Antibody Array Based N-glycan Imaging of Captured Immune Cells

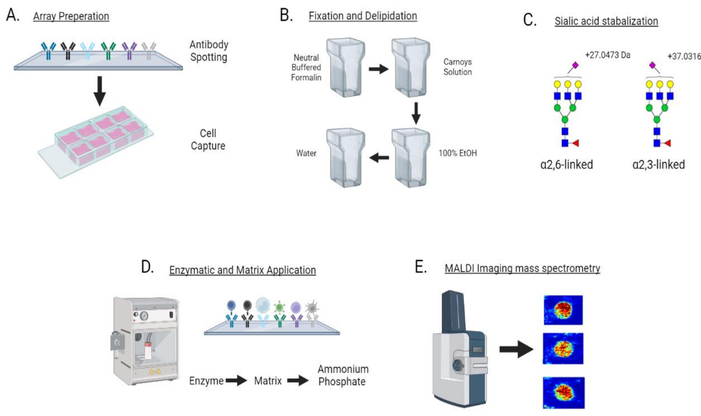
James W Dressman[1], Colin T McDowell[1], Xiaowei Lu, PhD[1], Peggi M Angel, PhD[1], Richard R Drake, PhD[1] and Anand Mehta, PhD[1]

[1]Department of Cellular and Molecular Pharmacology and Experimental Therapeutics, **Medical University of South Carolina**

Introduction

- Cell surface N-glycosylation plays an important role in both the innate and adaptive immune response through the modulation of cell surface receptors and cell to cell interactions. The study of immune cell N-glycosylation is increasingly becoming a field of interest, but hindered by the complexity of cell type specific N-glycan analysis. Analytical techniques such as chromatography, LC-MS/MS and lectin blotting are all standard methods for the study N-glycans. Pitfalls from each technique hinder either throughput or the acquisition of accurate structural data. Here, we are developing a rapid antibody array-based approach for the capture of nonadherent immune cells coupled with MALDI-IMS to analyze cell surface N-glycosylation, increasing the feasibility of immune cell N-glycan analysis.

Method



Antibody Specificity

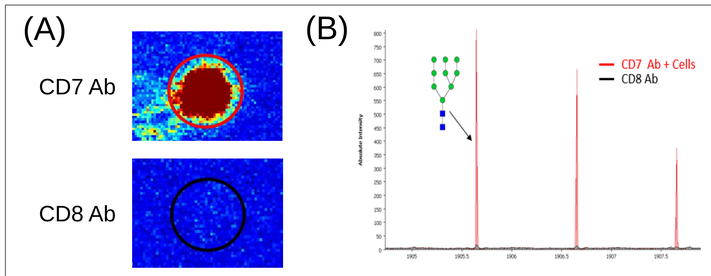


Figure 1. Antibody Capture Specificity. Jurkat cells, a CD4 acute T cell leukemia cell line, was used as a model immune cell to prove the concept of immune cell capture via antibody immobilization. A) CD7 is a costimulatory surface marker expressed by Jurkat cells. Spotted CD7 Ab captured Jurkat cells while spotted CD8 Ab showed no capture. B) Mass spectrum overlay of the highest abundant N-glycan found on Jurkat cells .

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Assay Sensitivity

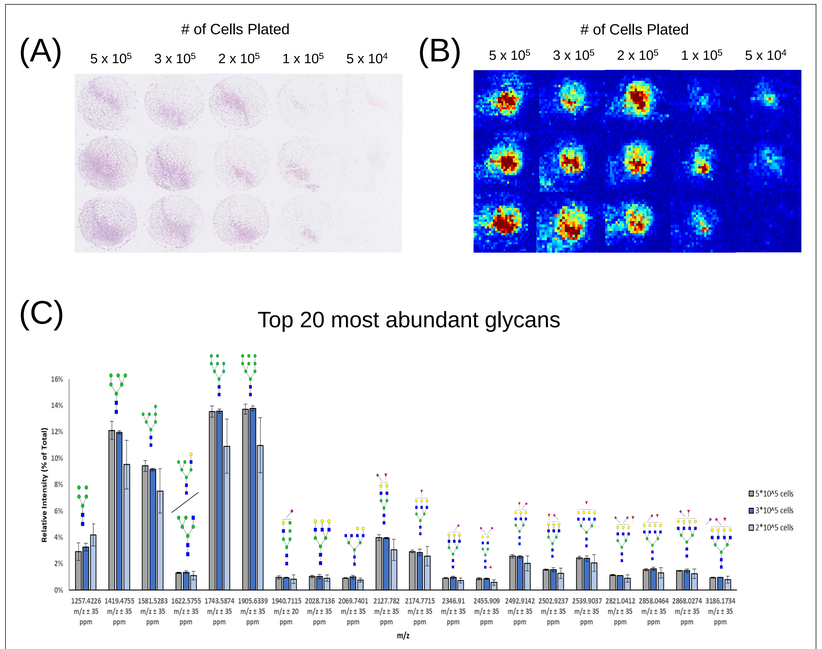


Figure 2. Assay Sensitivity. A Jurkat cell dilution series was incubated over 200ng of spotted CD7 Ab to test assay sensitivity. A) H&E stained captured Jurkat cells. B) MALDI-IMS N-glycan imaged Jurkat cells. C) Relative intensity of the top 20 most abundant N-glycans detected on immobilized Jurkat cells captured from dilution series.

Glycan Modulation

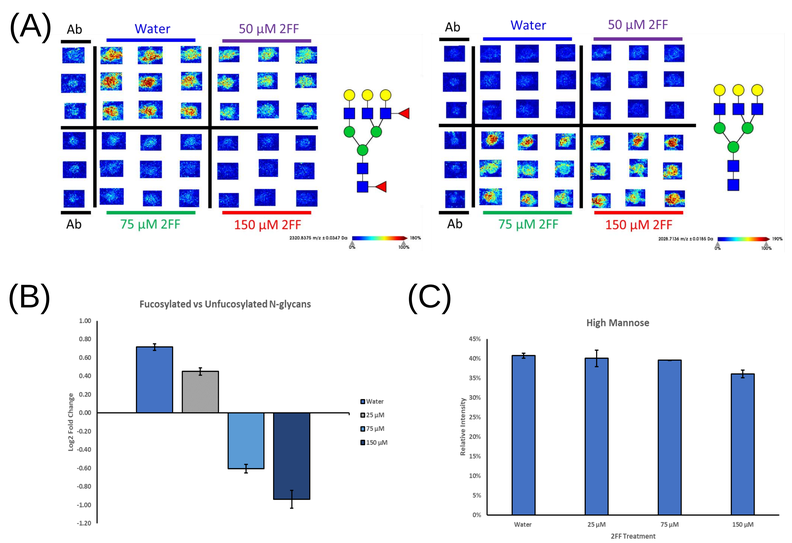
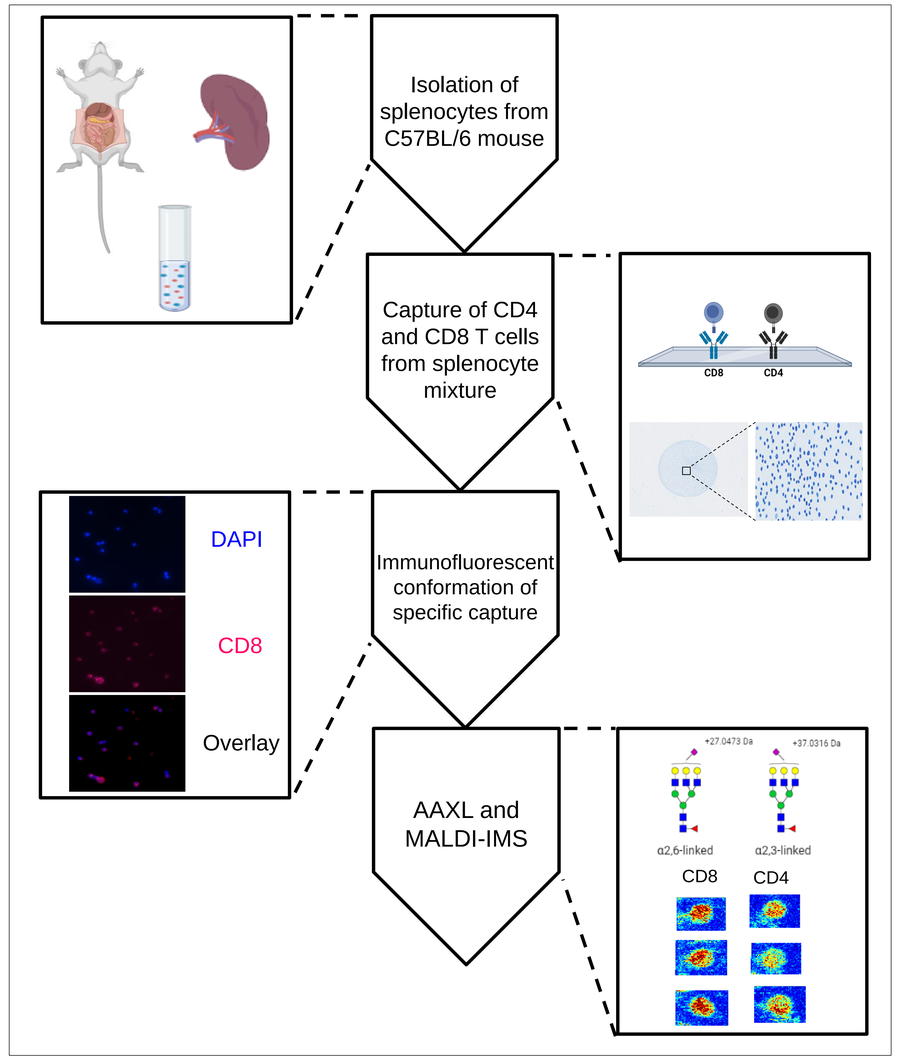


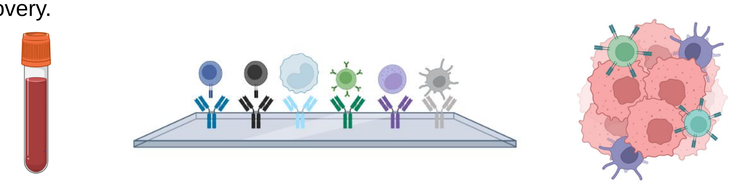
Figure 3. HepG2 Glycan Modulation by 2FF. HepG2 cells were treated with 2FF, an inhibitor fucosylation, to determine if the assay could detected changes in glycosylation. A) HepG2 cells were treated with 50-150μM of 2FF for 3 days. Cells were captured with a CD71 Ab prior to N-glycan imaging. B) Log2fold change comparing fucosylated N-glycans versus their unfucosylated counterparts exhibited a dose response reduction in fucosylation. C) Relative intensity of high mannose N-glycans across groups was not effected by 2FF.

Murine T Cell Capture



FUTURE DIRECTION

- Method optimization to enable capture of circulating T cells and other immune cells from blood and tumor homogenate.
- Analyze changes in T cell glycosylation in relation to disease state for biomarker discovery.



References:
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- Angel, P. M. et al. A Rapid Array-Based Approach to N-Glycan Profiling of Cultured Cells. (2019) J. Proteome Res. 18 (10), 3630-3639
- Blaschke, C. R. K., Black, A. P., Mehta, A. S., Angel, P. M. & Drake, R. R. Rapid N-Glycan Profiling of Serum and Plasma by a Novel Slide-Based Imaging Mass Spectrometry Workflow. J Am Soc Mass Spectrom 31, 2511–2520 (2020).