CD38 Identifies a Subset of Natural Killer Cells with Differential Phenotype and Function

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Abstract

Natural killer (NK) cells are involved in surveillance and killing of tumor cells. Although classically part of innate immunity, we and others have identified a subset of NK cells with adaptive properties that are present in many healthy individuals, which are longer lived and possess enhanced antitumor activity. We have previously identified that adaptive NK cells express lower levels of CD38. Therefore, we hypothesize that CD38 regulates NK cell function.

To study the functionality of these cells, we compared the activation of NK cells with low CD38 expression (putative adaptive NK cells) vs those with high CD38 expression (i.e. canonical NK cells) present in healthy blood donors. We used flow cytometry to measure NK cell degranulation by CD107a staining and cytokine production (IFN-γ and TNF) when co-cultured with leukemia (K562) and lymphoma (Raji +/- rituximab) tumor target cells. We also measured the expression of perforin and granzyme b, proteins involved in direct tumor cell killing.

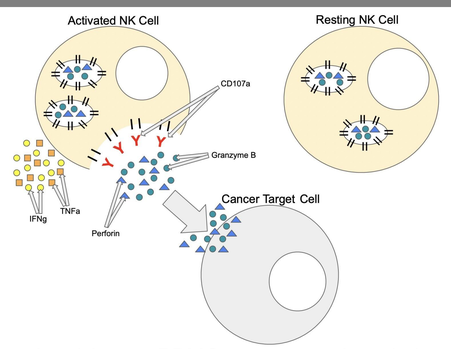
We found that CD38 low/negative NK cells express higher levels of granzyme b, but lower levels of perforin. This subset also demonstrated decreased activation markers when co-cultured with tumor cells. Future studies aim to characterize other modes of NK cell activation, phenotype, and tumor cell killing using NK cells with varying expression of CD38.

Introduction

Adaptive NK cells are found in the peripheral blood of healthy individuals who have been exposed to a variety of viruses. One of the better characterized are found in the context of prior cytomegalovirus (CMV) infection. The expansion of CMV-induced adaptive NK cells after bone marrow transplantation has been associated with improved remission in patients with leukemia.

CMV-induced adaptive NK cells can be identified by co-expression of NKG2C and CD57. We have previously shown that these cells also have lower expression of CD38. CD38 is an enzyme which regulates the levels of NAD^+. Therefore, CD38 can regulate cell metabolism, protein activation, and gene expression; and may contribute to immunologic memory in the adaptive NK cells.

Methods



NK Cell Functional Assay: PBMCs were co-cultured alone or with tumor cells while being stained for CD107a as a marker for degranulation for 5 hours. Cells were treated with protein transport inhibitors to retain cytokines intracellularly, allowing staining with fluorescent antibodies. Results were then analyzed by flow cytometry.

Results

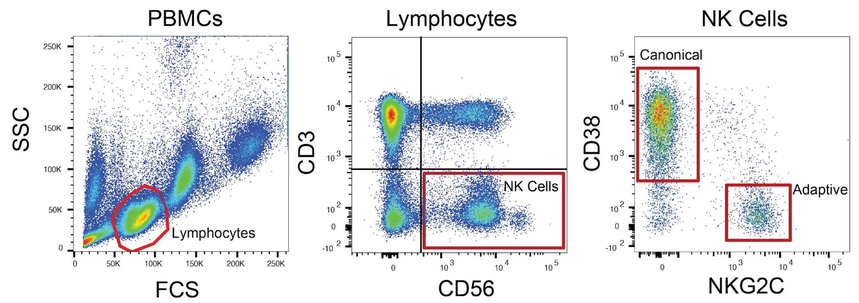


Figure 1. CMV-Induced Adaptive NK Cells Express Low Levels of CD38. Peripheral blood mononuclear cells were isolated from the blood of healthy donors and analyzed by flow cytometry. Natural killer cells were identified by gating on lymphocytes (left panel) followed by staining with CD56+ and CD3- (middle panel). NK cells were then analyzed for expression on adaptive (NKG2C+) vs canonical (NKG2C-) subsets.

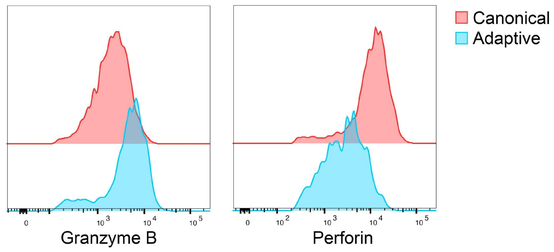


Figure 2. Expression of Perforin and Granzyme B in Adaptive vs Canonical NK cells. Expression levels in NK cells was measured by intracellular staining and analyzed by flow cytometry.

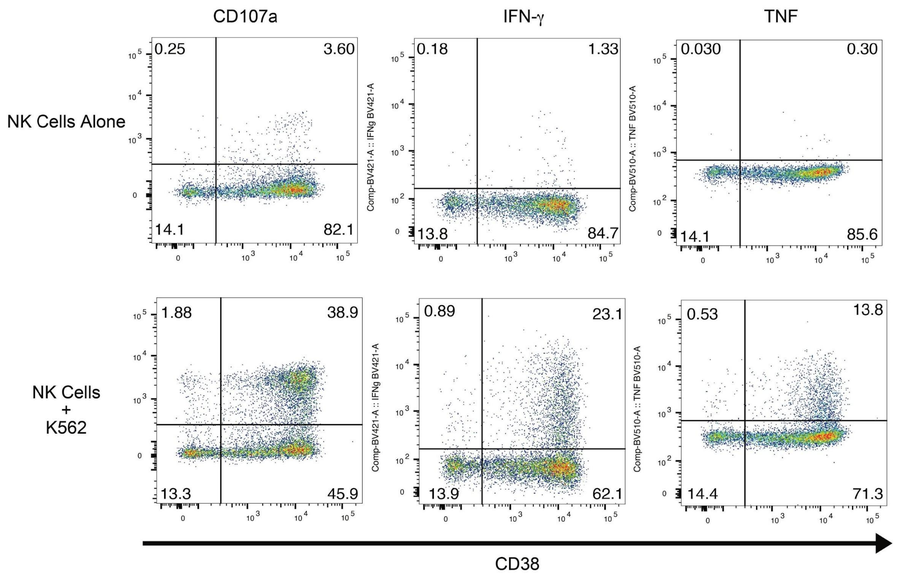


Figure 3. Activation of Natural Cytotoxicity in NK Cells. PBMCs were co-cultured alone or with K562. Cells were stained by fluorescently labeled antibodies and NK cells analyzed for degranulation (left panel) and cytokine production (middle and right panels).

Results

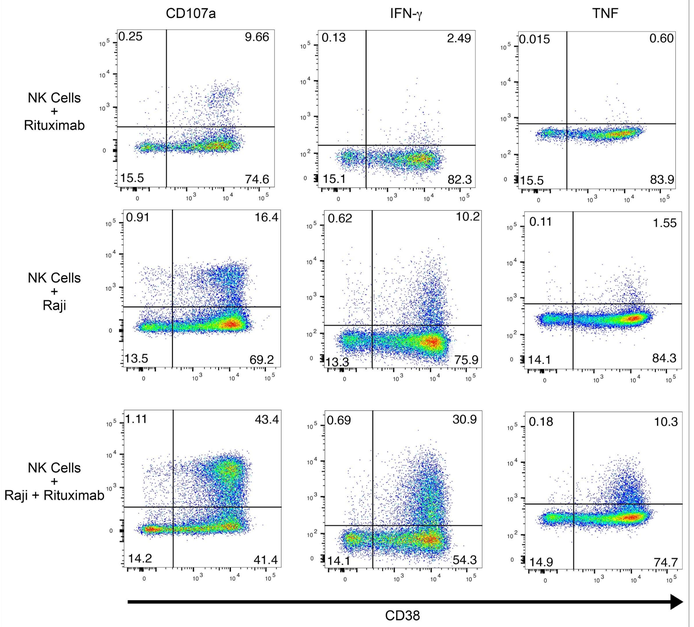


Figure 4. Activation of Antibody Dependent Cell Cytotoxicity in NK Cells. PBMCs were co-cultured with or without rituximab (an antibody against CD20) and with Raji. Cells were stained by fluorescently labeled antibodies and NK cells analyzed for degranulation (left panel) and cytokine production (middle and right panels).

Conclusions

When compared with canonical NK cells, adaptive NK cells express Higher levels of:
-Granzyme b
Lower levels of:
-CD38
-Perforin
Decreased degranulation and cytokine production following stimulation with tumor cells in assays for natural and antibody dependent cell cytotoxicity

It is unclear if these differences translate to actual tumor cell killing.

Future Direction

- Analyze additional PBMC donors
- Compare adaptive NK cells from donors with varying degrees of CD38 expression
- Perform more extensive phenotypic characterization (e.g. death receptors)
- Purifying adaptive vs canonical NK cells to assess tumor cell killing

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