Isolation of human NK subsets

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Introduction

Methodological details about sorting and purification of peripheral blood and decidual human NK subsets.

Reagents

Decidual and placental waste material from elective pregnancy termination procedures.

Lymphocytes from parts of decidua basalis and parietalis as previously described (1).

CD56brightCD16-CD3- dNK cells, purified afterwards as described below.

Mouse anti-human mAbs conjugated with FITC, PE, APC or Cychrome for cell sorting: anti-CD56, anti-CD16, anti-CD3 (all obtained from BD biosciences) and matching conjugated isotype controls.

Mouse anti-human mAbs against IL-8 (mouse IgG1 clone 6217), IP-10 (Mouse IgG1 clone 33036), VEGF (mouse IgG2a clone 23410 – recognizes all isoforms of VEGF), PLGF (mouse IgG1 clone 37203.111), SDF1 (mouse IgG1 clone 79014) and matching isotype controls (all obtained from RnD systems) were used for intracellular staining.

Procedure

1) Isolate peripheral blood lymphocytes from different healthy donors using Ficoll gradients.

2) Isolate NK cells using the NK isolation kit II (Miltenyi Biotec), according to manufacturer's instructions.

3) Sort 100 X 10^6 cells enriched for CD56+CD3-NK cells from each donor, as described below, in order to purify CD56dimCD16+CD3- and CD56brightCD16-CD3- subsets.

4) For each experimental with NK subsets, also perform mock isolation of pbNK cells as if they were dNK cells to rule out the possibility that differences between NK subsets was due to methodological differences in isolation procedures. Achieve this by subjecting isolated pbNK cells to the same isolation protocol used for the isolation of dNK cells(2).

Staining and cell sorting

1) Wash cells in PBS supplemented with 2% FCS and incubate with mAb on ice for 30 min.

2) Wash twice.

3) Cell sort and measure fluorescence on a MoFlo high performance cell sorter (DakoCytomation).

4) Collect data from single cell events using a standard FACScaliburTM flow cytometer

(Immunocytometry systems; Beckton Dickinson).

5) Highly purify peripheral blood NK subsets to obtain 2 subsets: CD56dimCD16+CD3- NK (pbCD16+ NK) and CD56brightCD16-CD3- (pbCD16- NK). Decidual NK cells should be uniformly of the CD56brightCD16-CD3-phenotype (dNK) cells. Subset purity should be routinely higher than 99%.

6) Perform intracellular staining with a Cytoperm-Cytofix Intracellular Staining kit (Pharmingen) according to the manufacturer's instructions.

References

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Decidual NK cells regulate key developmental processes at the human fetal-maternal interface

by Hanna, J. et al.

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