Growth of IL-2 Expanded NK cells in Glutamine Deprivation

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INTRODUCTION

Natural Killer (NK) cells are innate lymphocytes that have the ability to control virus and tumor infected cells. NK cell activity against target cells is regulated by balances of signals from activating and inhibitory receptors. These cells can be activated when placed in an environment with high concentrations of Interleukin (IL)-2. IL-2-Activated Killer cells reprogram the NK cells' metabolic system to increase nutrient uptake and energy levels required for cell proliferation and effector functions. Therefore, once activated by IL-2, the expanded NK cells are able to expand rapidly and function with higher efficiency.

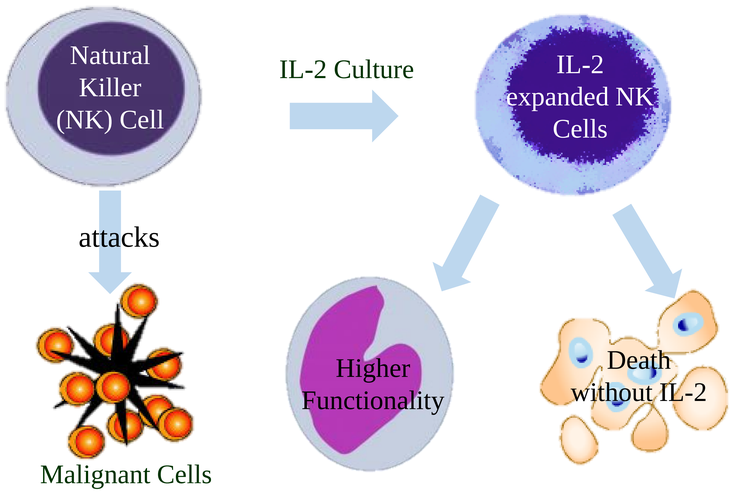

Figure 1. The growth of NK cells into IL-2 expanded NK cells, and each of the functions they are responsible for. It is important to note the two outcomes for the resulting cells.

However, IL-2 activated and expanded NK cells become dependent on high concentrations of IL-2, and thus fail to survive in low IL-2 conditions. Furthermore, absence of glutamine reduces the metabolic activity of IL-2-nurtured NK cells. Previous study indicates that pre-activation of naïve NK cells from IL-12/15/18 induces IL-2Ra (CD25) expression that maintains NK cells in vivo. Combining these factors, our study ultimately aims to determine whether LAK cells cultured in an environment with very low glutamine levels is able to survive under conditions of low IL-2.

OBJECTIVES

- Reprogram the metabolic system of IL-2 expanded NK Cells
- Investigate further usage and applications in cancer immunotherapies
- Investigate the possible cytotoxicity of the newly metabolically reprogrammed NK cells

HYPOTHESIS

We hypothesize that treatment of IL-2 on NK cells induces a greater rate of expansion and highly activated metabolic state, demonstrating more efficient effector functions.

METHODS

Harvesting & Enriching

- Isolate spleen cells from mouse
- Discard supernatants with ACK lysis buffer
- Add NK enrichment buffer and antibody cocktail
- Separate NK cells using magnet

Purity Checks

- Flow cytometry to ensure predominance of NK cells
- Direct microscopic Counting
- Stain with tryptan blue & counted using Petroff-Hauser counting chamber

RESULTS

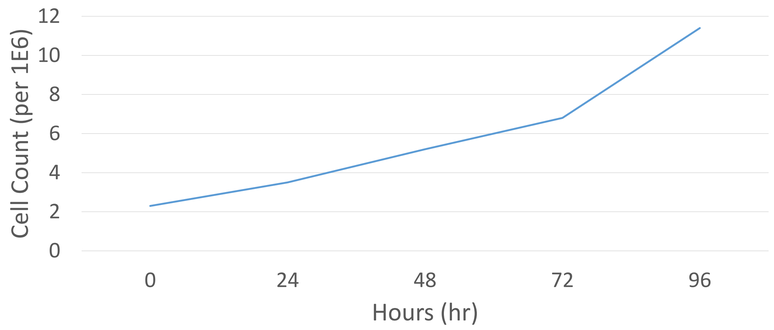

Figure 2. IL2-expanded NK cell counts when grown under RP-10 media with IL-2 rich environment, observed for 96 hours before splitting. The cell counting was carried out through direct microscopic counting procedure with Petroff-Hauser counting chamber, and was counted twice for the values to be averaged to ensure higher accuracy.

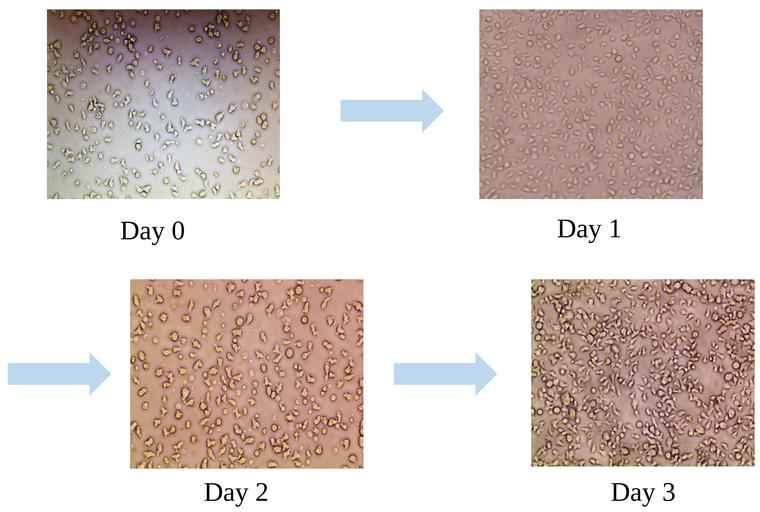

Figure 3. Progressive change in morphology of IL-2 expanded NK cells from days 0 to 3, observed under the light microscope. Depending on the metabolic states in the enriching environment and the cells' nutrient uptakes, the cells exhibit different characteristics that morphs each day.

CONCLUSIONS & SIGNIFICANCE

From the preliminary results gathered, it is evident that IL-2 expanded NK cells were able to greatly proliferate when cultured with RP-10 media with IL-2, demonstrating the possibility of efficient effector functions with further proliferation with glutamine-free RP-10 media. It is important to note the morphology transformation in the cells, for it is indicative of possible metabolic change derived from the change in culturing environment. The elongation of the cells clearly demonstrate that the cells are able to greatly proliferate within the given conditions. Since this study is limited to *in vitro* & *in vivo* conditions, it will be insightful to observe the cells under molecular levels as well.

Through the study, more effective treatments to enhance NK cells' activity and suppression of tumor cells will be developed. If the cells show great potential in proliferating under different types of stressful environments, the resulting treatment will be applicable to a wider range of patients for it is able to flourish in conditions that are not as favorable as normal cells would need in order to perform their base functions. It will greatly advance NK cell-based adoptive transfer therapy and allow treatments that break the present limitations to improve the conditions of patients suffering from terminal cancers.

FUTURE DIRECTIONS

Further experimentation is advised to fully test the hypothesis; the possibility of cell proliferation was only tested with IL-2 culturing, thus, the next step would be to culture the IL-2 expanded NK cells with glutamine-deprived RP-10 media.

After the results have been obtained, it is advised to study future possible applications, such as its relevance to adoptive transfer therapy or cancer immunotherapies.

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