Differential expression levels of activating and inhibitory receptors on NK cell subsets: relevance to NK cell function

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Introduction

- Natural killer (NK) cells are essential innate immune cells.
- NK cell cytotoxicity is regulated by the interplay between activating and inhibitory membrane receptors, mainly the Killer immunoglobulin-like receptors (KIRs) and their ligands.
- CD56 expression can be used to define two major subsets of NK cells (CD56 Dim and CD56 Bright) that appear to differ in their main function and tissue distribution. CD56 Dim cells are associated with cytotoxicity and CD56 Bright cells are associated with immune-regulation[1].

Hypothesis

KIR gene expression is different between NK cell subsets and reflects their main function in blood and other tissues.

Materials and methods

- PBMC sorting by flow cytometry (isolation of NK cell subsets using the cell surface markers CD3 – T cell, CD56 – NK cell, CD57 – maturation [2])
- DNA genotyping of samples using real-time PCR (screening for the presence or absence of *KIR* genes using SYBRGreen)
- RNA expression using Nanostring technology (allows absolute quantification without amplification)

Results

1. NK cell subset isolation from PBMCs by flow cytometry. Using anti-CD56, anti-CD3 and anti-CD57 antibodies, we were able to successfully isolate NK cell subpopulations of interest (Fig.1.).

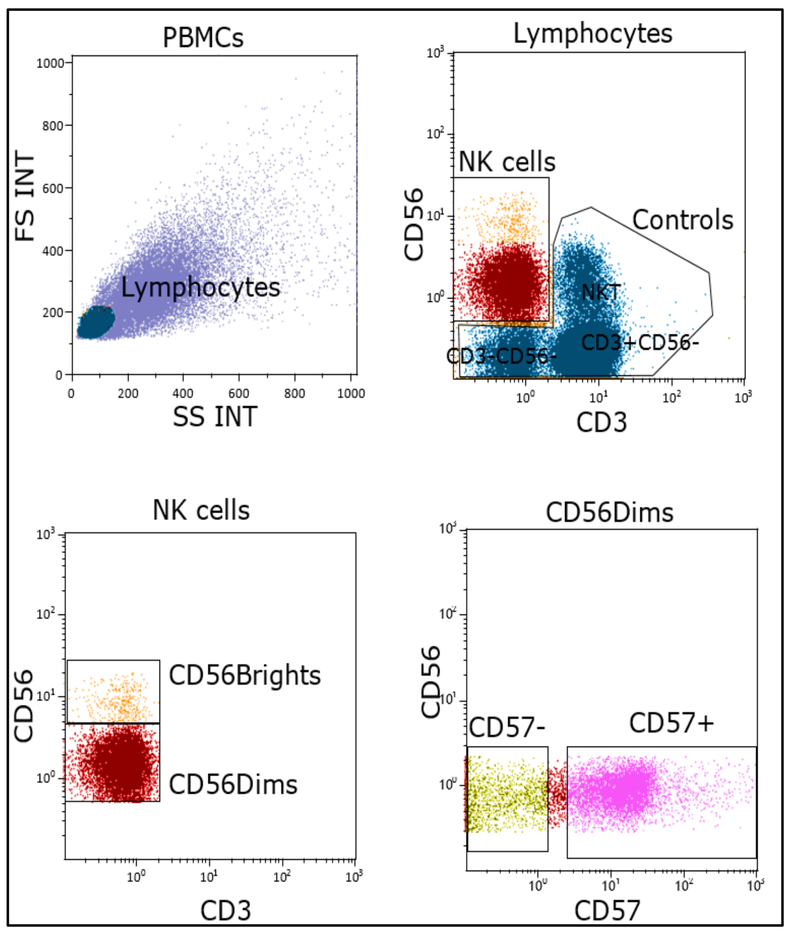

Fig.1. NK cell subsets flow cytometric sorting from PBMCs pool using: anti-CD3 antibody to differentiate NK cells from T-cells (CD3+), anti-CD56 antibody for NK cells isolation (CD56+) and anti-CD57 antibody for maturation distinction within NK cells.

2. DNA genotyping by real-time PCR DNA from PBMC pool was extracted and genotyped for the presence or absence of KIRs (Fig.2.).

Framework Genes			
3DL3	3DL2	3DP1	2DL4
+	+	+	+

Haplotype A genes			
2DL1	2DL3	2DS4	2DP1
+	+	+	+

Haplotype B genes							
2DL2	2DL5	3DL1	3DS1	2DS1	2DS2	2DS3	2DS5
-	-	+	+	-	-	-	-

Fig.2. Real-time PCR results obtained from PBMC pool DNA, screening for the presence (+) or absence (-) of the 16 *KIR* genes.

3. KIR Expression results using Nanostring Technology Greater inhibitory KIR (subgroup_1, in green, and subgroup_2 in blue) expression is observed within CD56 Bright compared to CD56Dim CD57+ and CD56DimCD57- cells, while activating KIRs (in red) are greatly expressed within CD56Dim CD57+ cells compared to CD56DimCD57- cells while CD56 BrightCD57- cells showed the lowest activating KIRs expression (Fig.3.).

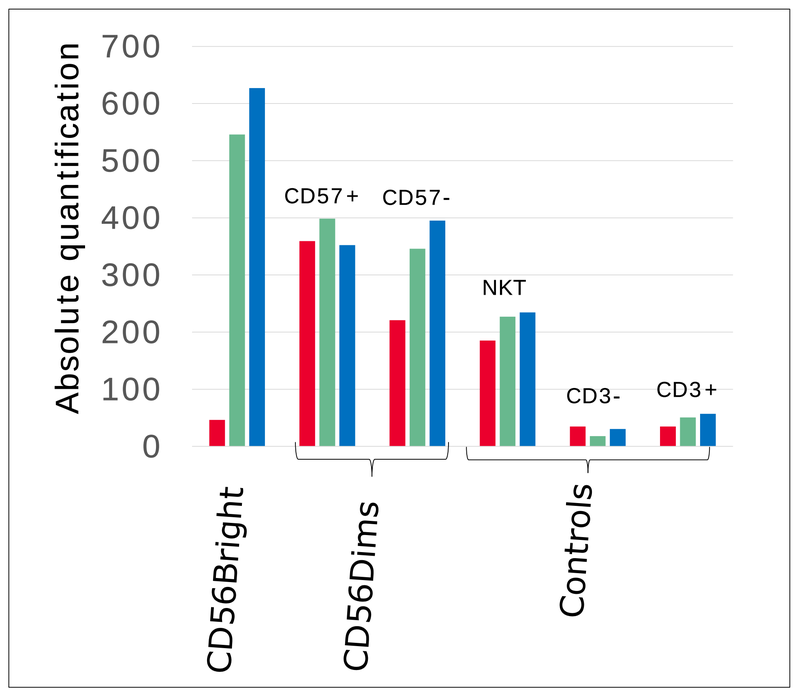

Fig.3. Differences in KIR expression between the three different NK subsets (CD56Bright, CD56DimCD57+ and CD56DimCD57-) and controls (NKT, CD3-CD56- and CD3+CD56-).

Conclusions

- There are differences in KIR expression between the NK cell subsets.
- The results support the hypothesis that CD56Dim NK cells are likely to have increased expression of activating *KIR* genes that reflects their cytotoxic function when compared to CD56Bright NK cells which are less cytotoxic and mainly cytokine producing NK cells.

References

1. Lopez-Verges, S., et al., CD57 defines a functionally distinct population of mature NK cells in the human CD56dimCD16+ NK-cell subset. *Blood*, 2010. **116**(19): p. 3865-74.
2. De Maria, A., et al., Revisiting human natural killer cell subset function revealed cytolytic CD56(dim)CD16+ NK cells as rapid producers of abundant IFN-gamma on activation. *Proc Natl Acad Sci U S A*, 2011. **108**(2): p. 728-32.

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