Published in final edited form as:

Biologicals. 2017 September ; 49: 15–22. doi:10.1016/j.biologicals.2017.07.006.

Recovery and assessment of leukocytes from LR Express filters

Abby K. Wegehaupt¹, Ellen K. Roufs¹, Cory R. Hewitt¹, Marisela L. Killian², Oxana Gorbatenko³, Cynthia M. Anderson³, and M. Scott Killian^{1,*}

¹Department of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion, SD, 57069

²Flow Cytometry Core Facility, Sanford Research, Sioux Falls, SD, 57104

³Western South Dakota DNA Core Facility, Black Hills State University, Spearfish, SD 57799

Abstract

Leukocytes, or white blood cells, are used for a variety of investigational purposes and they offer advantages over laboratory-adapted cell lines. Leukocytes that are typically discarded by blood banks during the collection of red blood cells, platelets, and plasma can often be obtained for research use. However, the available leukocytes are frequently contained within a blood filtration device, such as the Terumo LR Express (TLRE) filter. In this study, procedures were evaluated for the ability to elute viable leukocytes from TLRE filters. The recovered leukocytes were assessed for composition, growth, and functionality. The large majority (>70%) of leukocytes were eluted with a single reverse-elution procedure and the recovered cells contained representative populations of the major leukocyte subsets. Purified T cells exhibited diverse T cell receptor repertoires, characteristic growth upon mitogen stimulation, and CD4⁺ T cells were able to support HIV-1 propagation. Purified monocytes were able to be differentiated into phenotypically characteristic populations of macrophages and dendritic cells. Overall, TLRE filters offer an attractive source of primary human cells for research and possibly clinical purposes.

Keywords

blood filter; primary human cells; leukocytes; T cells; T cell receptor repertoire; monocytes; dendritic cells; HIV; immunoassays; reagent validation

---

*Corresponding author's address: 414 E. Clark St., Vermillion, SD 57069. m.scott.killian@gmail.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest

The authors report having no conflicts of interest related to this study.

Author Contributions

AW, ER, and CH performed experiments, analyzed data, and wrote parts of the manuscript. MLK contributed to the flow cytometric analyses. OG and CMA contributed to the design and conduct of the sequencing experiments. MSK designed the study, procured blood specimens, conducted experiments, analyzed data, and wrote parts of the manuscript. All authors reviewed and contributed to the final manuscript.

1. Introduction

Human blood is widely used as a source of primary cells for research [1, 2]. Blood is routinely obtained by veinipuncture of volunteer research participants. However, the collection of blood from research volunteers can be an involved process and requires a trained phlebotomist, a designated blood collection area, phlebotomy supplies, the recruitment of volunteers, and informed consent and institutional review board (IRB) approval [3]. Moreover, it is common for such blood collection attempts to be restricted to 90mls or less of blood per volunteer donor.

Leukocyte reduction filters (LRFs), used by blood banks to facilitate the large-scale collection of blood products from healthy donors, are an alternative source of human blood cells. LRFs are designed to trap leukocytes (i.e., white blood cells) while allowing erythrocytes (i.e., red blood cells), platelets, and plasma to flow through the device [4]. LRFs offer several advantages as a source of leukocytes for research. One advantage is that 400–450 ml (1 unit) is the typical blood donation and therefore LRFs contain many more leukocytes than can be obtained from a volunteer research participant under common guidelines. A second advantage is that the phlebotomy procedures have already been performed, eliminating the need for phlebotomy resources. A third advantage is that blood bank donors typically provide informed consent for their donated blood to be used for research. The LRFs are not usually labelled with donor information and therefore can be readily provided to research in an anonymous manner. Thus, the use of LRFs is usually determined to be “research exempt”. A fourth advantage is that the blood donated to blood banks is screened for the presence of several viral infections (e.g., HIV, HBV, and HCV). In addition, LRFs are waste products and can be provided by blood banks to researchers at little to no cost.

Commercially available LRFs vary in physical design and in the technology used to trap leukocytes [4]. The use of some LRFs as research resources has been previously described [5, 6], but not the use of the common Terumo LR Express (TLRE) filter that according to the manufacturer (A. Wegehaupt, personal communication, July 1, 2015) has unique physical properties [7]. Here we describe a procedure for recovering leukocytes from TLRE filters and demonstrate that the recovered cells are well-suited for a variety of research applications.

2. Materials & Methods

2.1 Blood donors

LR Express filters (Terumo Medical Corp., Somerset, NJ), that had been used to facilitate the collection and processing of peripheral blood from healthy donors, were procured from the Sanford Medical Center Blood Bank (Sioux Falls, SD). All blood donors signed informed consent forms and this study received approval from the University of South Dakota IRB (Project 2015.111).

2.2 Recovery of blood cells

The exterior surfaces of the TLRE filters and tubing were wiped with 70% ethanol and the following procedures were performed in a biosafety cabinet (Labconco, Kansas City, MO). Sterile scissors were used to cut the sealed ends of the tubing, leaving ample length for one end to be placed into a sterile 50ml collection tube and the other end to be connected to a sterile 60 ml syringe (BD). Importantly, the syringe was connected downstream of the arrow on the LRF (Fig 1). Various volumes of Hank's buffered saline solution (HBSS; Invitrogen) were moderately forced through (at a plunge rate of approximately 10 ml/s) the TLRE filter to counter-elute the trapped cells (i.e., to flow the cells out of the filter in the reverse direction). The resulting collection tubes were then centrifuged (1000×g, 10 min) to pellet the blood cells.

2.3 Isolation of PBMCs and leukocyte subsets

Leukocytes were isolated by density-gradient separation over ficoll (GE) according to the manufacturer's instructions. Platelets were depleted from the leukocyte preparations by washing and pelleting the cells using slow centrifugation (50×g for 5 min, thrice repeated). CD4⁺ T cells, CD8⁺ T cells, and CD14⁺ monocytes were purified using immunomagnetic beads (Miltenyi) as previously described [8]. The resulting cell subsets were routinely >95% pure upon flow cytometric analysis (see Fig 3). Cell counts and viability and were performed using trypan dye exclusion and a standard hemocytometer.

2.4 Cell culture

Immediately following their isolation, the purified T cells and monocytes were resuspended in growth medium (RPMI1640, 10% FBS, and antibiotics). The growth medium used to culture T cells was supplemented with 100U/ml rhIL2 (Invitrogen). T cells were stimulated with anti-CD3/CD28 beads (Invitrogen) according to the manufacturer's protocol or with phytohemagglutinin (PHA; Sigma; 3 ug/ml). After 3 days, PHA-stimulated CD4⁺ T cells were acutely infected with a primary HIV-1 isolate and the following outgrowth of virus was measured as previously described [8]. T cells were passaged each 2–3 days to maintain a density of 2–3 million cells/ml in fresh medium. The purified CD14⁺ monocytes were resuspended in growth medium supplemented with 40ng/ml GMCSF and with or without IL-4 (40ng/ml) and then placed into a 12-well tissue culture plate (BD) at a density of 5x10⁵ cells/ml. On days 2 and 4, 50% of the cell culture medium was replaced with fresh complete grow medium and cytokines. After 5 days of culture, lipopolysaccharide (LPS, Sigma; 1 ug/ml) was added to produce mature dendritic cells.

2.5 Flow cytometry

To characterize the expression of cell surface markers, cells were stained with combinations of fluorescently-labeled monoclonal antibodies, washed, and resuspended in isotonic buffer. The fluorescently labeled antibodies used for this study included IgG1 FITC, IgG2a PE, IgG1 PerCP-Cy5.5, IgG2a APC, CD14 PE, CD4 APC, CD3 FITC, HLA-DR FITC, CD4 PerCP-Cy5.5, and CD8 (BD Biosciences) and CD83 APC and CD86 FITC (Miltenyi). In some experiments, cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen) according to the manufacturer's protocol. Flow cytometric analyses were

performed using Accuri C6 and LSRFortessa cytometers (BD) and FlowJo v10 software (FlowJo).

2.6 T cell receptor sequencing

RNA was extracted from purified CD4⁺ and CD8⁺ T cells using RNeasy columns (Qiagen) and was assessed for quality using a Bioanalyzer. T cell receptor (TCR)-specific PCR libraries were created using a SMARTer Human TCR α/β Profiling Kit (Takara). Next generation sequencing (NGS) was performed using a MiSeq Sequencing System (illumina). Sequence analysis was performed using BaseSpace (illumina) and MiXCR software packages [9].

2.7 Data analysis

Laboratory data were compiled in Excel spreadsheets (Microsoft). Statistical analyses and charting was performed using SigmaPlot 11.0 (Systat) and Omics Explorer 3.2 (Qlucore). Statistical significance was set at the conventional level of $\alpha = 0.05$

3. Results

3.1 Recovery of leukocytes

We began this study by evaluating procedures for the recovery of leukocytes from TLRE filters (Fig 1). An important consideration is the orientation of the filter in the blood collection process; blood flows through the filter in the direction of the arrow molded on the filter (Fig 1A). Therefore, a counter-elution procedure was performed using a 60-ml syringe to force saline solution through the filter in the reverse direction of the blood collection orientation (Fig 1B). Leukocytes are not recoverable when elutions are performed in the direction of the arrow on the filter (data not shown).

To determine the recoverable number of leukocytes, following the ficoll separation, cell counts and viability assessments were performed (Fig 1C). Among 10 TLRE filters, the median yield was 356×10^6 cells (range = $200 - 549 \times 10^6$ cells). Viability of the recovered cells was routinely $> 95\%$. While the pre-ficoll volume of eluted red blood cells was not reliably predictive of the post-ficoll leukocyte count between filters, the ratio of the volume of packed red blood cells to the number of peripheral blood mononuclear cells (PBMCs) recovered in each eluate from an individual filter was generally consistent (data not shown), indicating that the cellular compositions in each of the two washes were similar.

To optimize the procedure for recovering blood cells from the TLRE filters, we compared the number of cells recovered with incremental 50ml washes (Fig 1D). On average, 75% of the recoverable cells were present in the first 50 ml eluate. An additional 25% could be recovered with subsequent washes and the majority of these residual cells were recovered in the second 50 ml eluate. Thus, two 50 ml collection tubes are needed for maximal recovery of leukocytes from each filter.

3.2 Cellular composition

To determine whether or not representative leukocyte subsets were present in the cells recovered from the TLRE filters, flow cytometric and molecular assessments were performed (Fig 2). Upon flow cytometric inspection, typical lysed whole blood light scatter properties were observed, including distinct lymphocyte, monocyte, and granulocyte populations (Fig 2A). Staining for cell surface markers revealed the presence of at least 5 mutually exclusive PBMC subsets: CD3⁺CD4⁺ and CD3⁺CD4⁻ lymphocytes (T cells), CD3⁻CD16⁺CD56⁺ lymphocytes (NK cells), CD3⁻CD16⁻CD56⁻ lymphocytes (mostly B cells) and CD14⁺ cells (monocytes). The proportions of these recovered leukocyte subsets (Fig 2B) were consistent with the proportions reported for whole blood. These results demonstrate that the cells from LR filters contain representative populations of the major cell subsets found in whole blood.

To determine the representation of T cells, T cell receptor (TCR) diversity was assessed by NGS (Fig 3). Highly purified populations of CD8⁺ and CD4⁺ T cells were isolated from leukocytes recovered from LRFs (Fig 3A). Sequencing analysis of RNA transcripts revealed that the T cell receptor repertoires of both CD4⁺ and CD8⁺ T cells were broadly representative, as indicated by the diverse presence of rearranged TCR- β chain genes containing different variable-joining (V-J) segment combinations (Fig 3B). These results indicate that the T cells recovered from TLRE filters are representative of those found in peripheral blood [10].

3.3 Functional assessments

To assess the replicative capacity of leukocytes recovered from TLRE filters, we evaluated the effects of mitogen stimulation (Fig 4) [11]. In comparison to the unstimulated cell cultures, lymphocytes in cultures containing PHA and IL-2 exhibited a 'blasting' phenotype as evidenced by appreciable increases in cell size and complexity (Fig 4A). Cell proliferation in PHA-stimulated bulk leukocyte cultures was detected after 7 days (Fig 4B). The replicative capacity of PHA-stimulated purified CD8⁺ T cells was measured using a CFSE-based cell proliferation assay (Fig 4C) [12]. In this assay, the fluorescence intensity of dye-labeled cells decreases uniformly with each round of cell division. During a 12 day cell culture period, we were able to detect at least 7 rounds of cell division. Together these results demonstrate that leukocytes recovered from the TLRE filters, particularly the T lymphocytes, are able to respond and proliferate to mitogenic stimulation.

As an additional functional assessment, CD4⁺ T cells purified from TLRE filters were evaluated for their ability to support the propagation of human immunodeficiency virus 1 (HIV-1) (Fig 5). HIV-1 replicated in cultures of acutely-infected PHA-stimulated CD4⁺ T with kinetics similar to those previously reported for cultures of CD4⁺ T cells purified from buffy coats or whole blood (Fig 5A) [8, 13]. Moreover, the combination of CD4⁺ T cells from 2 different blood donors resulted in the enhanced production of HIV, consistent with a prior report [14]. The characteristic cytopathic effects of HIV-1 replication were visible in the CD4⁺ T cell cultures after 3 days of infection (Fig 5B). Because HIV-1 only replicates at appreciable levels in actively dividing CD4⁺ T cells [15], these results demonstrate that the CD4⁺ T cells recovered from TLRE filters are functional in this regard.

3.4 Production of macrophages and dendritic cells

To evaluate the recovery of functional monocytes from TLRE filters, we assessed the ability of CD14⁺ cells to be differentiated into monocyte-derived macrophages (moM ϕ) and monocyte-derived dendritic cells (moDC) in vitro (Fig 6). Microscopic inspection revealed that purified CD14⁺ cells cultured in the presence of GM-CSF developed into large adherent cells that often featured extended protrusions (Fig 6A). In comparison, purified CD14⁺ cells cultured with GM-CSF and IL-4 were relatively smaller in size, nonadherent, and exhibited limited protrusions. These observations are consistent with the established properties of moM ϕ and moDC, respectively [16, 17]. Upon analysis by flow cytometry (Fig 6B), moDC exposed to LPS exhibited substantial increases in the cell surface expression of HLA-DR, CD83, and CD86, indicating that they had developed into mature dendritic cells [18].

4. Discussion

Increasing attention has been given to the need to validate experimental methods and resources [19]. Accordingly, different sources of primary human cells require independent validation. In this study we evaluated Terumo LR Express blood filters for their utility as a source of primary human leukocytes for use in a variety of potential research applications.

Based on our experimental results, we present optimized procedures for the recovery of leukocytes from TLRE filters (Fig 1). The maximal recovery of cells is obtained with two counter-flow elution steps using a syringe to force buffered saline solution through the downstream side of the filter, resulting in two 50 ml collection tubes. However, the collection of a single 50 ml tube will generally yield 75% of the recoverable leukocytes. This consideration can be useful for efficiency purposes.

Despite their selective retention of leukocytes, TLRE filters contain abundant quantities of red blood cells (up to 20 mls of packed RBCs) and platelets. While we did not evaluate the integrity of these non-leukocyte components, we presume that they remain biologically functional and could be useful for some applications. For example, the RBCs could be used in hemagglutination assays [20]. Nonetheless, post-elution procedures are needed to purify leukocytes from the non-leukocyte components recovered from TLRE filters, such as a ficoll step to eliminate RBCs and a subsequent slow centrifugation step (e.g. 75 RPM for 10 minutes) to reduce platelet levels.

Our flow cytometric analyses revealed that the ficoll-purified leukocytes from TLRE filters exhibited light scatter properties similar to those of lysed whole blood in other studies [21, 22], evidencing the presence of granulocytes, monocytes, and lymphocytes (Fig 2). Finer inspection of lymphocytes demonstrated the presence of representative frequencies of T cells, B cells, and NK cells. Moreover, the T cells exhibited broad diversity as determined by NGS of the T cell receptor repertoires of CD4⁺ and CD8⁺ T cells (Fig 3). These findings demonstrate that the leukocytes recovered from TLRE filters are similar in composition to those found in whole blood.

Finally, we assessed the functionality of the leukocytes recovered from TLRE filters. Bulk leukocytes as well as purified T cells proliferated in response to mitogenic stimulation (Fig

4). In addition, purified CD4⁺ T cells were able to support the replication of HIV (Fig 5), evidencing the overall biologic integrity of these cells. Likewise, purified monocytes could be differentiated into macrophages and dendritic cells in response to the appropriate external stimuli (Fig 6). These findings establish that the leukocytes recovered from TLRE filters can be useful for applications requiring functional cells.

A limitation of the present study is that we were unable to obtain whole blood from the blood bank for direct comparisons with the cells recovered from the TLRE filters. However, the cell subset frequencies and cellular functions of the recovered cells were consistent with our prior observations of whole blood [8, 13, 23] and those of others. Nonetheless, subtle differences in blood collection procedures can alter laboratory results [24] and additional validation studies may be needed for specific applications.

In summary, a variety of viable and functional leukocytes are recoverable from TLRE blood filters. Our findings demonstrate that these cells can be used for diverse areas of research and commercial applications. In addition, our approach to evaluating TLRE filters should be helpful for establishing standardized criteria for validating different sources of primary human leukocytes.

Acknowledgments

Research reported in this publication was supported in part by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103443. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This research was also supported in part by funding from the USD-SSOM Medical Student Research Program. We thank Lisa Greenfield and the Sanford Medical Center Blood Bank staff for making available the LR filters used in this study.

References

1. Earnshaw CJ, Pecaric-Petkovic T, Park BK, Naisbitt DJ. T cell responses to drugs and drug metabolites. *Exs.* 2014; 104:137–63. [PubMed: 24214623]
2. Becker K, Schroecksnadel S, Gostner J, Zaknun C, Schennach H, Uberall F, et al. Comparison of in vitro tests for antioxidant and immunomodulatory capacities of compounds. *Phytomedicine.* 2014; 21:164–71. [PubMed: 24041614]
3. WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy. Geneva: 2010.
4. Bruil A, Beugeling T, Feijen J, van Aken WG. The mechanisms of leukocyte removal by filtration. *Transfusion medicine reviews.* 1995; 9:145–66. [PubMed: 7795332]
5. Peytour Y, Villacreces A, Chevaleyre J, Ivanovic Z, Praloran V. Discarded leukoreduction filters: a new source of stem cells for research, cell engineering and therapy? *Stem Cell Res.* 2013; 11:736–42. [PubMed: 23743265]
6. Pfeiffer IA, Zinser E, Strasser E, Stein MF, Dorrie J, Schaft N, et al. Leukoreduction system chambers are an efficient, valid, and economic source of functional monocyte-derived dendritic cells and lymphocytes. *Immunobiology.* 2013; 218:1392–401. [PubMed: 23932569]
7. Lozano ML, Perez-Ceballos E, Rivera J, Paunovic D, Candela MJ, Vicente V. Evaluation of a new whole-blood filter that allows preparation of platelet concentrates by platelet-rich plasma methods. *Transfusion.* 2003; 43:1723–8. [PubMed: 14641870]
8. Killian MS, Teque F, Walker RL, Meltzer PS, Killian JK. CD8(+) lymphocytes suppress human immunodeficiency virus 1 replication by secreting type I interferons. *J Interferon Cytokine Res.* 2013; 33:632–45. [PubMed: 23402527]

9. Bolotin DA, Poslavsky S, Mitrophanov I, Shugay M, Mamedov IZ, Putintseva EV, et al. MiXCR: software for comprehensive adaptive immunity profiling. *Nat Methods*. 2015; 12:380–1. [PubMed: 25924071]
10. Robins HS, Campregher PV, Srivastava SK, Wachter A, Turtle CJ, Kahsai O, et al. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. *Blood*. 2009; 114:4099–107. [PubMed: 19706884]
11. O'Donovan MR, Johns S, Wilcox P. The effect of PHA stimulation on lymphocyte subpopulations in whole-blood cultures. *Mutagenesis*. 1995; 10:371–4. [PubMed: 7476275]
12. Quah BJ, Warren HS, Parish CR. Monitoring lymphocyte proliferation in vitro and in vivo with the intracellular fluorescent dye carboxyfluorescein diacetate succinimidyl ester. *Nat Protoc*. 2007; 2:2049–56. [PubMed: 17853860]
13. Killian MS, Ng S, Mackewicz CE, Levy JA. A screening assay for detecting CD8(+) cell non-cytotoxic anti-HIV responses. *Journal of Immunological Methods*. 2005; 304:137–50. [PubMed: 16129447]
14. Lai-Hipp C, Goldberg T, Scott E, Ziman A, Vyas G. Pooled peripheral blood mononuclear cells provide an optimized cellular substrate for human immunodeficiency virus Type 1 isolation during acute infection. *Transfusion*. 2011; 51:333–7. [PubMed: 20796251]
15. Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen IS. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell*. 1990; 61:213–22. [PubMed: 2331748]
16. Zarif JC, Hernandez JR, Verdone JE, Campbell SP, Drake CG, Pienta KJ. A phased strategy to differentiate human CD14+ monocytes into classically and alternatively activated macrophages and dendritic cells. *Biotechniques*. 2016; 61:33–41. [PubMed: 27401672]
17. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *The Journal of experimental medicine*. 1994; 179:1109–18. [PubMed: 8145033]
18. Zhou LJ, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93:2588–92. [PubMed: 8637918]
19. Anonymous. The long road to reproducibility. *Nat Cell Biol*. 2015; 17:1513–4. [PubMed: 26612570]
20. Katz JM, Hancock K, Xu X. Serologic assays for influenza surveillance, diagnosis and vaccine evaluation. *Expert Rev Anti Infect Ther*. 2011; 9:669–83. [PubMed: 21692672]
21. Killian MS, Fujimura SH, Hecht FM, Levy JA. Similar changes in plasmacytoid dendritic cell and CD4 T-cell counts during primary HIV-1 infection and treatment. *AIDS*. 2006; 20:1247–52. [PubMed: 16816552]
22. Bossuyt X, Marti GE, Fleisher TA. Comparative analysis of whole blood lysis methods for flow cytometry. *Cytometry*. 1997; 30:124–33. [PubMed: 9222098]
23. Killian MS, Monteiro J, Matud J, Hultin LE, Hausner MA, Yang OO, et al. Persistent alterations in the T-cell repertoires of HIV-1-infected and at-risk uninfected men. *AIDS*. 2004; 18:161–70. [PubMed: 15075532]
24. Bowen RA, Hortin GL, Csako G, Otanez OH, Remaley AT. Impact of blood collection devices on clinical chemistry assays. *Clin Biochem*. 2010; 43:4–25. [PubMed: 19822139]

Figure 1. Recovery of leukocytes

A) In the blood bank, the TLRE filter is placed in a unidirectional position downstream of a whole blood collection bag. An arrow is molded into the construction of the filter shell indicating the proper orientation for blood collection. **B)** To recover cells from the TLRE filter, a counter-elution procedure is used. Optimally, a 60 ml sterile syringe is attached to the filter tubing and used to force saline solution in the reverse direction through the filter as shown. **C)** Shown are the post-ficoll cell counts from 10 individual TLRE filters. For each filter, 100 ml total eluate was collected in two sequential 50 ml tubes. **D)** Box plots show the percentage of total leukocytes recovered in the first and second 50 ml eluates. The boxes mark the interquartile ranges, while the whiskers show the 90th and 10th percentiles.

Figure 2. Composition of recovered cells
A) Flow cytometric analysis shows the light scatter properties and cell surface marker expression of ficoll purified cells from a TLRE filter. **B)** A pie chart shows the relative frequencies of mutually exclusive leukocyte subsets. Results are representative of 3 independent experiments.

Author Manuscript

Figure 4. Cell growth

A) Shown are the light scatter properties of leukocytes from TLRE filters cultured without (left) or with PHA stimulation (right). **B)** Longitudinal cell counts are shown for parallel leukocyte cultures in the presence and absence of PHA and IL-2. **C)** A single-parameter histogram shows the CFSE profiles for cultured CD8⁺ T cells without PHA stimulation (shaded grey) and with PHA stimulation (open). Results are representative of 3 independent experiments.

Figure 5. Growth of HIV-1 in purified CD4⁺ T cells

A) Shown are the temporal HIV replication levels, as measured by p24 ELISA, in acutely HIV-1-infected CD4⁺ T cells purified from TLRE filters from 2 different donors and cultured alone or together at a 1:1 cell input ratio. **B)** A light microscopy (200X) photograph of acutely HIV-1-infected CD4⁺ T cells isolated from a TLRE filter. Arrow denote pronounced CPE characteristic of the HIV-1 isolate used. Results are representative of 3 independent experiments.

Figure 6. Monocyte cultures

A) Light microscopy images (200X) are shown for CD14⁺ cells upon initial isolation from a TLRE filter (day 0) and after 5 days of culture in the presence of GM-CSF or GM-CSF and IL-4. **B)** Shown are the fluorescence intensities of cell surface markers (HLA-DR, CD83, and CD86) on monocytes freshly isolated from a TLRE filter (upper histograms) and monocytes culture in GM-CSF and IL-4 for 5 days and then LPS for 1 day (lower histograms). Shaded traces indicate unstained control cells. Results are representative of 2 independent experiments.