Development of an isolation method for CD3$^+$/CD56$^+$ NKT cells

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Introduction

Natural Killer (NK) cells (CD56$^+$/CD3$^-$) are currently under investigation for cellular immunotherapy. As it is presently not clear which role Natural Killer T (NKT) cells (CD3$^+$/CD56$^+$) might play in such grafts, they are usually removed before NK cell infusion. To achieve NK cell grafts with low content of conventional CD3$^+$/CD56$^-$ T cells and CD3$^+$/CD56$^+$ NKT cells, T and NKT cells are first depleted according to the expression of CD3 before NK cells are enriched according to CD56 expression.

Knowledge on NKT cell function is limited and more investigations are needed for their further characterisation. These investigations might propose that the purification of NK cells could be reduced to just one step – a CD56 enrichment. This would not only decrease the processing time for NK cell products by reducing the amount of processing steps but would probably also enhance the recovery of the NK cells due to a reduced amount of processing steps and potential lack of overnight storage before processing.

Methods

We developed a small–scale procedure to isolate NKT cells for basic research by MACS® Technology. This method is based on magnetic labeling and depletion of monocytes and NK cells in the first step followed by magnetic labeling and enrichment of remaining NKT cells (see figure 1).
The monocytes and NK cells are depleted from the PBMC sample according to their expression of CD14, NKp46 and/or CD16 with a cocktail of these antibodies conjugated to biotin in combination with Anti-Biotin MicroBeads. This depletion step is performed by using an LD Separation Column in the magnetic field of a MidiMACS Separator. In the following step, the remaining NKT cells are enriched according to their expression of CD56: the non-labeled fraction of the depletion step – containing the NKT cells – is labeled with CD56 MicroBeads and separated over an MS or LS Column.

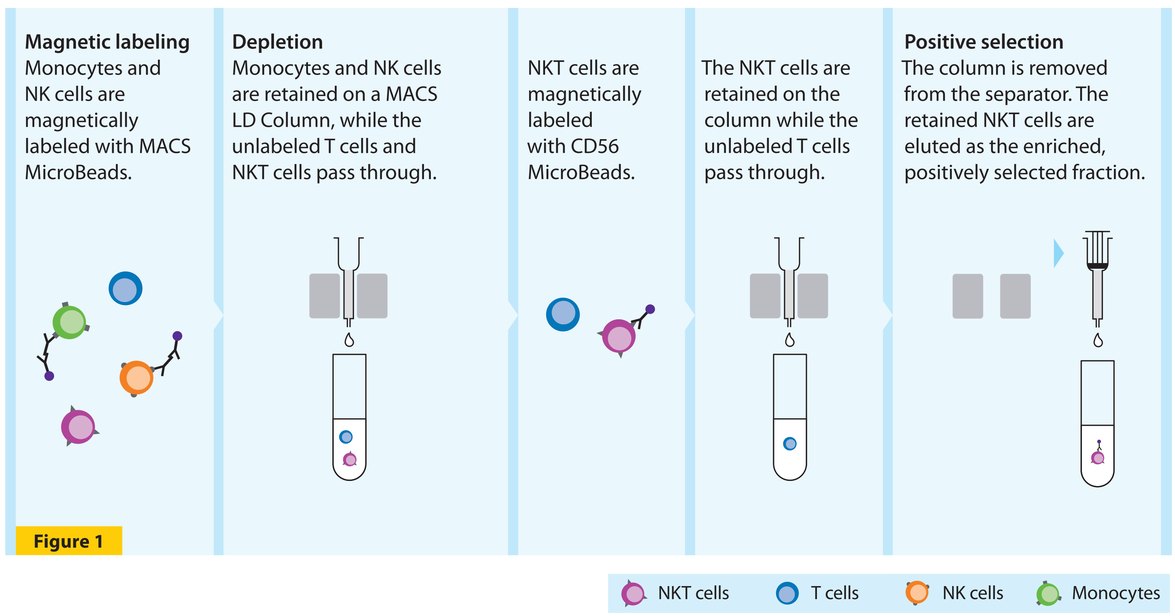

Figure 1

Results

1 NKT cell separation by CD14, NKp46, CD16 depletion followed by CD56 enrichment

Figures 2 and 3 show typical results of the described procedure achieved with two different samples. After the first step nearly all of the NK cells and monocytes are depleted from the sample and most of the NKT cells are in the non-labeled target fraction (yield = 70% ± 8.4%; n = 10). As especially most of the NK cells are depleted the enrichment of the NKT cells using their expression of CD56 is possible. The performance seems to correlate with the frequency of the NKT cells in the unseparated sample. The lower the NKT cell concentration in the sample, the lower the final purity in the target cell fraction after CD56 enrichment. We achieved purities of 89.5% and 99% in the enriched target fraction for samples with low (7%) and high (12%) starting frequencies of NKT cell (see figures 2c and 3c). Nevertheless, final purity may not only be a function of starting frequency of NKT cells but also depend on CD56 expression. Figure 3 shows that real purity may be higher than that obtained by the initial gating.

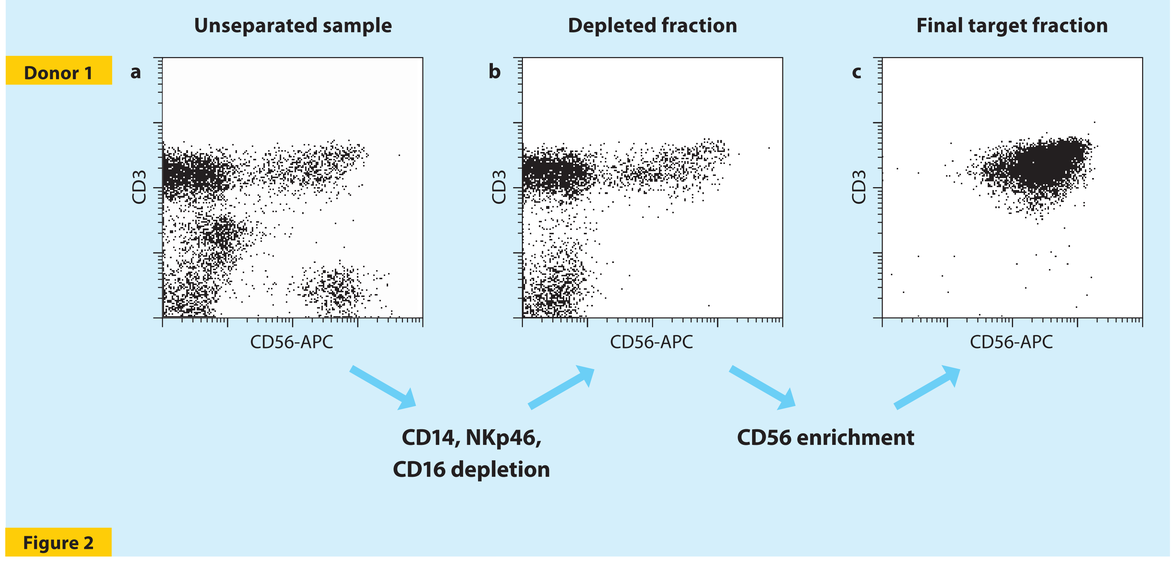

Figure 2

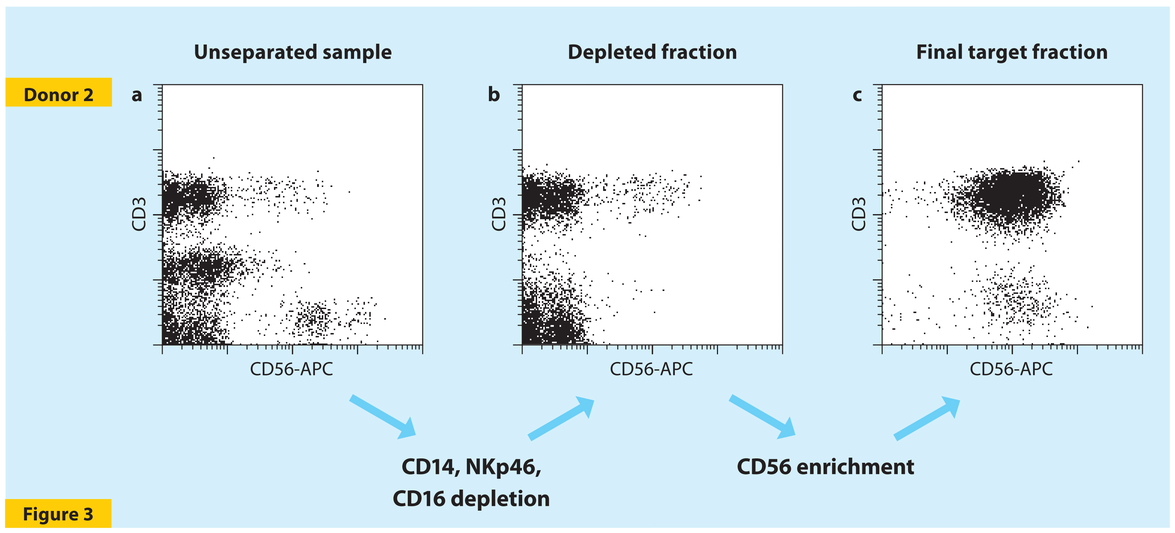

Figure 3

2 Competition with CD56 MultiSort followed by CD3 enrichment

NKT cell separation is also possible with the CD56 MultiSort Kit (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). First, NK and NKT cells are enriched with CD56 MultiSort MicroBeads. The beads are then released and depleted from the cells with the aim to allow a second positive selection. NKT cells are finally enriched according to their CD3 expression with CD3 MicroBeads.

Both selection methods led to comparable final purities and yields (see figure 4). The new method, however, has the advantage of being less time-consuming and requiring only two instead of three separation columns.

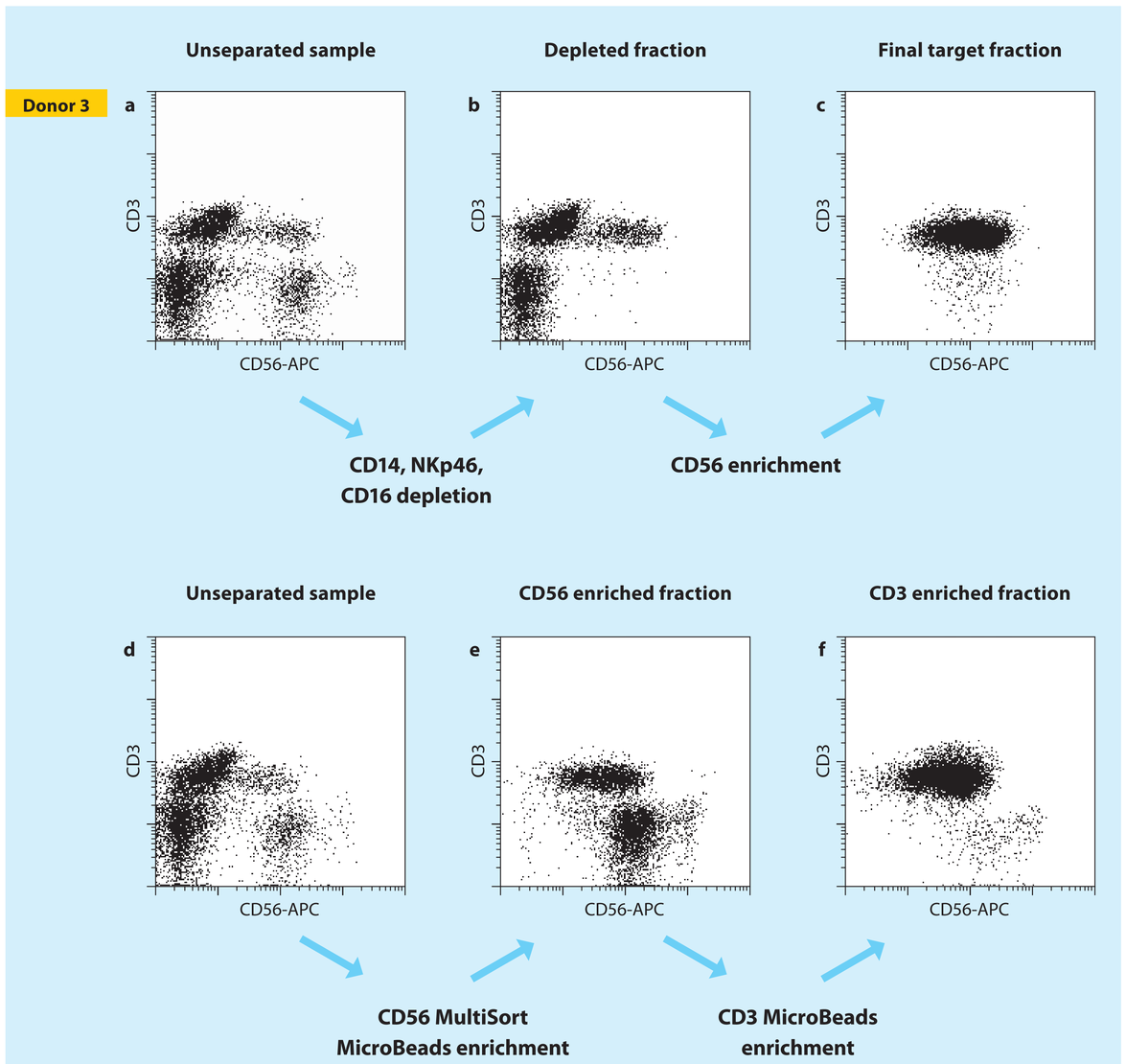

Figure 4

Conclusion

A suitable small-scale two-step method based on MACS® Technology was developed for the separation of NKT cells from a PBMC sample. In this process, monocytes and NK cells are first depleted according to their expression of CD14, NKp46 and/or CD16. Then the remaining NKT cells are enriched by a positive selection with CD56 MicroBeads.
It was demonstrated that it is possible to achieve final purities of more than 90%, depending on the NKT cell frequency in the unseparated sample. Additionally, the results of this new method are comparable to results obtained with the already available MACS CD56 MultiSort Kit.

In the next development step the method has to be evaluated with many different donor samples for method validation. Furthermore, we will demonstrate that the autoMACS Separator (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) can be used for this NKT cell separation procedure.
A commercially available "NKT Cell Isolation Kit" (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) may provide a useful tool for NKT cell research. Data on NKT cell function achieved by using this method may provide arguments for either maintaining the CD3$^-$/CD56$^+$ NK cell isolation procedure or simplifying to NK/NKT isolation by CD56 enrichment only for clinical-scale NK cell preparation.