Effect of Expansion and Tumor Challenge on Chemokine Receptor Expression in Cord Blood-Derived CAR-NK Cells

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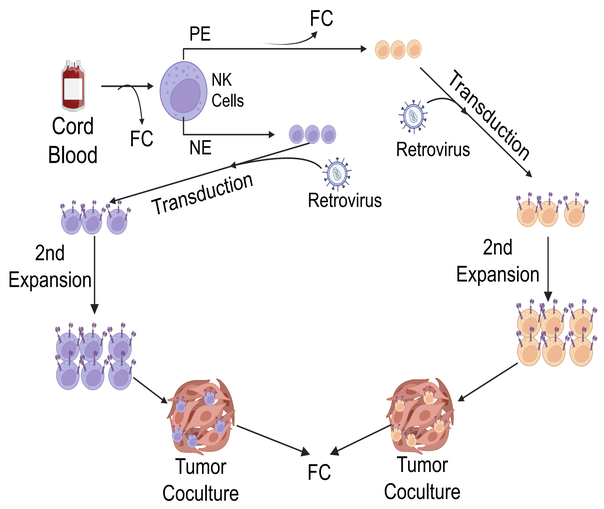
Background

NK cells are innate lymphoid cells that have a nonredundant functional role in tumor surveillance[1]. CAR NK-cell therapy has emerged as a promising cellular immunotherapy for cancer[1]. A persistent challenge, however, involves insufficient trafficking to tumor sites[2]. NK cell trafficking to organs and tumors is governed by chemokine and adhesion receptors/ligands[2]. The chemokine receptor profile of NK cells can be modulated by a variety of factors including expansion techniques, cytokine exposure, and interactions with tumors[3,4].

In this project, we analyzed the effect of **expansion**, **cytokine exposure**, and **tumor interactions** on the expression on CXCR3, CXCR4, and CD62L. These receptors have been shown to be very relevant to NK cell homing and infiltration. Cells either underwent normal expansion (NE) or were first pre-stimulated with IL-12/IL-15/IL-18 and subsequently expanded (PE). After transduction and secondary expansion, the NK cells where cocultured with UMRC3 kidney cancer cells to determine the effect of tumor interaction on the expression of these receptors/ligands.

Methodology



NK cell conditions:
1) NT
2) IL-15
3) CAR.70/IL-15
4) CAR.70

Expansion conditions:
1) NE: normal expansion with universal antigen presenting cells (uAPCs).
2) PE: pre-stimulated with IL-12/IL-15/IL-18 and subsequently expanded with uAPCs.

Results

Gating Strategy

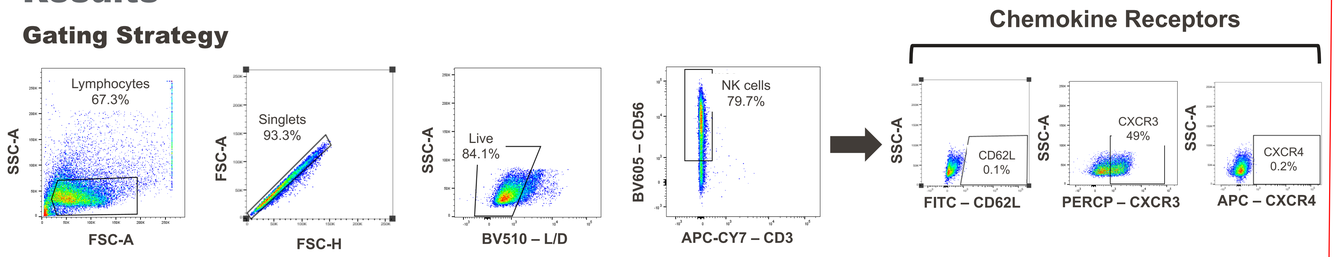


Fig. 1. Gating Strategy. After gating on the lymphocyte population and filtering out the doublets, live dead stain was used to gate on the live cells. Subsequently, NK cells were gated as the $CD56^{+}CD3^{-}$ population. The CD62L, CXCR3, and CXCR4 positive populations where gated from NK cells using negative controls (fluorescence minus one; FMO) and positive controls (Jurkat and Raji cells).

Expression through Expansion

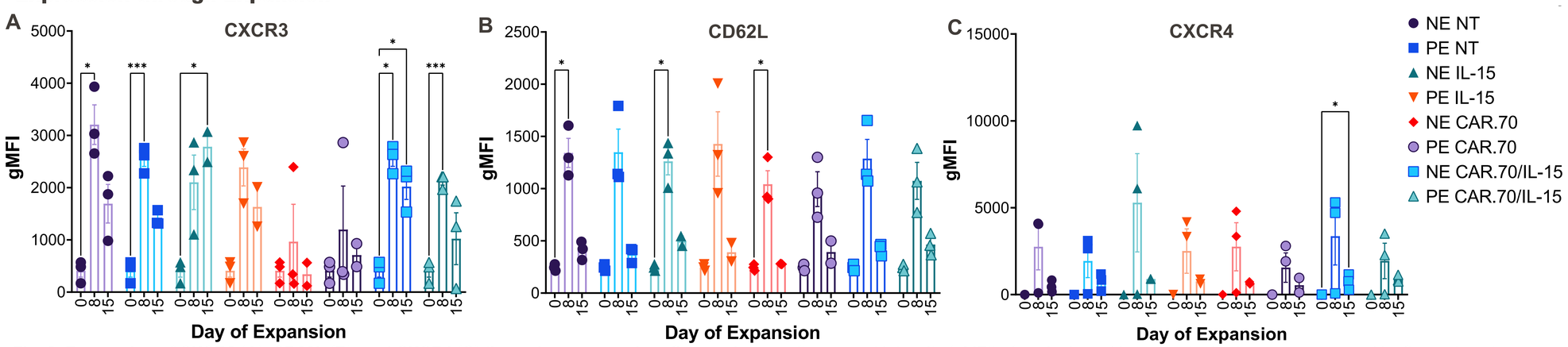


Fig. 2. Expression of CXCR3 (A), CD62L (B), and CXCR4 (C) throughout expansion. Geometric mean fluorescent intensity (gMFI) of each chemokine receptor is shown as measured by flow cytometry on Day 0, 8, and 15 of expansion for each NK cell condition. Statistical analysis was performed on Prism v9.0.0 with *=p<0.05, ** = p<0.01, *** = p < 0.001.

Tumor Coculture Assay

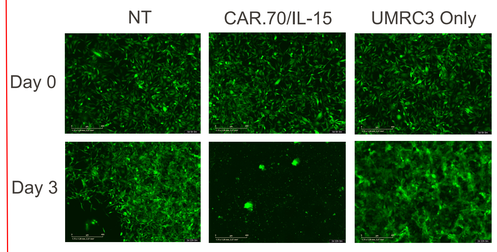


Fig. 3. Cytotoxicity assay of CAR.70/IL-15 NK cells vs. NT NK cells against the GFP-transduced UMRC3 cell line. UMRC3 cells have naturally high levels of CD70 (target of CAR.70) expression. Representative pictures taken by real-time imaging through Incucyte are shown.

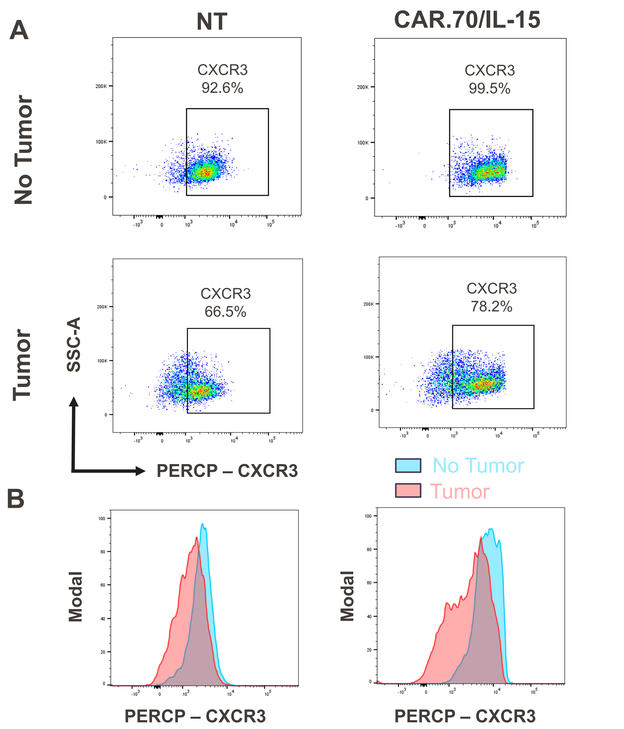


Fig. 5. Representative examples of changes in CXCR3 expression due to tumor coculture. A) FACS plots of NT and CAR.70/IL-15 NK cells with or without UMRC3 coculture. B) Summary histograms.

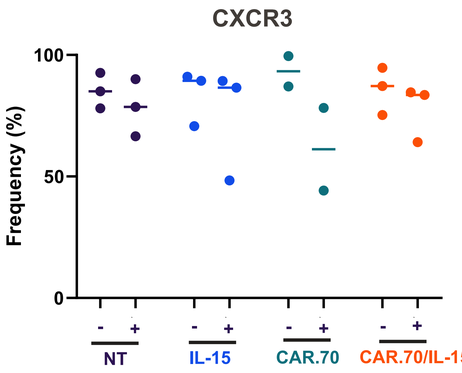


Fig. 4. Expression of CXCR3 on NK cells upon coculture. One cord lost a PE CAR.70 condition due to cell death. The – sign indicates no tumor coculture while the + sign indicates with tumor coculture. Statistical analysis was performed on Prism. No differences were statistically significant.

Conclusions

- Expression of CXCR3, CXCR4, and CD62L all had a trend of increase from Day 0 to 8 and then decreased from Day 8 to 15 to various degrees.
- Frequency of CXCR3+ NK cells decreased in a tumor coculture model although the difference was not statistically significant.
- There were no statistically significant differences due to expansion protocol or cytokine exposure.

Limitations

- Limited number of biological replicates.
- Cord-to-cord variability.
- Only one tumor cell line used.

Future Directions

- More biological replicates.
- Use mass cytometry to profile a larger panel of chemokines receptors.
- Functional analyses to correlate changes in chemokine receptor expression with tumor homing and infiltration capacity of NK cells.

Acknowledgement

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References

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