

Pediatric Infectious Disease; Dr. Nayak

Summer 2019 Abstract

## Evaluation of Influenza-Specific AIM Responses in Adult and Pediatric CD4+ T Cells

### Background

The CDC estimated the disease burden of influenza during the 2017-2018 year was the highest it's been since the 2009 pandemic, resulting in an estimated 48.8 million infected and 79,000 deaths. To combat the growing threat of influenza, seasonal vaccines are released to prime the immune system against probable strains. The key to creating more effective flu vaccinations is a better understanding of the anti-influenza response of the immune system, including both cellular and serologic immunity.

### Objective

The purpose of this research project was to investigate the upregulation of cytokine-independent activation-induced markers (AIM) on CD4+ T cells upon stimulation with various influenza peptide pools and controls in order to evaluate methods of detecting flu-specific immune reactions independent of cytokines.

### Methods

Peripheral blood mononuclear cells (PBMCs) from healthy donors collected outside of the period of active influenza circulation were thawed and examined. In addition, samples from 3 pediatric subjects collected at baseline and at days 8 to 10 post-IIV administration were assayed. All PBMCs were stimulated using complete peptide pools representing the major influenza proteins or SEB for 21 hours, with cytokine secretion blocking during the last 4 hours, stained, and evaluated using multiparameter flow cytometry.

### Results

Initial experiments evaluated different AIM on CD4+ T cells, with OX40 CD25 and CD69 CD71 proving to be the most specific and supported by the literature. It was experimentally determined that OX40 and CD25 identified a population of activated cells that would not be identified using traditional cytokine screening. In a majority of cases, the phenotype of OX40 CD25 cells was observed to be consistent with cells that are responding to antigen addition\*. Analysis of stimulated patient samples 1 and 10 days post-vaccination revealed an upregulation of flu-specific AIM as well as Tfh cells.

### Conclusion

Cytokine-independent AIM is a valuable resource in healthy donor as well as post-vaccination samples regarding flu studies to better understand the specificity, phenotype, and function of the responding influenza-specific CD4 T cell response. In order for this technique to be applied more broadly to the evaluation of influenza-specific immunity, stimulation conditions that minimize nonspecific upregulation of these markers will need to be further investigated.