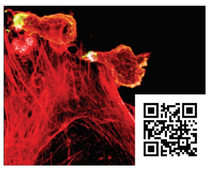
The role of ROCK inhibitor in the polarization of human natural killer cells



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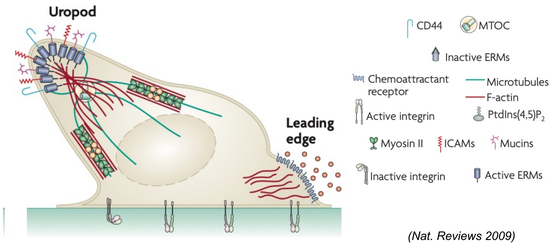
Introduction

- Natural killer (NK) cells of the innate immune system fight newly introduced infections.
- NK cells develop and migrate through different locations, including the bone marrow and secondary lymphoid tissue, displaying stage-specific migratory phenotypes in each phase of development.[1]
- On developmentally supportive stromal cells in vitro, developing NK cells undergo a period of arrest, and adhere to developmentally supportive cells via polarization, with unique morphology, termed the developmental synapse. [2]
- The polarized morphology is characterized by a leading edge and a uropod (displayed figure below).[3]
- The uropod has been demonstrated to play an important role in forming the DS, making cell-cell contact, as the site of CD56 and CD62L clustering in $CD56^{bright}$ NK cells, as well as displaying increased Ca^{2+} levels. [2]
- Polarization is characterized by formation of actomyosin bundles and receptor clustering.[3]
- Actomyosin bundles and receptor clustering is mediated by the activity of Rho family proteins.
- Rho-dependent coiled-coil containing protein (ROCK) is an effector kinase of RhoA, and results in the formation of actomyosin bundles at the cell rear, as well as activation of ezrin-radixin-moesin (ERM) proteins, which facilitate receptor clustering.[4]



(Nat. Reviews 2009)

- ROCK inhibition has resulted in impaired migration of granulocytes, the loss of stress fibers and focal adhesion complexes in the migrating cell.[5]
- This experiment aims to understand the effect of ROCK inhibition on the polarization of human NK cells when cultured on an Fc-Fibronectin surface and treated with IL-15.
- Human NK cells were isolated from healthy donor blood. The isolated NK cells were treated with IL-15 and ROCK inhibitor, and NK cells treated with only IL-15 were used as controls.

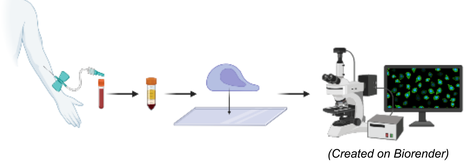
Goals & Hypotheses

In this experiment, we aimed to understand polarization, one of the essential steps of forming the DS. We targeted ROCK, an essential effector kinase protein, in order to understand how disruption of the Rho-ROCK pathway affects NK cell motility and formation of the DS.

We hypothesized that treatment of NK cells with ROCK inhibitor will result in poorly-adhered NK cells, with a morphology that is classically "non-polarized," and an even distribution of uropod markers, in contrast to a "polarized distribution" in the control condition.

Methods

- Human NK cells were isolated from healthy donor blood.
- The isolated NK cells were plated on fibronectin surfaces on glass coverslips, fixed, permeabilized, and stained for CD56, Phalloidin (Actin), DAPI, and CD44 (uropod marker).



(Created on Biorender)

- The isolated NK cells were validated using flow cytometry with a compensation control matrix.
- Confocal imaging was performed on cells after fixing and staining, using 100X oil.
- Confocal imaging results were analyzed by scoring for the presence of a clear uropod and CD44 localization using Fiji[6] and Prism. n=19 untreated condition, n=57 in the treated condition.

Results

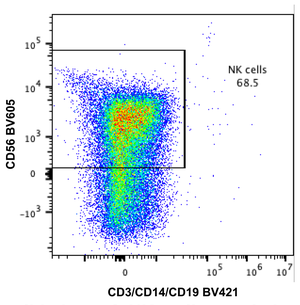


Figure 1. Flow validation of NK cell population. The isolated NK cell suspension was validated using flow cytometry. Gating for CD56 (BV605) + and Lin- (CD3, CD14, CD19, BV421) cells demonstrated that the isolated population was 68.5% NK cells.

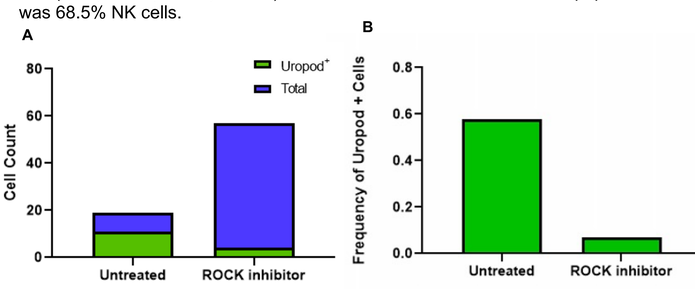


Figure 2. Counts of cells with a clear uropod treated with ROCK inhibitor versus untreated population. [A] Total cells with a clear uropod as a portion of total cells. In the untreated condition, 11 out of 19 cells displayed a clear uropod, whereas 4 out of 57 cells treated with ROCK inhibitor displayed a clear uropod. [B] Frequency of cells with a clear uropod in the treated versus untreated population. NK cells in the untreated population displayed a greater percentage of polarized cells.

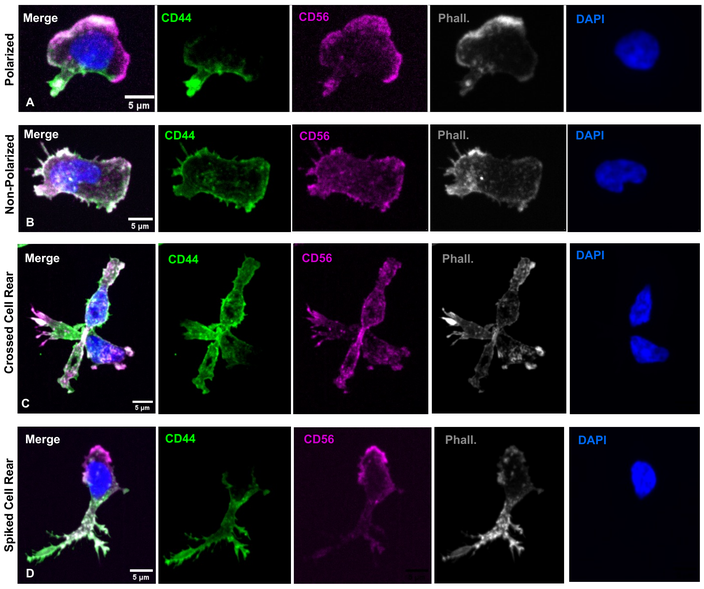


Figure 3. Select examples of polarized, non-polarized, and other notable phenotypes. [A] Example of a polarized cell, based on morphology and CD44 localization (untreated). [B] Example of a non-polarized cell (untreated). [C] Example of a notable phenotype, "crossed" trailing edge projections (treated). [D] Example of a notable phenotype, "spiked" cell rear projections (treated).

Conclusions & Next Steps

- A greater percentage of NK cells in the untreated condition were polarized based on morphology and CD44 localization than the treated condition.
- ROCK inhibitor plays an inhibitory role in polarization of NK cells.
- Imaging experiments revealed unique phenotypes for NK cells treated with ROCK inhibitor, including "spikes" and overlapping cell rears.
- ROCK inhibitor may disrupt the development of a clear uropod, due to its integral role in receptor clustering (by activating ERM proteins) and in forming actomyosin bundles that characterize the thin projection of the uropod.
- Based on the absence of the uropod as well as "clustered" CD44 in the non-polarized cells, I propose that ROCK inhibitor has a role in disrupting uropod development and receptor clustering in NK cells.
- Further investigation is required as to the role of Rho-family proteins in establishing signaling polarity in human NK cells.
- Future steps include determining if ROCK inhibition affects the migration and differentiation of NK cells.

References

1. Freud, A. G. & Calgiuri, M. A. Immunol Rev. 2006; 214(1): 56-72.
2. Mace EM., Gunesch, J.T., Dixon, A & Orange, J. S. Nat Commun 2016; 7(1)
3. Vicente-Manzanares, M., and Sanchez-Madrid, F.. Nat Rev Immunol. 2004; 4(2):120-122.
4. Sanchez-Madrid, F., Serrador, J. M. Nat. Rev Mol Cell Bio. 2009; 10(5):353-359.
5. Albas et al. Mol Bio of the Cell. 2001; 12(7): 2137–2145.
6. Schindelin et al. Nat Methods. 2012; 9(7):676-82.