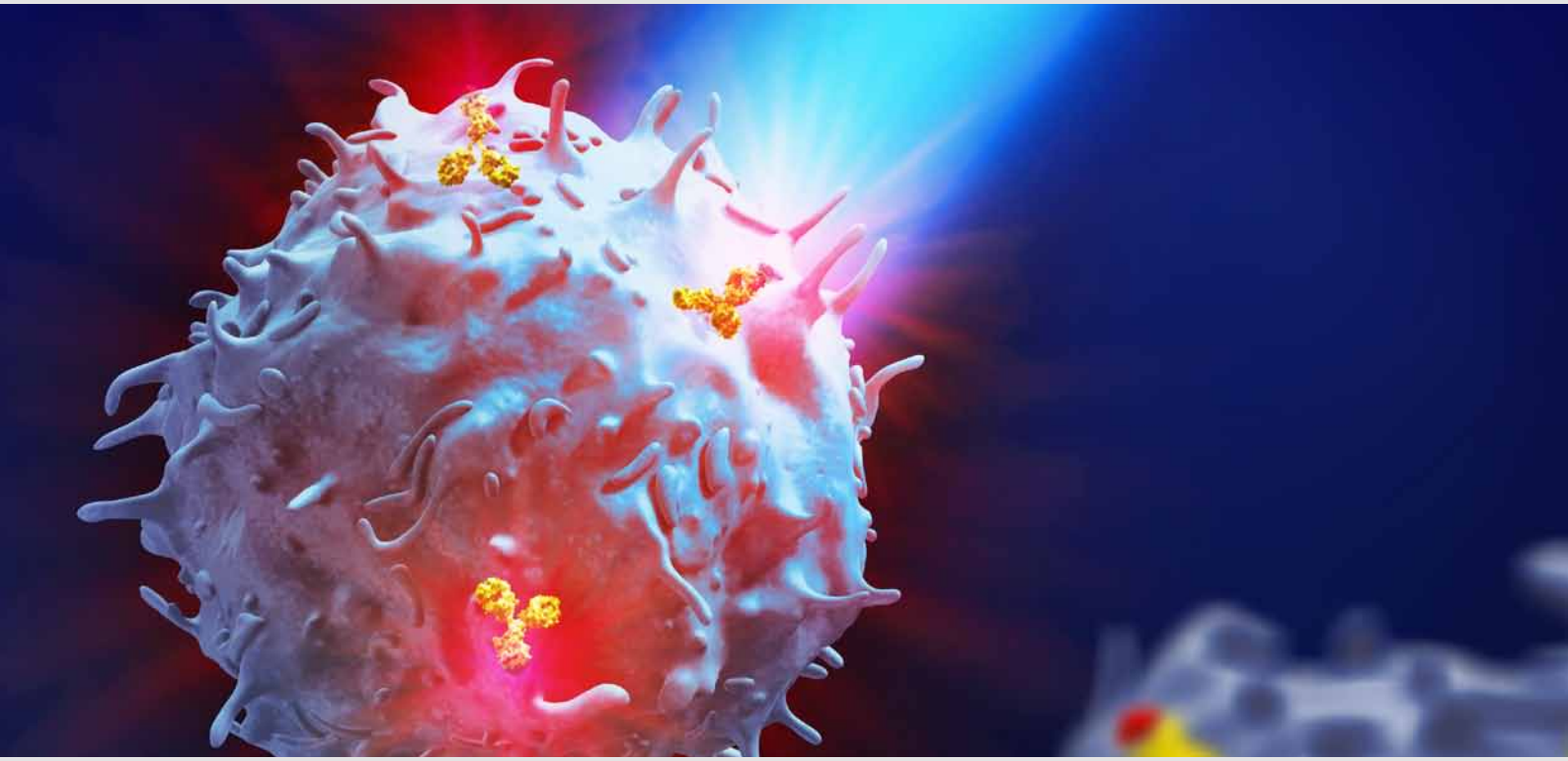
MACS&more

Excerpt from MACS&more Vol 14 – 1/2012

High-sensitivity detection and analysis of rare cells by integrated magnetic enrichment and flow cytometry using the MACSQuant® Analyzer

Dorothee Köhler, Petra Bacher, Alexander Scheffold, Mario Assenmacher, and Manuela Herber

Miltenyi Biotec GmbH, Bergisch Gladbach, Germany



High-sensitivity detection and analysis of rare cells by integrated magnetic enrichment and flow cytometry using the MACSQuant® Analyzer

Dorothee Köhler, Petra Bacher, Alexander Scheffold, Mario Assenmacher, and Manuela Herber
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

The detection of rare cell types in standard flow cytometric assays is restricted by the total number of cells acquired during a single measurement. Pre-enrichment of a rare target population dramatically increases the sensitivity of the subsequent flow cytometric analysis. Enrichment can be achieved, e.g., by magnetic cell separation, which allows rapid processing of large cell numbers.

To gain such high sensitivity, the MACSQuant® Analyzer integrates magnetic pre-enrichment and flow cytometric analysis in a single automated workflow. Simultaneous quantification of absolute target cell numbers is achieved by volumetric measurement.

Typically in flow cytometry, the detection level for rare cells is about one event in 1,000 (0.1%), corresponding to the frequency of, for example, hematopoietic stem cells in peripheral blood. Other cell types, such as endothelial progenitor cells, antigen-specific T cells, or circulating tumor cells (CTCs) may occur at considerably lower frequencies (table 1). Detection of these extremely rare cells requires acquisition and analysis of large cell numbers. Achieving this by conventional flow cytometry is very time-consuming or even impossible due to data file limitations. However, a pre-enrichment step prior to analysis facilitates processing of large cell numbers and increases the ratio between the cells of interest and non-target cells. In addition, avoiding false positive cells by doublet exclusion, using a dump channel etc., improves the detection of rare cells.

Materials and methods

Cell enrichment

SK-BR3 cells spiked into 1×10^8 white blood cells (WBCs) at different amounts were either enriched automatically by using the MACSQuant Analyzer's Cell Enrichment Unit in combination with CD326 (EpCAM) MicroBeads, or manually by using MS Columns and CD326 (EpCAM) MicroBeads.

Cell type	Frequency in peripheral blood
Circulating tumor cells	1 cell per 10^7 cells
Antigen-specific T cells	1 cell per 10^6 cells
Endothelial progenitor cells	1 cell per 10^5 cells
Hematopoietic stem cells	1 cell per 10^3 cells

Table 1 Frequency of rare cell types in peripheral blood.

CMV-positive cells from an HLA-A2$^+$ donor, spiked into 1×10^8 PBMCs from a CMV$^-$ HLA-A2$^-$ donor, were automatically enriched by using the MACSQuant Cell Enrichment Unit. Cells were magnetically labeled by using a combination of CMVpp65$_{495_503}$/HLA-A2-tetramer-PE and Anti-PE MicroBeads.

Aspergillus-specific CD4$^+$ T cells were enriched from human PBMCs (previously stimulated for 6 hours with *Aspergillus fumigatus* lysate) by using the MACSQuant Cell Enrichment Unit. Cells were magnetically labeled by using a combination of CD154-PE and Anti-PE MicroBeads. For cytokine analysis, CD154$^+$ cells were enriched by using CD154-Biotin and Anti-Biotin MicroBeads and two consecutive MS Columns. To reduce cell loss during intracellular cytokine staining, surface staining with CD4-APC and Anti-Biotin-VioBlue was performed on the first column, followed by fixation and intracellular staining of IFN-γ and IL-10 on the second column.

Flow cytometry

Cells were analyzed by flow cytometry using the MACSQuant Analyzer. Additionally, height was selected for doublet exclusion using FSC-H vs. FSC-A.

Results

Enrichment and analysis of CD326⁺ tumor cells

The challenge of reliably detecting circulating tumor cells (CTCs) is to find one CTC among 10^6–10^8 normal blood cells. CTCs express CD326, which allows their magnetic enrichment. Using the MACSQuant Cell Enrichment Unit, up to 10^8 cells can be processed in a single workflow. This significantly increases sensitivity, allowing the detection of 10 cells among 10^8 WBCs. In our experiment we spiked 0, 10, and 100 cells from the breast cancer cell line, SK-BR3, into 1×10^8 WBCs. Magnetic enrichment was performed by MACS® Technology with CD326 MicroBeads, using the MACSQuant Analyzer's Cell Enrichment Unit in comparison to an MS Column (fig. 1). Following either automated or manual pre-enrichment, spiked CD326⁺ cells could be reliably recovered by flow cytometry using the MACSQuant Analyzer.

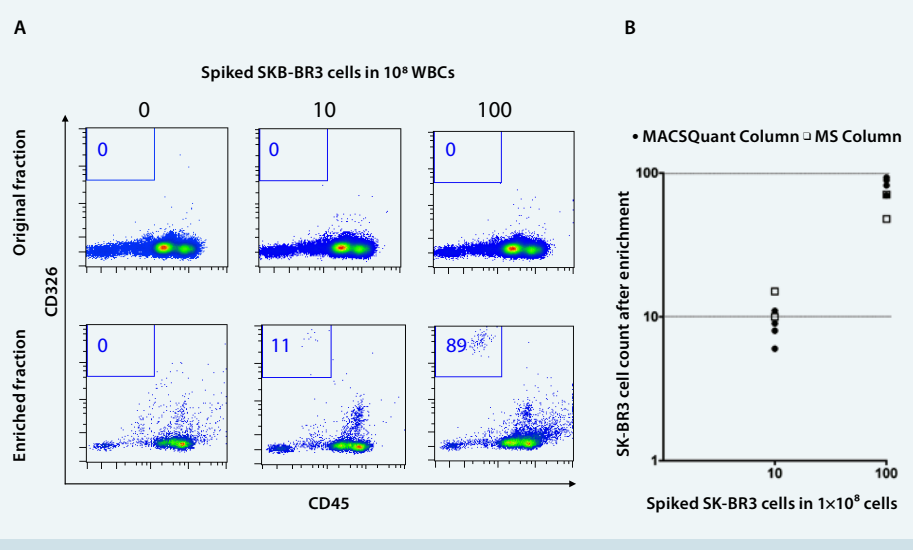


Figure 1 Enrichment and analysis of CD326⁺ tumor cells. CD326⁺ SKB-BR3 cells were spiked into WBCs. CD326⁺ cells were enriched by using the MACSQuant Analyzer's Cell Enrichment Unit prior to flow cytometric enumeration.

Enrichment and analysis of CMV-specific T cells

Likewise, quantification of antigen-specific CD8⁺ T cells can be limited by their extremely low frequencies. In order to define the detection limit, we diluted 0, 5, 10, 50, 100, and 500 CMV⁺ cells from an HLA-A2⁺ donor with 1×10^8 PBMCs from a CMV–HLA-A2⁻ donor. CMV⁺ cells were enriched via CMVpp65$_{495_503}$/HLA-A2-tetramer-PE and Anti-PE MicroBeads using the Cell Enrichment Unit (fig. 2). To reduce background signals, a gate on single cells and $CD3^+CD14^-CD20^-PI^-$ cells was applied. Enrichment prior to analysis greatly increased sensitivity of detection for antigen-specific CD8⁺ T cells.

Enrichment of *Aspergillus*-specific CD4⁺ T cells

Following a 6-hour stimulation with antigen, antigen-specific CD4⁺ T cells express CD154, which allows their detection and enrichment. In our experiment, stimulation with *Aspergillus* lysate induced a defined population of 0.35% CD154-expressing CD4⁺ T cells. Since this frequency was too low for further characterization, the cells were enriched via a CD154-PE antibody and Anti-PE MicroBeads by MACS Technology (fig. 3A). Pre-enrichment allowed further dissection of the

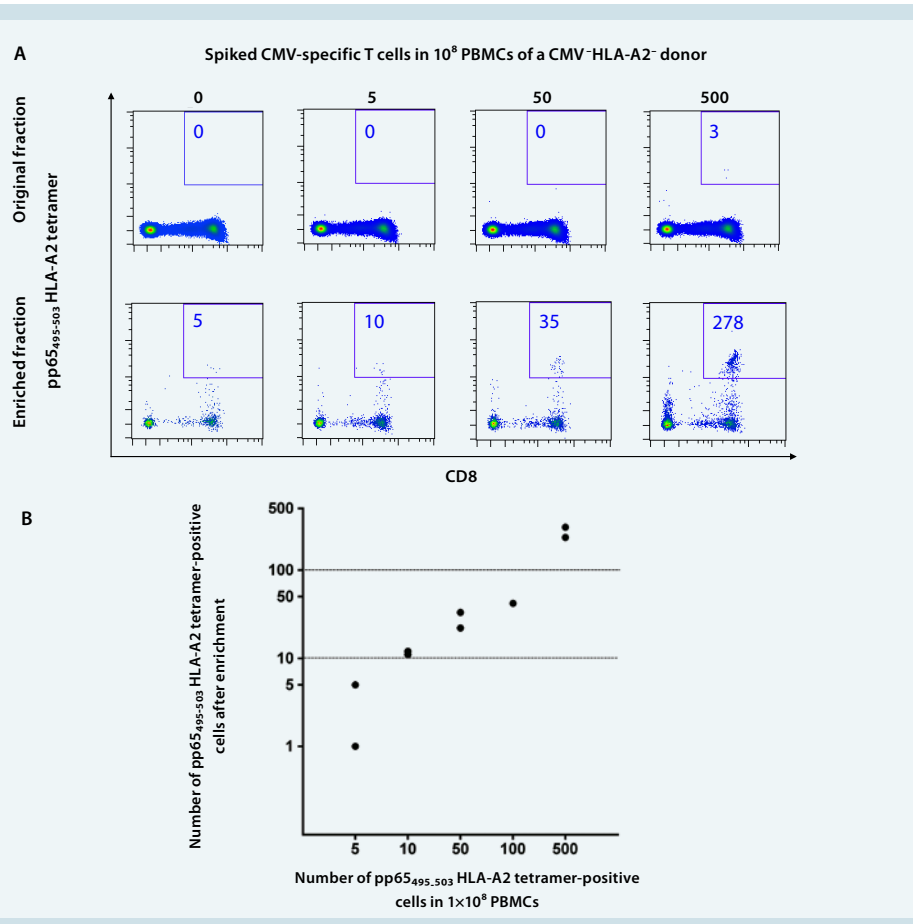


Figure 2 Enrichment and analysis of CMV-specific T cells. Various amounts of CMV-specific T cells were spiked into PBMCs from a CMV-negative donor. CMVpp65$_{495\text{-}503}$/HLA-A2-tetramer⁺ cells were enriched by using the MACSQuant Analyzer's Cell Enrichment Unit prior to flow cytometric enumeration.

total population of rare *Aspergillus*-specific T cells into small, functionally distinct subpopulations producing defined cytokines. Intracellular staining of IL-10 and IFN-γ was performed following enrichment of CD154⁺ T cells via MS Columns (fig. 3B).

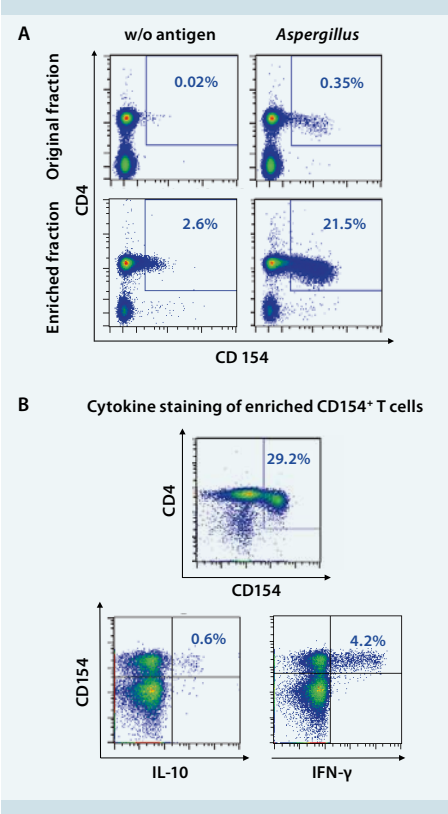


Figure 3 Enrichment of *Aspergillus*-specific CD4⁺ T cells. Human PBMCs were stimulated with *Aspergillus* lysate for 6 hours. Activated CD4⁺CD154⁺ T cells were enriched by using the MACSQuant Analyzer's Cell Enrichment Unit prior to flow cytometric enumeration (A). Pre-enriched CD154⁺ cells were subsequently analyzed for IL-10 and IFN-γ production by intracellular cytokine staining on MS Columns (B). Flow cytometry was performed with the MACSQuant Analyzer.

Conclusion

The MACSQuant Analyzer facilitates both automated enrichment and flow cytometric analysis of rare target cells. This procedure allows the rapid and reproducible identification, quantification, and characterization of rare cells, and thus may provide a technical basis for the development of standardized diagnostic assays. This approach will be an invaluable tool for the routine analysis of rare cells, such as CTCs or antigen-specific lymphocytes.

MACS Product	Order no.
MACSQuant Analyzer	130-092-197
CD4-APC, human	130-091-232
CD154-PE, human	130-092-289
CD154-Biotin, human	130-092-690
Anti-Biotin-VioBlue	130-094-669
Anti-PE MicroBeads	130-048-801
Anti-Biotin MicroBeads	130-090-485
CD326 (EpCAM) MicroBeads, human	130-061-101
MS Columns	130-042-201