

Investigating the relationship between donor NK cell cytotoxicity profiles and NK cell killing capacity

PROJECT DESCRIPTION

Background & Description

The human immune system has two lines of defense against pathogens, disease, and other disorders: the active and innate immune systems. In adaptive immunity, antigen-presenting cells (APCs), such as dendritic cells (DCs), prime effector T and B cells for killing by giving them antigen recognition of a diseased cell [1]. This process produces an effective, cell-specific response which can be reinitiated at a later time by memory T and B cells: however, adaptive responses take time and can be evaded by some pathologies such as immune-derived B cell lymphomas [2]. Innate immunity does not require this antigen specificity and instead relies on molecular signals—including pathogen-associated molecular patterns (PAMPs) and “missing self” molecules—to identify diseased cells [3]. As such, innate immunity takes a more broad-spectrum, fast-acting approach that can be better utilized against lymphomas and other such diseases.

Natural killer (NK) cells, the innate immune counterparts to cytotoxic T cells, are the primary effectors of innate immunity. They kill diseased cells by two methods: direct killing and antibody-dependent cellular cytotoxicity (ADCC). Direct killing occurs when previously described molecular signals indicate the presence of a diseased cell: in response, NK cells excrete cytotoxic molecules such as granzymes and perforin, lysing the cell [4]. In ADCC, antibodies unique to diseased cells enable NK cell recognition and killing—essentially enabling NK cells to kill like T cells by utilizing antigen specificity [4]. Because of the multifaceted approach by which NK cells kill diseased cells, they provide robust defense against cancers with immunoevasive capacities, including leukemias.

NK cell-mediated killing is a verified strategy for immunotherapeutic treatment of cancers and other pathologies [5]. The Denton Immunobiology Lab at the University of Nebraska at Omaha (of which I have been a member from Jan. 2020 onward) is currently focused on studying NK-mediated killing (both direct and via ADCC) and combination immunotherapeutic strategies in B cell lymphoma and leukemia. To study these processes, we obtain peripheral blood mononuclear cells (PBMCs)—a cell population which contains many types of human immune cells—from human donors for NK cell enrichment and subsequent combination immunotherapeutic testing. Due to the variability of the human immune system, human immunology research comes with multiple challenges. One such challenge is the wide range of NK cell composition in human donors; NK cells account for 5-15% of human PBMCs [4]. Within that range, NK cells can be made up of two predominant phenotypes, cytotoxic NK cells—activated NK cells with the capacity to kill diseased cells—and cytokine-producing NK cells [6]. While this variability can cause high variability in assay testing, it can also provide valuable insight into individual immunity profiles. I aim to analyze the human donor NK cells used in our assays in the context of two factors: cytotoxic NK cell composition, and NK cell killing capacity. I hypothesize that human donors with a high composition of cytotoxic NK cells from total NK cells will show heightened killing capacity in direct and ADCC assays.

Aim 1: To develop a flow cytometry panel to distinguish between cytotoxic and cytokine-producing NK cells within donor NK cell populations.

Aim 2: To determine the relationship between donor cytotoxic NK cell composition and killing capacity.

Completion of these aims should provide greater insight into donor NK killing capacity in pre-clinical and clinical settings as well as contribute to greater knowledge of individual immune profiles.

Methodology

NK Cell Isolation: Human blood products are obtained (e.g., American Red Cross via an existing MTA) and PBMCs isolated using standard Ficoll isolation methods. NK cells are enriched from PBMCs using the Miltenyi Biotec NK Cell Isolation Kit, human, (Cat. #130-092-657) and corresponding protocol.

Killing Assay: Target cells (ADCC: ATCC Daudi, Cat. # CCL-213™; direct: K562, Cat. # CCL-243™) are stained with CFDA-SE (CFSE) (Sigma-Aldrich Cat. # 216275-M) for effector-target discrimination in flow cytometric analysis. NK cells are cocultured with target cells at a 5:1 effector-target ratio for two hours with α -CD20 (ADCC) (Selleck Chemicals Cat. #A2009) or without α -CD20 (direct).

Flow Cytometry: Post-incubation, experimental and control samples are stained with 7-AAD for live-dead discrimination in flow cytometric analysis. Samples are then analyzed using the Beckman Coulter CytoFLEX flow cytometer by the following gating strategy: Singlet \rightarrow CFSE+ \rightarrow 7-AAD+. Literature review will indicate pertinent markers for cytotoxic vs. cytokine-producing NK cell discrimination.

Donor Composition/Killing Analysis: NK cell killing efficacy is determined by the following formula: $\% \text{ killing} = \% \text{ killing (experimental)} - \% \text{ killing (control)}$. Analysis techniques (e.g., k -means clustering ($k=3$)) will be used to establish “high-killing”, “moderate-killing”, and “low-killing” donor killing classifications (**Aim 1**). Cytotoxic NK cell composition is determined by the ratio: $\text{cytotoxic NK cells} / \text{total NK cells}$. Analysis techniques (e.g., k -means clustering ($k=3$)) will be used to establish “highly-cytotoxic”, “moderately-cytotoxic”, and “low-cytotoxic” donor composition classifications (**Aim 1**). A proper statistical test (e.g., Chi-square test or ANOVA) will be performed to establish a statistical relationship between cytotoxic composition and killing subcategorizations (**Aim 2**).

Project Timeline

| Task | Spring 2022 | Summer 2022 | Fall 2022 | Spring 2023 |
|-------------------------------------|-------------|-------------|-----------|-------------|
| <i>NK Cell Isolation</i> | X | X | X | |
| <i>Killing Assay</i> | X | X | X | |
| <i>Flow Cytometry</i> | X | X | X | |
| <i>Donor Yield/Killing Analysis</i> | | X | X | X |
| <i>Present at RCAF</i> | | | | X |

Student/Faculty Mentor Roles

Dr. Paul W. Denton will be my primary mentor for this research project. I will review pertinent literature to identify verified markers for cytotoxic/cytokine-producing discrimination before Summer 2022. I will assist in running all NK cell-mediated killing assays (data generation) in conjunction with Anna Mahr, a graduate assistant in the Denton Lab. All proposed analytical work will be performed by myself and reviewed by Dr. Denton for scientific and statistical accuracy. I will meet with Dr. Denton regularly to go over new developments and findings, and will continue to attend weekly Denton Lab meetings. I will conclude my research by presenting at UNO’s Research and Creativity Fair.

Previous Internal Funding

My previous FUSE funding investigated the impact of helper innate lymphoid cells (ILCs) on NK-mediated ADCC in B cell lymphoma. It entailed development and testing of an ILC isolation methodology and implementation of ILCs as a possible combination immunotherapeutic approach to our NK ADCC assay, to varied results. This project was highly engaging and provided new avenues for investigation: however, continuing that work is currently cost-prohibitive and would be best pursued in a graduate capacity.

My proposed project relates to my previous project, as it revolves around the same NK mediated killing assay which provided the base for my ILC combination immunotherapy work. As such, I already have a robust knowledge of the assays and data that I will be analyzing in the current project. Completion of the proposed project will provide valuable insight a different facet of NK cell biology.

BUDGET & BUDGET JUSTIFICATION

| Item | Cost (FUSE) |
|--|--------------------|
| Reagents (<i>e.g., blood products, Miltenyi Biotec NK Cell Isolation Kits</i>) | \$500.00 |
| Student Stipend | \$2,000.00 |
| Total Funds Requested | \$2,500.00 |

I am requesting \$500 for the material costs of research investigating a correlation of donor yield and killing capacity of NK cells. This sum will be used to purchase blood products for subsequent NK cell isolation. Currently, each blood product costs \$300 to obtain: \$500 will allow us to obtain approximately two blood products. These blood products will supplement those ordered using other Denton lab funding for analysis in **Aims 1-2**. I am requesting the remaining sum, \$2,000, be allotted as a student stipend. This funding will enable me to pursue NK cell research (\$10/hour, 20 hours/week for 10 weeks) from June-August 2022 in pursuit of **Aims 1-2**.

CITATIONS

- [1] F.A. Bonilla and H. C. Oettgen, "Adaptive immunity," *J. Allergy Clin Immunol.*, vol. 125, iss. 2, pp. S33-S40, Feb. 2010, doi: 10.1016/j.jaci.2009.09.017.
- [2] T. Menter and A. Tzankov, "Mechanisms of immune evasion and immune modulation by lymphoma cells," *Front. Oncol.*, vol. 8, March 2018, doi: 10.3389/fonc.2018.00054.
- [3] S.E. Turvey and D.H. Broide, "Innate immunity," *J. Allergy Clin Immunol.*, vol. 125, iss. 2, pp. S24-S32, Feb. 2010, doi: 10.1016/j.jaci.2009.07.016.
- [4] S. Paul and G. Lal, "The molecular mechanism of natural killer cells function and its importance in cancer immunotherapy," *Front. Immunol.*, vol. 8, no. 1124, Sept. 2017, doi: 10.3389/fimmu.2017.01124.
- [5] A. Jewett et. al., "Natural killer cells: Diverse functions in tumor immunity and defects in pre-neoplastic and neoplastic stages of tumorigenesis," *Molecular Therapy: Oncolytics*, vol. 16, pp. 41-52, March 2020, doi: 10.1016/j.omto.2019.11.002.
- [6] Krzywinska, E., Allende-Vega, N., Cornillon, A., et al. Identification of Anti-tumor Cells Carrying Natural Killer (NK) Cell Antigens in Patients With Hematological Cancers. *EBioMedicine*, vol. 2, iss. 10, pp. 1364, Oct. 2015, doi: 10.1016/j.ebiom.2015.08.021



College of Arts and Sciences / Department of Biology

January 31, 2022

Dear FUSE Review Committee,

I am writing to express my strongest support for Maia Bennett's FUSE Grant application, "Investigation the relationship between donor NK cell cytotoxicity profiles and NK cell killing capacity."

Maia is a Bioinformatics student in the Department of Information Science & Technology. I have worked with Maia in a professional capacity since December of 2019, when she joined my fledgling lab in the UNO Biology Department. Quickly, Maia established herself as a major presence in the lab and her efforts are highly valued. She has been a driving force in the development of rigorous protocols, assay implementation, and solid data generation. Importantly, she has done this within the context of her prior FUSE Grant project as well as in the greater lab context. Maia has shown great drive in her research as well, often running her assays over eight-plus-hour days with minimal assistance in addition to her routine lab duties. I believe Maia's previous work and current research trajectory set her up to excel in her second FUSE Grant project and beyond - when she attends graduate school.

The research Maia is proposing is instrumental to better understanding the data produced from NK-mediated cancer cell killing assays that comprise the primary research focus of my lab. The flow cytometry panel she is planning to establish will provide crucial insight into the phenotypic profiles of the effector NK cells in the NK-mediated killing assays performed in my lab. Results gained from the use of this panel will be incorporated into further analytical directions as well. These include planned downstream RNA-Seq and proteomics analysis of donor NK cells for potential genetic markers of killing efficacy.

As Maia's mentor, I will be providing both intellectual and material support for the project. This will include approximately weekly meetings to encourage progress and address any questions the research may bring, as well as financial responsibility for material costs not covered by the FUSE budget allocation.

Sincerely,

A handwritten signature in black ink that reads "Paul W Denton".

Paul W. Denton, Ph.D.
Assistant Professor of Biology
University of Nebraska at Omaha
Allwine Hall 514AA
6001 Dodge Street
Omaha, NE 68182-0040
Office: 402-554-3781
E-mail: pdenton@unomaha.edu

University Communications / 102 Eppley Administration Building / 6001 Dodge Street / Omaha, NE 68182
402.554.1234 / FAX: 402.554.1234 / unomaha.edu