New technology for fast isolation of NK cells from human whole blood

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Introduction

NK cells are an important population of the innate immune system and play a crucial role in a variety of physiological and pathophysiological processes. Therefore, NK cells hold great potential for clinical applications, e.g., cancer therapy. To better understand specific roles of NK cells in the immune system and to scrutinize their function, it is essential to use pure, functional cell populations. Preparation of these cells is often time consuming, and analyses of functional NK cells can be challenging and may be influenced by the separation procedure. Therefore, we developed an easy and convenient technique (MACSxpress® Technology) for the efficient purification of untouched immune cells from human whole blood within 20 minutes. Up to 30 mL of whole blood can be processed in a single tube, enabling the fast isolation of a large number of NK cells for downstream applications. The procedure does not require density gradient centrifugation, minimizing pipetting steps and aerosol formation for a high level of safety.

Methods

1 Fast large-scale isolation of NK cells from whole blood

The cell isolation reagent (MACSxpress NK Cell Isolation Kit, human) is added to a tube containing up to 30 mL of anticoagulated whole blood. After a 5-minute incubation, 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).

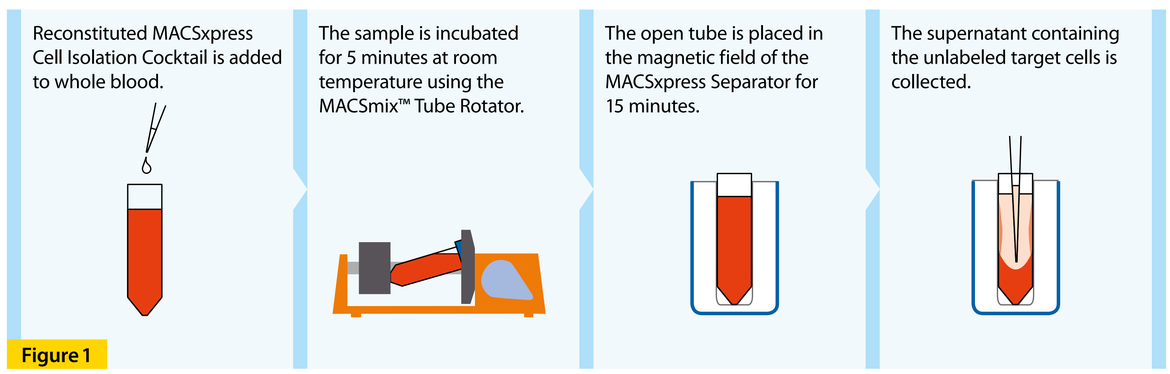

Figure 1

2 Activation and expansion of NK cells

For NK cell expansion PBMCs or isolated NK cells were cultured for up to 20 days in the presence of MACSiBead™ Particles loaded with CD2 and CD335 (NKp46) antibodies (fig. 2).

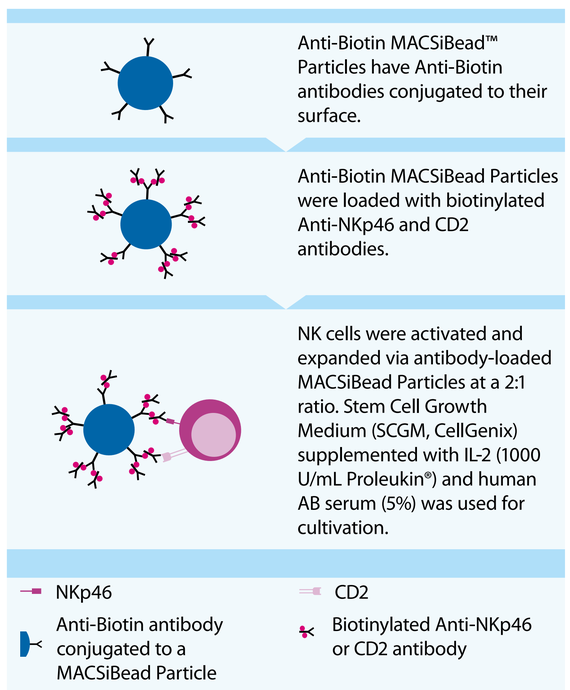

Figure 2

3 Cytotoxicity assay

The cytotoxicity of NK cells was determined by analyzing their capacity to specifically lyse K562 target cells (fig. 3).

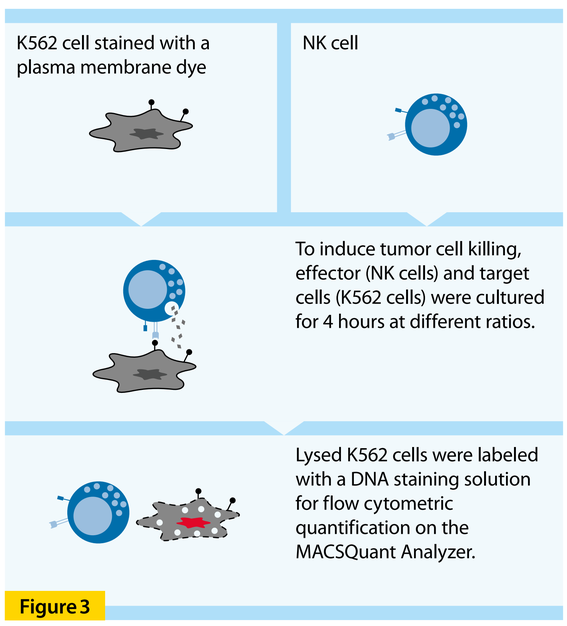

Figure 3

Results

1 Isolation of NK cells

The mean NK cell frequency in whole blood samples from seven healthy donors was 4.8%. NK cells were isolated from 30 mL of anticoagulated whole blood using MACSxpress Technology. Cells were recovered in a volume of 25–30 mL of supernatant. The average purity of isolated NK cells in relation to white blood cells amounted to 88.9% with a recovery of 75%. Red blood cells and platelets were reduced by ~99.7% and >99.9% respectively (fig. 4). Proportions of NK cells were determined by flow cytometry using the MACSQuant® Analyzer. Removal of erythrocytes and platelets was quantified using a Sysmex® KX-21N automated hematology analyzer.

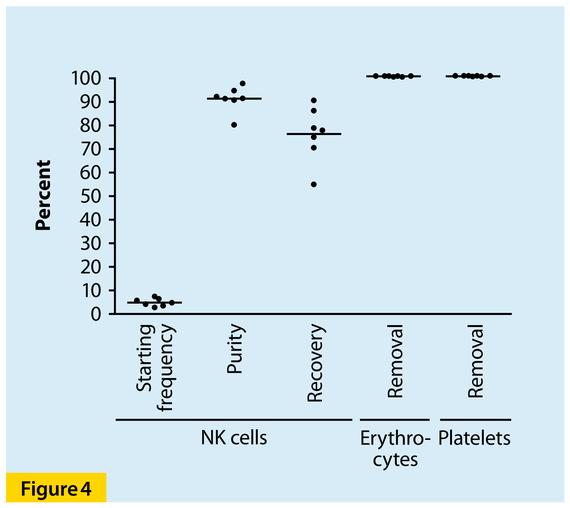

Figure 4

2 Expansion of isolated NK cells with MACSiBead™ Particles

Culturing isolated NK cells for 20 days in the presence of MACSiBead™ Particles loaded with CD2 and CD335 antibodies resulted in an approximately 300-fold expansion (fig. 5A). Starting with PBMCs we achieved an NK cell expansion of 500–1,000-fold within 16 days (fig. 5B), regardless of whether PBMCs were isolated by MACSxpress Technology or density gradient centrifugation. Data shown are the means +/– SEM from two experiments with samples from two healthy donors.

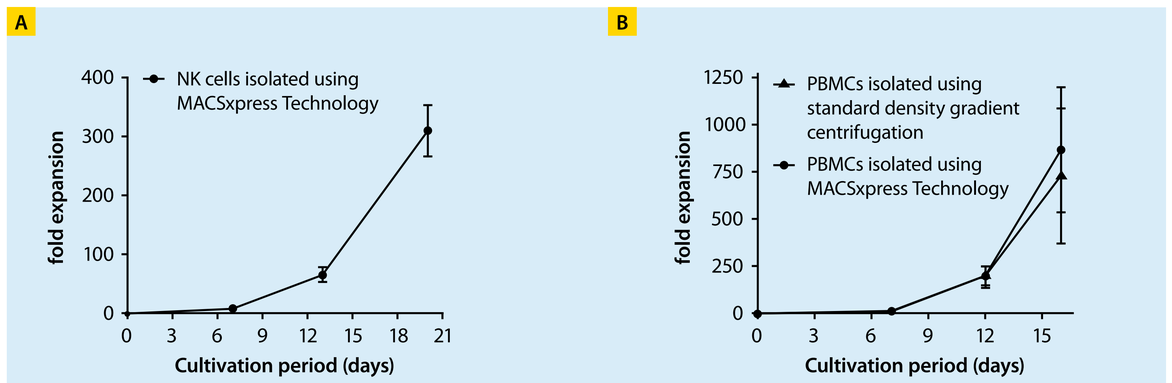

Figure 5

3 Cytotoxicity of NK cells

NK cells were fully functional as shown by their strong capacity to lyse K562 cells. Figure 6 shows the percentages of lysed K562 cells after four hours of coculture with isolated NK cells or PBMCs. Isolated NK cells showed a high lysis capacity at low effector:target (E:T) cell ratios.

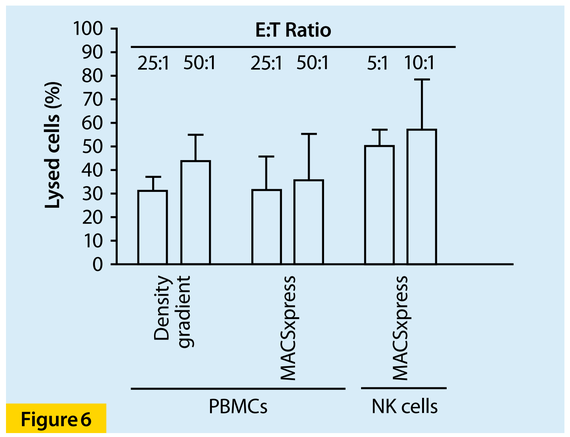

Figure 6

Conclusion

- MACSxpress Technology enables effective isolation of NK cells directly from whole blood within 20 minutes.
- No density gradient centrifugation required.
- Volumes of up to 30 mL of whole blood can be processed in a single tube.
- Isolated cells can be expanded efficiently and exhibit strong cytotoxic capacity.
- MACSxpress Technology requires a minimum of laboratory equipment.