A simple and fast method for enrichment of lymphocyte subsets for complement-dependent cytotoxicity assays

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Introduction

Serological cross-match analysis such as complement-dependent cytotoxicity (CDC) assay is routinely done before solid organ transplantation to detect donor-specific antibodies that may lead to graft rejection or dysfunction. This analysis is based on isolated donor lymphocyte subpopulations and recipient serum. Enrichment of cells for CDC can be exceedingly time consuming or result in co-enrichment of non-target or dead cells. With MACSprep™ HLA Cell Isolation Kits, untouched lymphocyte subsets can be isolated from anticoagulated whole blood or spleen cell suspensions within 20 minutes. While erythrocytes are aggregated and sedimented, non-target cells are removed by immunomagnetic depletion with MACSxpress® Beads, yielding untouched and viable target cells.

Methods

1 Magnetic separation of untouched cells from whole blood using MACSxpress® Technology

For the enrichment of B, T cells, or a combination of both cell types, anticoagulated whole blood or cell suspensions obtained from rinsed donor spleens were incubated with the respective bead cocktails for 5 min at room temperature. Then the open tube was placed in the magnetic field of a MACSxpress® Separator for 15 min (fig. 1A). With the tube inside the magnetic field, the supernatant containing the enriched target cells was collected and transferred into a new tube. Magnetically labeled non-target cells as well as aggregated erythrocytes were retained in the tube (fig. 1B).

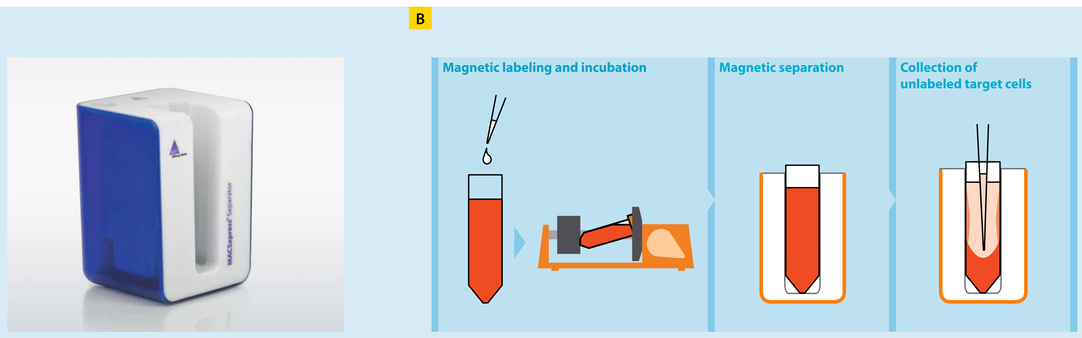

Figure 1

2 Flow cytometric analysis of magnetically enriched lymphocytes

Purity, recovery, and viability of magnetically enriched cell populations were determined by flow cytometric analysis using a MACSQuant® Analyzer. T cells and B cells were identified based on CD3 and CD19 expression on the cell surface, respectively (fig. 2). CD56 counterstaining was used to exclude NK and NKT cells, propidium iodide to analyze viability of enriched cells. Shown are pre-gated CD45⁺ leukocytes after dead cell exclusion.

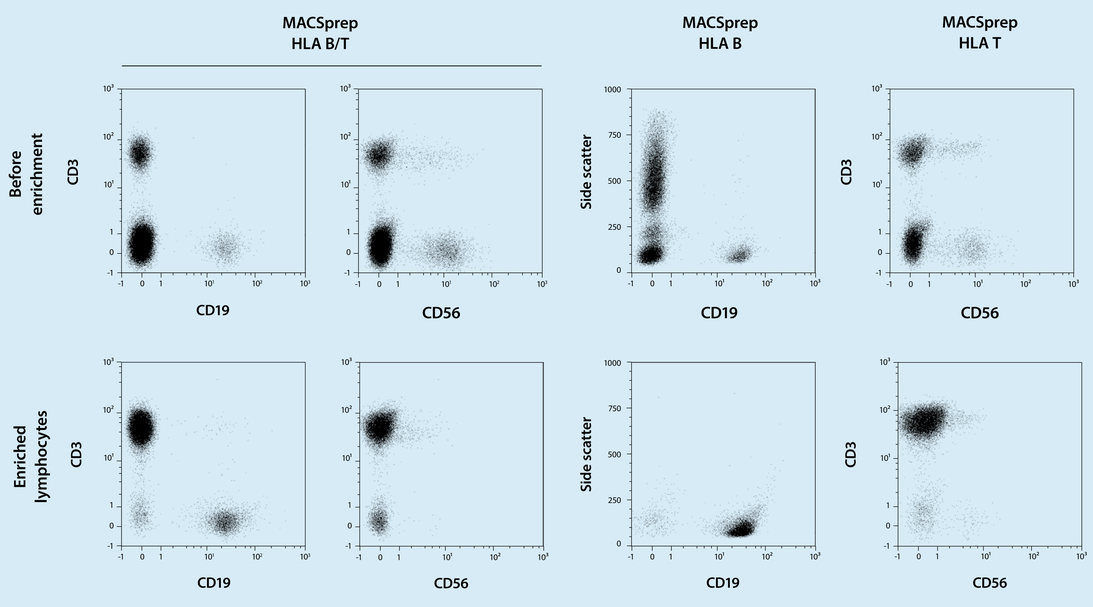

Figure 2

Results

1 Yield, purity, and viability of lymphocyte subpopulations obtained with the new separation technology

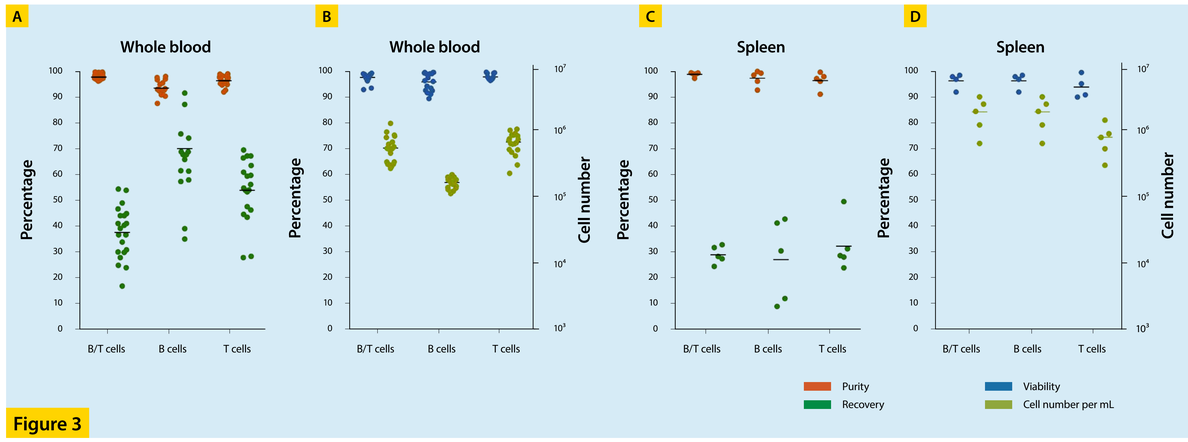

Figure 3

Whole blood samples from healthy donors were used to enrich lymphocyte subpopulations. Purities and recoveries were assessed by flow cytometry using a MACSQuant Analyzer. Magnetically enriched lymphocyte subsets (B/T, B, and T cells) from whole blood had average purities of 98, 94, and 96% with yields of 40, 70, and 54%, respectively (n = 21, 17, and 19; fig. 3A). Viability analysis of enriched fractions showed that the frequency of dead cells was below 5% for all samples (fig. 3B). The figure further depicts the yield as cell number per milliliter of starting material to illustrate that for a subsequent CDC assay it is sufficient to start with whole blood volumes as low as 1–2 mL.

To test the enrichment from human spleen samples, cells were flushed out of the tissue using RPMI medium to obtain cell suspensions. The cell suspensions were then filtered and used directly for MACSxpress Separation without any further processing. Enrichment of B/T, B, and T cells resulted in purities of 98, 97, and 96% with recoveries of 29, 27, and 32%, respectively (n = 5; fig. 3C). Viability of the enriched fractions varied due to the age of the samples but was never below 90%. Numbers of enriched cells reflect the frequency of lymphocytes in the spleen with a higher B cell / T cell ratio (fig. 3D).

2 CDC assay with lymphocytes enriched by MACSprep™ HLA Cell Isolation Kits

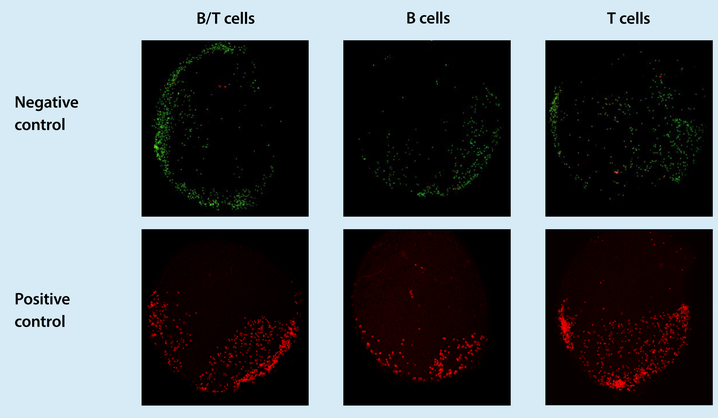

Figure 4

The suitability for functional applications of lymphocyte subpopulations enriched by MACSprep™ HLA Cell Isolation Kits was analyzed in CDC assays. Enriched cells from healthy donors were incubated in Terasaki plates with Anti-HLA–positive or –negative control sera from Bio-Rad® (fig. 4) before supplementation with rabbit complement. FluoroQuench™ AO/EB was used as detection reagent for fluorescence microscopy analysis. Viability of cells in negative controls was comparable to flow cytometry analysis (fig. 3) confirming that magnetically enriched cells can be used for CDC assays. One representative data set from a single donor is shown.

Conclusion

This new procedure for the enrichment of lymphocyte subsets from whole blood and spleen tissue represents the fastest, simplest, and most convenient method currently established.

- The MACSprep HLA Cell Isolation Kits based on MACSxpress Technology are best suited for the rapid cell isolation from small samples of whole blood or spleen samples without the need for centrifugation equipment and time-consuming PBMC preparation.
- The isolation procedure is completed within only 20 min, which cannot be achieved with any other method available.
- The enriched lymphocytes can be used for all downstream applications like CDC assays