CELLPLY

Characterization of NK cell donors for allogeneic cell therapy using single cell serial killing

TECHNICAL NOTE

INTRODUCTION

The ability to evaluate supercharged subpopulations within a heterogeneous sample which retain a phenotype capable of serial killing can support identification of sustainable and potent cell sources. By identifying such sub-populations, it may be possible to develop a process which favors expansion of these phenotypes. Here we demonstrate how NK cells have been analyzed using the unique VivaCyte® single cell serial killing assay which can rank order optimized donors relative to bulk cytotoxicity.

METHODS

NK cells and target K562 (ATCC) cells, were loaded onto the CC-Array® microfluidic device to create multiple co-cultures in each channel's 1200 microwells. Cell delivery was made automatically by the integrated liquid handling system and controlled by the proprietary workflow assay, Figure 1. Cells were added in each microchannel and after stopping the fluid flow, cells were randomly deposited in the microwells by gravity. Cell concentration was determined empirically to achieve a significant number of co-cultures with single NK cells.

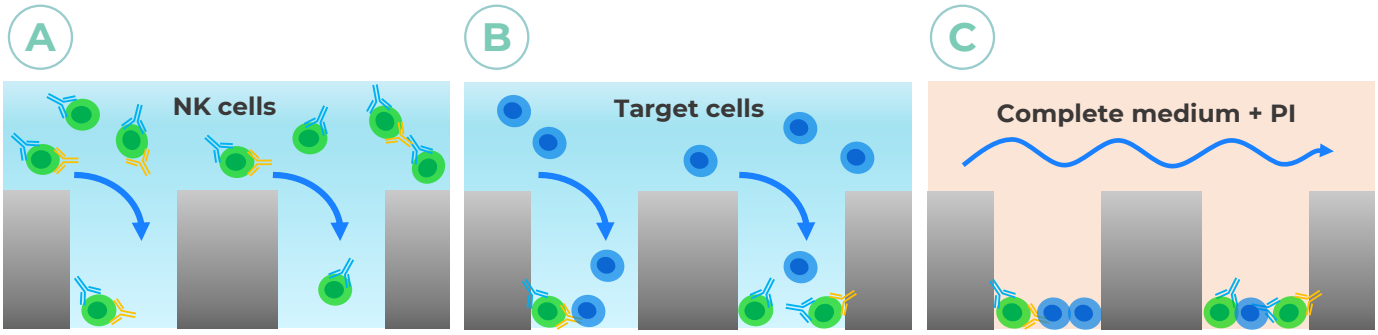

Figure 1: Workflow showing (A) loading of NK cells tracked in DAPI, FITC and CY5 channels followed by (B) loading of the target cells tracked in DAPI to create co-cultures and (C) staining of the co-cultures in time-lapse using PI as cell death marker.

NK and target cells were tracked using Calcein AM and CMAC (both Thermo Fisher), respectively. In addition, NK cells were specifically marked with anti-CD336 (AF647, Biolegend) and anti-CD56 (BV421, BD Biosciences). Fluorescent imaging was performed on a subset of 900 co-cultures per microchannel at time points 0h, 2h, 4h, 6h, 8h, 12h, 18h and 24h. When concentration is optimized for single NK cell occupancy, cell delivery can be approximated by a Poisson distribution and controlled by adjusting the input concentration. Images were analyzed, and co-cultures clustered based on the number of NK and target cells per microwell.

RESULTS AND DISCUSSION

Using the VivaCyte Analysis Software, the wells in each channel were clustered into three types of co-cultures: a) target cells without NK cells, b) target cells co-localized with a single NK cell, and c) target cells co-localized with multiple NK cells. An average of 814, 956 and 717 microwells with a single NK cell were obtained per experiment for donor A, B and C, respectively. Figure 2 shows how the activity of a single NK cell can be followed during the assay to determine killing ability by counting the number of target cells killed at a specific time point.

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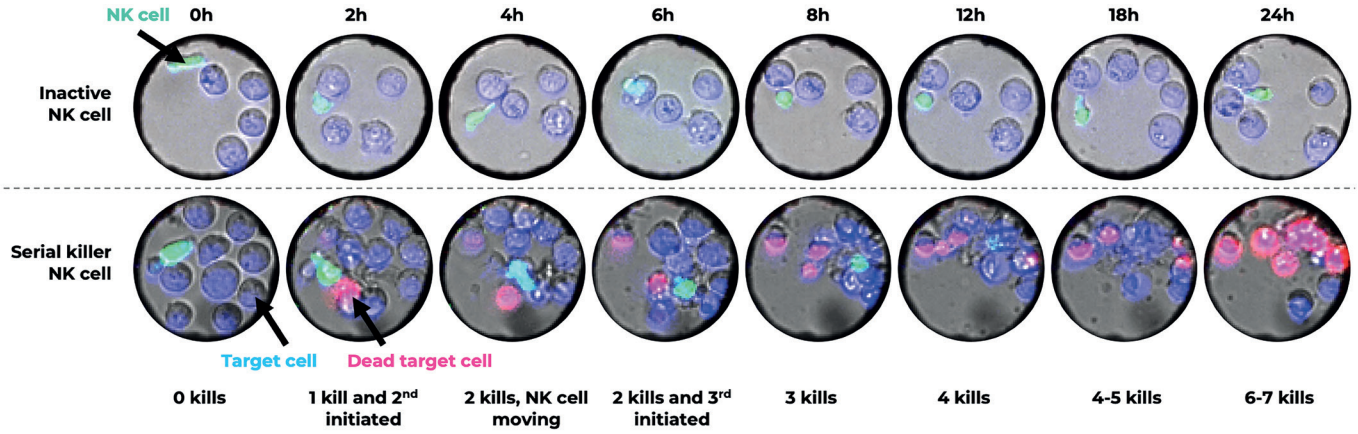

Figure 2: The images show a microwell containing an inactive NK cell (top) and a serial killer NK cell (bottom) from the same donor. The inactive NK cell (green) touched the target cells (blue) at different times without inducing any cytotoxicity, with cells growth observed between 12h and 18h. The serial killer NK cell (green) is in contact with more than one target cells (blue) resulting in the death of almost all target cells in the microwell (pink).

NK cell serial killing ability was assessed after 12 hours of co-culturing inside VivaCyte, Figure 3.a. The experiment was repeated 3 times on 3 different days to determine the reproducibility. NK cells were classified according to the ability to kill 0, 1, 2, 3, 4, 5, 6 or more target cells. An average activity of 30% was seen in the wells with single NK cells in each of the 3 donors. These single active NK cells were broken down into two subpopulations: (1) able to kill one or more target cells indicated as “killers”, and (2) able to kill two or more target cells, indicated as “serial killers” (Figure 3b). In the “killer” cell group, significant differences were seen between donor A and C (p=0.0123) and donor A and B (p=0.0227) indicating that donor B and C both have a higher killing activity than donor A. The killer cell count had a coefficient of variation (CV) between 11-16%. The serial killer cell count had a CV of 20-33%. The higher CV values for the serial killing analysis are due a lower frequency of NK cells.

Figure 3a: Quantification of the activity of single NK cells for three different donors after 12h incubation

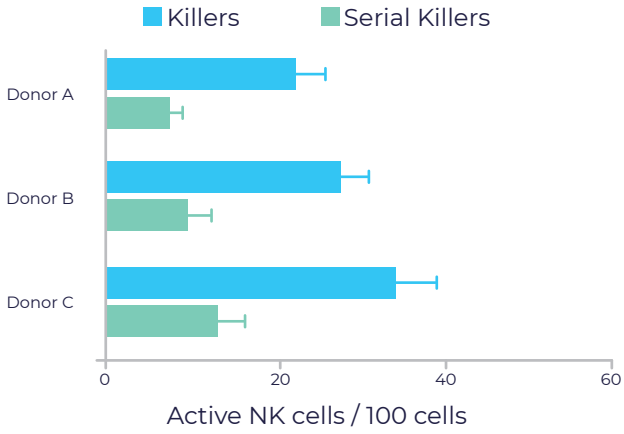

Figure 3b: Quantification of the single cell killers and serial killers for each donor after 12h incubation of co-cultures

CONCLUSION

A single-cell serial killing method can provide greater insight into a heterogenous population of immune cells than bulk analyses. With VivaCyte, killing and serial killing ability of multiple donor samples can be readily determined and the data used to identify subpopulations of the best killer NK cells. Using this approach, good reproducibility was achieved with minimal effector cells per condition, saving valuable donor sample. This approach can be used to reproducibly identify sustainable and potent cell sources and help inform donor selection.