Natural killer cell function is altered by freezing in DMSO.

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We have analysed the effect of freezing on NK cell viability, activation and function of peripheral blood mononuclear cells populations (PBMC) taken from normal individuals. PBMC were obtained by standard methods using heparinised blood and separated by density gradient centrifugation using Ficoll. Cells were frozen in 10% DMSO and thawed at 37°C. Using this standard method of collecting, freezing and thawing PBMC, we showed no change in the percentage of CD56⁺CD3⁻ NK cells with freezing. The percentages of viable NK (CD56⁺CD3⁻) cells in fresh and frozen samples were 8.8 ± 1.6% and 12.4 ± 6.5% (mean ± SD) respectively. Although there were some changes in the percentages of viable NK cells after freezing, the changes were very small and inconsistent, and there was no significant difference (by 2 tailed paired t-test) between the NK cell percentage fresh and frozen samples (P=0.32). Similarly the percentage of viable activated NK cells was not altered by the freezing process (P= 0.19). The mean percentage of CD69⁺ NK cells was 14.5 ± 1.5 and 22.6 ± 7.6 pre and post freezing respectively. It could be assumed from this data that since neither the frequency of NK cells per se nor the frequency of activated NK were altered with our freezing protocol, NK cells were not affected by our freezing procedure. However, with further analysis we showed that this was not the case. The percentage of K562 killing by the 5 PBMC was significantly altered after freezing (P<0.05). The ability to kill was reduced in 4/5 samples, in the one sample where the ability to kill was not reduced by freezing there was no change in cytotoxicity (percentage killing ± SD) pre and post freeze for this samples was 22.5 ± 5.2 and 25.8 ± 2.8 respectively). Prior to assessing the ability of the cells to kill K562 target cells we routinely 'rest' the PBMC for 24 hours in RPMI plus serum. This increases the proportion of activated NK cells. We showed that this was still the case when we 'rested' our frozen samples (P<0.05). However, in contrast to fresh samples, resting frozen samples decreased the percentage of CD3⁺ (T) cells (P<0.005). Previous reports have implied that NK cells require T cells for the initial stages of the killing process. These results suggest that the reduction in the percentage of T cells after freezing may be, at least in part, responsible for the reduced NK cell function. These results are important in interpreting the analysis of NK cell function of frozen samples and raise questions concerning how we can effectively freeze PBMC populations when intending to analyse NK cell function.