A rapid, flow cytometry-based assay for the determination of natural killer cell activity in isolated periphery blood mononuclear cells.

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Natural killer cell activity (NKCA) is an important assessment of innate immunity in humans. Natural killer (NK) cells are known to be affected by exercise; therefore it is of interest to have an efficient method for measuring NKCA in pre- and post-exercise blood samples. Assays for NKCA include incubating human NK cells (effector cells) with stained target cells. The target cells are typically from the human leukemia cell line K562. The number of K562 target cells killed can be determined using a dead cell dye. Control target cells incubated alone provide a measure of spontaneous cell death. PURPOSE: The purpose of this study was to optimize a flow cytometry protocol for testing natural killer cell activity in human blood. METHODS: In a series of experiments, different variables were altered or held constant to find a method that yielded consistent, easily-distinguishable results. Variables tested included using freshly-stained target cells (K562 cells), or previously-stained K562 cells that had been stored in liquid nitrogen; number of cell washes; ratios and concentrations of target and effector cells; co-incubation time of target and effector cells; concentration and incubation time with dead cell dye (7AAD); and source of effectors cells (whole blood or isolated periphery blood mononuclear cells (PBMC)). RESULTS: We have found that consistent results are obtained with the following protocol: K562 cells are stained with 0.15 µM CFSE in 1 ml for 15-min, then incubated with 1ml complete-media containing 10% FCS for 30-min. The stained cells are washed three times with 15 ml PBS, resuspended in DMSO-containing complete media, aliquoted, and stored in liquid nitrogen. When needed, the target cells are thawed and washed three times with 50 ml PBS. The target cells are co-incubated with isolated PBMCs at concentration ratios of 1:20, 1:40, and 1:80 (target: effector) for 2 h, using 5000 target cells in a final volume of 100 µl. Following incubation, 30 µl 7 AAD-dye is added for 20-min, and then acquired on a flow cytometer. Representative results are shown above. Live target cells are shown in the upper left quadrant (CFSE+7AAD-), while dead target cells are found in the upper right quadrant (CFSE+7AAD+). Effectors cells have been gated out based on CFSE expression (CFSE-). Note the increase in dead target cells from incubation with PBS only (left figure), to incubation with effector cells (right figure). CONCLUSIONS: We have optimized a method of measuring NKCA in human PBMCs using flow cytometry.