Evaluation of Leukocyte Removal Filters Modeled by Use of HIV-Infected Cells and DNA Amplification

By Bhupat D. Rawal, Ron Schwadron, Michael P. Busch, Rosalind Endow, and Girish N. Vyas

The concept of reducing cell-associated blood-borne viruses (BBVs) by filtration of the vector leukocytes from blood collected for transfusion has led to the development of high efficiency filters. Improved filtration technology demands newer methodology to accurately estimate the residual cells. We have developed an experimental model based on the hemocytometer counts and the polymerase chain reaction (PCR), performed on the lymphocytes derived from the units of red cell mass inoculated with marker cells (H9) persistently carrying cell-associated human immunodeficiency virus DNA (CA-HIV). We measured the efficiency of 6 units of a prototype filter using our model and found an estimated mean of less than 4 residual cells per milliliter in the filtered blood. This represents a mean 5.84 log10 reduction of normal PBMC and CA-HIV in pre- and post-filtration aliquots and exemplifies the application of our model for evaluating a new generation of blood filters. Our model illustrates that a biological tracer (ie, DNA) is a better measure of the efficacy of a leukocyte filter than the hemocytometric enumeration of pre- and post-filtration PBMC concentrates.

© 1990 by The American Society of Hematology.

HUMAN immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human T-cell leukemia virus-1 (HTLV-I) all known to be transmitted by cellular blood products, persist in leukocytes as latent infections. Removal of these vectors from blood by filtering out the leukocytes has been reported to (1) reduce the in vitro HIV-1 infectivity of blood, (2) prevent seroconversion of CMV-negative persons transfused with blood from CMV-positive donors, (3) prevent primary CMV infection in patients with hematologic malignancies, and (4) prevent transmission of HTLV-I virus through blood transfusion.

Although bedside leukocyte filters have been generally used to avoid febrile transfusion reactions, the putative benefits of preparing leukocyte-depleted blood for reducing alloimmunization, and most recently for reducing the transmission of transfusion-mediated virus diseases, have led to the development of newer filters capable of a six-log reduction of leukocytes. Reductions of this magnitude cannot be measured by conventional counting methods, including the recent method using propidium iodide staining that detects 11 leukocytes per microliter of blood. This has led to the need for alternative approaches to assess filtration efficiency.

We have investigated the use of a cell-associated virus model that involves inoculation of HIV-infected lymphocytes into units of packed red cells as an independent marker. We estimated the efficiency of filtration by quantitating cell-associated HIV DNA (CA-HIV) in pre- and post-filtration concentrates using limiting-dilution titrations and gene amplification of HIV sequences by the polymerase chain reaction (PCR). Our model experimentally validates the hypothesis that biologic markers (nucleic acid) allow for more accurate and quantitative assessment of filter efficiency than do conventional automated or manual counting methods.

MATERIALS AND METHODS

Blood. Six anti-HIV negative blood units were selected from routinely screened normal blood donors accepted at the Irwin Memorial Blood Centers, San Francisco. Freshly drawn blood was collected in the CPD anticoagulant and processed according to standard procedures to make units of packed cells in ADSOL (Fenwal, Deerfield, IL). Units used in our experiments were obtained within 48 hours of blood collection. Before filtration, each of these units was inoculated with a predetermined number of cells containing CA-HIV.

HIV inoculum. CA-HIV inoculum was obtained from continuous cell line (H9), stably infected with HIV-1 (HTLV-IH9). Persistent infection of these cells was regularly confirmed by immunocytochemical detection of the p24 antigen in over 90% of the cells. Before inoculation into blood for filtration, the infected cells were washed 5 times in Hanks balanced salt solution to remove any cell-free HIV. Viable HIV infected cells were inoculated into the unit of blood prepared for filtration in a ratio of 1 cell to 100 total leukocytes counted in the unit of blood. The inoculated number of marker cells range between 1.14 x 107 and 3 x 107 with a mean of 2.07 x 107

Filters. A prototype experimental filter (Pall Biomed Products Corporation, Glen Cove, NY) was used for the evaluation of concurrent removal of total leukocytes and the CA-HIV marker cells.

Isolation, concentration, and enumeration of PBMC and CA-HIV from blood. After addition and adequate mixing of the CA-HIV inocula in the blood bag, 15.0 mL aliquot of blood was removed to obtain the pre-filtration sample. The entire volume of the filtered blood was divided into 15.0 mL aliquots and processed to recover the maximum possible residual cells. After mixing with an equal volume of Hanks, each 15.0 mL aliquot of both pre- and post-filtration samples was layered on 30.0 mL Lymphoprep (Robin Scientifics, Sunnyvale, CA). Following centrifugation, the erythrocytes and granulocytes were pelleted, while the peripheral blood mononuclear cells (PBMC) as well as the inoculated CA-HIV were concentrated as a single lymphocyte band in the Lymphoprep tube containing the prefiltration blood. No band was visible at the interface in the tubes containing the post-filtered blood. Therefore, about 10 mL of the interface fluid from each tube of post-filtered blood was pooled and centrifuged to recover any residual cells. Most of the supernatant was removed, leaving about 200 μL of the fluid containing any residual cells that were washed with 20.0 mL of Hanks, followed by centrifugation and resuspension in 1.0 mL Hanks. Similarly, cells

From the Department of Laboratory Medicine, University of California, San Francisco, and the Irwin Memorial Blood Centers, San Francisco, CA.

Submitted March 22, 1990; accepted July 12, 1990.

Supported by Research Grants ROI HL-41361 and PO1-HL-36589 from the National Heart, Lung, and Blood Institute.

Address reprint requests to Girish N. Vyas, PhD, Transfusion Research Program, Department of Laboratory Medicine, University of California, San Francisco, CA 94143.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1990 by The American Society of Hematology.

Blood, Vol 76, No 10 (November 15), 1990, pp 2159-2161
concentrated from the pre-filtration sample were also washed and suspended in 1.0 mL Hanks. Thus, the cells from 15.0 mL aliquot of pre-filtration blood, as well as the residual cells from the entire post-filtration unit were concentrated into separate 1.0 mL cell suspensions. The purpose of concentrating PBMC from the filtered blood was to remove red cells, as well as plasma, that inhibit the detection of HIV-DNA by PCR. The respective concentrates were corrected for the loss from Lymphoprep concentration. The reduction in the cell numbers was expressed as the difference between the log₁₀ of corresponding pre- and post-filtration numbers of cells.

**Quantitative detection of HIV-1 DNA by polymerase chain reaction (PCR).** An aliquot of the concentrated cell suspension containing 1,000,000 cells from the pre-filtration sample was serially diluted in 10-fold steps in Hanks containing 10⁶ normal carrier PBMC. Half of the post-filtered cell suspension that typically had undetectable cells was similarly diluted in Hanks with 10⁶ carrier cells; the remainder was used for viability test using trypan blue stain. Lysates of the cell suspensions containing 250,000 cells were prepared for PCR-mediated DNA amplifications using the SK38/39 primer pairs, Taq polymerase, 30 cycles of amplification and liquid hybridization with ³²P-labeled probes for the detection of amplified HIV gene products, as previously reported in detail.¹ The highest dilution of the cell concentrate giving a positive PCR signal was considered to contain 1 detectable unit of HIV-DNA, and is referred to hereafter as the polymerase chain reaction unit (PCRU). The number of PCRU/mL of blood was then calculated by dividing the number of PCRU/mL of the concentrate by the volume of blood used for the Lymphoprep concentration step.

**Estimation of residual PBMC in the filtered blood.** The numbers of residual PBMC in the filtered blood were estimated based on the assumption that the number of input PCRU per PBMC before filtration remained unchanged after filtration. Because neither PCRU nor PBMC were detectable in the post-filtered blood, the residual cell numbers were estimated to be less than the number of input PBMC corresponding to 1 PCRU in the prefiltration sample. The difference between log₁₀ of input cells and log₁₀ of the estimated PBMC in the filtered blood provided the log₁₀ reduction of the number of PBMC as a measure of efficiency of the filter (see footnote to Table 1 for an example of calculations and derivations of the estimate).

**RESULTS**

The volume of blood used for the six experiments (Table 1) ranged between 143 and 363 mL (mean 314 ± 79 mL). While the marker cells were inoculated into each unit of blood in the ratio of 1:100 leukocytes, the Lymphoprep gradient concentrated the mononuclear cell fraction. Hence the prefiltration input lymphocytes (column A) including both the donor PBMC and CA-HIV-bearing cells were corrected for Lymphoprep concentration efficiency in each experiment. Column B shows the titer of PCRU determined on the prefiltration blood samples. The data reveal that the total PCRU of CA-HIV-bearing marker cells per unit consistently ranged between 5.53 and 6.10 with a mean of 5.83 logs. The 5.83 logs of PCRU correspond to a mean of 7.25 logs of CA-HIV marker cells added as a tracer (see "HIV inoculum" in the Materials and Methods section), indicating that under the experimental conditions 1 PCRU was functionally equivalent to 26 marker cells. By dividing the number of lymphocytes (column A) by the corresponding PCRU (column B) we derived that 1 PCRU represents a mean of 965 lymphocytes including the 26 marker cells (column C). Because the marker cells were originally added

### Table 1. Concurrent Removal of Peripheral Blood Mononuclear and HIV-Infected Marker Cells by Filtration of Packed Red Cells Using a Prototype Filter

<table>
<thead>
<tr>
<th>Filter</th>
<th>Volume of Blood</th>
<th>Lymphocytes and Marker Cells A</th>
<th>PCRU B</th>
<th>Lymphocytes/PCRU C</th>
<th>Total D</th>
<th>E</th>
<th>Net Cell Reduction log₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>358</td>
<td>8.75</td>
<td>5.84</td>
<td>790</td>
<td>&lt;790</td>
<td>2.20</td>
<td>&gt;5.85</td>
</tr>
<tr>
<td>2</td>
<td>417</td>
<td>8.53</td>
<td>5.53</td>
<td>993</td>
<td>&lt;993</td>
<td>2.38</td>
<td>&gt;5.53</td>
</tr>
<tr>
<td>3</td>
<td>348</td>
<td>8.83</td>
<td>5.83</td>
<td>1001</td>
<td>&lt;1001</td>
<td>2.87</td>
<td>&gt;5.83</td>
</tr>
<tr>
<td>4</td>
<td>143</td>
<td>8.54</td>
<td>5.77</td>
<td>1003</td>
<td>&lt;1003</td>
<td>7.01</td>
<td>&gt;5.83</td>
</tr>
<tr>
<td>5</td>
<td>255</td>
<td>9.10</td>
<td>6.10</td>
<td>1001</td>
<td>&lt;1001</td>
<td>7.73</td>
<td>&gt;6.10</td>
</tr>
<tr>
<td>6</td>
<td>348</td>
<td>8.94</td>
<td>5.94</td>
<td>1002</td>
<td>&lt;1002</td>
<td>2.92</td>
<td>&gt;5.94</td>
</tr>
<tr>
<td>Mean</td>
<td>314</td>
<td>8.74</td>
<td>5.83</td>
<td>965</td>
<td>&lt;965</td>
<td>4.18</td>
<td>&gt;5.84</td>
</tr>
<tr>
<td>± 95% limit</td>
<td>79.1</td>
<td>0.20</td>
<td>0.14</td>
<td>35.0</td>
<td>35.0</td>
<td>1.99</td>
<td>0.07</td>
</tr>
</tbody>
</table>

An example of calculations and derivations in the table (log, Filter #2).

**Prefiltration:** The volume of blood/unit was 417 mL, with a total leukocyte count of 1.338 × 10⁸, to which 1.34 × 10⁷ CA-HIV marker cells (1.0%) were added. Differential leukocyte count was 24.4% lymphocytes. Therefore, lymphocytes/unit = 24.4% of 1.338 × 10⁸ = 3.264 × 10⁷ and total lymphocytes with marker cells/unit = 3.264 × 10⁸ + 1.34 × 10⁷ = 3.398 × 10⁸. Lymphocytes and marker cells/unit recovered after Lymphoprep concentration was 2.224 × 10⁸. Correction for the loss from concentration = 1/(recovered cells/total cells) = 1/2.224 × 10⁸/3.398 × 10⁸ = 0.528. Corrected (lymphocytes + marker cells) recovered cells = (2.224 × 10⁸) × 1.528 = 3.398 × 10⁸ = log 8.53 (column A). Total PCRU/unit were 2.24 × 10⁴ based on endpoint titration, corrected for Lymphoprep loss = (2.24 × 10⁴) × 1.528 = 3.42 × 10⁵ = log 5.53 (column B). Therefore, lymphocytes per 1 PCRU = 3.398 × 10⁸/3.42 × 10⁵ = 993 (column C).

**Postfiltration:** No PCRU or cells were detected. Therefore, the number of lymphocytes/unit after filtration are estimated to be less than the number equivalent to 1 PCRU observed before filtration = <993 (column D). Lymphocytes/mL blood = 8.53/993 = <0.01 (column E).

Net cell reduction (log₁₀) = log of input lymphocytes – log of estimated output lymphocytes, (column A) - log of 993 (column D) = 8.53 – 2.96 = >5.53.

*Recovery of cells corrected for the loss from Lymphoprep concentration.
in the ratio of 1:100 leukocytes, 1 PCRU corresponds to the detection of approximately 2,600 total leukocytes in a unit of blood.

Because PCRU and lymphocytes were undetectable in the filtered blood, we estimated that the total residual lymphocytes in the filtered blood (column D) was less than the number of lymphocytes corresponding to 1 PCRU detected in the blood before filtration (column C). In column E, we have expressed the estimated number of residual cells per milliliter, with a mean of less than 4.18 cells. We determined the filtration efficiency by subtracting the log_{10} of the estimated postfiltration lymphocyte counts shown in column D from the log_{10} of input lymphocytes shown in column A. The mean reduction in cells was greater than 5.84 ± 0.07 logs.

DISCUSSION

To evaluate the performance of any leukocyte removal filter, one needs to determine the number of leukocytes entering the filter (input cells) and then enumerate the residual cells (output) in the filtered blood. The difference between these two counts would reflect the reduction caused by the filtration process. However, this simplistic derivation gets complicated when a filter yields an effluent that contains residual cells in too small a number to be reliably counted by automated cell counters or hemocytometers. Recently, flow cytometric analysis of propidium iodide stained cells has been shown to detect an average of 11,000 leukocytes per milliliter, which computes to 3,300 lymphocytes per milliliter. The apparently higher sensitivity of our model possibly reflects the dual benefits of concentrating the supply of the prototype filters by Dr Judy Angelbeck of Pall Biomed Corporation is gratefully acknowledged.

REFERENCES


Evaluation of leukocyte removal filters modelled by use of HIV-infected cells and DNA amplification

BD Rawal, R Schwadron, MP Busch, R Endow and GN Vyas