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"The Correlation between the Percent of CD3- CD56+ Cells and NK Precursor Function"

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Abstract:

The number and function of human natural killer (NK) cells are generally assessed to monitor the baseline of immune function, the effect of treatment, the progress of malignancy or metastases and diseases. NK cells recognise and kill target cells in the absence of prior sensitisation and are able to defend the host from infection or prevent the progression of a disease. Human NK cells express CD16 and CD56 which are (massively) being used as a major hallmark for the NK cell. The purpose of this study was to identify the unique subsets of peripheral blood mononuclear cells (PBMC) (%CD3-CD56+ cells) by flow cytometry and to determine whether there is any correlation with functionally mature progeny of (NKp) precursor after five days of culture. The correlation was analysed using samples obtained from 120 Caucasian patients. 20-30ml of whole blood was collected in sterile tube containing preservative free sodium heparin and a similar sample was obtained after five days. Maturation of NKp required the continuous presence of recombinant interleukin 2 (rIL-2), or interleukin 15 (rIL-15) and functional maturity of NK cells was determined by their ability to lyse target cells from the K562 cell line. The NK precursor frequency was measured by limiting dilution analysis (LDA), which The NKpf assay was set up with a range of cell dilutions from 40,000 to 625 per 100ml/well in 96 well culture plates. At the end of the culture period the K562 cell line labelled with Europium (Eu-K562) was added and Eu release measured in culture supernatants using time-resolved fluorometry. The PBMC were set up in parallel cultures under various conditions .On day five cells were collected from culture plates and adjusted to 1x10 cells/ml and then mixed. The mixture was incubated and anti CD3 and anti CD56 were added. NK cells were enumerated in 120 patients by double staining with a combination of anti-CD3- and anti-CD56+. The results of these Immunophenotyping studies by flow cytometry showed no correlation between the NKpf (natural killer precursor frequency) and the percent of CD3-CD56+ cells expressed after five days confirming that CD56 was inadequate as a unique marker for functional NK cells.

Keywords:

CD3-CD56+ cell , Limiting dilution analysis , Natural killer cell precursor , rIL-2 , rIL-15

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