# DETECTION OF NATURAL KILLER T CELLS IN HUMAN PERIPHERAL BLOOD, SPLEEN AND LUNGS.

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## INTRODUCTION

The natural killer T (NKT) cells are a rare heterogeneous group of lymphocytes that express both a limited range of T cell receptors and markers common to natural killer cells (such as CD56 and CD161). NKT cells recognize lipid antigens, such as Alpha-GalactosylCeramide, which are presented by CD1d-expressing antigen presenting cells. Subsets of human NKT cells have previously been described based on expression of CD4 and CD8. NKT cells are thought to play a regulatory role in alloimmune responses after solid organ transplantation. However, whether all NKT cell subsets have a regulatory function is unclear.

**The purpose of this study is to set up and optimize a flow cytometric assay to detect and characterize human NKT cell subsets in human peripheral blood, spleen and lungs in order to investigate human NKT cell subsets in organ transplant recipients.**

## PROCEDURE

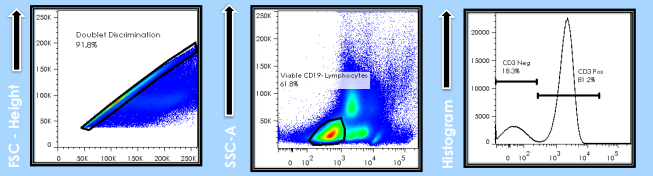
**PBMC/BAL/SPLEEN SAMPLES**

- ½ hour incubation with viability dye stain
- 1 hour incubation with CD1d tetramer loaded with Alpha-GalCer
- ½ hour incubation with other surface antibodies: CD3, CD19, CD4, CD8 and CD161
- Measure on the flowcytometer
- Analysis on Flowjo

Fluorescent human CD1d tetramers loaded with Alpha-GalCer in combination with CD3 expression were used to detect NKT cells in adult blood samples (n=7), spleen samples (n=2) and bronchoalvelar lavage samples (n=2) by flow cytometry. Subsequently, NKT cells were characterized by their surface expression of CD4 and CD8, and CD161 expression was determined within the latter subpopulations. To exclude non-specific binding, a viability dye stain and CD19 were used to gate out non-viable cells and B cells.

## FIGURES and RESULTS

GATING STRATEGY:



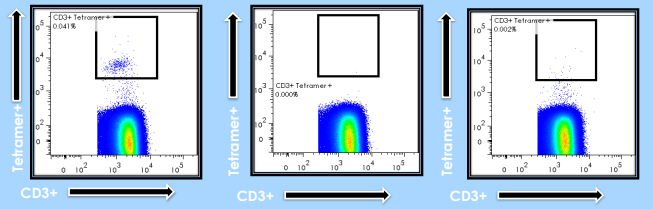

**Gate 1: Doublet discrimination**

This gate excludes cells which clumped together.

**Gate 2: Viable CD19- cells**

This gate excludes dead cells, as well as CD19+(B) cells.

**Gate 3: CD3+ cells within viable CD19- cells (Gate 2)**




**Gate 4-A: Natural killer T cells within CD3+ cells (Gate 3)**

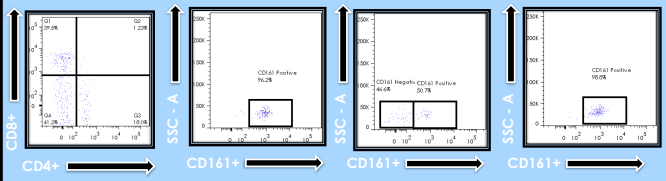
Cells were incubated with CD1d-loaded Alpha-GalCer. The FMO gate (Gate 4-B) was applied to the original staining in order to capture the natural killer T cell population. In this sample, the natural killer T cells comprised 0.041% of the CD3+ population.

**Gate 4-B: Background control 1 – fluorescence minus one (FMO)**

The FMO contained the same antibody cocktail as the original staining except for the CD1d-loaded Alpha-GalCer.

**Gate 4-C: Background control 2 – CD1d negative control**

Stainings were also conducted with CD1d mock-loaded tetramers to determine the non-specific binding of the tetramer to T cells.




**Gate 5: CD4+, CD8+ and double negative subsets within the natural killer T cells**

Quad 1 – CD8+
Quad 3 – CD4+
Quad 4 – Double negative

**Gate 6: CD161 expression within CD8+ natural killer T cells**

In this example, 93.6% of CD8+ natural killer T cells were CD161 positive.

**Gate 7: CD161 expression within CD4+ natural killer T cells**

In this example, 50.7% of CD4+ naatural killer T cells were CD161 positive.

**Gate 8: CD161 expression within double negative natural killer T cells**

In this example, 98.8% of double negative natural killer T cells were CD161 positive.

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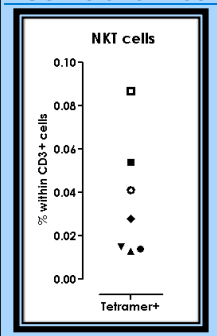
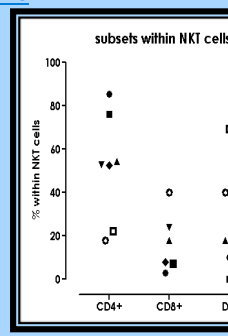



**Graph 1: Percentage of natural killer T cells within CD3+ cells in peripheral blood samples**




**Graph 2: Percentage of CD4+, CD8+ and double negative subsets within natural killer T cells**

*Different shapes represent different samples

### SUMMARY OF RESULTS:

NKT cells were detected in all peripheral blood samples (median: 0.028%, range: 0.013-0.087%). Within the NKT cell population, cell frequencies positive for either CD4 or CD8 varied among the samples (53%, 18-86% and 8%, 3-40%, respectively). CD4+ cells consistently formed a distinct population, while CD8 was dimly to highly expressed, rendering no clear separation between CD8+ and CD8- populations. A double negative (DN) subpopulation was noted in six samples (18%, range: 0-69%). CD4+ NKT cells were mainly CD161- (82%, 45-90%) while DN NKT cells were mainly CD161+ (96%, 73-100%). The frequency of CD161+ within the CD8+ subset varied among samples (64%, range: 5-97%). NKT cells were also detected in both spleen samples (0.04% and 0.016%). Only one BAL sample contained CD3+ cells; 0.032% of these cells were NTK cells.

## SUMMARY and FUTURE DIRECTIONS

In this preliminary study, we demonstrated the detection and characterization of various NKT cell subsets in human peripheral blood, spleen and lungs. These distinct populations may represent different functions. Future experiments will include characterizing the NKT cell populations in samples of healthy individuals and lung and heart transplant recipients. In addition, functional assays are being set up to investigate the cytokine profile for each subpopulation.

## ACKNOWLEDGEMENTS

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