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Article

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

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SUMMARY

Natural killer (NK) cells are involved in surveillance and killing of tumor cells. Although classically part of innate immunity, I 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. I have previously identified that adaptive NK cells express lower levels of CD38. Therefore, I hypothesize that CD38 regulates NK cell function. To study the functionality of these cells, I compared the activation of NK cells with low CD38 expression (putative adaptive NK cells) versus those with high CD38 expression (i.e. canonical NK cells) present in healthy blood donors. I 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. I also measured the expression of perforin and granzyme b, proteins involved in direct tumor cell killing. I found that CD38 low/negative NK cells express higher levels of granzyme b, but lower levels of performance. 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

Natural killer cells are a critical component of the innate immune system, serving as a crucial defense mechanism against infections and diseases by promptly responding to threats (Denman, Senyukov, et al., 2013). NK cells play a pivotal role in constraining the proliferative capacity of tumor cells, thus assuming a paramount role in combating the progression of cancer within the human body. Canonical human NK cells can usually be identified by low expression of CD3 and high expression of CD56.

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 adaptive NK cells is 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 (Cichoki, Woan, et al., 2019). Studies have previously shown that these cells also have lower expression of CD38. CD38 is an enzyme which regulates the levels of NAD+ (Mallone, Funaro, et al., 2001). Therefore, CD38 can regulate cell metabolism, protein activation, and gene expression; and may contribute to immunologic memory in the adaptive NK cells (Forrest, Gomes, et al., 2020).

Because of the previously shown effects of the CD38 cell marker and its absence in the CMV-induced adaptive NK cell population, I will be researching this subset of cells' functionality and cancer killing activity. By comparing the killing activity and functionality of the CMV-induced adaptive NK cells to canonical NK cells that have vastly different levels of CD38 expression, I will explore future implications of CD38 and adaptive NK cells as a cancer treatment.

My project will involve phenotyping human peripheral mononuclear blood cells to identify populations of canonical and adaptive NK cells. I will be using antibodies to stain the cells of

interest and using flow cytometry to analyze my results. After phenotyping, I will be performing killing assays, exposing the natural killer cells to leukemia and lymphoma cells to test their killing activity and functionality.

To test killing activity and functionality, I will be measuring levels of Perforin and Granzyme B, two proteins that NK cells use to kill target cancer cells. When an NK cell comes across a target cell, lysosomes perform exocytosis, releasing Perforin, which creates a hole in the target cell membrane. It then releases Granzyme B into the target cell. Granzyme B then causes apoptosis in the target cell (Veugelers, Motyka, et al., 2006). The area of exocytosis is also a binding site for CD107a, making it a marker I used for functionality. Additionally, NK cells release cytokines including IFNg, which activates macrophages for phagocytosis or lysis of the target cells, and TNFa, which promotes direct NK cell killing (Figure 1.1). By measuring levels of these proteins and markers in both the canonical and CMV-induced natural killer cells after exposure to the cancer cells, I will be able to measure the difference in effectiveness against cancer cells of the two populations.

I hypothesize that the CMV-induced adaptive NK cells will show higher levels of all the killing and functionality markers.

While my project will not directly answer the question of whether or not CMV-induced adaptive NK cells can be a future cancer treatment, it will provide valuable data that can be further investigated to eventually make strides towards more effective and safer cancer treatment.

RESULTS

When measuring the killing markers in the canonical and CMV-induced adaptive NK cells (Figure 1.3), I found that the canonical cells had lower expression of Granzyme B but had higher expression of Perforin compared to the CMV-induced adaptive NK cells. The CMV-induced adaptive NK cells expressed much lower levels of Perforin compared to the canonical NK cells, and only slightly higher expression of Granzyme B. When measuring the functionality markers of the NK cells alone (Figure 1.4), flow cytometry results showed that the CMV-induced adaptive NK cells made up about 0.25% of CD107a expression, 0.18% of IFN-y expression, and 0.03% of TNF expression. In the NK cells and K562 population, the CMV-induced adaptive NK cells made up 1.88% of CD107a expression, 0.89% of IFN-y expression, and 0.53% of TNF expression. In the NK cells and rituximab population, the CMV-induced adaptive NK cells made up 0.25% of CD107a expression, 0.13% of IFN-y expression, and 0.015% of TNF expression. In the NK cells and raji population, the CMV-induced adaptive NK cells made up 0.91% of CD107a expression, 0.62% of IFN-y expression, and 0.11% of TNF expression. In the NK cells, raji, and rituximab population, the CMV-induced adaptive NK cells made up 1.11% of CD107a expression, 0.69% of IFN-y expression, and 0.18% of TNF expression.

DISCUSSION

My data had varying results. The killing markers did not provide any conclusive evidence on whether the canonical or CMV-induced adaptive NK cell populations were more effective in killing the cancer cells because each population had higher expression of one killing marker. Contrary to my hypothesis, the CMV-induced adaptive NK cell populations expressed lower levels of all functionality markers as well. The canonical NK cells made up the bulk of the expression of all the functionality markers in almost every experiment, while the CMV-induced adaptive NK cells showed almost no activation.

It is unclear if the unexpectedly low expression of functionality and some killing markers in the CMV-induced adaptive NK cells translate to actual tumor cell killing. This is a future direction that should be taken to further explore whether the CMV-induced adaptive NK cells are more effective against cancer cells as expected based on bone marrow transplant patients who contracted CMV.

I hope to perform more research on adaptive NK cells in the future to continue searching for better cancer treatments. Further researching this topic will allow for more data that will lead to a better understanding of the difference between the canonical and CMV-induced adaptive NK cells and applications in the medical world.

MATERIALS AND METHODS

Materials:

Human peripheral blood mononuclear cells; K562 leukemia cells; raji lymphoma cells; CD56 antibody; CD3 antibody; CD38 antibody; NKG2C antibody; Granzyme B antibody; Perforin antibody; CD107a antibody; IFN-y antibody; TNF antibody; rituximab cancer therapy

Methods:

1) Phenotype the human peripheral mononuclear blood cells to isolate the canonical and CMV-induced natural killer cell populations. Place cells in a well and add CD56, CD3, CD38 and NKG2C antibodies. Use a centrifuge to bind antibodies to cells. Place cells in vials and buff them to prepare for flow cytometry. Use flow cytometry to get a scatterplot of cells of interest and their markers.
2) Analyze data (Figure 1.2). Cells with high CD56 and low CD3 are natural killer cells. From that NK cell population, high NKG2C and low CD38 expression represents CMV-induced adaptive NK cells and low NKG2C and high CD38 expression represents the canonical NK cells.
3) Measure killing markers in both canonical and CMV-induced adaptive NK cell populations. Stain cells previously used for phenotyping with Perforin and Granzyme B antibodies. Use a centrifuge to bind antibodies to cells. Place cells in vials and buff them to prepare for flow cytometry. Use flow cytometry to get a scatterplot of cells of interest and their markers.
4) Analyze data. Measure levels of Perforin and Granzyme B in two cell populations.
5) Measure functionality markers in two populations. Using a new well, create four sets of cells. In the first well, place NK cells alone. In the second well, place NK cells and K562 leukemia cells. In the third well, place NK cells and rituximab lymphoma therapy. In the fourth well, place NK cells and Raji lymphoma cells. In the fifth well, place NK cells, raji lymphoma cells, and rituximab lymphoma therapy.
6) In each well, place CD107a, IFN-y, and TNF antibodies in cells.
7) Use centrifuge to spin down cells and bind antibodies. Place cells in vials and buff them to prepare for flow cytometry. Use flow cytometry to get a scatterplot of cells of interest and their markers.
8) Analyze data. Create three graphs for each well with cells on the y-axis and expression of CD107a, TNF-y, and TNF respectively on the x-axis. Separate each graph to make a distinction between canonical and CMV-induced adaptive NK cells.
9) Measure expression of functionality markers for each well and the difference in functionality between the canonical and CMV-induced adaptive NK cells within each population.
10) I will be following safety precautions by wearing gloves, wearing goggles, wearing a mask, and wearing long sleeves, so that I can avoid any potential hazards.

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APPENDIX

All pictures courtesy of author

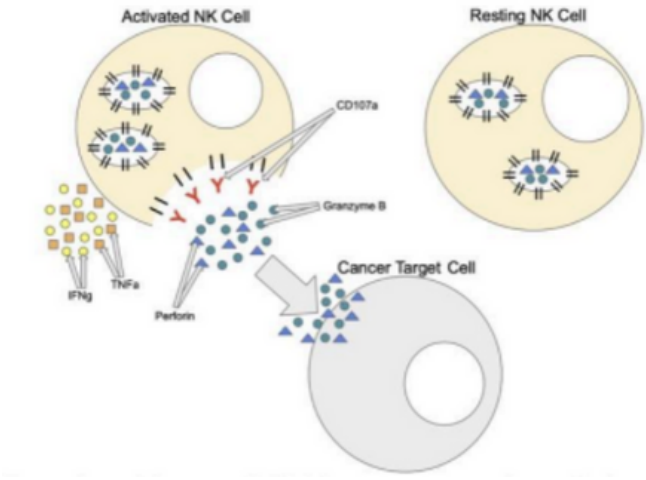

Figure 1.1 Visual representation of canonical vs CMV-induced adaptive NK cells reaction in the presence of a cancer cell

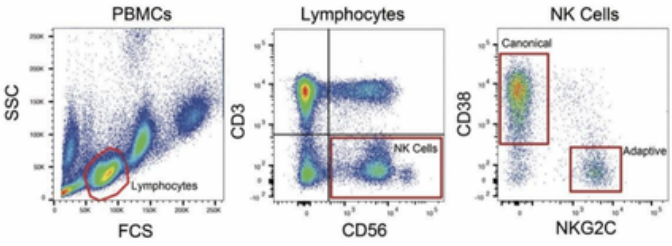

Figure 1.2 Flow cytometry results of phenotyping of human peripheral mononuclear blood cells

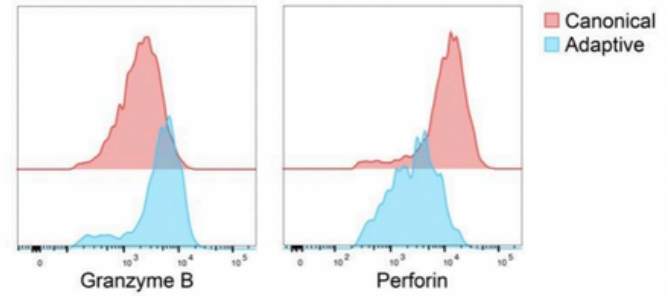

Figure 1.3 Flow cytometry results of killing activity

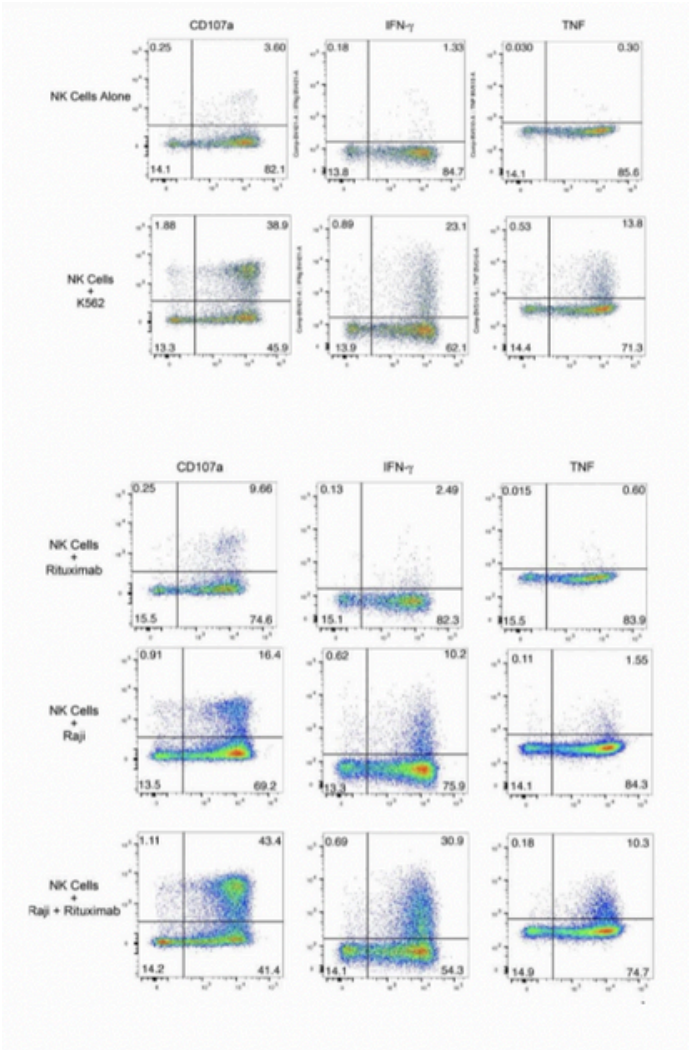

Figure 1.4 Flow cytometry results of functionality markers (top right quadrant is CMV-induced adaptive NK cells and bottom right quadrant is canonical NK cells