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MACSxpress® Technology allows isolation of B cells with high purity for sensitive and concordant microarray-based gene expression profiling

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Introduction

Microarray-based gene expression profiling is an excellent tool for the identification of biomarkers in blood cells. However, whole blood and PBMCs, which are frequently used for biomarker research, are complex mixtures of many different cell types and subsets. Therefore, subtle changes in gene expression within a particular subpopulation can escape detection, if these complex mixtures are used for analysis. There is also a large variability in the proportions of blood cell subsets among different donors, which can further mask expression changes within a particular cell type. The use of isolated blood cell subsets has greatly enhanced biomarker research. Lyons *et al.*[1] used purified leukocyte subsets isolated by MACS® Technology for their biomarker research in systemic lupus erythematosus. The authors identified disease-associated differentially expressed genes in purified CD4+ cells and monocytes that were not detectable in PBMCs.

Similarly, Lee *et al.*[2] identified signatures in magnetically purified CD8+ T cells in their biomarker research on Crohn's disease and ulcerative colitis, which they otherwise would not have detected in unseparated PBMCs.

For omics research, in general, it is desirable to have simple and short procedures that are highly reproducible and require minimal handling. The novel MACSxpress® Technology for the fast isolation of cells directly from whole blood meets all these requirements. Using this technology, we isolated B cells from different donors to high purity. The obtained gene expression patterns were distinct from the corresponding whole blood and PBMC samples and showed enrichment of B cell–specific gene transcripts as well as depletion of non-B cell–related gene transcripts.

Materials and methods

B cell isolation

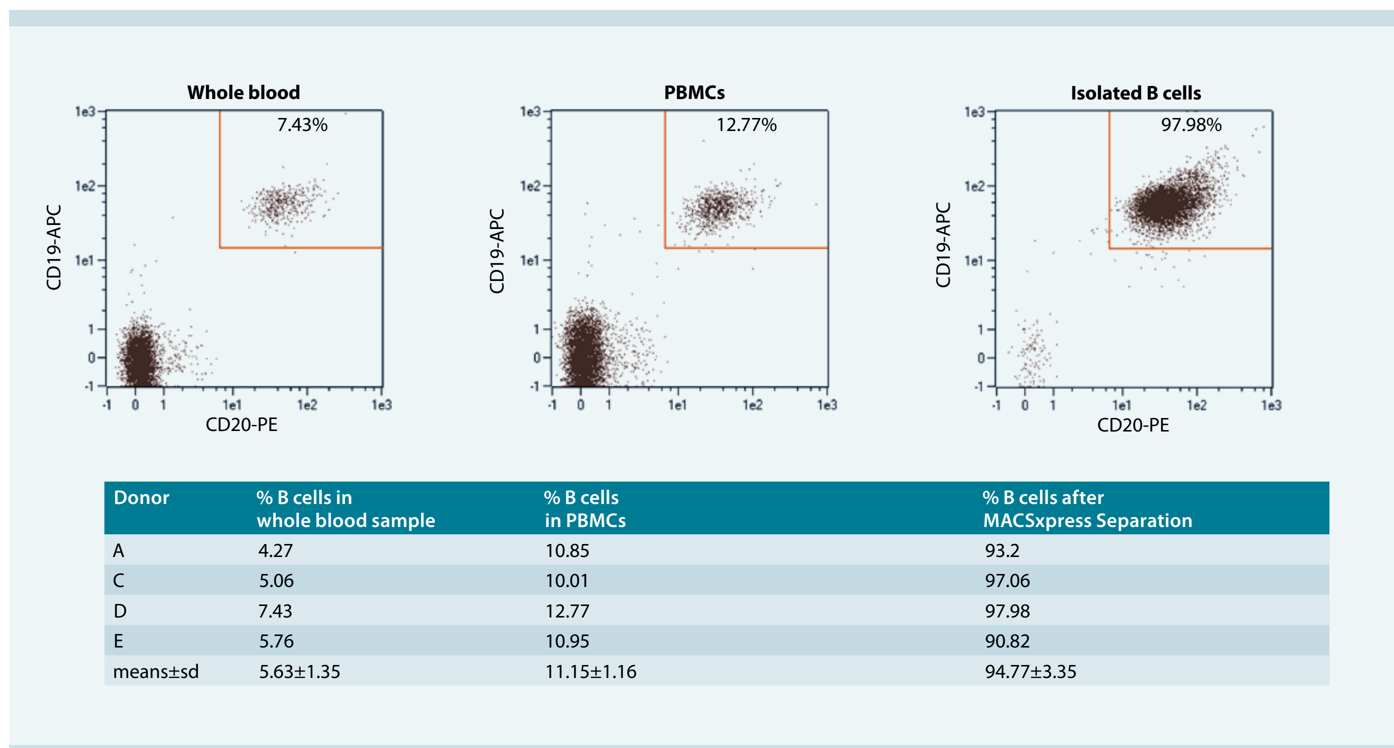
For the isolation of B cells, 30 mL of EDTA-anticoagulated whole blood were incubated with the MACSxpress B Cell Isolation Cocktail for 5 min in a 50-mL tube. During the incubation step, the tube was gently rotated using the MACSmix™ Tube Rotator. The B Cell Isolation Cocktail contains antibody-conjugated MACSxpress Beads magnetically labeling the non-target cells. Following the labeling step, the tube was placed in the magnetic field of a MACSxpress Separator. The labeled non-target cells adhered to the tube wall and the aggregated erythrocytes sedimented to the bottom, whereas the supernatant contained pure unlabeled B cells, which could immediately be used for further experiments. Samples obtained from four healthy donors were processed.

Flow cytometry

To assess the purity of B cells after isolation with MACSxpress Technology, cells were labeled with CD45-VioBlue®, CD19-APC, and CD20-PE, before and after separation. Flow cytometry was performed on the MACSQuant® Analyzer. For comparison, PBMCs prepared by density gradient centrifugation from the same whole blood sample were analyzed.

Sample preparation for microarray analysis

RNA was extracted from whole blood (stabilized with PAXgene® Blood RNA Tubes) using the PAXgene Blood RNA Kit (Qiagen), and from PBMCs and isolated B cells using the NucleoSpin® RNA II system (Macherey-Nagel). RNA quality, i.e., RNA integrity number (RIN), was assessed using the Agilent 2100 Bioanalyzer platform and the integrated software. For linear T7-based amplification, 50 ng of total RNA were used. Cy™3-labeled cRNA was prepared



Donor	% B cells in whole blood sample	% B cells in PBMCs	% B cells after MACSxpress Separation
A	4.27	10.85	93.2
C	5.06	10.01	97.06
D	7.43	12.77	97.98
E	5.76	10.95	90.82
means±sd	5.63±1.35	11.15±1.16	94.77±3.35

Figure 1 **Flow cytometric analysis of whole blood, PBMCs, and isolated B cells.** Cells were stained as indicated in the materials and methods section, and analyzed on the MACSQuant Analyzer. Data were gated on leukocytes. Dot plots are shown for one representative donor. The table summarizes the results for samples from four different donors.

by using the Agilent Low Input Quick Amp Labeling Kit (Agilent Technologies) following the manufacturer's protocol.

Hybridization of Agilent Whole Genome Oligo Microarrays

Hybridization was performed according to the Agilent 60-mer oligo microarray processing protocol using the Agilent Gene Expression Hybridization Kit (Agilent Technologies). Briefly, 1.65 µg of Cy3-labeled fragmented cRNA in hybridization buffer were hybridized overnight (17 hours, 65 °C) to Agilent Whole Human Genome Oligo Microarrays 4×44K V1 using Agilent's recommended hybridization chamber and oven. Subsequently, the microarrays were washed once with the Agilent Gene Expression Wash Buffer 1 for 1 min at room temperature followed by a second wash with pre-heated Agilent Gene Expression Wash Buffer 2 for 1 min at 37 °C. The last washing step was performed with acetonitrile for 30 s at room temperature.

Microarray analysis

Fluorescence signals of the hybridized Agilent Microarrays were detected using Agilent's Microarray Scanner System (Agilent Technologies). The Agilent Feature Extraction Software was used to read out and process the microarray image files. The software determines feature intensities (including background subtraction), rejects outliers, and calculates statistical confidences.

	Whole blood	PBMCs	Isolated B cells
RIN	7.93±0.19	8.63±0.25	8.18±0.51
RNA yield (µg/mL whole blood)	2.6 ±1.96	0.77±0.34	0.064±0.012

Table 1 **RIN values and yields for RNA extracted from different sample materials.** RNA was extracted as described in the materials and methods section. Values represent samples from four different donors (means±sd).

Results and discussion

Isolation of B cells with MACSxpress Technology

MACSxpress Technology allows the isolation of B cells to excellent purities up to 98% (fig. 1). The average purity was about 95% (n=4). The recovery of B cells isolated from whole blood ranged between 68% and 83%.

RNA quality

A RIN value of greater than 5 is considered to indicate that RNA quality is sufficient for gene expression profiling experiments[3]. In our experiments, RNA prepared from whole blood, PBMCs, and purified B cells consistently showed RIN values of approximately 8. RNA yields were sufficient for gene expression profiling experiments (table 1).

Microarray analysis

Expression analysis of various cell markers in whole blood vs. the purified B cell fraction allowed us to further validate the purity of the isolated B cells (fig. 2). As anticipated $CD19^+$ cells were efficiently enriched, as indicated by a 20-fold increase in light units (≈3,000 LU in whole blood vs. ≈60,000 LU in purified B cells).

Figure 2 Microarray-based analysis of cell type–specific gene expression. Gene expression analysis was performed as indicated in the materials and methods section. Data indicate quantile-normalized raw data (light units, LU) for whole blood samples, PBMCs, and isolated B cells, from four different donors (A,C,D,E) each.

Likewise, the lymphocyte-associated marker TCL1A showed an increase from ≈10,000 LU to 100,000 LU, whereas CD14 expression was almost absent (≈10,000 LU vs. ≈50 LU). The reticulocyte/erythrocyte–associated marker HBA1 showed high values of ≈450,000 LU in whole blood samples, which was in the range of saturation, while the purified B cells showed a reduced value of ≈5,000 LU. The values for the neutrophil marker MPO were reduced from ≈250 LU to ≈30 LU, and the platelet marker PF4 from ≈400 LU to ≈2 LU. These results confirm that MACSxpress Technology allowed the efficient removal of non-B cells from whole blood.

We compared expression profiles of whole blood samples, PBMCs, and purified B cells in an unsupervised cluster analysis with 4,100 genes as input. Genes not associated with

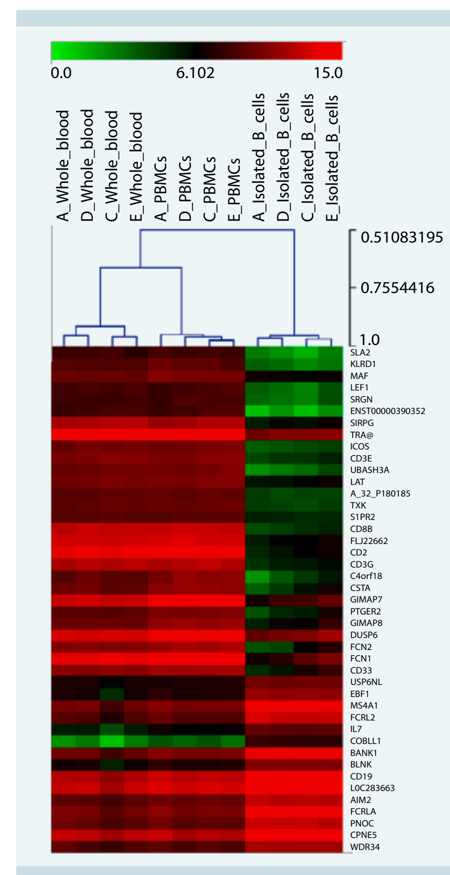


Figure 3 Gene expression profiling of cells isolated by MACSxpress Technology. Gene expression analysis was performed as indicated in the materials and methods section. Data were extracted from an unsupervised heatmap (input 4,100 genes) and are shown for whole blood samples, PBMCs, and isolated B cells, from four different donors each.

B cells, such as CD3, CD8, and CD33, were not represented in the purified B cell fraction. In contrast, the B cell marker CD19 was highly abundant in this fraction (fig. 3). Overall, the heat map reveals considerable differences between purified B cells and the unpurified fractions (whole blood and PBMCs). A two-way analysis of variance showed that 2,111 of 4,100 genes were significantly differentially expressed in purified B cells vs. unpurified cells. This suggests that subtle yet significant gene expression changes within the B cell fraction might escape detection if unpurified cells are used for analysis.

Conclusion

- Gene expression analysis of isolated cell populations is more sensitive and conclusive than analysis of whole blood or PBMCs.
- MACSxpress Technology is a fast method (as little as 20 min) for the efficient isolation of cells from large whole blood volumes (up to 30 mL) requiring minimal handling steps.
- Isolated B cells show high purities.
- The isolation procedure is gentle to cells. Cells can be immediately used for downstream applications.
- RNA extracted from isolated cells is of high quality and suitable for gene expression analysis.
- MACSxpress Technology is an ideal method for the isolation of cells prior to gene expression analysis.

References

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2. Lee, J.C. *et al.* (2011) J. Clin. Invest. 121: 4171–4179.
3. Fleige, S. and Pfaffl, M.W. (2006) Mol. Aspects Med. 27: 126–139.

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MACSxpress Cell Isolation Kits are also available for naive B cells, NK cells, pan T cells, $CD4^+$ T cells, and $CD8^+$ T cells. For further information visit www.macsxpress.com

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