Leukoreduction System (LRS) Chambers Provide More Human Lymphocytes And Monocytes than Buffy Coats

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Executive Summary

Peripheral blood mononuclear cells (PBMCs) are integral in immunology and cell therapy research. These cells are often sourced from buffy coats derived during whole blood processing, however, leukoreduction system (LRS) chambers—typically discarded components of platelet apheresis kits—represent an underutilized yet superior alternative.

This study evaluated and compared the cellular composition, PBMC subtype distribution, and viability of cells from LRS chambers versus buffy coats. LRS chambers were found to contain slightly fewer total white blood cells (WBCs), but significantly more PBMCs. The PBMCs included higher counts of lymphocyte subsets (T cells, B cells, NK cells) due to their minimal granulocyte content.

Moreover, PBMCs from LRS chambers maintained high viability (>90%) for up to four days post-collection. LRS Chamber PBMC viability outperformed buffy coats, which showed declining viability likely due to granulocyte-induced cell death.

Another advantage of LRS chambers includes fewer processing steps. This advantage increases accessibility while reducing technical variability.

These findings support the use of LRS chambers as a more efficient, reliable, and cost-effective source of high-quality PBMCs for laboratory research.

Introduction

A buffy coat blood component is a layer of white blood cells (WBCs) and platelets that forms between the plasma and red blood cell (RBC) fractions when a unit of whole blood is centrifuged. Buffy coats can be extracted during the whole blood manufacturing process at community blood centers[1], provided this is planned in advance. Buffy coats serve as a valuable source of WBCs for laboratory research, particularly for isolating peripheral blood mononuclear cells (PBMCs), which include lymphocytes and monocytes.

Buffy coats have traditionally been the only choice for researchers who need PBMCs for their work in immunology or cell therapy. However, not all blood centers manufacture buffy coats or make them available for researchers to purchase.

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Isolating PBMCs from buffy coats is time-consuming and requires specialized training. Additionally, the use of density gradient media, such as Ficoll-Paque, to isolate PBMCs from granulocytes and RBCs can alter flow cytometry-based phenotyping[2] and enhance cell activation[3], potentially affecting research outcomes[2-4].

Leukoreduction system (LRS) chambers are small cone-like chambers attached to tubing sets used in platelet apheresis for the Trima Accel from Terumo BCT. They facilitate the separation of platelets and WBCs through counterflow centrifugal elutriation (CCE)[5], pushing WBCs and platelets in opposite directions. While platelets are collected in a bag, WBCs are returned to the donor. However, the platelet collection apheresis system intentionally traps PBMCs inside the chamber to ensure low PBMC numbers in the platelet collection bag. For this reason, LRS chambers are an excellent source of PBMCs when salvaged[5-8].

The studies referenced in this paper highlight the composition and viability of cells isolated from LRS chambers and compares them with cells isolated from buffy coats collected at Our Blood Institute (OBI), a community blood center.

In this study, we demonstrate that whole blood-derived buffy coats contain slightly more WBCs overall than LRS chambers. However, LRS chambers are almost devoid of granulocytes, resulting in higher PBMC counts compared to buffy coats. Consequently, LRS chambers contain more lymphocyte subtypes, including T cells, B cells, and NK cells. Additionally, cells inside LRS chambers remain viable for longer periods than those inside buffy coats.

LRS chambers are typically easier to source and more readily available without the lead time required for the planned manufacturing steps buffy coats require. PBMCs eluted from LRS chambers can be isolated easily from the RBC fraction using a gentle isotonic RBC lysis buffer. Previous studies demonstrated strong functionality of PBMCs isolated from LRS chambers[5,8].

We recommend the use of LRS chambers over whole blood-derived buffy coats as a source of PBMCs for research and cell therapy development.

Results and Discussion

Two studies were conducted at Our Blood Institute to evaluate the cellular composition and viability of LRS chambers. The first study examined 62 LRS chambers over a six-month period, salvaged from platelet apheresis collection kits of healthy donors. Some chambers were analyzed on the day of collection (Day 0), while others were stored at 2-8°C for 1-4 days before analysis. The second study, conducted over one week, compared buffy coats from eight whole blood units against LRS chambers from ten platelet collections. Samples were collected on the same day, at the same center, and analyzed simultaneously to directly compare their contents and viability.

WBCs in LRS Chambers and Buffy Coats

In the first study, 62 LRS chamber eluates were analyzed for cellular content using an automated hematology analyzer to obtain complete blood count (CBC) reports with differential. The total number of WBCs in each blood sample was calculated by multiplying the WBC concentration (cells/mL) by the volume (mL) of each LRS chamber eluate. On average, $1.56x10^9$ WBCs were measured per LRS chamber, with a range of $4.35x10^8$-$2.76x10^9$ (Figure 1A). Of these, 95.5% ($1.49x10^9$) were peripheral blood mononuclear cells (PBMCs), and 4.5% were granulocytes such as neutrophils, basophils, and eosinophils (Figure 1A).

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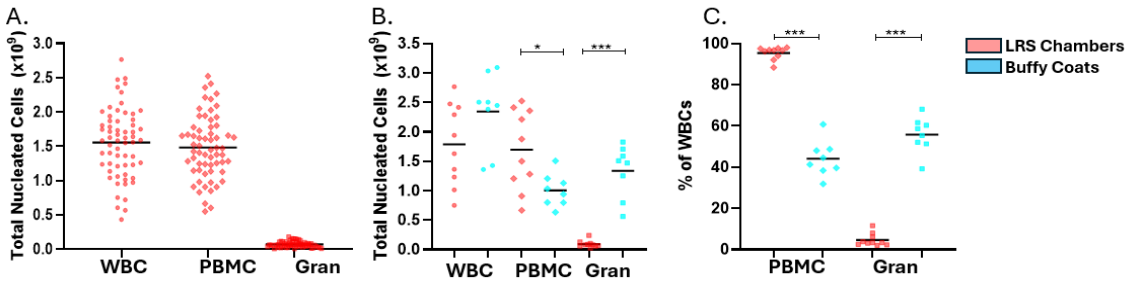

Figure 1: **White Blood Cell Count and Subtypes in LRS Chambers and Buffy Coats.** Number of white blood cells (WBCs), peripheral blood mononuclear cells (PBMCs) and granulocytes (Gran) in (**A**) 62 LRS chambers and (**B**) comparing 10 LRS Chambers and 8 buffy coats. (**C**) Percentage of PBMCs and Gran of the total WBC cells in the 10 LRS chambers and 8 buffy coats. An automated hematology analyzer was used to measure the total number and percentage of each cell population. * = $p<0.05$; *** = $p<0.0001$

The second study comparing buffy coats to LRS chambers indicated that buffy coats had slightly more WBCs on average ($2.34x10^9$ vs. $1.79x10^9$), although this difference was not statistically significant (p=0.097, Figure 1B). Despite this, the PBMC count was higher in LRS chambers compared to buffy coats ($1.69x10^9$ vs. $1.01x10^9$) which was statistically significant (p=0.018, Figure 1B).

The disparity between WBC and PBMC counts in the two samples can be attributed to their granulocyte content. Buffy coats contain 44.2% PBMCs and 56.8% granulocytes on average, while LRS chambers have >95% PBMCs ($p<0.0001$, Figure 1C). The CCE process used during platelet collection separates WBCs by size and sedimentation characteristics[5,9] inside the LRS chamber, which pushes granulocytes out of the opposite end of the chamber from the platelets. This results in low granulocyte count in LRS chambers at the end of the platelet collection procedure (Figure 1).

Buffy coat manufacturing involves simple centrifugation to separate plasma and RBC layers of a whole blood unit. The buffy coat at the interface between the layers includes granulocytes and some platelets. Harvesting the buffy coat layer results in the transfer of 40-60 mL of plasma and some RBCs. RBC contamination in an LRS chamber is approximately 8 mL of packed RBCs, whereas a manufactured buffy coat has 4-6 mL, resulting in 25-50% fewer RBCs than the average LRS chamber (data not shown).

PBMC Subtypes in LRS Chambers and Buffy Coats

PBMCs, comprising of lymphocytes and monocytes, are used most commonly in immunology and cell therapy research. The first 62 LRS chambers analyzed had an average of $1.05x10^9$ lymphocytes and $4.36x10^8$ monocytes per chamber (Figure 2A).

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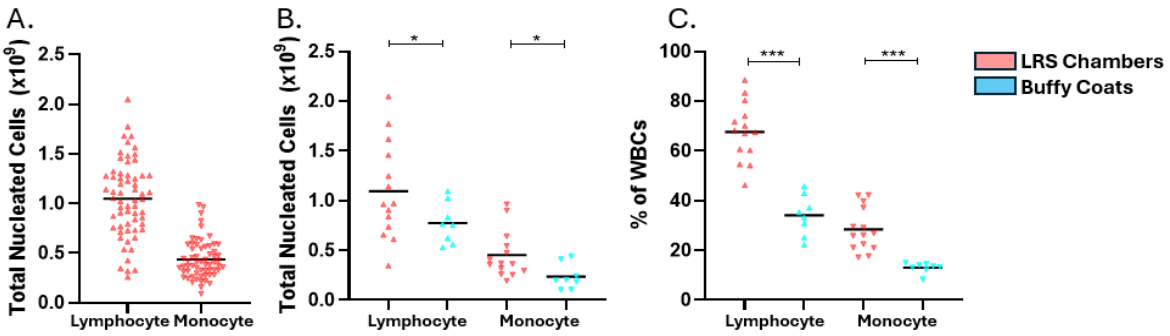

Figure 2: Peripheral Blood Mononuclear Cell Subtypes in LRS Chambers and Buffy Coats. Number of lymphocytes and monocytes in (**A**) 62 LRS chambers and (**B**) comparing 10 LRS Chambers and 8 buffy coats. (**C**) Percentage of lymphocytes and monocytes the total WBC cells in the 10 LRS chambers and 8 buffy coats. An automated hematology analyzer was used to measure the total number and percentage of each cell population. * = $p<0.05$; *** = $p<0.0001$

The smaller group of LRS chambers analyzed with the buffy coats showed that LRS chambers contain more lymphocytes ($1.11x10^9$ vs. $7.73x10^8$, p=0.043) and more monocytes ($4.53x10^8$ vs. $2.36x10^8$, p=0.011) (Figure 2B). LRS chambers contain almost double the percentage of each PBMC population compared to buffy coats, with 67.6% lymphocytes and 28.5% monocytes in LRS chambers compared to 34.1% lymphocytes and 13.0% monocytes in the buffy coats (Figure 2C).

Using density gradient centrifugation and media like Ficoll-Paque to remove RBCs and granulocytes from buffy coats requires time and skill. Poor technique affects cell yield, as do excess vibrations and small temperature changes. However, since LRS chambers are already devoid of most granulocytes, RBCs can be removed by incubating the LRS chamber eluate with a gentle, isotonic RBC lysis buffer, saving time and requiring less expertise. Here is a sample RBC lysis procedure for isolating PBMCs from LRS chamber eluates https://bcaadvancedtherapies.com/wp-content/uploads/2025/07/RBC-Lysis-Protcol-LRS-Chamber-PBMC-Isolation-BCA.pdf.

A recent study by Kronenberg, et al.,[13] examined LRS chambers from platelet apheresis procedures performed with Trima software version 7, utilizing either plasma only or platelet additive solution (PAS). LRS chambers from procedures using plasma demonstrated similar results to those described in our study. However, LRS chambers from procedures using PAS showed significantly reduced numbers of total WBCs and PBMCs (both lymphocytes and monocytes) but showed increased numbers of RBCs and neutrophil populations compared to the chambers from platelet collections using plasma only. Another study published in 2024 by Kelly et al.,[14] also analyzed the effects of PAS on LRS chamber cellular content, but in their study, no differences in cellular content were seen between LRS chambers collected with plasma or PAS.

The LRS chambers used the study detailed herein, were obtained from platelet collection procedures using Trima Software version 7, which was started in 2023. The collection procedure used only plasma, not PAS. Due to the differing results available on the cellular content in LRS chambers from platelet collection procedures using PAS, we do not recommend those for immunology or cell therapy research.

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Lymphocyte Subtypes in LRS Chambers and Buffy Coats

Three types of lymphocytes, T cells, B cells, and NK cells, are most commonly used in immunology and cell therapy research. Flow cytometry was randomly performed on harvested LRS chambers resulting in flow analysis on 25 of the first 62 LRS chambers. The analysis showed averages of $7.64x10^8$ $CD3^+$ T cells, $1.63x10^8$ $CD19^+$ B cells, and $1.64x10^8$ $CD3^-CD56^+$ NK cells (Figure 3A). Comparing lymphocyte subtypes in LRS chambers and buffy coats revealed statistically more $CD3^+$ T cells ($6.77x10^8$ vs. $3.06x10^8$, p=0.0216), $CD19^+$ B cells ($1.35x10^8$ vs. $3.28x10^7$, p=0.0032), and $CD3^-CD56^+$ NK cells ($1.21x10^8$ vs. $4.09x10^7$, p=0.0052) in LRS chambers (Figure 3B). Percentages of each population were higher in LRS chambers than buffy coats (Figure 3C).

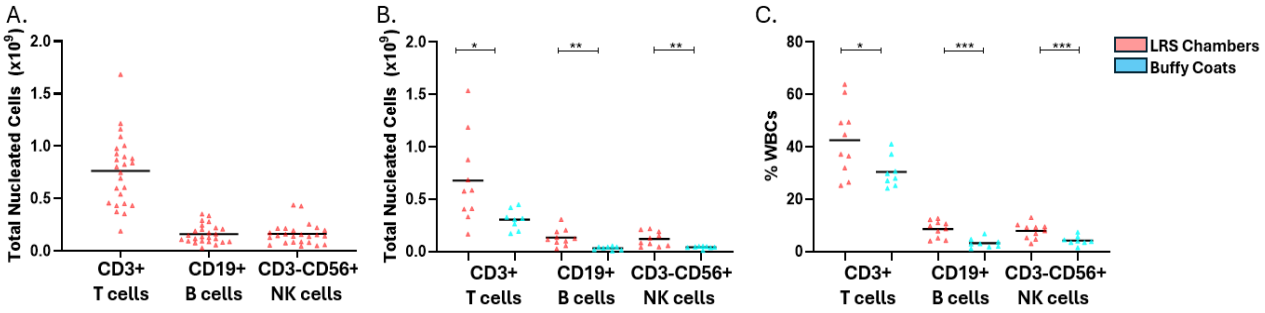

Figure 3: **Lymphocyte Subtypes in LRS Chambers and Buffy Coats.** Number of $CD3^+$ T cells, $CD19^+$ B cells, and $CD3^-CD56^+$ NK cells in (**A**) 25 LRS chambers and (**B**) comparing 10 LRS Chambers and 8 buffy coats. (**C**) Percentage of $CD3^+$ T cells, $CD19^+$ B cells, and $CD3^-CD56^+$ NK cells of the total WBC cells in the 10 LRS chambers and 8 buffy coats. A flow cytometer was used to determine the percentage of each cell population and the total number of each population was calculated by multiplying the percentage by the total nucleated cell number from the automated hematology analyzer. * = p<0.05; **= p<0.001 *** = p<0.0001

It should be noted that variability in cell counts and types between buffy coat products and LRS chambers can be largely attributed to donor variability. Additionally, during platelet collection, the Trima Accel instrument randomly rinses out the LRS chamber 1-3 times per procedure[10], affecting final cell counts somewhat unpredictably. Consequently, some LRS chambers may contain fewer cells than buffy coats (Figures 1-3). However, once granulocytes are removed from buffy coats, the remaining PBMC fraction contains fewer lymphocytes and monocytes than the average LRS chamber (Figure 2), resulting in fewer lymphocytes (T cells, B cells, NK cells) in buffy coats (Figure 3).

Viability of PBMCs in LRS Chambers and Buffy Coats

Cell viability is a crucial factor to consider when choosing between LRS chambers and buffy coats as a PBMC source. LRS chambers were tested for PBMC viability using flow cytometry and 7-aminoactinomycin D (7AAD) dye exclusion on the day of collection (Day 0) or after storage at 2-8°C for 1-4 days. Average viability decreased each day but remained over 90% at 4 days post-collection (Figure 4A).

LRS chambers and buffy coats analyzed together were eluted into 50 mL conical tubes the day after collection (Day 1) and

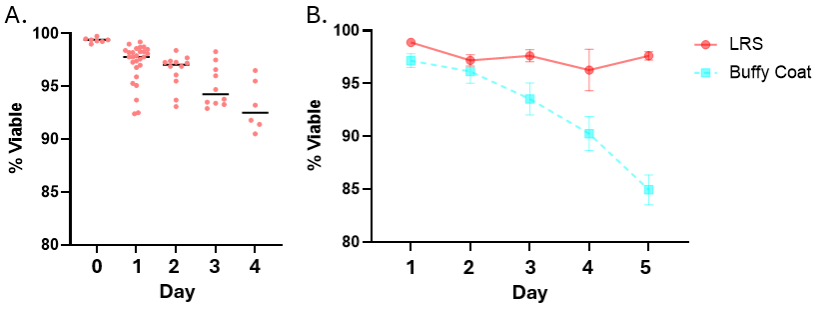

Figure 4: **PBMC Viability in LRS Chambers and Buffy Coats.** (**A**) Viability of the PBMC population in 62 LRS chambers tested by flow cytometry on the day of collection (Day 0) or stored at 2°-8°C for number of days indicated (Day 1-4). (**B**) Viability time course of 10 LRS chambers and 8 buffy coats. Eluates were created from LRS chambers (red line) and buffy coats (blue line) the day after collection (Day 1) and tested for total viable PBMCs by flow cytometry. The eluates were stored at were 2°-8°C and tested again for viable PBMCs each day for 5 days. Error bars represent standard error of the mean.

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stored for up to 5 days at 2-8°C. PBMC viability was measured daily using flow cytometry and 7AAD dye exclusion. PBMC viability in LRS chambers remained steady, decreasing slightly from Day 1 (99.0%) to Day 5 (97.6%) (Figure 4B, red line). In contrast, PBMC viability in buffy coats decreased steadily from Day 1 (97.1%) to Day 5 (84.9%) (Figure 4B, blue line).

These results indicate that PBMC viability in LRS chambers remains high (>90%) for at least 4 days post-collection, while PBMC viability in buffy coats decreases over the same period. Granulocytes, which die easily and affect surrounding PBMC viability[11], likely contributed to accelerated cell death in buffy coats due to their high granulocyte content (average 57%), while the overall lack of granulocytes in LRS chambers helped sustain viability over time (Figure 4).

Conclusions

The comparative analysis of LRS chambers and buffy coats revealed significant differences in cellular composition and viability. LRS chambers, salvaged from platelet apheresis kits, demonstrated a higher percentage of peripheral blood mononuclear cells (PBMCs) and lower granulocyte content compared to buffy coats. This resulted in a greater PBMC count and higher viability over time in LRS chambers. Buffy coats, while containing slightly more white blood cells overall, had a higher granulocyte content that negatively impacted cell viability. The findings suggest that LRS chambers are a superior source of PBMCs for research and cell therapy development due to their ease of provision, higher PBMC concentration, and sustained cell viability. Consequently, LRS chambers offer a more efficient and reliable alternative to buffy coats for obtaining PBMCs used in immunology and cell therapy research.

Materials and Methods

Donor Qualification and Consenting

Volunteer donors at Our Blood Institute were qualified for blood or platelet donation using FDA required screening criteria[12], which included a general health assessment and behavioral or travel questions to assess the risk of transmitting relevant infectious agents. All donors were consented to the blood or platelet donation process, which included a description of the risks and permission for their donation to be used for transfusion, research, or further manufacturing. It is important to note that all LRS chambers and buffy coats were de-identified of all donor information for their use within this study.

Manufacturing Buffy Coat from Whole Blood

Whole blood from these qualified and consented donors was collected at Our Blood Institute into a blood bag kit containing citrate-phosphate-dextrose (CPD) anticoagulant (Fresenius Kabi, Germany) and stored at ambient temperature (18-26^{o}C). Within 8 hours of collection, the blood bag (450-660 mL) was centrifuged in a blood bank centrifuge such as ROTIXA 500 RS centrifuge (Hettich Instruments, Germany), J6-MI centrifuge (Beckman Coulter, California), or similar centrifuge at 800 x g for 10 minutes to separate plasma, WBC, and RBC fractions, as previously described[1]. The plasma fraction was diverted into a labeled satellite bag and flash frozen for future use. The thin layer of white blood cells (buffy coat) was diverted into a labeled satellite bag, along with 40-60 mL of residual plasma and RBCs. The buffy coat was stored at 2-8^{o}C until processing. The remaining RBC fraction was filtered and stored in preservative at 2-8^{o}C for future use in patient transfusion.

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Generation of LRS Chambers from Platelet Apheresis

Platelet apheresis was performed at Our Blood Institute on consented, eligible and suitable donors using a Trima Accel (Terumo Blood and Cell Technologies, Colorado) with closed system collection kits containing anticoagulant citrate dextrose, solution A (ACD-A). All data shown are from LRS chambers collected under version 7 of the Trima software utilizing plasma for platelet suspension. Following the completion of the collection procedure, LRS chambers were sterilely sealed off and removed from the platelet collection kit before the rest of the kit was discarded as biohazard waste. The LRS chamber was placed at 18-26^{o}C immediately after collection and transferred to 2-8^{o}C for overnight storage.

Creating White Blood Cell Eluates

Buffy Coat: The prepared buffy coats were drained by cutting the sealed tubing and allowing the eluate to drip into 50 mL Falcon conical tubes (Corning, New York). Buffy coat eluates were sampled for complete blood count (CBC) and flow cytometry analysis and stored at 2-8^{o}C for 5 days.

LRS Chamber: The collected LRS chambers were mounted on a ring stand over 15 mL or 50 mL Falcon conical tubes (Corning) and drained by cutting the lower tubing then the upper tubing (to break the vacuum), then the eluate was allowed to drip into the tube. LRS chamber eluates were approximately 8 mL ± 1 mL total volume. When the LRS eluate was to be stored overnight, phosphate buffered saline (PBS, ThermoFisher Scientific, Massachusetts) containing 2% human serum (ThermoFisher Scientific) was added. LRS eluates were sampled for CBC and flow cytometry analysis and stored at 2-8^{o}C for various times, as indicated.

CBC Analysis

Samples of eluates from buffy coats and LRS chambers were analyzed on a Sysmex Hematology Analyzer XE2100D (Symex, Japan) or similar instrument to obtain the WBC concentration and differential of the lymphocyte, monocyte, and granulocyte (neutrophil, basophil, and eosinophil) counts. When necessary, the eluate samples were diluted 1:5, 1:10, or 1:20 in PBS to get an accurate reading, within the linear range of the instrument's capabilities.

Flow Cytometry Analysis

Flow cytometry analysis was conducted on buffy coat and LRS eluates to examine the PBMC subpopulations ($CD45^+$), including total T cells ($CD45^+CD3^+$), B cells ($CD45^+CD19^+$), and NK cells ($CD45^+CD3^-CD56^+$). Cell viability was assessed using 7-aminoactinomycin D (7AAD) to exclude dead cells.

Briefly, approximately $1x10^6$ PBMCs were placed in sample tubes (BD Biosciences, California). Cells were washed by adding 1 mL of PBS+2% human serum and spinning in a tabletop centrifuge such as GP ST Plus (ThermoFisher Scientific) at 1100 RPM for 10 minutes at 2-8^{o}C. After decanting the wash buffer from the pelleted cells, 1 µg of the following antibodies were added according to package directions to corresponding Panel tubes to measure the populations listed above. Panel 1: 7AAD only; Panel 2: CD45-FITC (BD Biosciences), CD3-PE (BD Biosciences), CD56-PerCP-Cy5.5 (BD Biosciences); Panel 3: CD45-FITC (BD Biosciences), CD19-PE (BD Biosciences), 7AAD (BD Biosciences). Panel 1 was run on all LRS chambers and buffy coat eluates, while Panels 2 and 3 were only run on 25 LRS chambers out of the 62 from the first study and all LRS chambers and buffy coats from the second study. Only 25 LRS chambers were analyzed by flow due to multiple constraining factors such as reagent and instrument availability. Compensation controls were prepared at the same time as the samples, each tube containing only one of the following antibodies: CD45-FITC, CD3-PE, CD56-PerCP-Cy5.5, or no antibody. Cells were incubated with antibodies for 20-30 minutes at room temperature and washed as described above. Pelleted cells were

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resuspended in RBC lysis buffer (BD Biosciences) and incubated for 10 minutes before running on the flow cytometer.

A FACSCalibur flow cytometer (BD Biosciences) was prepared for daily operations by running BD Calibrite beads (BD Biosciences). CellQuest Software was used to acquire and analyze samples. Compensation controls were acquired first and compensation was manually adjusted. Next, 100,000 cells were acquired from each sample. Sample files were analyzed using CellQuest for the percentage of each population as described above.

Data Analysis and Statistics

Data from CBC and flow cytometry analyses were analyzed using Prism software v10.5.0 (GraphPad, California). Mean and Standard Error of the Mean were calculated using the software. Buffy coat and LRS chamber data were compared in the software using Welch's unpaired t test to generate a p value at a 95% confidence level. A p value of ≥0.05 was considered not statistically significant (not different from each other) while a p value <0.05 was considered statistically significant (different from each other).

Acknowledgements

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