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Rapid purification of billions of circulating CD19+ B cells directly from leukophoresis samples

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ABSTRACT

The study of the biology and function of B cells, or the dissection and *in vitro* creation of enormous recombinant antibody repertoires, requires the isolation of large numbers of pure CD19+ B cells. The StraightFrom® Leukopak CD19 MicroBead Kit was recently introduced as a fast and robust kit to isolate human CD19+ B cells. This uses paramagnetic microbeads conjugated to high-affinity anti-CD19 monoclonal antibodies to bind B cells in leukapheresis (Leukopak) samples. The overall purity of the isolated cells, together with the characterization of the different CD19+ subclasses, was assessed by flow cytometry using a recombinant (REAffinity) antibody panel, revealing that the method allowed the recovery of over 93% of CD19+ B cells without any pre-purification step. This enables the relatively straightforward purification of all the circulating CD19+ B cells in a single donor.

Introduction

B cells were discovered in the mid-1960s, when Cooper and Good described their antibody secreting role, in the chicken bursa (hence B cells) of Fabricius [1]. Since then, B cells have been found to be responsible not only for the generation and expression of antibodies, but also for the presentation of antigen to T-cells [2,3] and the modulation of numerous essential immune homeostatic functions [4], mediated by cytokine secretion and the expression of specific cell surface receptors [5,6]. While in Hodgkin's lymphoma the malignant cells themselves are derived from different stages of the B cell lineage, the general roles of B-cells in oncogenesis, tumor progression and tumor resistance are poorly understood, with B cells thought to be involved in anti-tumor immune responses ranging from interactions with T cells and humoral immune responses [7,8] to tumor promoting functions [8].

In order to study the biology and molecular function of human B cells, pure populations are required, since the presence of different cell types can indirectly influence B-cell function, either through cell-cell interaction or by the production of cytokines. This is particularly challenging when working with peripheral blood since only 5% to 10% of blood mononuclear cells are B cells, with remaining cells (T cells, macrophages/monocytes and natural killer (NK) cells) having strong indirect effects on B cells. Beyond their physiology, B cells are also important sources of rearranged antibody genes, which are useful in the generation of antibody libraries [9–12] from which therapeutic antibodies have been derived.

Historically, B cells have been purified by the sequential depletion of monocytes, NK cells and T cells from peripheral blood. Although effective, this multistep negative selection procedure was, for many years, remarkably laborious, requiring first the isolation of buffy coat mononuclear cells by density gradient (e.g. Ficoll-Paque) centrifugation, the chemical elimination of NK cells with L-leucine methyl ester [13], and two rounds of neuraminidase-treated sheep red blood cell (SRBC) rosetting to eliminate T cells [14]. More recently, simple, rapid, negative isolation techniques using antibody cocktails targeting unwanted cells have been developed that are able to isolate B cells far more rapidly at up to 90% purity [15,16]. The negative selection approach has been considered essential to the isolation of untouched B cells, where the presence of bound antibodies recognizing cell surface targets may affect B cell physiology.

However, when large numbers of pure B cells are required, and the presence of bound antibodies is immaterial, positive selection strategies, involving either antibodies labeled with paramagnetic beads or cell sorting by flow cytometry, can be more effective. Positive selection

Abbreviations: LP, leukopak; PBMC, peripheral blood mononuclear cell; FACS, fluorescent activated cell sorting; CD, cluster of differentiation; FSC, forward scatter; SSC, Side Scatter

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Table 1
Cell composition before and after cell isolation with Miltenyi StraightFrom® LP CD19 MicroBead Kit.

Cell type	Gating strategy	Subject 1		Subject 2		Subject 3		Subject 4		Subject 5		Mean of all subjects	
		% pre	% post	% pre	% post	% pre	% post	% pre	% post	% pre	% post	% pre	% post
T cells	$CD45^+$, $CD3^+$	48.95	5.56	66.67	7.48	51.18	12.66	59.19	1.67	48.48	5.29	54.89 ± 7.03	6.53 ± 3.59
$CD4^+$	$CD45^+$, $CD3^+$, $CD4^+$	34.65	1.61	36.28	0.49	32.08	4.75	44.36	0.26	27.15	2.29	34.90 ± 5.65	1.88 ± 1.61
$CD8^+$	$CD45^+$, $CD3^+$, $CD8^+$	11.56	3.93	24.80	6.97	17.29	6.45	11.52	1.41	18.40	2.97	16.71 ± 4.94	4.35 ± 2.10
B cells	$CD45^+$, $CD19^+$	10.28	93.98	9.33	91.72	6.62	84.84	12.97	97.98	12.03	94.19	10.25 ± 2.22	92.54 ± 4.34
Monocytes	$CD45^+$, $CD14^+$	22.97	0.03	11.62	0.04	30.30	0.09	17.47	0.02	17.83	0.07	20.04 ± 6.26	0.05 ± 0.03
NK cells	SSC-low, $CD45^+$, $CD14^-$ $CD16^+$, $CD56^+$, $CD3^-$	11.21	0.68	9.05	0.97	9.29	1.81	7.62	2.57	14.21	1.72	10.28 ± 2.28	1.55 ± 0.67
Eosinophils	SSC-high, $CD45^+$ $CD14^-$, $CD16^-$	0.53	0.13	0.76	0.16	0.98	2.03	0.69	0.08	0.76	0.45	0.744 ± 0.14	0.57 ± 0.74
Neutrophils	SSC-high, $CD45^+$ $CD14^-$, $CD16^+$	1.61	0.01	0.17	0.00	0.35	0.00	0.57	0.00	1.10	0.01	0.76 ± 0.53	0.004 ± 0.005

methods have the advantage that only cells characterized by B cell–specific markers are isolated, with the possibility of obtaining high levels of purity. Antibodies are labeled with fluorescent dyes for fluorescent activated cell sorting (FACS) or superparamagnetic nanoparticles (SPNPs) in magnetic activated cell sorting (MACS). Cells labeled with SPNPs are separated from unlabeled cells by passing them through a matrix of ferromagnetic spheres. As the space between spheres is far larger than the cells, when there is no magnetic field or cells are unlabeled they flow freely through the column, minimizing cell stress. When placed in a magnetic separator, the magnetic field is amplified 10,000 times by the ferromagnetic spheres, creating a powerful gradient within the column. This holds magnetically labeled cells in suspension between the spheres, without binding to the column matrix, preventing cell activation and aggregation and allowing efficient washing.

In order to reduce the activation effects of antibodies targeting B cells for positive purification, B cell–specific antibodies with relatively low signaling potential are usually used. Antibodies recognizing CD19 have proved to be particularly effective [17]. As a consequence of cross-linking during the positive selection process, the CD19 antigen is internalized and temporarily downregulated, inducing a resting state in the B cells, with no interference, activation, proliferation or differentiation of CD19-selected cells having been reported [18]. Signaling is further attenuated by using shorter processing times and handling cells in chilled calcium-free buffers. SPNP-based protocols usually use indirect procedures to conjugate B cells to the magnetic microbeads (i.e. incubating the cells with biotinylated anti-CD19 antibodies and then linking the B cells with SPNP labeled anti-biotin) and generally require additional steps to lyse the red blood cells and/or density gradient purification before actual separation is able to take place.

B-cells can also be purified by FACS. This yields greater purity (> 99%) than other methods, but is far more time consuming, due to the relatively low abundance of B cells in blood, and the ability of (even) high speed sorters to process no more than 5000–20,000 events per second. For these reasons, only a small number of cells may be processed at any one time and the purity may drop down to 95%. Moreover, the physical stresses placed upon the cells during the sorting process means that if sorting of large numbers is attempted, cells are inevitably damaged or die, and may become contaminated if the sorter is not maintained within a sterile environment.

For both MACS- and FACS-based purification methods, peripheral blood mononuclear cells (PBMCs) are required. While the preparation of small numbers of PBMCs is relatively straightforward, the purification of large numbers is far more challenging. This can be addressed by the use of Leukopaks (LP), which are derived from the leukapheresis of donors, in which blood is separated into its constituent cells extracorporeally. Leukocytes are retained, while the rest are returned to the donor. Three blood volumes are usually processed, with each LP containing ~20 times more B-cells than a single unit of blood, and almost all a donor's circulating B-cells ($\leq 3 \times 10^9$). In the present work, we have tested a new direct positive selection method based on the use of a novel SPNP labeled anti-CD19 formulation developed by Miltenyi specifically for LPs. While other positive selection methods based on SPNP labeled antibodies are optimized for 1–2 billion cells, this new StraightFrom method is able to purify from up to 10^{10} starting PBMCs (MNCs) cells directly from LPs without the need for prior density gradient purification. The purification of the isolated B cells was tested by flow cytometry using traditional monoclonal antibodies, as well as recombinantly generated REAfinity flow antibodies. Recombinant antibodies, which provide higher lot-to-lot consistency and are further engineered to abolish any non-specific binding to Fc receptors, showed performance equivalent to the traditional monoclonals.

Materials and methods

B-cell purification

B cells were purified using the StraightFrom Leukopak CD19 Microbead Kit, human (Miltenyi Biotec Inc. 6125 Cornerstone Court East San Diego, CA 92121, USA) according to the manufacturer's protocol. In order to test the broad applicability of the protocol, LPs from five different donors were used. However, rather than use full LPs ($< 3 \times 10^9$ cells), each donor was represented by a ¼ LP ($\leq 7.5 \times 10^8$ cells), comprising ~100 mL. If the initial ¼ LP volume was less than 100 mL, it was adjusted to 100 mL using MACS buffer (phosphate buffered saline (PBS), 0.5% bovine serum albumin (BSA), 2 mM EDTA). The magnetic labeling was achieved by adding 2 mL of StraightFrom Leukopak CD19 Microbeads, mixing the sample, and incubating for 15 min in a refrigerator (2–8 °C). Cell separation was then performed using a Multi-24 Column Block (Miltenyi Biotec) in a MultiMACS Cell24 Separator Plus (Miltenyi Biotec) according to the manufacturer's protocol (program POSSEL2 on the instrument). Briefly, 8.5 mL of the sample was loaded per column in two stages (4.25 mL each). The columns were then washed 3 times with 1 mL of MACS buffer for each wash. Finally, the target B cells were eluted using 1 mL of Whole Blood Column Elution buffer.

Flow cytometry analysis

After purification, 100 µL of each fraction (positive, negative, original) was stained by adding 100 µL of MACS buffer, 10 µL of antibody cocktail from the 7-Color Immunophenotyping Kit, human, containing CD56 conjugated to PE, CD16 conjugated to PE, CD4 conjugated to PerCP, CD19 conjugated to PE-Vio® 770, CD3 conjugated to APC, CD8 conjugated to APC-Vio 770, CD45 conjugated to VioBlue® (Miltenyi Biotec) and 10 µL of CD20-PE-Vio770 monoclonal antibody (clone LT20), mixing well, and incubating for 10 min in a refrigerator (2–8 °C). MACS buffer was then added to bring the total volume to 2 mL and

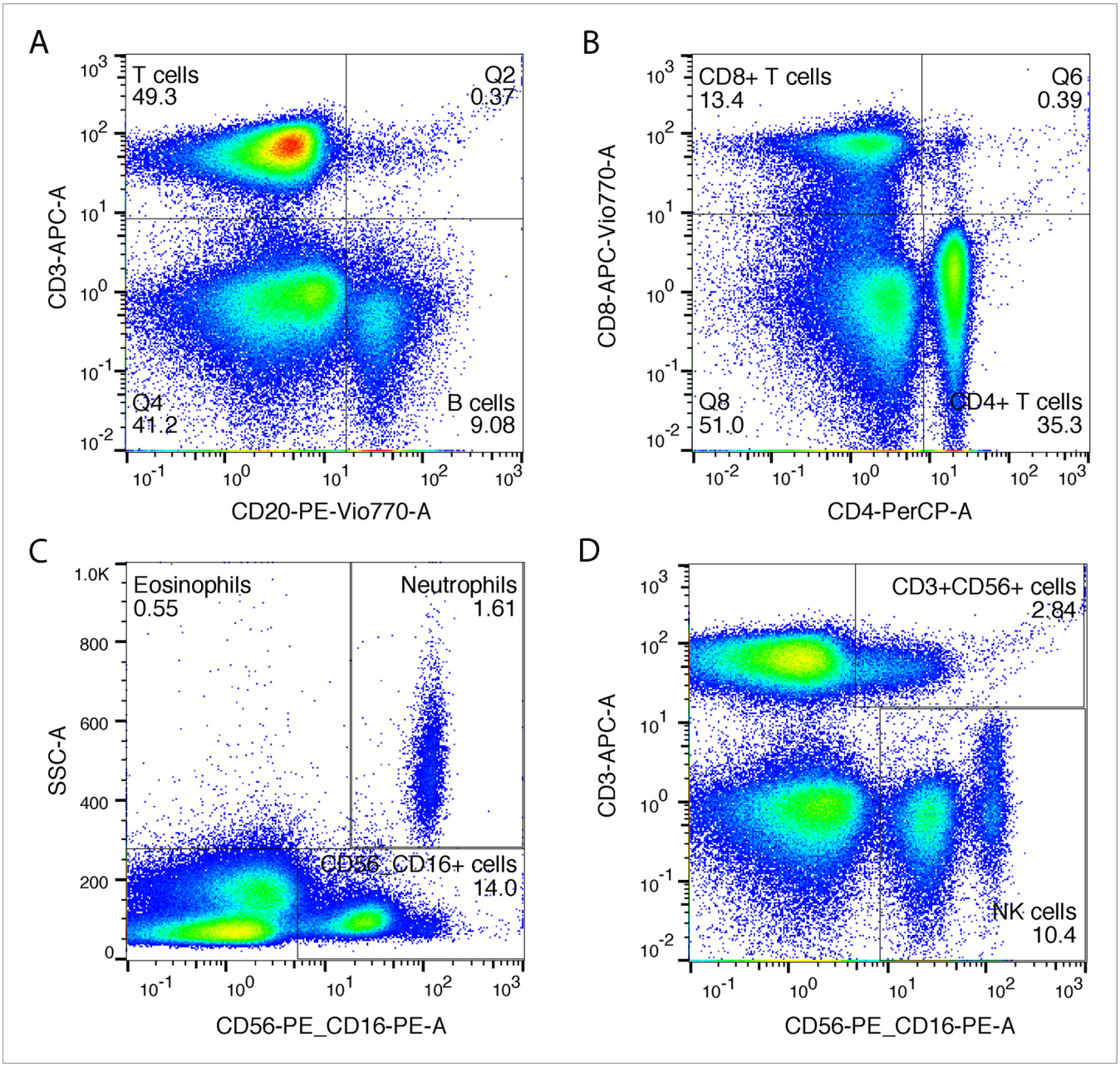

Fig. 1. Staining of an LP obtained from one healthy donor with the 7-Color Immunophenotyping Kit (Miltenyi). (A) Single CD45$^+$ cells were analyzed and CD19 was used to define B cells and CD3 for T cells. (B) The T cell population was further separated into CD4$^+$ and CD8$^+$ T cells. (C) CD16_56/SSC were selected to identify mature neutrophils (CD16_56/SSC^{high}) and eosinophils with absence of CD56 expression. (D) A CD56/SSC^{low} gate was used for further sub-characterization, gating on CD3/CD56$^+$ T cells and CD56/CD16$^+$ NK cells.

samples were analyzed on the MACSQuant 10 Analyzer. Based on the cell counts obtained from the staining using the 7-Color Immunophenotyping Kit, 10^6 cells were removed from each sample in duplicate for subsequent staining with REAffinity panels. Briefly, each sample of 10^6 cells was made up to 1 mL with PBS and washed by centrifuging at 300 × *g* for 10 min and removing the supernatant. The sample was then resuspended in 100 µL of PBS with Viobility 405/520 Fixable Dye (Miltenyi Biotec) diluted to the working concentration per the manufacturer's protocol. Samples were each mixed and incubated at room temperature in the dark for 15 min. The samples were subsequently washed by adding 1 mL MACS buffer and centrifuging at 300 × *g* for 10 min and removing the supernatant. Each sample was then stained with REAfinity antibody clones from Miltenyi Biotec for the following markers (REAffinity clone information shown in parentheses for each marker): CD45-APC-Vio770 (REA747), CD10-VioBlue (REA877), CD20-PerCP-Vio700 (REA780), IgD-PE-Vio770 (REA740), CD27-FITC (REA499), CD38-PE (REA572) according to the manufacturer's recommended titers. Samples were each mixed and incubated for 10 min in a refrigerator (2–8 °C). The samples were then washed by adding 1 mL of MACS buffer, centrifuging at 300 × *g* for 10 min and removing the supernatant. The cells were resuspended in 1 mL of MACS buffer and were immediately analyzed on the MACSQuant 10 Analyzer.

RNA extraction and quality control

Total RNA was extracted from the purified B-cells preserved in RNAlater® (Thermo Fisher Scientific) to help stabilize RNA within the

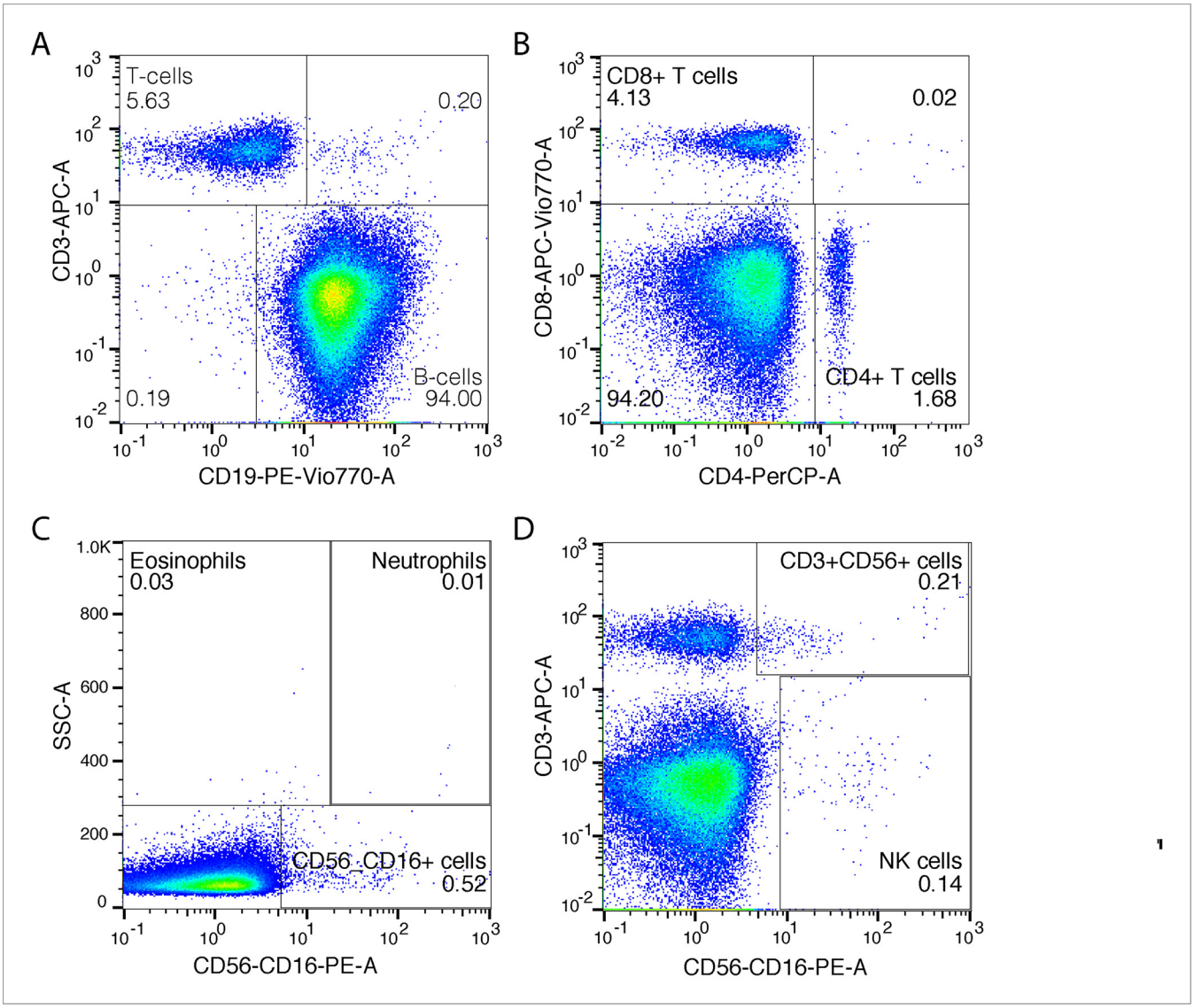

Fig. 2. Staining of a fraction purified with the StraightFrom® Leukopak CD19 MicroBead Kit (Miltenyi) using with the 7-Color Immunophenotyping Kit (Miltenyi). The figure focuses on the B cells defined by CD19/CD3 staining (A) and T cells separated in $CD4^+$ and $CD8^+$ T cells (B).

Table 2
Analysis of the number of purified cells obtained using the Miltenyi StraightFrom® LP CD19 MicroBead Kit.

Subjects	Starting cell numbers	Number of purified cells	recovered B cells
1	5.0×10^9	2.5×10^8	49%
2	4.3×10^9	2.6×10^8	64%
3	3.6×10^9	1.5×10^8	63%
4	5.1×10^9	5.0×10^8	76%
5	3.2×10^9	3.4×10^8	88%
Total	2.1×10^{10}	1.6×10^9	68%

cells and prevent degradation. Centrifuged cells were resuspended in lysis buffer and homogenized using the Qiagen TissueRuptor II homogenizer. Total RNA was purified using the RNeasy Maxi kit (Qiagen) following manufacturer's instructions. The quality of RNA was assessed with the Agilent 2100 Bioanalyzer on RNA 6000 Nano chips.

Results

Purity of B-cells purified with miltenyi StraightFrom® leukopak CD19 MicroBead kit

Flow cytometry analysis of LPs from 5 donors using a panel of recombinant fluorescently labeled REAfinity antibodies recognizing different surface antigens (Table 1) was performed. The analysis was first focused on singlets and CD45 was used to identify all leukocytes. Although cells were originally purified with magnetic beads coated with anti-CD19 antibodies, for the flow cytometry analysis we used an antibody recognizing CD20, an orthologous pan-B cell marker, to stain the purified cells, since the use of a CD19 antibody in the flow cytometry analysis can be inhibited by the presence of the anti-CD19 magnetic beads. CD20 and CD3 were used to discriminate between B and T cells respectively (Fig. 1 panel A), and T cells were further separated into $CD4^+$ and $CD8^+$ subsets (Fig. 1 panel B). To identify mature neutrophils, we gated on $CD16^+$/sideward scatter (SSC) high cells, which could be separated from eosinophils that lack CD16 expression (Fig. 1 panel C). By gating CD56_CD16/SSC low cells, a subset of cells was identified comprising CD3/$CD56^+$ T cells and CD56_$CD16^+$ natural

Table 3
B cell subset composition of the starting Leukopacks samples, the purified cells obtained using the Miltenyi StraightFrom® LP CD19 MicroBead Kit and the remaining fractions. Cell staining performed with recombinant (REAffinity) fluorescent antibody panel.

Subjects	Viable			CD20			Memory CD38+/CD10-/CD27+			Plasma CD20low/CD45-			Naïve CD38+/CD10-/IgD+/CD27-		
	Starting LP	CD19 purified fraction	Remaining fraction	Starting LP	CD19 purified fraction	Remaining fraction	Starting LP	CD19 purified fraction	Remaining fraction	Starting LP	CD19 purified fraction	Remaining fraction	Starting LP	CD19 purified fraction	Remaining fraction
1	93.8	96.4	97.6	11.9	91.3	1.2	27.7	23.4	38.9	0.015	0.023	0.004	48.7	54.3	36.1
2	97.5	95.9	97.5	9.4	86.1	2.4	26.2	23.8	32.2	0.026	0.095	0.031	50.4	51.6	41.1
3	94.7	92.1	92.8	6.1	79.3	1.4	28.1	28.0	35.2	0.078	0.240	0.100	46.0	47.1	36.2
4	97.9	94.3	98.0	14.5	97.8	2.2	18.2	12.9	26.4	0.020	0.026	0.026	65.5	69.2	52.3
5	97.2	92.8	97.2	9.9	85.7	2.5	22.3	22.4	30.0	0.021	0.045	0.110	55.3	54.4	46.2
Mean subjects	96.22	94.3	96.62	10.36	88.04	1.94	24.5	22.1	32.54	0.032	0.086	0.0542	53.18	55.32	42.38

killer (NK) cells (Fig. 1 panel D). The analysis revealed that, prior to purification, the 5 samples contained a mean of 10.25 ± 2.22% B cells, and 92.54 ± 4.34% B cells after purification with the StraightFrom® Leukopak CD19 MicroBead Kit.

After purification, the vast majority of non-B cells in the purified population corresponded to T cells ($CD3^+$), ~30% of which were CD4+ and ~70% $CD8^+$ (Table 1). An example of the flow cytometric analysis of the enriched B cell population is shown in Fig. 2 (the slight modification of the gates in panel A compared to Fig. 1 is to include all the enriched B cells), while Table 2 shows the total number of B cells purified from a ¼ LP. There was significant subject-to-subject variability in the number of recovered B-cells (from 49 to 88%), probably reflecting inherent biological differences since all the purifications were performed the same day using the same experimental setting.

Characterization of the purified $CD19^+$ cell population

Directly after purification, 10^6 cells from each of the $CD19^+$ enriched sample were simultaneously stained with 6 different recombinant antibodies directed against different CDs and a viability dye distinguishing live and dead cells (Table 3). Fig. 3 shows an example of the flow cytometry gate selection strategy from one of the subjects stained with the recombinant antibodies. In detail, to determine the different B cell subsets of interest, the isolated cells were stained for specific subset markers and a multistep gating strategy was established. First, cell debris was gated out based on the cell depiction on the forward and sideward scatter (FSC-A, SSC-A, Fig. 3 panel A). Next, doublets were excluded by gating on single cells (Fig. 3 panel B) followed by excluding dead cells which can give false positive signals (Fig. 3 panel C). The remaining cells were analyzed and classified as plasma, memory/naive and transitional cells according to their expression of CD38 and CD10. Plasma cells display high expression of CD38 and no CD10 expression. Memory cells show a heterogeneous CD38 expression ranging from none to intermediate while transitional B cells are CD38 as well as CD10 positive (Fig. 3 panel D). Plasma cells can subsequently be distinguished as immature plasmablasts, which exhibit a heterogeneous CD20 and CD45 expression pattern, and mature plasma cells, which show low to no CD20 and CD45 expression (Fig. 3 panel E). To discriminate memory cells from naïve B cells the $CD38^{+/-}$ $CD10^-$ cells were analyzed for their IgD and CD27 expression. Naïve B cells display IgD on their surface, but no CD27, while memory cells are positive for CD27. Memory cells can exhibit a switched phenotype indicated by missing IgD on their surface or a non-switched phenotype being positive for IgD (Fig. 3 panel F).

The $CD19^+$ population of live cells comprised 88.04 ± 6.92% $CD20^+$ cells, reflecting the known overlap of these two markers. Further analysis of the cells making up the $CD20^+$ purified fraction showed that the majority were naïve B cells (55.32 ± 8.30%), followed by memory B cells (22.1 ± 5.57%) and very low levels of plasma cells (0.0858 ± 0.090%). Fig. 3 shows an example of flow cytometry-based analysis of the purified $CD19^+$ cell population from one of the subjects stained with the recombinant antibodies.

As further quality control on the purified B-cells, total RNA was extracted from the cells preserved in RNAlater® and the subsequent quality assessment by microchip-based electrophoretic run (Agilent 2100 Bioanalyzer) showed excellent quality of the extracted RNA, thus indicating that the purification process did not affect the cellular or RNA integrity (Fig. 4).

Discussion

In this report, we tested the new StraightFrom® Leukopak CD19 MicroBead Kit, which allows the purification of large numbers of $CD19^+$ B cells directly from LPs, without the need for pre-purification. Leukopaks are leukapheresis samples obtained from normal peripheral blood. A single full Leukopak (~300 mL) contains 1–3 × 10^{10} cells, and

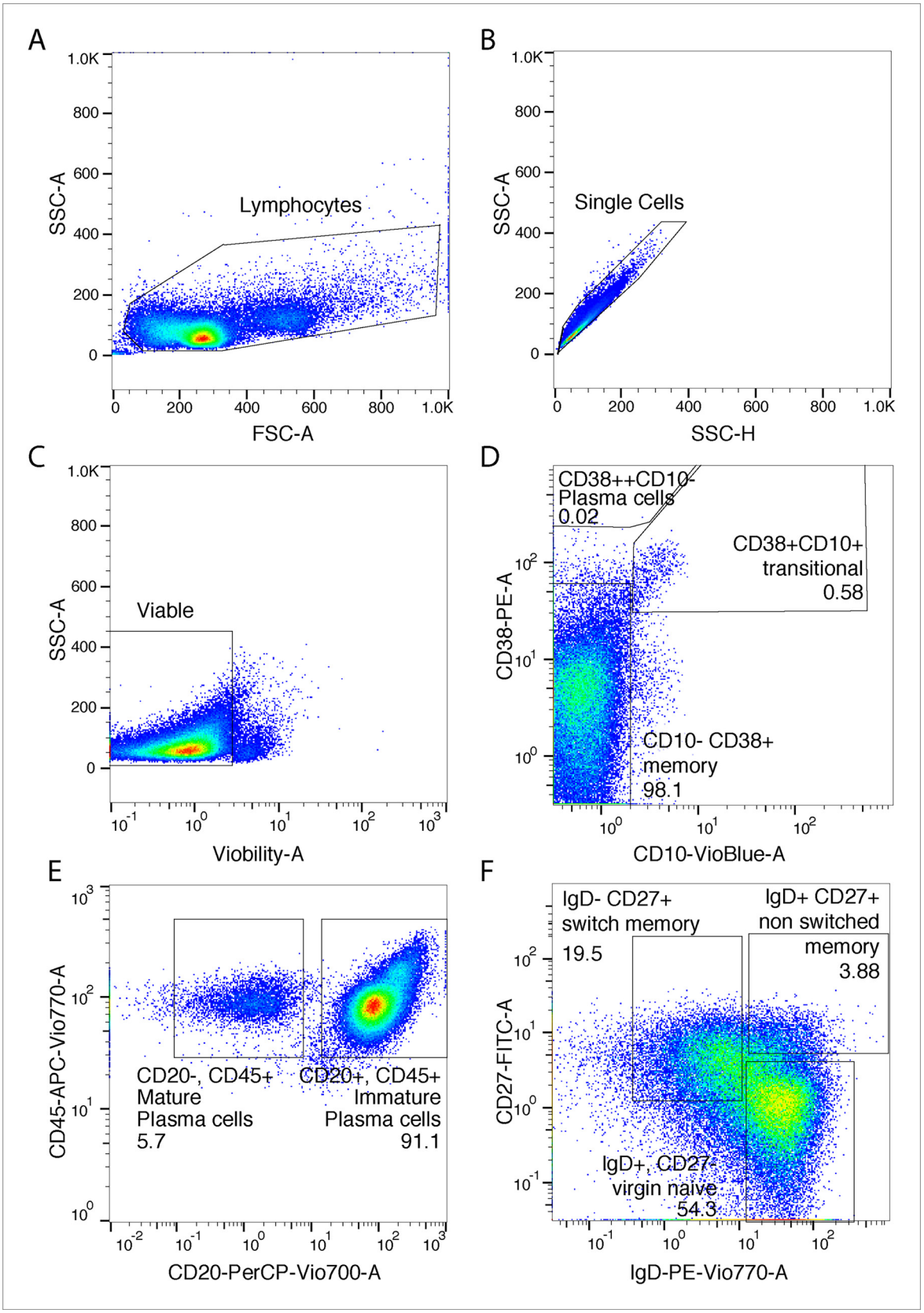

Fig. 3. Determination of the different isolated B cell subsets. (A) Cell debris was gated out using forward and side scatter (FSC-A, SSC-A). (B) Doublets were excluded by gating on single cells, followed by (C) excluding dead cells which could give false positive signals. (D) The remaining cells were analyzed and determined as plasma, memory/naïve and transitional cells according to their expression of CD38 and CD10. Plasma cells express high CD38 and zero CD10. Memory cells show a heterogeneous CD38 expression ranging from zero to intermediate while transitional B cells are CD38 and CD10 positive. (E) Plasma cells can subsequently be distinguished as immature plasmablasts exhibiting heterogeneous CD20 and CD45 expression and mature plasma cells which show low to zero CD20 and CD45 expression. (F) To discriminate memory cells from naïve B cells the $CD38^{+/-}$ $CD10^{-}$ cells were analyzed for their IgD and CD27 expression.

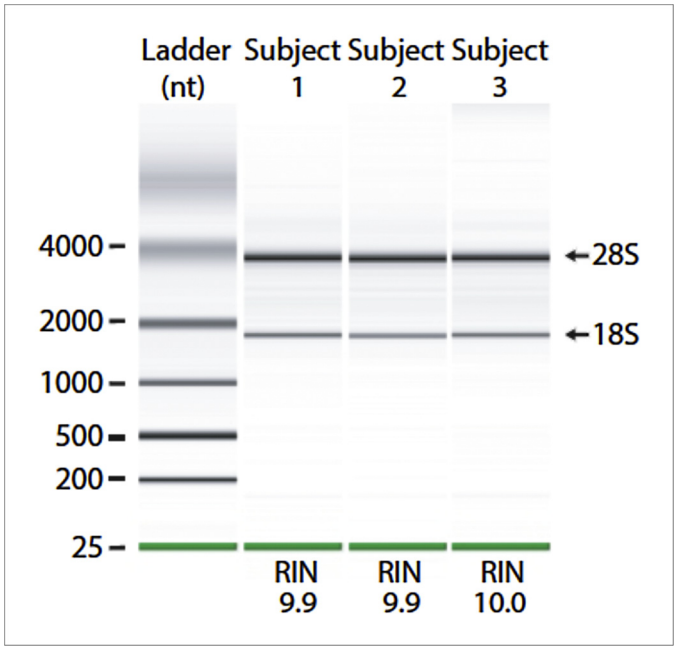

Fig. 4. Quality control of the total RNA extracted from the purified B-cells as assessed by chip-based electrophoretic run. The RNA integrity number (RIN) is reported.

comprises virtually all the circulating PBMCs in a given donor. LPs are highly enriched for PBMCs, which are found at far higher concentrations compared to standard venepuncture collection methods or buffy coat products. In the present study, we started with ¼ LPs from 5 different donors, for a total of $\sim 2.1 \times 10^{10}$ PBMCs. In ~2.5 h we were able to obtain a total of 1.5×10^{9} B cells from the 5 donors (Table 2) with an average purity close to 93%. The final number of purified cells represent 7.1% of the total number of starting cells, indicating that we were able to purify ~68% of the B cells present in the starting cell population.

Although the purity of the isolated population is lower than that theoretically obtainable by FACS (~99%), this scale of purification is practically unachievable by FACS, and would require approximately one week of sorting, without taking into account pre-processing steps such as red cell lysis, gradient centrifugation and elimination of cell aggregates. This estimate assumes sorting for 24 h a day at a maximum speed of 30,000 events per second, with no breaks or breakdowns and cells surviving unharmed for so long, none of which are practically feasible.

Our need for such large numbers of purified B cells is related to our interest in the dissection and *in vitro* creation of enormous recombinant antibody repertoires [9–12]. This makes the enrichment of B cells over plasma cells particularly important, since plasma cells are oligoclonal and express high levels of immunoglobulin mRNA that would bias any libraries. The results obtained here show that the average number of plasma cells in the final purified B cell population was ~0.0867%, similar to the percentage found in peripheral blood, but representing significantly fewer total plasma cells due to the reduction in volume after purification. Consequently, we expect antibody libraries generated from LP-purified B cells to be diverse, and unbiased.

The analysis of cells in the B lineage of the purified population was performed by flow cytometry using REAfinity recombinant antibodies, which are able to discriminate between the different cell populations. We performed these experiments in parallel using antibodies produced by hybridoma cell lines with no statistically significance difference between results obtained with recombinant or hybridoma antibodies (Table 4), demonstrating that recombinant antibodies can be produced as commercially viable replacements for more traditional antibodies

Table 4
B cell subset composition of the starting Leukopacks samples, the purified cells obtained using the Miltenyi StraightFrom® LP CD19 MicroBead Kit and the remaining fractions. Cell staining performed with hybridoma derived antibody panel.

Subjects	Viable			CD20			Memory CD38+/CD10-/CD27+			Plasma CD20low/CD45-			Naïve CD38+/CD10-/IgD+/CD27-		
	Starting LP	CD19 purified fraction	Remaining fraction	Starting LP	CD19 purified fraction	Remaining fraction	Starting LP	CD19 purified fraction	Remaining fraction	Starting LP	CD19 purified fraction	Remaining fraction	Starting LP	CD19 purified fraction	Remaining fraction
1	94.5	95.7	96.9	10.6	92.3	1.02	24.2	21.4	34.0	0.016	0.014	0.014	59.3	59.6	47.1
2	94.9	96.7	93.1	10.3	88.2	2.75	23.6	22.1	29.5	0.026	0.056	0.031	55.1	55.6	45.5
3	93.6	92.0	91.2	6.4	81.5	1.18	25.7	25.1	33.5	0.100	0.150	0.120	52.1	53.6	38.6
4	93.4	93.3	92.0	16.0	96.4	2.41	15.6	12.6	23.6	0.024	0.017	0.038	71.0	74.5	57.6
5	95.3	94.6	91.8	11.4	90.1	2.56	21.3	21.8	25.2	0.037	0.060	0.038	58.6	57.3	52.3

produced by hybridomas, with the advantages of reproducibility and minimal lot to lot variation [19,20], and without the problems associated with hybridoma instability and additional functional V regions [21].

In conclusion, the procedure described represents a straightforward, manageable method to directly purified large numbers of pure B cells directly from leukapheresis treated cells.

Declaration of interest

Martin Kolnik and Daniela Vorholt are employed by Miltenyi, the makers of the StraightFrom® Leukopak CD19 MicroBead Kit used in this study.

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