ELSEVIER

Journal of Immunological Methods 307 (2005) 150–166

JIM
Journal of
Immunological Methods

www.elsevier.com/locate/jim

Research paper

Filter Buffy Coats (FBC): A source of peripheral blood leukocytes recovered from leukocyte depletion filters

T.P.H. Meyer [a,*], I. Zehnter [c], B. Hofmann [a], J. Zaisserer [a], J. Burkhart [a], S. Rapp [a], F. Weinauer [a], J. Schmitz [c], W.E. Illert [b]

[a] *Blood Donor Service, Bavarian Red Cross (BRK Blutspendedienst), Herzog-Heinrich-Str. 4, 80336 Munich, Germany*
[b] *Blood Donor Service, Bavarian Red Cross, Wiesentheid, Germany*
[c] *Miltenyi Biotec, Bergisch Gladbach, Germany*

Received 20 April 2005; received in revised form 23 September 2005; accepted 6 October 2005
Available online 17 November 2005

Abstract

In compliance with federal regulations, blood banks routinely use leukocyte depletion filters to eliminate contaminating leukocytes from blood products such as red blood cell and platelet concentrates. We developed and optimized conditions to elute leukocytes adsorbed to these filters; resulting in leukocyte suspensions which we termed Filter Buffy Coats (FBCs). These Filter Buffy Coats can replace standard buffy coats for various research applications.

After optimizing both the filter elution medium as well as elution protocols, we compared commonly used leukocyte depletion filters from four different manufacturers. Relative fractions as well as total recoveries of leukocyte subsets, such as lymphocytes, monocytes and granulocytes, found in Filter Buffy Coats were identified and compared among the filters as well as to standard buffy coats and whole blood. Flow cytometric analysis of Filter Buffy Coats confirmed the presence of T- and B-lymphocytes, NK cells and monocytes. Furthermore, a significant quantity of $CD34^+$ hematopoietic stem or progenitor cells (HSC/HPC) was detected in Filter Buffy Coats prepared from different filters, thus making FBCs a valuable source for research on HSC/HPC. Colony assays revealed that most of these $CD34^+$ cells are functional. Using immunomagnetic cell sorting (MACS), we isolated a variety of leukocyte populations from FBC mononuclear cells (Filter-PBMCs) including T lymphocytes ($CD4^+$, $CD8^+$, $CD3^+$), B lymphocytes ($CD19^+$), NK cells ($CD56^+$), HSC/HPC ($CD34^+$, $CD133^+$) or dendritic cells ($BDCA\text{-}4^+$). Functional properties of Filter-PBMCs, as well as of some of these isolated leukocyte populations, were confirmed using standard assays.

In summary, Filter Buffy Coats are a valuable and convenient source of different peripheral leukocyte populations and can replace standard buffy coat preparations for research applications.

Keywords: Buffy coat; Leukocyte depletion filter; Filter Buffy Coat; PBMC; $CD34^+$ cells

1. Introduction

Allogeneic leukocytes present in blood cell products such as red blood cell and platelet concentrates are known to cause a variety of adverse transfusion reactions like HLA-induced incompatibilities or viral infections. Furthermore, leukocytes contained in blood

Abbreviations: FBC, Filter Buffy Coat; MNCs, mononuclear cells; HSC/HPC, hematopoietic stem cells/hematopoietic progenitor cells; PDCs, plasmacytoid dendritic cells.

* Corresponding author. Tel.: +49 89 5399 429; fax: +49 89 5399 434.
E-mail address: t.meyer@blutspendedienst.com (T.P.H. Meyer).

0022-1759/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jim.2005.10.004

products can release compounds (such as enzymes or cytokines) upon disintegration, which negatively affect the quality and shelf life of the blood products (Goldman and Delage, 1995; Dzik, 1996; British Committee for Standards in Haematology, 1998; Seghatchian, 2003). Thus, leukocytes should be removed from blood products prior to use and in Germany leukocyte depletion by in-line filtration has been implemented as a regulatory requirement by the national authorities. Subsequently, a variety of different leukocyte depletion filters have been developed and are now routinely used at blood banks for leukocyte removal. These filters are capable of reducing the number of leukocytes in blood products by a factor of up to 10,000. These circumstances led to a shortage of standard buffy coats derived from whole blood after component fractionation by centrifugation, which has been widely used in the past as a leukocyte source in scientific and technical development.

We developed a method to elute leukocytes aseptically from leukocyte depletion filters, thus creating a convenient source of leukocytes (termed Filter Buffy Coat, or FBC) for research purposes. In this study, we show that viable peripheral blood leukocytes can be retrieved from a variety of leukocyte depletion filters currently implemented in blood banking. Filters back-flushed with physiological buffer solutions generated Filter Buffy Coats containing viable peripheral blood mononuclear cells in sufficient amounts for scientific purposes, with cellular compositions similar to standard buffy coats. Furthermore, a European patent application has been submitted.[1] By using immunomagnetic cell sorting (MACS), we isolated a variety of leukocyte populations from Filter Buffy Coat mononuclear cells (Filter-PBMCs), including T- and B-lymphocytes, hematopoietic progenitor cells, monocytes, dendritic cells and NK cells. Further characterization of Filter-PBMCs as well as of MACS isolated cell populations revealed that these cells are functional.

2. Materials and methods

2.1. Leukocyte depletion filters

The following four different leukocyte depletion filters were used to prepare Filter Buffy Coats, to analyze leukocyte recovery and to characterize cellular fractions: Compoflex T3908 (Fresenius Hemocare, Friedberg, Germany), Leukoflex LST-1 (MacoPharma, Tourcoing, France), Leukotrap WBF-3 (Pall Medical, Ascoli, Italy), and Optipure RZ 2000 (Baxter, Unterschleißheim, Germany). These filters are used in routine blood banking and are designed either as hard case (WBF-3, RZ 2000, T3908) or soft case (LST-1) filters. Each filter contains about 30 to 40 different filter layers consisting of unwoven PVC, PP or PET fibers (Bruil et al., 1995). After initial experiments with all four filters, optimal back-flushing conditions were developed with T3908 and LST-1 filters. For all of the following experiments, leukocyte depletion filters were used after leukodepleting 500 ml of whole blood from normal healthy donors.

2.2. Filter elution medium

Chemicals (research purity grade) were obtained from Merck (Darmstadt, Germany).

PBS buffer (pH 7.2–7.4) was prepared according to standard procedures (Mishell and Shiigi, 1980). Alternatively, Dulbecco's PBS (D-PBS) without $MgCl_2$ and $CaCl_2$ (Invitrogen, UK) was used for back-flushing of filters. To obtain the filter elution medium, 5 mM Na_2–EDTA and 2.5% [w/v] sucrose was added to the PBS. Sterile filtration of the filter elution medium was performed prior to use. The pH of the filter elution medium was determined by glass electrode measurement according to the European Pharmacopeia.

2.3. Filter back-flushing

Filters were flushed at room temperature with leukocyte elution medium by attaching a sterile 100 ml syringe to the tubing of the blood bag system using a luer lock adapter. The flow direction of the leukocyte elution medium during filter back-flushing was opposite to the primary blood flow during the leukocyte depletion step. Filter back-flushing was conducted in steps of 50 ml each up to a total volume of 200 ml elution medium between 2 and 8 h after the leukocyte depletion step of whole blood. Applying high pressure and thus increasing the speed of back-flushing was avoided since this may lead to cell loss, cell disruption and, in the case of soft case filters, even to filter leakage. It took approximately 1 to 3 min to flush hard case filters (T3908, WBF-3, RZ 2000), although partially clogged filter units may take longer. Soft case filters, e.g. LST-1, can inflate during back-flushing and thus lead to reduced cell recoveries and increased flushing times. Therefore, soft case filters were trapped in an

[1] European patent application No. 03012551.2: Methods of Preparing Peripheral Stem Cells from Leukocyte Reduction Filters and the uses thereof. Blutspendedienst des Bayerischen Roten Kreuzes.