A novel source of viable peripheral blood mononuclear cells from leukoreduction system chambers

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BACKGROUND: Buffy coats are becoming less available as a source of research-grade peripheral blood mononuclear cells (PBMNCs). Therefore, alternative sources of these cells were investigated.

STUDY DESIGN AND METHODS: PBMNCs isolated from the cells retained in leukoreduction system chambers (LRSCs) and those eluted from white blood cell filters were compared. From LRSCs (1.88 ± 0.40) $\times 10^9$ PBMNCs ($n = 13$) versus (0.43 ± 0.15) $\times 10^9$ PBMNCs were isolated from leukofilter eluates (LFEs, $n = 8$; $p < 0.0001$).

RESULTS: Cells from LRSCs and LFEs produced similar numbers of burst-forming unit–erythroid, colony-forming unit (CFU)–granulocyte-macrophage, and CFU–granulocyte-erythrocyte-monocyte-macrophage-megakaryocyte colonies. The percentages of cells positive for CD3, CD4, CD8, CD14, CD19, and CD56 in the PBMNCs isolated from LRSCs and LFEs were indistinguishable. Cells isolated from LRSCs expressed higher levels of CD69 and CD25 in reaction to staphylococcal enterotoxin B than the cells isolated from LFEs. The source of cells affected neither the yield and purity of immunomagnetically isolated CD3+ cells, CD14+ cells, and CD56+ cells nor the function of T cells, natural killer cells, and in vitro matured dendritic cells (DCs). DC yield from LRSC-derived CD14+ cells, however, was higher.

CONCLUSION: LRSCs are a novel source of fully functional PBMNCs that can replace the more traditional sources of research-grade cellular products.

Traditionally, the most common source of human primary white blood cells (WBCs) for laboratory use has been buffy coats, the cells separated from red blood cells (RBCs) by centrifugation. Recently, however, blood banks have been introducing filtration for WBC removal to minimize HLA alloimmunization, to reduce the risk of febrile nonhemolytic transfusion reaction, and as a more effective method of removing WBC-borne viruses from blood components.[1-4] In addition, because of the increasing demand for blood components, blood donor centers are using apheresis machines to collect blood components such as platelets (PLTs),

ABBREVIATIONS: 7-AAD = 7-aminoactinomycin D; CFU-GEMM = colony-forming unit–granulocyte-erythrocyte-monocyte-macrophage-megakaryocyte; DC(s) = dendritic cell(s); HABS = human AB serum; LFE(s) = leukofilter eluate(s); LRSC(s) = leukoreduction system chambers(s); MDC(s) = mature dendritic cell(s); NK = natural killer; SEB = staphylococcal enterotoxin B.

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RBCs, or plasma. These machines have built in leukoreduction systems or filters.

This change in blood processing methods necessitated the development of alternative sources of cells for research purposes. Thus, investigators have eluted WBC filters and found the cells to be viable, functional, and appropriate for laboratory use.[5-8] The numbers of peripheral blood mononuclear cells (PBMNCs) recovered from filters, however, were smaller than the numbers obtained from standard buffy coats.[5-7] Consequently, we developed a novel, more abundant source of WBCs—the cells retained in the leukoreduction system chambers (LRSCs) after plateletpheresis. On average, we isolated four times as many cells from one LRSC residue than from eluates of WBC filters retaining the cells from 1 unit of blood. The cells isolated from LRSCs were fully viable and functional. Cells from both sources responded to activation with staphylococcal enterotoxin B (SEB), but CD4+ T cells isolated from LRSCs expressed higher levels of CD25 and CD69 upon activation. In addition, yields of dendritic cells (DCs) matured from CD14+ cells isolated from LRSCs were higher. Thus, the cells retained in LRSCs after plateletpheresis provide a novel and abundant source of viable research-grade WBCs obtained in compliance with the current blood bank practices.

MATERIALS AND METHODS

Blood and PLT donors

Volunteers donated blood at the Division of Transfusion Medicine, Mayo Clinic, Rochester, Minnesota, in accord with the current regulations by the AABB and the US Food and Drug Administration. Donors were eligible for plateletpheresis if they exhibited at least 150×10^9 PLTs per L of blood and were free of aspirin for at least 36 hours. Donor's antecubital fossa was cleaned with an iodine tincture and the vein was accessed with a 16-gauge sterile needle.

PBMNCs from whole blood

Whole blood, 500 mL, was collected in less than 15 minutes into a triple-bag system containing citrate phosphate-2-dextrose (CP2D) anticoagulant (LeukoTrap RCPL, Pall Corp., East Hills, NY). During collection blood was agitated on a shaker (CompoGard, Fresenius Hemocare, Redmond, WA). We further processed the cells according to the LeukoTrap system manufacturer's guidelines. Briefly, after initial centrifugal separation of RBCs and PLT-rich plasma, the blood collection set was placed in a plasma extractor. The whole-blood bag port was opened to allow PLT-rich plasma to flow through the white blood cell filter. Filtration was terminated when RBCs contaminated the filter's inlet side. The filter inlet and outlet tubing was sealed and the filter removed from the set. We eluted leukofilter eluates (LFEs) by gently pushing 50 mL of phosphate-buffered saline (PBS), pH 7.4, in the direction opposite to the one employed at blood filtration. Subsequently, we layered two parts of the cell suspension over one part of a Lymphoprep solution (ICN Biomedicals, Aurora, OH) and centrifuged at $425 \times g$ for 30 minutes at room temperature with no brake applied. The PBMNC layer was aspirated and transferred into a 50-mL conical tube, and the cells were collected by centrifugation. The cell pellet was resuspended in PBS and centrifuged at $450 \times g$ for 5 minutes followed by a second wash and centrifugation at $300 \times g$ for 5 minutes. The cells were resuspended in PBS containing 0.5 percent bovine serum albumin (Sigma-Aldrich, St. Louis, MO) and 2.0 mmol per L ethylenediaminetetraacetate (Sigma-Aldrich). We used a hemocytometer to enumerate the cells and assessed viability by trypan blue exclusion.

PBMNCs from residue of plateletpheresis

PLTs were collected with an apheresis apparatus (Trima Accel, Gambro BCT, Lakewood, CO) controlled by software Version 5.1 with the following settings: anticoagulant management, 4; draw management, 3; return management, 1; maximal draw flow, fast; infusion draw ramp, yes; and anticoagulant ratio, 13:1. Draw rate and return rate were set automatically unless problems in venous access or donor comfort made adjustments necessary. Target yields were 3.0×10^{11}, 3.5×10^{11}, 4.0×10^{11}, 6.2×10^{11}, 6.5×10^{11}, and 6.8×10^{11} PLTs in up to 100 minutes of processing time. Coagulation of the blood and the product was prevented with acid citrate dextrose-A. Once collection had been completed, the PLT collection bag was separated from the disposable set by a heat sealer. The disposable set was removed from the apparatus and the leads surrounding the LRSC were heat-sealed. The kit was removed and discarded and the LRSC (Fig. 1A) stored at room temperature. Within 2 hours, the tubing was cut at both ends of the LRSC and the cells were drained into a 50-mL conical tube. We diluted the cells from LRSC with PBS to a final volume of 50 mL. Subsequently, we processed the LRSC with density centrifugation and washing exactly as described above for the LFEs.

Immunomagnetic isolation of cells

To isolate CD14+ cells, we incubated 200 µL of CD14-specific immunomagnetic reagent (all immunomagnetic reagents were from Miltenyi Biotec, Auburn, CA) per 4×10^8 PBMNCs. For isolation of T cells and natural killer (NK) cells, we incubated the PBMNCs with CD3- or CD56-specific immunomagnetic reagent (at one-half of the amount of reagent recommended by the manufacturer). After incubation and washing, the labeled cells were separated on a separator (AutoMACS, Miltenyi Biotec)

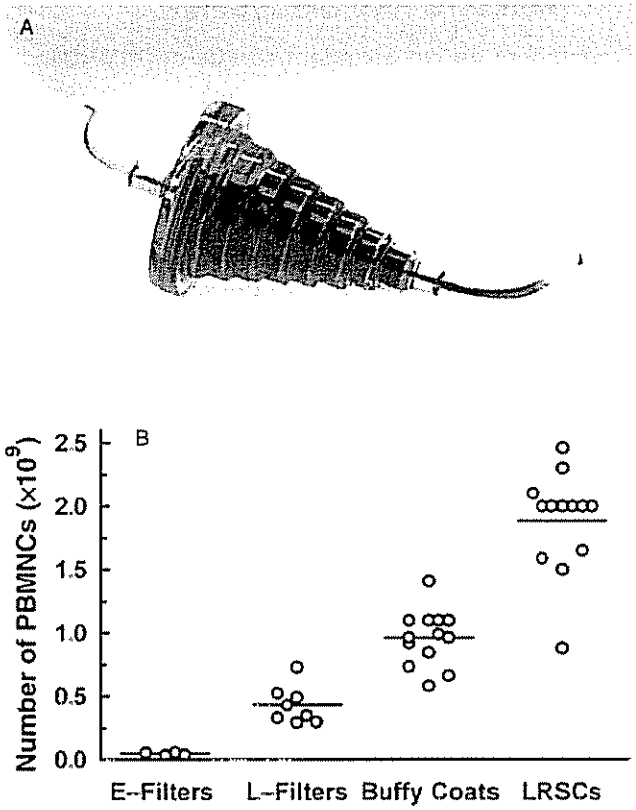

Fig. 1. (A) LRSC separated from the PLT collection kit employed on Gambro Trima Accel apheresis apparatus. (B) The number of PBMNCs isolated by density gradient centrifugation from the eluate of the RBC filters (E-Filters), WBC filters (L-Filters), or buffy coats from 1 unit of blood and from the cellular residue in the LRSCs after normal plateletpheresis.

running the POSSEL program. Purity of isolated cells was accessed by flow cytometry with antibodies listed in Table 1.

Preparation of mature DCs

We matured the cells as described previously.[9,10] Briefly, in six-well plates we seeded 6.0×10^6 immunomagnetically purified CD14+ cells in 3.0 mL of X-VIVO 15 medium (Cambrex, East Rutherford, NJ) containing 1.0 percent pooled human AB serum (HABS; Cambrex), granulocyte-macrophage–colony-stimulating factor (GM-CSF, 800 IU/mL; Berlex, Montville, NJ), interleukin-4 (IL-4, 1000 IU/mL; R&D Systems, Minneapolis, MN) and 1.0 percent penicillin-streptomycin (Gibco, Grand Island, NY). One milliliter of fresh medium (containing the same components, but with GM-CSF increased to 1600 IU/mL) was added per well on Day 3 of incubation. On Day 5, the cells were collected by centrifugation and resuspended at 1.0×10^6 cells/mL in the fresh maturation medium (X-VIVO 15, 1.0% HABS, 800 IU/mL GM-CSF, 1000 IU/mL IL-4, 1100 IU/mL tumor necrosis factor-α [R&D Systems] and 1.0 μg/mL prostaglandin E_2 [Sigma-Aldrich]). Nonadherent mature DCs (MDCs) were collected 2 days later and characterized for viability, yield, and expression of CD80 and CD83.

TABLE 1. Characteristics of immunoreagents used in this study*

Antibody specificity	Fluorescent label	Manufacturer†
CD3	FITC	Biosource
CD3	PE	Biosource
CD3	APC	eBioscience
CD4	FITC	eBioscience
CD8	FITC	eBioscience
CD14	PE	eBioscience
CD16	PE	Becton Dickinson
CD19	PE	eBioscience
CD25	APC	PharMingen
CD45	APC	eBioscience
CD56	FITC	Becton Dickinson
CD69	APC	eBioscience
CD80	FITC	PharMingen
CD83	PE	Immuntech
Live/dead	7-AAD	PharMingen
Annexin V	PE	PharMingen
IgG	PE	Biosource

* All cells were analyzed live except when stained for CD80 and CD83.
† Biosource, Camarillo, CA; eBioscience, San Diego, CA; Becton Dickinson, San Jose, CA; PharMingen, San Diego, CA; Immuntech, Marseille, France.
FITC = fluorescein isothiocyanate; PE = phycoerythrin; APC = allophycocyanin; 7-AAD = 7-aminoactinomycin D.

Cell characterization by flow cytometry

We characterized the cells by flow cytometry with a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA) and the fluorophore-conjugated monoclonal antibodies with specificity indicated in Table 1. By multiple immunostaining we monitored CD3+CD45+ T cells, CD3+CD4+CD45+ T-helper cells, CD3+CD8+CD45+ cytotoxic T cells, CD14+CD45+ monocytes, CD19+CD45+ B cells, and CD56+CD45+ NK cells. Cells were incubated with 7-aminoactinomycin D (7-AAD) to exclude dead cells from analysis. Before analysis of DCs, we fixed the cells in 1.0 percent paraformaldehyde. For each analysis we recorded 100,000 counts. Data were analyzed with computer software (CellQuest, BD Biosciences). Generally, we gated on the PBMNC populations (based on the characteristic patterns of forward and side scatter and the absence of 7-AAD fluorescence) and quantified the cells by binding of specific antibodies.

Activation of lymphocyte subsets

To determine the responsiveness of WBC subsets to activation, we stimulated PBMNCs with SEB and measured

the effects by the expression of activation markers CD25 and CD69.[11,12] We incubated the PBMNCs with SEB (1.0 μg/mL in RPMI 1640 [Sigma-Aldrich] supplemented with 5.0 percent HABS [Sigma-Aldrich] and 1.0 percent penicillin-streptomycin [Gibco]) in a humidified atmosphere of 5 percent carbon dioxide at 37°C for 18 hours, collected the cells by centrifugation, stained them for CD25 or CD69 and for antigens characteristic of particular WBC subsets, and analyzed by flow cytometry.

In vitro function of T cells, NK cells, and DCs

We evaluated the function of T cells and NK cells purified from the two cell sources by measuring the proliferative response to allogeneic MDCs as model antigen-presenting cells. A mixture of MDCs derived from four donors was plated at 1.0×10^4 per well in 96-well plates containing X-VIVO 15 supplemented with 1.0 percent HABS and 1.0 percent penicillin-streptomycin. A total of 100,000 T cells or NK cells were added to wells containing the MDCs in a final volume of 200 μL. The cells were coincubated for 84 hours. Twelve hours before cell collection with a semiautomatic cell harvester (Skatron, Sterling, VA), [^{3}H]thymidine (1.0 μCi in 100 μL) was added to each well. Radioactivity incorporated into DNA was measured by a scintillation counter (LS 6000SC, Beckman-Coulter, Fullerton, CA). To evaluate the capacity of individual MDC preparations derived from monocytes isolated from the two sources, we followed the same procedure except that we used CD3+ cells as responder cells.

Quantifying hematopoietic progenitors

We suspended the PBMNCs in MethoCult GF H4434 medium (StemCell Technologies, Vancouver, BC, Canada) at final densities of 2×10^5 per mL. Duplicate 1-mL samples were plated into 35-mm culture dishes and incubated for 14-17 days under standard tissue culture conditions. With the aid of an inverted microscope we identified and scored erythroid colonies (burst forming units-erythroid [BFU-E]), granulocyte-macrophage colonies (colony-forming unit–granulocyte-macrophate [CFU-GM]), and mixed colonies (CFU–granulocyte-erythrocyte-monocyte-macrophage-megakaryocyte [CFU-GEMM]) according to StemCell Technologies instructions.[13]

Statistical analysis

Flow cytometry data represent percentages of live cells labeled by a particular antibody. We analyzed all data by computer software (Prism, GraphPad, San Diego, CA) and tested the significance of differences between and among groups by the two-tailed t test for unpaired samples or analysis of variance. The probability ($p < 0.05$) that the difference was due to chance was taken as significant.

RESULTS AND DISCUSSION

LRS chambers are an abundant source of PBMNCs

To compare the numbers of PBMNCs eluted from the filters following filtration of 1 unit of blood (approx. 450 mL), we cut off the RBC and WBC filters from normal donor collections and passed 50 mL of PBS in the direction opposite to the one used for blood filtering. The numbers of PBMNCs obtained from RBC filters, WBC filters, and LRSCs are shown in Fig. 1B. The numbers of PBMNCs eluted from RBC filters were expectedly negligible, but the numbers eluted from WBC filters were high ($0.43 \times 10^9 \pm 0.15 \times 10^9$) and similar to the value reported by Meyer and coworkers.[5] (The slight difference between the two studies may result from the use by Meyer et al. of larger volumes of sucrose-replete filter-eluting PBS.)

The number of PBMNCs isolated from LRSCs ($[1.88 \pm 0.40] \times 10^9$, $n = 13$) was four times larger than the number of PBMNCs isolated from LFEs ($0.43 \pm 0.15 \times 10^9$, $n = 8$, $p < 0.0001$; Fig. 1B) and twice as large as the number of PBMNCs obtained from buffy coats ($0.96 \pm 0.22 \times 10^9$, $n = 13$, $p < 0.0001$). Although the three methods are not comparable either in the amount of treated blood or in the manner of WBC isolation, this result does establish that LRSCs are a useful source of substantial numbers of PBMNCs from the hitherto discarded material.

As buffy coats are becoming increasingly unavailable, we chose to compare the PBMNCs isolated from LFEs and LRSCs in more detail. Hence, we quantified the relative amounts of CD4+, CD8+, CD14+, CD19+, and CD56+ cells and found no difference between the amounts of analogous cells isolated from the two sources (Fig. 2). Our results for LFEs are in overall agreement with the results of Meyer and colleagues.[5] Importantly, the cell composition in LRSC isolates appears fully comparable to the cell composition in LFEs.

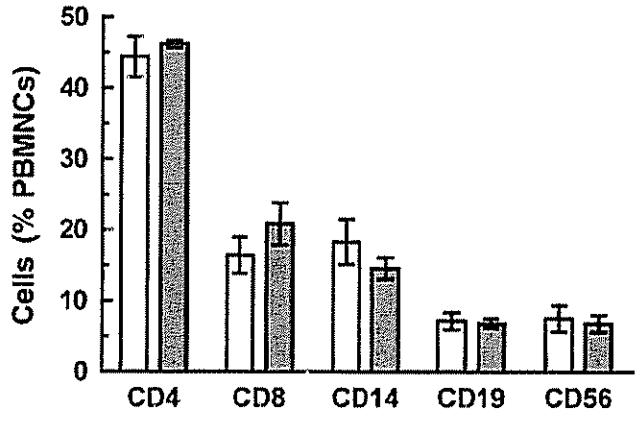

Fig. 2. Percentage of CD4+, CD8+, CD14+, CD19+, and CD56+ cells in PBMNCs isolated from LRSCs (□) and LFEs (▨). n = 4 for all groups. No difference between analogous cells isolated from LRSCs and LFEs was significant ($p > 0.05$).

Hematopoietic stem cells and progenitors in PBMNCs isolated from LRSCs and LFEs retain similar differentiation potential

We used the colony formation assay to determine the presence of hematopoietic stem cells and early progenitors within the PBMNCs. The numbers of BFU-E colonies, CFU-GM colonies, and CFU-GEMM colonies, differentiated from PBMNCs prepared from LFEs and LRSCs, were indistinguishable (Fig. 3). In addition, these values were similar to those reported for PBMNCs isolated from normal buffy coats employing the same culture conditions (cf., Table 7 in Reference 13). Thus, LRSCs provide viable hematopoietic stem cells and progenitors in the numbers typically found in PBMNCs.

SEB activates PBMNCs isolated from LRSCs and LFEs

To assess the functional status of major cell populations in the PBMNCs isolated from the two WBC sources, we incubated the PBMNCs with SEB and measured the levels of activation markers CD25 and CD69 in the viable CD3+, CD4+, CD8+, CD14+, CD19+, and CD56+ cells. We found that SEB strongly affected the levels of CD25 and CD69 in all cells, but that the effect was higher in the cells isolated from LRSCs (Fig. 4). The difference in the cell source (LRSCs vs. LFEs) accounted for 13 percent of total variance in CD25 ($p < 0.0001$) and 3.7 percent for CD69 ($p = 0.001$; while, expectedly, the difference in response among different cell types accounts for the rest). We found no such differences between control cells from the two sources. Thus, cell subpopulations isolated from LRSCs were fully functional as ascertained by their susceptibility to activation by SEB.

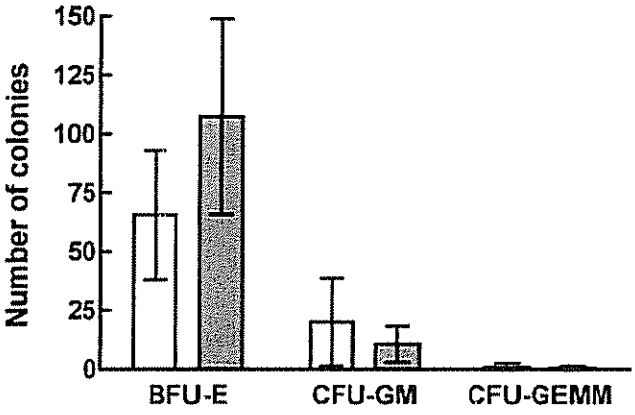

Fig. 3. Hematopoietic stem cells and early progenitors in PBMNCs isolated from LRSCs (□) and LFEs (▨). The numbers of BFU-E, CFU-GM, and CFU-GEMM were indistinguishable ($p > 0.05$).

In a more detailed analysis, we observed that CD4+ T cells isolated from LRSCs responded to SEB by expressing more CD25 (Fig. 4B) and CD69 (Fig. 4D) than the T cells isolated from LFEs ($p < 0.05$; $n = 4$ for all groups). Importantly, this finding parallels the observation by others that CD4+ T cells eluted from filters responded to SEB to a lesser extent than the cells isolated from buffy coats.[5] Apparently, filtration and elution affected the potential of CD4+ cells to respond to SEB, while the cells isolated from LRSCs retained their activation potential at levels comparable to the cells from buffy coats. Although we did not find that the isolation method affected the activation of NK cells, we did observe that more control (i.e., SEB-free) LRSC-derived NK cells expressed CD25 and CD69 in comparison to the LFE-borne NK cells ($p < 0.01$, $n = 4$).

PBMNCs from LRSCs and LFEs yield highly pure cell subpopulations upon isolation by immunomagnetic adsorption

We used immunomagnetic adsorption to isolate CD3+ cells, CD14+ cells, and CD56+ cells from PBMNCs and determined cell yield, purity, and viability. Data in Table 2 show no difference in efficiency of cell isolation from the LFE- and LRSC-derived PBMNCs. In addition, we measured the ability of CD3+ T cells and CD56+ NK cells to synthesize DNA in response to allogeneic MDCs and found that there was no difference between the cells from the two sources either (Tables 3 and 4). Thus, all isolated cell populations were highly pure and viable indicating that the cells isolated from LRSCs and LFEs are similarly

TABLE 2. Purity and yield of cells isolated by immunomagnetic selection

Specificity of immunomagnetic reagent	Source of PBMNCs	Presence in PBMNCs (%)	Purity of isolated cells (%)	Yield of isolated cells (% of respective cells in PBMNCs)
CD3	LRSCs	55.4 ± 1.7	99.4 ± 0.2	70.2 ± 16.0
	LFEs	57.7 ± 12.5	99.4 ± 0.3	65.0 ± 23.7
CD14	LRSCs	17.4 ± 5.6	Not done	91.0 ± 17.2
	LFEs	14.3 ± 2.0	Not done	98.9 ± 2.4
CD56	LRSCs	8.0 ± 4.0	90.5 ± 4.9*	96.0 ± 8.1
	LFEs	4.9 ± 1.4	92.4 ± 3.1	89.1 ± 12.7

* $n = 4$, except for the group designated this group where $n = 3$.

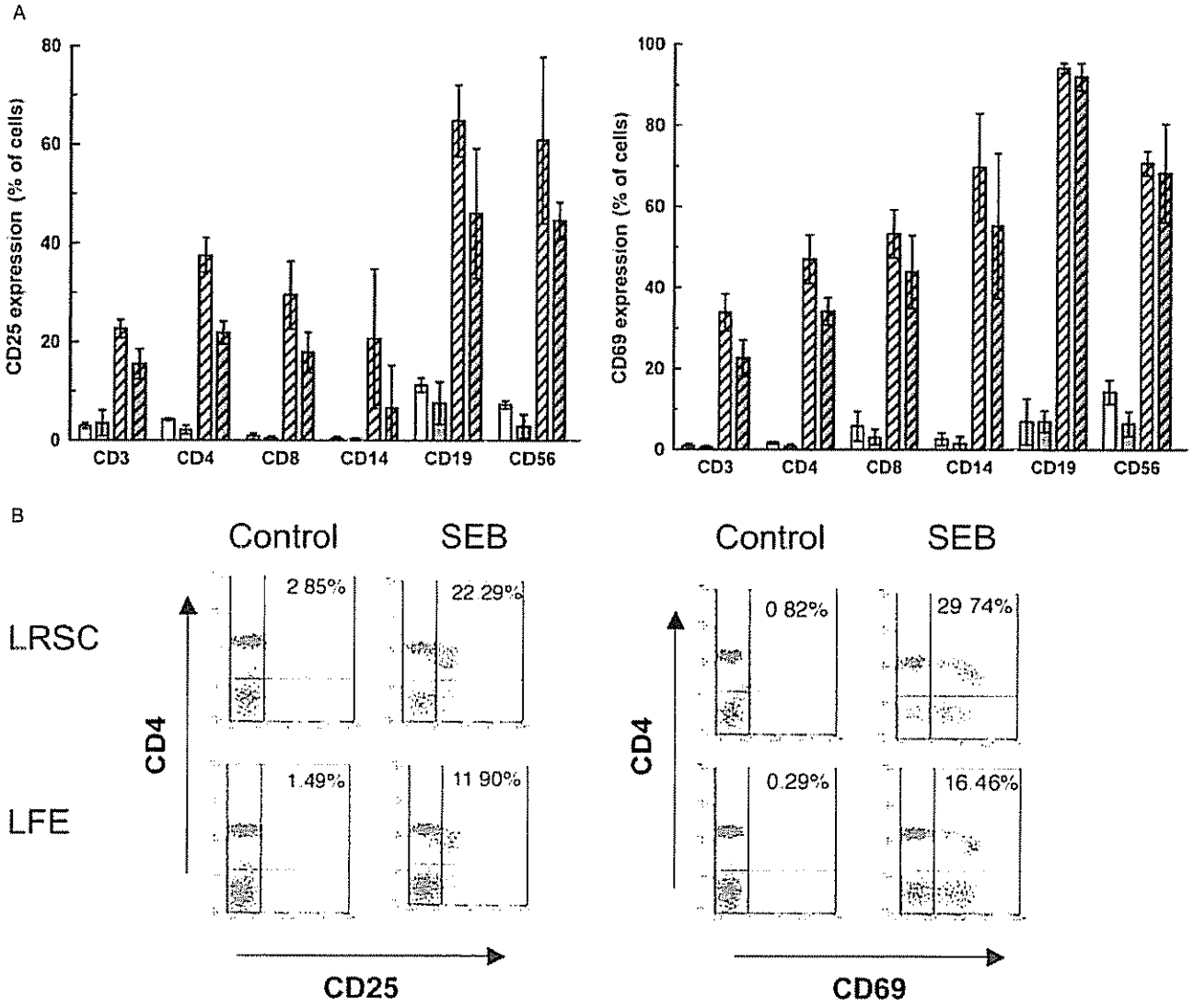

Fig. 4. Response of PBMNCs isolated from LRSCs and LFEs to SEB. (A) Expression of CD25 (left) and CD69 (right) by CD3+, CD4+, CD8+, CD14+, CD19+, and CD56+ cells isolated from LRSCs (□) and LFEs (▨). Hatching indicates the presence of SEB in the medium. See text for statistical details; n = 4 for all groups. (B) Representative flow cytometric dot plots measured in PBMNCs isolated from LRSCs (top row) or LFEs (bottom row). Control cells were incubated without SEB (control) or with SEB and stained with CD4 and CD25 (left panels) or CD4 and CD69 (right panels). The numbers show the percentage of cells in the top right quadrant, that is, the cells stained with both respective antibodies.

TABLE 3. DNA synthesis by CD3+ T cells and CD56+ NK cells isolated from LRSCs and LFEs in response to stimulation by allogeneic MDCs

Stimulator cells	Responder cells	Responder cells isolated from	[^{3}H]Thymidine incorporated (1000 × cpm)
MDC*	CD3+ T cells	LRSCs	283.6 ± 83.1
		LFEs	286.1 ± 53.2
MDC*	CD56+ NK cells	LRSCs	6.5 ± 3.8
		LFEs	14.9 ± 15.5

* A mixture of equal numbers of mature DCs from eight individuals.

TABLE 4. Efficiency of MDCs derived from CD14+ cells isolated from LRSCs and LFEs in stimulating DNA synthesis by allogeneic T cells

Stimulator cells	Responder cells	Stimulator cells matured from CD14+ cells isolated from	[^{3}H]Thymidine incorporated (1000 × cpm)
MDCs	CD3+ T cells*	LRSCs	266.6 ± 100.0
		LFEs	238.0 ± 65.5

* A mixture of equal numbers of cells from eight individuals.

amenable to immunomagnetic separation into highly pure and highly viable subpopulations

CD14+ cells isolated from LRSCs are a superior source of mature DCs

We evaluated CD14+ cells isolated from LRSCs and LFEs for their ability to differentiate into functional MDCs in vitro. We matured the cells and measured their yield from CD14+ cells and their ability to stimulate the proliferation of allogeneic T cells. After 7 days in culture, 29.7 ± 14.6 percent of LRSC-derived CD14+ cells matured into DCs ($n = 7$); in contrast, CD14+ cells isolated from LFEs yielded only 10.0 ± 9.1 percent DCs ($n = 4$, $p = 0.038$). This observation is at variance with the data by Ebner and coworkers[6] who found no difference in DC yields from PBMNCs isolated from buffy coats and LFEs. The reason for the discrepancy between the data by Ebner and coworkers and ours may reside in the differences in the composition of the elution buffer, purity of DC precursors, and method of DC culture. Nonetheless, our DCs, differentiated from LRSC- and LFE-derived cells, were equipotent in stimulation of allogeneic T cells (Tables 3 and 4).

In conclusion, we have demonstrated that PBMNCs isolated from the cellular residue contained in the LRSC after plateletpheresis are a plentiful source of viable and functional WBCs. This novel source compares favorably with the cells eluted from the filters introduced recently for WBC removal from blood. The advantages of cell isolation from LRSCs are simplicity (because it, unlike isolation from WBC filters, requires no elution) and bounty in comparison to the cells isolated from single units of blood.

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Recovery of white blood cells and platelets from leukoreduction system chambers of Trima Accel and COBE Spectra plateletpheresis devices

Recently, Dietz and coworkers[1] described a new source of viable peripheral blood mononuclear cells (PBMNCs) that they recovered from leukoreduction system chambers (LRSCs) of Trima Accel apheresis devices (Gambro BCT, Lakewood, CO) after routine donor plateletpheresis procedures. These white blood cells (WBCs), which are usually discarded, functioned excellently in conventional in vitro assays and therefore represent a potential and novel source for research-grade cellular products.

In the following report, we describe our results comparing the yields of WBCs recovered from LRSCs in Trima Accel (software version 5.1, Gambro BCT) and COBE Spectra (software version 7.0, Gambro BCT) plateletpheresis devices. After a storage period of 2 hours at room temperature, we drained the contents of the LRSCs into 20-mL conical tubes. We counted WBCs, platelets (PLTs), and red blood cells with a blood cell counter (ADVIA 120, Bayer HealthCare Diagnostics Division, Tarrytown, NY) and quantified CD14+ monocytes by flow cytometry (FACSCalibur, BD, San Jose, CA), as previously described.[2] WBC and PLT yields were significantly different for the plateletpheresis devices (Table 1). Lymphocyte yields were greater and more consistent than monocyte yields. The minimum and maximum monocyte yields differed by 10-fold. WBC yields from Trima Accel LRSCs were 30 times greater than those from COBE Spectra LRSCs. Dietz and coworkers recovered 1.88×10^9 PBMNCs from Trima Accel LRSCs. We recovered a mean of 66.5 percent fewer PBMNCs (0.69×10^9 PBMNCs) from Trima Accel LRSCs.

These different results may reflect actual differences in recoveries between the two plateletpheresis devices or, possibly, different instrument settings during plateletpheresis collections. For our study, we used an anticoagulant ratio of 11:1, whereas the ratio was 13:1 in the study by Dietz and coworkers.[1] Also, the draw management (6 vs. 3), return management (4 vs. 1), and maximal draw flow (medium vs. fast) were different in our study compared with that by Dietz and colleagues. Thus, direct comparison of the cell yields of both studies is limited. The wide range of CD14+ monocytes recovered in LRSCs could impair the consistency of the results of monocyte-derived dendritic cells (DCs). Typically, monocyte yields from the Trima Accel LRSCs consisted of only approximately 10 percent of the cell yield of a leukapheresis unit.[2] With current culture techniques, the

TABLE 1. Comparison of Trima Accel and COBE Spectra LRSC cell yields

Measure*	Median†	Range	Mean ± SD
Donation time (min)	(1) 54	38-91	57 ± 15
	(2) 68	43-90	67 ± 18‡
Separation volume (L)	(1) 3.20	1.95-4.19	3.21 ± 0.68
	(2) 4.56	3.10-6.44	4.61 ± 1.25§¶
Product volume (mL)	(1) 8.50	5.8-9.5	8.0 ± 1.1
	(2) 9.35	8.8-10.0	9.3 ± 0.4‡§
Preapheresis WBC count ($\times 10^9$/L)	(1) 5.6	3.4-7.5	5.52 ± 1.01
	(2) 5.9	4.5-7.3	6.01 ± 0.89¶
Postapheresis WBC count ($\times 10^9$/L)	(1) 6.4	3.7-10.4	6.40 ± 1.44
	(2) 6.6	4.6-8.3	6.60 ± 1.17¶
PLT yield ($\times 10^{11}$)‖	(1) 5.65	2.29-6.67	4.82 ± 1.47
	(2) 5.15	2.37-7.58	5.18 ± 2.06‡
WBC concentration ($\times 10^9$/L)	(1) 112.1	65.2-205.2	122.6 ± 38.8
	(2) 3.09	0.85-8.86	3.37 ± 2.31‡§
WBC yield ($\times 10^9$)	(1) 0.93	0.54-1.76	0.98 ± 0.33
	(2) 0.03	0.008-0.084	0.032 ± 0.022§¶
CD14+ product (%)	(1) 17.6	1.25-25.3	16.7 ± 5.6
	(2) 10.3	2.15-25.1	11.5 ± 6.2**¶
CD14+ yield ($\times 10^6$)	(1) 1.60	0.22-3.16	1.60 ± 0.74
	(2) 0.03	0.008-0.12	0.037 ± 0.036§‡
Lymphocytes (%)	(1) 56.3	40.7-64.2	54.8 ± 6.79
	(2) 53.3	30.3-73.6	54.1 ± 14.2¶
Lymphocyte yield ($\times 10^6$)	(1) 5.33	2.50-8.38	5.33 ± 1.72
	(2) 0.14	0.023-0.62	0.186 ± 0.168‡§
PBMNCs (%)	(1) 72.9	42.0-83.6	71.5 ± 8.5
	(2) 68.9	35.5-82.9	65.5 ± 15.7‡
PBMNC yield ($\times 10^6$)	(1) 7.14	3.47-11.0	6.93 ± 2.16
	(2) 0.16	0.03-0.70	0.22 ± 0.19§‡
PMCs†† (%)	(1) 9.0	1.7-14.5	8.85 ± 3.11
	(2) 18.6	4.2-47.7	19.87 ± 13.79¶**
PMC†† yield ($\times 10^9$)	(1) 0.80	0.28-1.53	0.83 ± 0.34
	(2) 0.03	0.172-0.168	0.052 ± 0.046‡§
PLT concentration ($\times 10^{12}$/L)	(1) 2.33	1.40-4.90	2.51 ± 0.97
	(2) 0.53	0.32-0.63	0.50 ± 0.10‡§
PLT yield ($\times 10^{10}$)	(1) 1.74	0.86-4.21	2.04 ± 0.92
	(2) 0.48	0.28-0.58	0.47 ± 0.09‡§
Hematocrit (%)	(1) 58.0	27-64.8	52.1 ± 17.7
	(2) 6.60	4.5-26.5	8.7 ± 6.4§‡
RBC ($\times 10^{12}$/L)	(1) 5.99	0.30-7.31	5.53 ± 1.90
	(2) 0.68	0.39-2.87	0.90 ± 0.71§‡

* Mean ± 1 SD
† (1) Trima Accel (n = 27); (2) COBE Spectra (n = 10)
‡ U test.
§ $p < 0.01$.
¶ t Test
‖ Product.
** $p < 0.05$.
††PMCs = polymorphic cells (granulocytes)

production of a therapeutic DC vaccination series requires more than 10^9 monocytes. WBC elutriation to enrich monocytes also requires a minimum of 10^9 monocytes as starting population for DC cell culture.[3] Currently, WBC yields from Trima Accel LRSCs are insufficient for the production of a whole therapeutic DC vaccine series. With improved ex vivo monocyte expansion protocols, the required minimum cell yield for DC vaccines could decrease. Given the mean loss of 10^9 WBCs per plateletpheresis collection by the Trima Accel, a 24-apheresis-procedures-per-year donor could lose more than 2×10^{10} WBCs. In comparison, the same number of plateletpheresis procedures with the COBE Spectra LRSCs would remove only 3.2×10^7 WBCs per donation or a total of 7.7×10^8 WBCs for 24 collections per year. Strauss[4] reported high losses of lymphocytes per PLT donation with previous plateletpheresis methods. This observation of immediate and long-term decreases in donors' lymphocyte counts lead to limits on the frequency of plateletpheresis. The number of WBCs retained by Trima Accel LRSCs after plateletpheresis is considerably less than the calculated WBC loss of these earlier studies. Only 10.8 percent of the PLT donors of our study (4/37) had decreased WBC counts immediately after plateletpheresis. For 89 percent of donors there was an increase in the postdonation WBC count (Table 1). The difference between predonation and postdonation WBC counts was slightly higher ($p = 0.32$) for Trima Accel procedures. A possible reason for that observation could be WBC recruitment, which may be greater during increased WBC loss.[2] Rock and coworkers found only minimal changes of donor WBC counts after repeated plateletpheresis.[5] Reasons for the variable number of WBCs retained in the Trima Accel LRSCs should be subject to further investigation to get a better standardization of this new WBC product.

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Reply

We recently described apheresis LRS chambers as an efficient source of research-grade PBMNCs.[1] Strasser and colleagues[2] could not replicate our data and challenged their reproducibility. They are correct that the type and settings of the apheresis machine can influence the number of recovered cells. The machine-borne differences are expected as the COBE Spectra uses a two-stage channel for blood separation whereas the Trima Accel uses a single-stage channel. We did not use the COBE Spectra and cannot comment on the findings of Strasser and colleagues.

The reported differences between the results of Strasser and colleagues and ours with the Trima Accel are most likely associated with differences in the volume of processed blood. Strasser and colleagues processed a mean of 3.21 L during 54-minute platelet (PLT) collections.[2] In contrast, we routinely process a mean of 4.25 L in 80 minutes in plateletpheresis collections (not including triple-PLT-product collections, which were not performed with LRS chambers in our report). Thus, the 66.5 percent difference in recovered PBMNCs between Strasser and colleagues and us can largely be attributed to the fact that Strasser and colleagues processed only 77.5 percent of our typical volume. It is noteworthy that we process the volume of 4.2 L because the Trima Accel LRS chamber is considered full at 4.7 L of blood processed for a donor with a body mass index (BMI) of less than 30 and 4.1 L for a donor with a BMI of more than 30; the machine automatically clears the LRS chamber at these volumes. Thus, the settings we used maximized the number of cells in the LRS chamber.

Other discrepancies between the results of Strasser and colleagues and us may be attributed to different collection procedures including different draw management, return management, or maximal draw flow settings. Additional factors influencing LRS chamber cell content—not mentioned by Strasser and colleagues—include the BMI of donors and concurrent plasma collection; we did not