Flow cytometric multicolor NK cell phenotyping as a potential tool for donor selection and NK cell reconstitution analysis in different hematopoietic stem cell transplantation setups

Introduction

NK cells are lymphocytes of the innate immune system and show the capacity to kill cancer cells. Their function is regulated by the balance of inhibition and activating signals transmitted mainly by killer-cell immunoglobulin-like (KIR), killer-cell lectin-like (KLL), and natural cytotoxicity (NCR) receptors. Generating, phenotyping, and haplotypeing of NK cell receptors are performed in different allelogeneic hematopoietic stem cell transplantation (HCT) settings to correlate donor and recipient-specific NK cell characteristics with the incidence of graft-versus-host disease (GVHD), graft-versus-leukemia effects (GVL), graft rejection, and, with the aim to improve donor selection. Furthermore, NK cell receptor phenotyping is a powerful tool to analyze NK cell reconstitution after allogeneic HCT to further improve the understanding of the complex NK cell biology. We have therefore performed a robust and high-frequency flow cytometry. NK cell reconstitution of allogeneic NK cells was evaluated using a panel applicable to EDTA-whole blood, peripheral blood mononuclear cells (PBMCs), and isolated NK cells. Panels focus on three inhibitory KIRs, three other inhibitory and activating KIRs, two KLRs and NCR receptors, and the NCRs. Panels include recombinant REAfinity™ Antibodies, which provide the advantages of high brightness, high-kern consistency for greater reproducibility and high specificity, so that full blocking is not required. Additionally, the brighter VioBlue™ FITC fluorescence is used to substitute brighter VioBright™ FITC to improve donor selection and NK cell reconstitution before HSCT and monitoring of NK cell receptors after HSCT with haploidentical TCRα/β cells.

Methods

Preparation of cellular material

Our study was performed on EDTA- and saline containing whole blood from healthy donors. PBMCs were isolated from EDTA- and saline containing whole blood using the autoMACS® Pro Separator, the MACSQuant Analyzer 10. Non-specific binding of antibodies was blocked by addition of Fc block Signal Enhancer. Also, for RETRUK and RETC distributions, the median fluorescence intensity (MFI) of the respective fluorochrome was used to perform retrospective compensation of spectral overlap using the FlowLogic Software (version 7). Compensation of spectral overlap was performed for each panel separately by flowlogic on single-stained beads in a retrospective manner. For each fluorochrome-conjugated antibody, the main bead population was identified (fig. 2A), unstained and stained beads were distinguished and compensated automatically by the FlowLogic Software for each flowlogic channel against all other channels (fig. 2B). The overlay of compensation resulted in compensated flowlogic channels. As an example, figure 2 shows the analysis of PBMCs with these panels. Analysis was also feasible for EDTA-whole blood and isolated NK cells. All samples were measured without compensation of spectral overlap using the MACSQuant Analyzer 10. The compensation matrices were applied retrospectively during analysis with FlowLogic Software. The bead compensation of each antibody panel was based on the exclusion of debris, doublets, dead cells, and CD3+ T and NK cells, as well as gating on CD56+ NK cells (fig. 3). MFI values were observed on isolated NK cells compared to PBMCs. Data were generated in independent experiments on six different whistle donors. While half of the PBMCs were directly stained and analyzed by flow cytometry, the other half was used for the isolation of label-free NK cells (NK Cell Isolation Kit, human) from the autoMACS® Pro Separator, followed by NK cell receptor phenotyping from the different cellular material that was then compared to each other.

Results

We established four antibody panels with bead staining of CD1, CD3, and CD56, and CD2, CD5, and CD56 (fig. 4). The antibody panels were chosen to match the configuration of the MACSQuant® Analyzer 10. Non-specific binding of antibodies was blocked by addition of Fc block Signal Enhancer. Antibody panels included recombinant REAfinity™ Antibodies in bold and brighter VioBright™ FITC fluorophore is used to substitute and  relapse, with the aim to improve donor selection.

Figure 1

Table 1

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Figure 2

1 Antibody panels for the comprehensive flow cytometric analysis of 24 NK cell receptors

We established four antibody panels with bead staining of CD1, CD3 and CD56 to analyze 24 different NK cell receptors. As an example, figure 2 shows the analysis of PBMCs with these panels. Analysis was also feasible for EDTA-whole blood and isolated NK cells. All samples were measured without compensation of spectral overlap using the MACSQuant Analyzer 10. The compensation matrices were applied retrospectively during analysis with FlowLogic Software. The bead compensation of each antibody panel was based on the exclusion of debris, doublets, dead cells, and CD3+ T and NK cells, as well as gating on CD56+ NK cells (fig. 3). MFI values were observed on isolated NK cells compared to PBMCs. Data were generated in independent experiments on six different whistle donors. While half of the PBMCs were directly stained and analyzed by flow cytometry, the other half was used for the isolation of label-free NK cells (NK Cell Isolation Kit, human) from the autoMACS® Pro Separator, followed by NK cell receptor phenotyping from the different cellular material that was then compared to each other.

Figure 3

2 Antibody panels of EDTA–whole blood, PBMCs, and isolated NK cell samples from healthy donors

Figure 4

3 Conclusion

The automatic PROCepSOR II system allows walk-away isolation with fully automated labeling and separation, resulting in high purity and recovery of target cells. Furthermore, the MACS™ column technology together with isolation kits enable trouble free unattended isolation of target cells with minimal labeling. Unlike column-free methods, this approach leaves all target cells exposed free of labeling and allows for unbiased phenotypic analysis of surface markers using flow cytometry.

References