS chamber eluates were not matching blood normal ranges for WBCs but PBMNCs range usually observed after removal of granulocytes.[12]

TABLE 1. Hematologic analysis of LRS chamber content (n = 20)*

WBC populations†	Parameters: Cell count ($\times 10^9$ cells/L)	Parameters: Proportion of WBCs (%)
WBCs	17.1 ± 9.2 (4.2-38.2)	NA
Neutrophils	0.68 ± 0.33 (0.05-1.35)	4.99 ± 2.59 (2.31-12.8)
Lymphocytes	11.7 ± 6.7 (2.9-27.8)	67.78 ± 8.63 (47.6-79.0)
Monocytes	4.2 ± 2.8 (0.6-11.8)	24.62 ± 8.94 (13.9-46.1)
Eosinophils	0.05 ± 0.07 (0.01-0.3)	0.39 ± 0.49 (0.07-1.87)
Basophils	0.38 ± 0.25 (0.08-0.99)	2.22 ± 0.72 (1.07-3.26)

* Data are reported as mean ± SD (range).
† Complete blood counts were done on the cells extracted from LRS chambers with a Cell-Dyn 3200 hematologic analyzer.

TABLE 2. Phenotype and distribution of CD45+ populations present in PBMNCs eluted from LRS chambers (n = 10)

Population	Phenotype	Frequency* (%): Mean ± SD	Frequency* (%): Range (%)
Lymphocytes†	CD3+	66.7 ± 6.4	56.9-79.8
	$CD14^{neg}CD16+$	12.7 ± 2.5	9.1-17.1
	CD19+	13.0 ± 5.8	6.0-21.9
	CD34+CD38+	0.3 ± 0.2	0.1-0.7
Monocytes‡	$CD14+CD16^{neg}$	71.6 ± 7.2	57.7-80.4
	$CD14^{lo}CD16+$	7.6 ± 2.9	3.6-11.4
	CD14+CD16+	8.0 ± 3.2	4.4-14.6
CD3+ CD45+ cells§	CD4+	61.6 ± 8.9	47.5-72.4
	CD8+	33.7 ± 9.5	21.2-42.8
	CD27+	89.1 ± 9.0	68.0-99.9
	CD4+CD27+	63.4 ± 9.8	45.0-76.0
	CD8+CD27+	29.1 ± 8.8	16.0-45.0
CD19+ CD45+ cells‖	CD5+	17.2 ± 4.2	11.9-22.7
	CD27+	35.3 ± 12.9	10.9-58.5
	IgM+	80.6 ± 9.8	64.2-95.3
	IgG+	7.6 ± 2.9	2.4-17.6
	CD27+IgM +	18.5 ± 8.0	6.5-32.4
	CD27+IgG +	7.2 ± 4.6	1.9-15.4
	$CD27^{neg}$IgM +	61.2 ± 13.4	39.3-87.8
	$CD27^{neg}$IgG +	1.9 ± 1.4	0.3-4.1

* Thawed PBMNCs prepared from LRS chamber content issued from single or double apheresis procedures were at least 98 percent CD45+ and viability was greater than 95 percent. Flow cytometry analyses were performed within each population, as indicated, with quadruple staining.
† The proportions of CD3, CD16, and CD19 single-positive cells were determined within the lymphocyte region (namely, gate R1; Fig. 2).
‡ The proportions of cells expressing CD14 and/or CD16 were evaluated within the monocyte region (namely, gate R2; Fig. 2).
§ The proportions of cells expressing CD4, CD8, and CD27 were evaluated within the gated CD3+ cells.
‖ The proportions of cells expressing IgG, IgM, CD5, and CD27 were evaluated within the gated CD19+ cells.

LRS chamber content is representative of the major human blood cell populations

The phenotypes of CD45+ populations of the PBMNCs eluted from the LRS chambers were determined after one freezing-thawing cycle by flow cytometry (Table 2 and Fig. 3A). Flow cytometry analysis of CD3, CD14, CD16, CD19, and CD45 expression was used to delineate T cells, monocytes, natural killer (NK) cells, and B cells, respectively, in 10 samples (Table 2 and Fig. 3). According to 7-AAD staining, the lymphocyte R1 gate and monocyte gate R2 (Fig. 3A) contained viable (>95%) CD45+ (>98%) cells. The mean proportion of B, NK, and T cells of 13, 12.7, and 66.7 percent, respectively, were in accordance to reference range expected for lymphocyte populations in blood[13,14] or leukoreduction filters.[1] Analysis of monocyte populations showed three distinct populations according to CD14 and CD16 expressions (Fig. 3A). The major cells population (72 ± 7.2%) corresponding to $CD14+ CD16^{neg}$ cells and a minor population (7.6 ± 2.9%) including $CD14^{lo}CD16+$ cells, matched the normal proportions of monocytes previously reported in human blood samples.[15-18] Additionally, an intermediate population of CD14+CD16+ cells, representing approximately 4 percent of cells in R2 gate, was also observed (Fig. 3A). This population represented a mean of 8.0 ± 3.2 percent in 10 samples analyzed by flow cytometry (Table 2). Such population coexpressing CD14 and CD16 was previously reported, albeit in a lower frequency among the monocyte populations of peripheral blood of healthy individuals.[15,16,18,19] Overall, our results indicated that LRS chamber content is representative of the major peripheral blood cell populations.

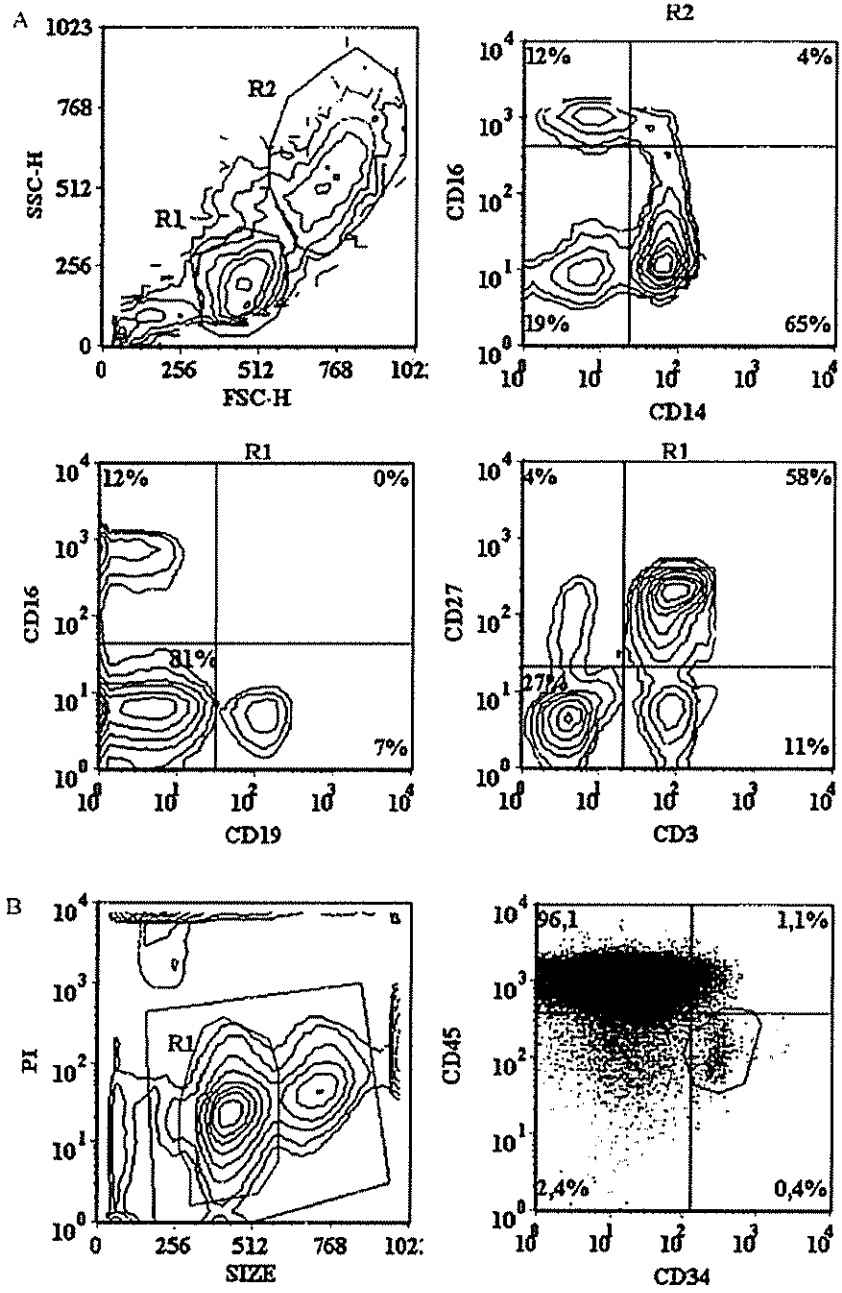

Fig. 3. Phenotype of mononuclear populations recovered from LRS chambers. Quadruple staining was used to analyze the PBMNCs after one freezing-thawing cycle. According to 7-AAD staining, the gates R1 and R2 contained only viable cells, which were all expressing CD45. (A) PBMNCs were analyzed for the proportion of cells expressing CD3, CD14, CD16, and CD19 in regions corresponding to lymphocytes (R1) and monocytes (R2) as indicated. (B) For CD34 analysis, cells were not fixed and PI staining was used to gate the viable cells in lymphocyte region (R1). The profiles, presented in contour plots (A and B), show the populations observed based on analysis performed on 150,000 to 250,000 events and are representative of 10 independent samples.

Enriched cell population of CD34+CD45^{lo} cells

The frequency of CD34+ cells within the CD45+ populations with PI staining to exclude dead cells (Table 2 and Fig. 3B) were done on PBMNCs eluted from LRS chambers; cells expressing high level of CD34 were detected in the eluate at an average proportion of 0.3 ± 0.2 percent (Fig. 3B). These CD34+ cells expressed three- to fivefold lower levels of CD45 (MFI, 176 ± 111) than the overall CD45+ populations (MFI, 983 ± 483). This CD45^{lo}-positive cell population matched up well with the reported phenotype of hematopoietic stem and progenitor cells of the human blood.[7,20] These CD34+CD45^{lo} cells were also characterized by low expression of CD38 (data not shown; MFI, 53 ± 22). WBCs usually show a frequency of 0.01 to 0.06 percent CD34+ cells[7,21] representing approximately 0.15 percent of mononucleated cells.[22] A mean of 0.16 percent CD34+ cells was also observed in PBMNCs isolated from WB leukoreduction filters,[8] and thus the frequency observed in the LRS chamber contents matched these expected frequencies.

B- and T-cell subpopulations isolated from LRS chambers

To further characterize the major B- and T-cells populations, more refined flow cytometry analysis was performed with gates adjusted to analyze the CD19+ and CD3+ cells, respectively (Table 2 and Fig. 4). Proportions of CD4+ and CD8+ T cells, respectively, of 62 and 34 percent, were similar to those observed in the peripheral blood of healthy individuals[13] as for the CD27 expression with a mean of 89 percent on the majority of T cells.[23,24] In addition, there was no difference in the distribution of CD3+CD27+ cells in function of CD4 and CD8 subpopulations[25] (Table 2 and Fig. 4). Similarly, the mean proportions of B cells expressing CD5 (17%), CD27 (35%), IgG (9%), or IgM (81%) were also in accordance with the described naive CD19+CD27– (60%) and memory CD19+CD27+ (40%) cell proportions of

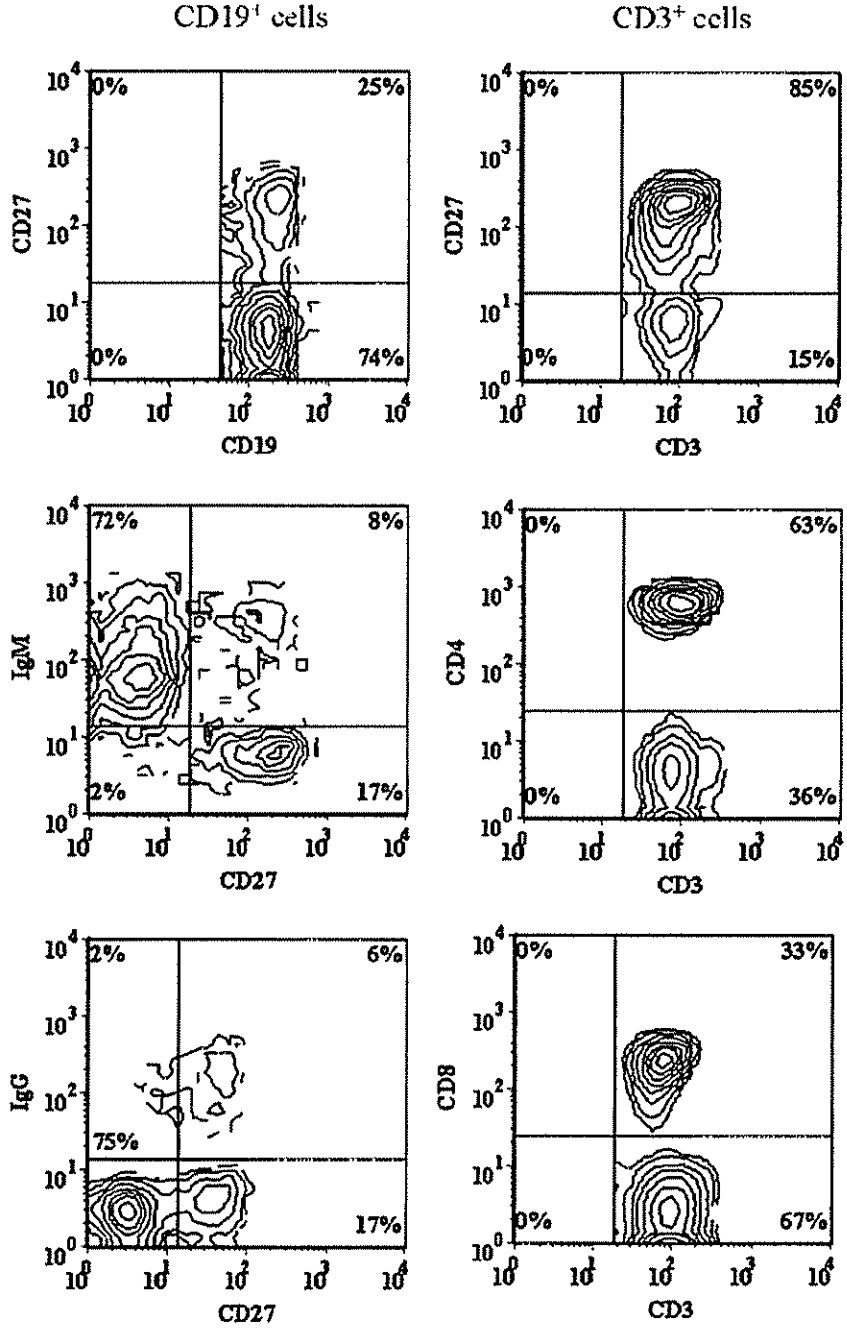

Fig. 4. Analysis of B and T lymphocyte populations after one freezing-thawing cycle. The CD19+ or CD3+ cells, as indicated, were delineated within the lymphocyte region (gate R1; Fig. 2), and their phenotypes were analyzed with quadruple staining as described under Materials and Methods.

the peripheral blood populations.[24,26-28] These frequencies were also similar to those previously observed in PBMNCs isolated from blood or WB leukoreduction filters.[1] Finally, the frequencies of naive and memory B cells expressing IgG or IgM were also similar to that previously reported in PBMNCs and peripheral blood.[27-29] The characterization of B and T cell subpopulations further indicated that PBMNCs isolated from LRS chambers were representative of major peripheral blood populations and subpopulations.

DISCUSSION

Investigations on human blood cells frequently require the use of normal cells as control cells. Obtaining these cells in adequate quantity and quality can be logistically and ethically challenging. Recently, we have demonstrated that cells recovered from WB leukoreduction filters represent a good source of viable PBMNCs because each unit can provide 2×10^8 to 3×10^8 PBMNCs.[1] These filters, however, were obtained several hours after collection (>4 hr), which can significantly reduce the cell yield.[1,7] In this study, we have characterized the residual WBC content and viability of 38 leukoreduction systems (LRS chambers) obtained with Trima Accel collection systems after single and double PLT donations. The LRS chambers are available immediately after plateletpheresis procedures. The recruitment of donors is done during the predonation interviews by nurses simplifying the logistics of volunteer recruitment. The retrieval of the LRS chambers was well accepted by our blood donors who considered that as an improved value to their blood donation. Also, these donors are regularly tested for blood-borne virus, are usually phenotyped for several blood group antigens and HLA, and can be recruited repeatedly for a study with a current interval of 14 days between two donations. Consequently, freezing and storing of those cells offers the possibility to obtain large quantities of viable cells from a single individual. Even though the majority of the WBCs and RBCs are returned to donors by the apheresis instrument at the end of the PLT collection procedure, we show that the residual cell content of the LRS chambers was approximately fivefold higher than what would be extracted from WB leukoreduction filters with a mean of 0.9×10^9 to 1.4×10^9 viable PBMNCs per chamber. This quantity represented approximately 2 to 5 percent of the total WBC content of the blood volume processed by the Trima Accel instrument. Recovery of cells from the LRS chambers was easy and fast because WBCs

can be eluted and frozen within 90 minutes after its reception.

Importantly, these isolated cells have a good representation of all major blood cells such as monocytes and B, NK, and T cells. Furthermore, the repartition of subpopulations within monocytes and B and T cells was also typical of normal human blood values. B cells isolated from these PBMNCs were physiologically active when stimulated in the CD40-CD154 system[30] and could be kept in culture for up to 14 days (data not shown). In addition, the high proportion of CD34+ cells and recovery rate of PBMNCs eluted from these LRS chambers, with ranges of 1×10^6 to 4×10^6 cells per unit, makes these chambers an interesting source to other scientific studies. The possibility to expand these progenitor cells in vitro as shown previously for CD34+ cells isolated from WB leukoreduction filters[8] and reported recently[10] could also have a valuable potential in cellular therapy applications and is currently under investigation. The same conclusion could be drawn for dendritic cells where CD14+CD16–, $CD14^{lo}CD16+$, and CD14+CD16+ subpopulations of monocytes could be used to prepare large numbers of human dendritic cells as previously proposed with WB leukoreduction filters.[3,7] To our knowledge, this is the first report on the characterization of the populations present in viable residual WBCs content of LRS chambers of Trima Accel instruments, which are used to produce approximately 50 percent of North America's leukoreduced PLT concentrates. Given that LRS chambers are discarded immediately after apheresis, their utilization adds an extra value to blood donations. The large numbers of PBMNCs that can be easily extracted from LRS chambers could also be an asset in many other investigations necessitating human normal cells as controls. Because of the ease and principally high cell yields, PBMNCs isolated from these LRS chambers are now used in our laboratory to isolate B lymphocytes for investigations on in vitro production of therapeutic immunoglobulins.

ACKNOWLEDGMENTS

We thank Jocelyne Dion, Lise Bourgoin, and all the quality control technologists for their assistance with the hematology analyzer. Also, we acknowledge the work of Serey-Phorn Sea and Claudia Racine for the data collection and Geneviève Boyer from Gambro BCT for her helpful discussion. We also thank Lucie Boyer for help in the analysis of CD34+ cells. Finally, we are thankful to our dedicated blood donors who agreed to participate in this study and to Claudine Côté for the recovery of all these LRS chambers.

REFERENCES

1. Néron S, Dussault N, Racine C. Whole-blood leukoreduction filters are a source for cryopreserved cells for phenotypic and functional investigations on peripheral blood lymphocytes. Transfusion 2006;46:537-44.
2. Cook MA, Jobson SE, Atkinson DC, et al. Used leucodepletion filters as a source of large quantities of DNA suitable for the study of genetic variations in human populations. Transfus Med 2003;13:77-81.
3. Ebner S, Neyer S, Hofer S, et al. Generation of large numbers of human dendritic cells from whole blood passaged through leukocyte removal filters: an alternative to standard buffy coats. J Immunol Methods 2001;252:93-104.
4. Weitkamp JH, Crowe JE. Blood donor leukocyte reduction filters as a source of human B lymphocytes. Biotechniques 2001;31:464-6.
5. 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.
6. Sowemimo-Coker SO, Zinn F, Kim JA, Angelbeck J. A simple procedure for recovery of viable leukocyte from Pall filters. Transfusion 2002;42(9s):62S.
7. Meyer TP, Zehnter I, Hofmann B, et al. Filter buffy coats (FBC): a source of peripheral blood leukocytes recovered from leukocyte depletion filters. J Immunol Methods 2005; 307:150-66.
8. Ivanovic Z, Duchez P, Morgan DA, et al. Whole-blood leuko-depletion filters as a source of CD34+ progenitors potentially usable in cell therapy. Transfusion 2006;46:118-25.
9. Svensson A, Adamson L, Pisa P, et al. Monocyte enriched apheresis for preparation of dendritic cells (DC) to be used in cellular therapy. Transfus Apher Sci 2005;33:165-73.
10. Dietz AB, Bultur PA, Emery RL, et al. A novel source of viable peripheral blood mononuclear cells from leukoreduction system chambers. Transfusion 2006;46:2083-9.
11. Vasconcelos E, Sousa AP, Seghatchian J, et al. The content of the LRS chamber provides a new quality tool for the characterization of the donor platelet profile. Transfus Apher Sci 2005;32:221-5.
12. Bain BJ. Normal ranges. In: Khan M, editor. Blood cells: a pratical guide. Oxford: Blackwell Publishing; 2006. p. 198-216.
13. Santagostino A, Garbaccio G, Pistorio A, et al. An Italian national multicenter study for the definition of reference ranges for normal values of peripheral blood lymphocyte subsets in healthy adults. Haematologica 1999;84:499-504.
14. Seidi OA, Semra YK, Sharief MK. Expression of CD5 on B lymphocytes correlates with disease activity in patients with multiple sclerosis. J Neuroimmunol 2002;133:205-10.
15. Tanaka M, Honda J, Imamura Y, et al. Surface phenotype analysis of CD16+ monocytes from leukapheresis collections for peripheral blood progenitors. Clin Exp Immunol 1999;116:57-61.
16. Kawanaka N, Yamamura M, Aita T, et al. CD14+,CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. Arthritis Rheum 2002;46:2578-86.

17. Passlick B, Flieger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood 1989;74:2527-34.
18. Tacke F, Randolph GJ. Migratory fate and differentiation of blood monocyte subsets. Immunobiology 2006;211:609-18.
19. Grage-Griebenow E, Zawatzky R, Kahlert H, et al. Identification of a novel dendritic cell-like subset of CD64(+)/CD16(+) blood monocytes. Eur J Immunol 2001;31:48-56.
20. Rivadeneyra-Espinoza L, Perez-Romano B, Gonzalez-Flores A, et al. Instrument- and protocol-dependent variation in the enumeration of CD34+ cells by flow cytometry. Transfusion 2006;46:530-6.
21. Anderlini P, Korbling M. The use of mobilized peripheral blood stem cells from normal donors for allografting. Stem Cells 1997;15:9-17.
22. 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.
23. Hamann D, Baars PA, Rep MH, et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. J Exp Med 1997;186:1407-18.
24. Agematsu K, Hokibara S, Nagumo H, Komiyama A. CD27: a memory B-cell marker. Immunol Today 2000;21:204-6.
25. Lens SM, Tesselaar K, van Oers MH, van Lier RA. Control of lymphocyte function through CD27–CD70 interactions. Semin Immunol 1998;10:491-9.
26. Klein U, Rajewsky K, Kuppers R. Human immunoglobulin (Ig) M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells. J Exp Med 1998;188:1679-89.
27. Kuppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B-cell lymphomas. N Engl J Med 1999;341:1520-9.
28. Klein U, Goossens T, Fischer M, et al. Somatic hypermutation in normal and transformed human B cells. Immunol Rev 1998;162:261-80.
29. Fecteau JF, Côté G, Néron S. A new memory CD27-IgG+ B cell population in peripheral blood expressing VH genes with low frequency of somatic mutation. J Immunol 2006;177:3728-36.
30. Néron S, Racine C, Roy A, Guerin M. Differential responses of human B-lymphocyte subpopulations to graded levels of CD40–CD154 interaction. Immunology 2005;116:454-63.