Immunomagnetic enrichment of regulatory T cells directly from whole blood, without the need for density gradient centrifugation

Philipp Steinbrück[1], Anne Richter[1], Sindy König[1], Thordis Hohnstein[2], Ramona Siemer[1], Tobias Schenk[1], Petra Bacher[2], Alexander Scheffold[2], and Christian Dose[1]
[1] Miltenyi Biotec GmbH, Bergisch Gladbach, Germany | [2] Department of Cellular Immunology, Clinic for Rheumatology and Clinical Immunology, Charité – University Medicine Berlin, Berlin, Germany

Introduction

CD4$^+$CD25^{high}CD127$^{dim/-}$ regulatory T (Treg) cells play a pivotal role in maintaining peripheral tolerance of non-specific immune responses or the suppression of excessive immune reactions. Thus, the detailed phenotypic and functional characterization of these cells is becoming more and more important for basic and translational research. Accordingly, there is a considerable need for continually improved and reliable cell isolation protocols. We introduce here a rapid, easy, and convenient process for the isolation of Treg cells directly from anticoagulated whole blood. Enriched Treg cells were capable of expanding *in vitro* for several weeks while maintaining stable FoxP3 expression. The new cell separation strategy provides the basis for the MACSxpress® Treg Isolation Kit.

Methods

Enrichment of CD4$^+$CD25^{high}CD127$^{dim/-}$ Treg cells from whole blood combining MACSxpress® and MACS® MicroBead Technologies

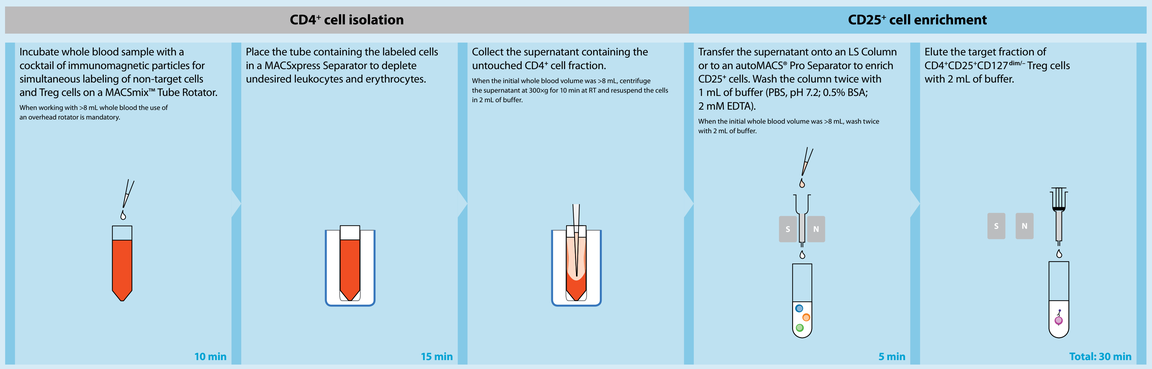

Figure 1

Flow cytometry analysis for evaluation of Treg cell enrichment
The following gating strategy was applied to detect CD4$^+$CD25$^+$CD127$^{dim/-}$ Treg cells by flow cytometry before and after enrichment: CD45$^+$ cells (left), CD4$^+$ T cells (middle), CD25$^+$CD127$^{dim/-}$ cells (right).

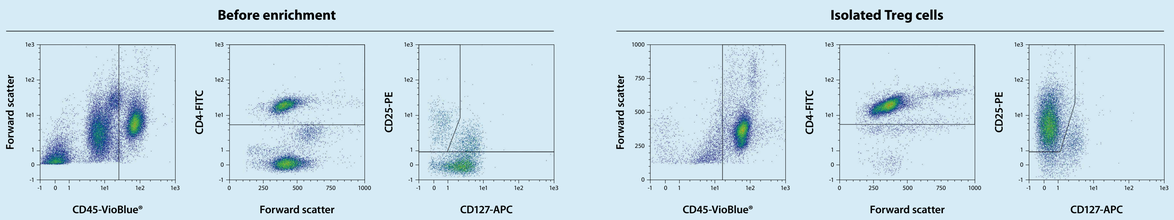

Figure 2

Results

1 Treg cell isolation directly from whole blood within just 30 min

We developed a new cell separation strategy offering a variety of advantages compared to conventional protocols. The process does not require density centrifugation for PBMC preparation prior to magnetic separation. Additionally, the combination of MACSxpress and MACS MicroBead Technologies enables the simultaneous immunomagnetic labeling of both non-target and Treg cells in a single step. For precious and time-sensitive samples this easy-to-perform protocol therefore provides the method of choice: 30 min after starting the cell separation process the enriched Treg cells are ready for functional downstream assays without any further washing/preparation steps (fig. 3). The process is implemented in the MACSxpress Treg Isolation Kit.

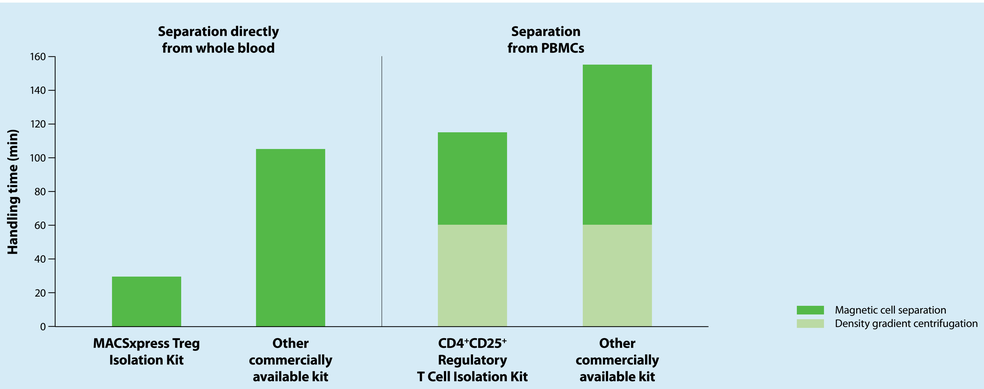

Figure 3

2 Yield and purity of CD4$^+$CD25$^+$CD127$^{dim/-}$ Treg cells obtained with the new separation strategy

Whole blood samples from healthy donors (n=11) were used to directly enrich Treg cells with the newly developed process (MACSxpress Treg Isolation Kit). In parallel, PMBCs were isolated from the same samples using density gradient centrifugation. Treg cells were then enriched from PBMCs using Miltenyi Biotec's CD4$^+$CD25$^+$CD127$^{dim/-}$ Regulatory T Cell Isolation Kit II or CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit.

High yield of isolated CD4$^+$CD25$^+$CD127$^{dim/-}$ Treg cells
With the new isolation procedure we obtained Treg cell numbers of around 3.5×10^4 cells per mL of whole blood, which were even higher than the numbers obtained with the CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit or the CD4$^+$CD25$^+$CD127$^{dim/-}$ Regulatory T Cell Isolation Kit II (fig. 4). This increase in cell yield was mainly due to the omission of the density centrifugation step.

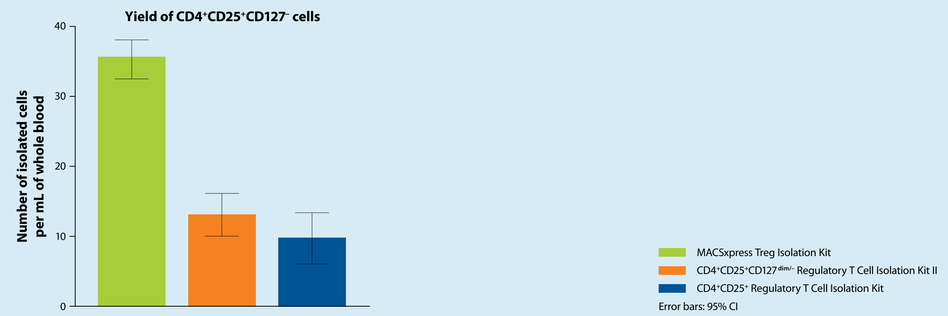

Figure 4

High purities of CD4$^+$CD25$^+$CD127$^{dim/-}$ Treg cells
Next we compared the purities of Treg cells directly isolated from whole blood and Treg cells enriched from PBMCs using Miltenyi Biotec's kits for the isolation of CD4$^+$CD25$^+$ and CD4$^+$CD25$^+$CD127$^{dim/-}$ cells. The new MACSxpress Regulatory T Cell Isolation Kit provided a purity of 90% for CD4$^+$CD25$^+$ cells and 75% for CD4$^+$CD25$^+$CD127$^{dim/-}$ cells (fig. 5). Only the CD4$^+$CD25$^+$CD127$^{dim/-}$ Regulatory T Cell Isolation Kit allowed for an even higher purity of 95% and is recommended when purity matters most.

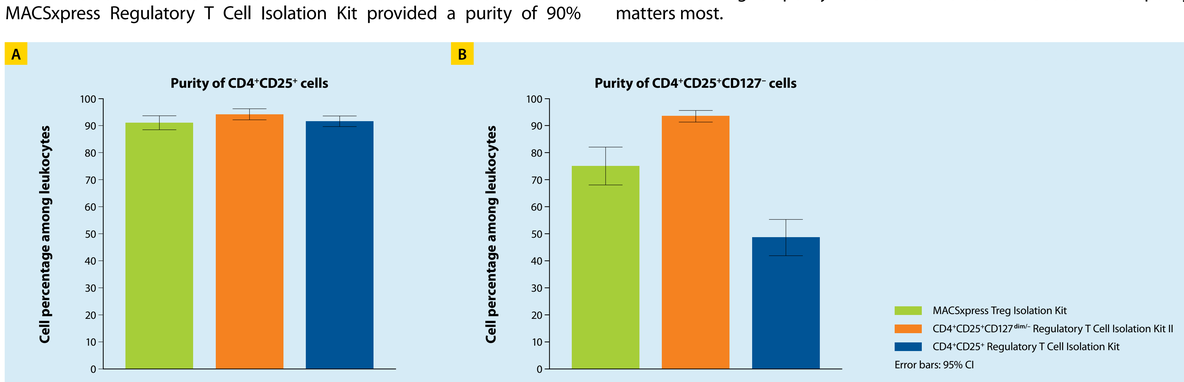

Figure 5

3 Expansion of enriched Treg cells

Isolated Treg cells were expanded in the presence of Rapamycin for 21 days, using the Treg Expansion Kit, human from Miltenyi Biotec. Purity of FoxP3$^+$ cells prior to expansion amounted to 65.9%. The expression of FoxP3 was stable during the entire expansion process. The expansion rate was 22-fold after 14 days and 40-fold after 21 days, which was within the expected range of expansion of Treg cells isolated with other seperation methods.

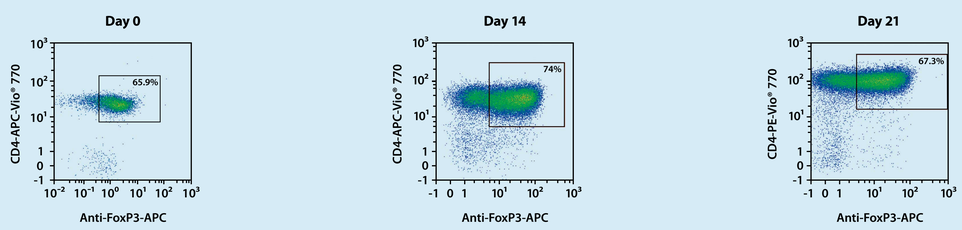

Figure 6

Conclusion

- The new strategy for the enrichment of Treg cells from whole blood combines the advantages of MACS MicroBead Technology with the benefits of MACSxpress Technology, resulting in the fastest, simplest, and most convenient immunomagnetic enrichment of Treg cells. The process is implemented in the MACSxpress Treg Isolation Kit.
- The MACSxpress Treg Isolation Kit is best suited for the fast Treg cell isolation from small samples of whole blood without the need for centrifugation equipment and time-consuming PBMC preparation.
- The isolation procedure is completed within only 30 min, which cannot be achieved with any other method available.
- The enriched Treg cells can be used for various downstream applications, e.g., cultivation, expansion experiments, and functional analysis.