Fast isolation of NK cells directly from human whole blood yields reproducibly high NK cell purities

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Introduction

Natural killer (NK) cells play a central role in the innate immune system and modulate various immune responses. They hold great potential for the development of clinical applications including anticancer therapy. To gain a better understanding of NK cell functionality it is important to investigate pure cell populations. However, isolation of these cells from blood samples is usually time consuming, and long separation processes may affect subsequent functional analyses.

Methods

The recently developed MACSxpress® Technology allows for a fast (20 min) isolation of NK cells directly from whole blood, without the necessity of performing density gradient centrifugation. The cell isolation cocktail (MACSxpress NK Cell Isolation Kit, human) is added to a tube containing up to 30 mL of anticoagulated whole blood. In our small-scale experiments we used only 2 mL of whole blood. After a 5-minute incubation period, the tube is placed in the magnetic field of a MACSxpress Separator. Aggregated erythrocytes and platelets sediment, and magnetically labeled non-target cells are retained in the strong magnetic field. The supernatant, containing the target cells, is collected and transferred into a new tube (fig. 1). The cells are immediately ready for downstream assays.

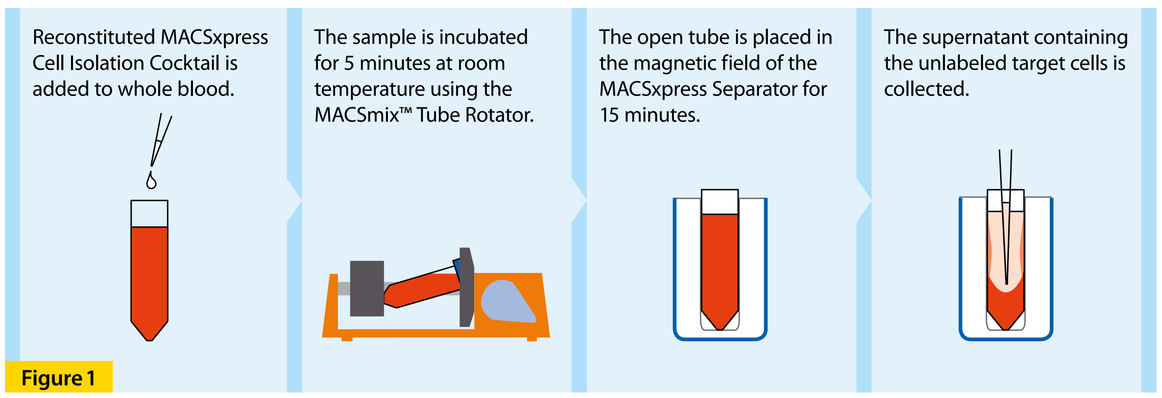

Figure 1

1 Flow cytometry analysis reveals high purity of isolated $CD3^{-}CD56^{+}$ NK cells

Unseparated whole blood samples and isolated NK cells were fluorescently stained with CD45-VioBlue®, CD3-APC, and CD56-PE and analyzed by flow cytometry using the MACSQuant® Analyzer. Non-leukocytes were excluded from the analysis based on CD45 expression, cell debris and dead cells based on scatter signals and propidium iodide fluorescence. In the example shown the purity of isolated $CD3^{-}CD56^{+}$ NK cells amounted to 93% (in relation to leukocytes).

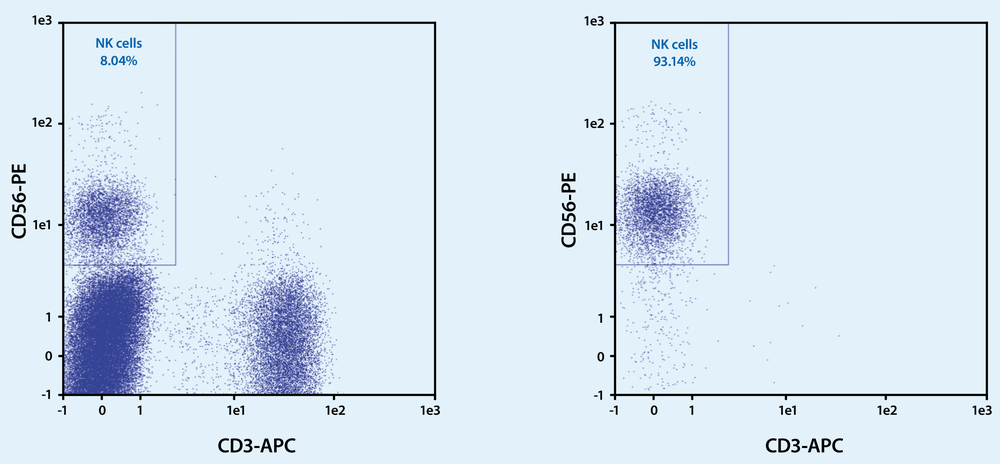

Figure 2

2 Cell separation results are highly reproducible – comparison of fresh and stored whole blood as starting material for NK cell isolation

We tested the robustness of MACSxpress Technology on 59 whole blood samples (47 fresh, 12 stored for 1 day), using only 2 mL of whole blood from healthy donors. Flow cytometry analysis of all samples processed revealed that the separation results in terms of yield and purity were highly reproducible. However, best results were achieved with fresh blood samples: Median values for yield and purity were 89% and 86% in fresh samples, and 75% and 79% in stored samples. For cells isolated from fresh whole blood, the 25th and 75th percentiles for purities were 81% and 90%, for yield 75% and 99%. In total, 5.4×10^5 NK cells (median) could be isolated from 2 mL of fresh whole blood (range: 1.6×10^5 to 9.5×10^5), and 2.7×10^5 NK cells (median) were isolated from blood stored for 1 day (range: 1.6×10^5 to 6.8×10^5).

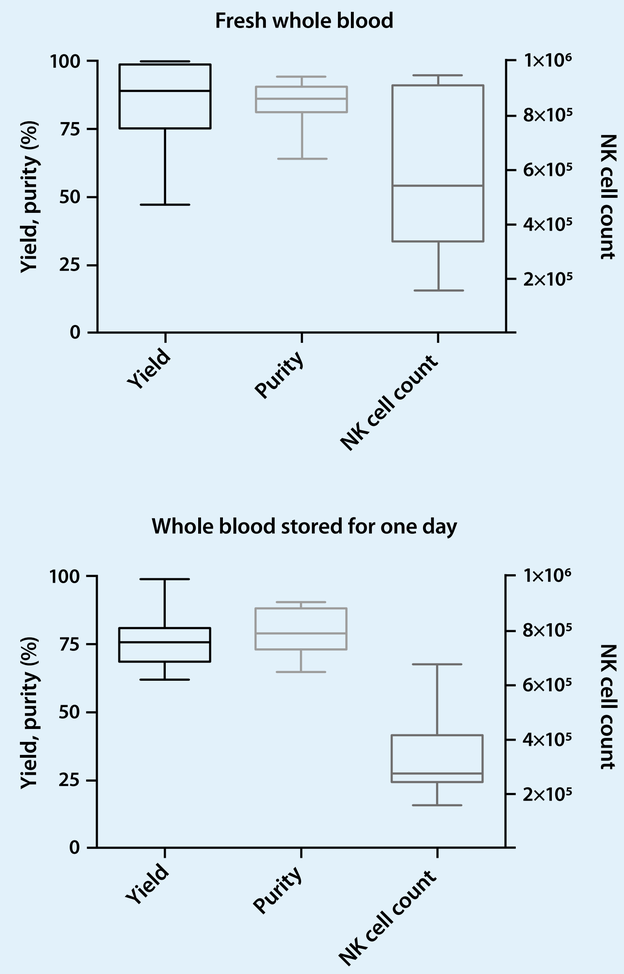

Figure 3

Conclusion

- Isolation of NK cells with MACSxpress Technology is easy to perform without the need for complex equipment and does not require a specialized laboratory environment.
- The procedure is fast (20 min) and can be performed with small starting volumes of anticoagulated blood.
- The use of fresh blood samples is recommended to achieve optimal results.
- Separation results in terms of purity and yield are reproducible between samples from different donors.
- MACSxpress Technology is a useful tool to isolate NK cells for functional and phenotyping assays, especially in settings where more time-consuming methods requiring complex laboratory equipment would hamper purification of NK cells for sensitive downstream assays.

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