Human vaccine response signatures revealed through simultaneous transcriptome and protein expression profiling in single cells

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Molecular and cellular signatures of vaccination responses derived from comprehensive measurements of immune cells in bulk are well described for a number of vaccines, including the elevation of inflammatory/interferon gene expression signatures 24 hours post vaccination. These studies have also consistently shown strong inter-individual heterogeneity in certain vaccine response signatures, including those that are predictive of antibody responses, such as the extent of plasmablast cell increases at day 7.

Bulk whole-blood (or PBMC) transcriptomic measurements are highly informative, yet measure RNA levels from a heterogeneous mix of cells and therefore may miss changes involving low frequency cell populations. The cellular origin of bulk transcriptomic signatures is thus a major unresolved issue. While single cell data generated by multi-parameter flow and mass cytometry is becoming widely available, the number of measured proteins remains relatively small and linking single cell (cell subset) protein expression phenotypes to bulk transcriptomic signatures remains challenging.

Here we adopted a recently developed technology, CITE-seq, to simultaneously profile the transcriptome and 83 cell surface proteins in 3000-5000 cells per sample at baseline, and days 1, 7, 100 post vaccination from multiple subjects selected from two NIH human cohorts involving adjuvanted and non-adjuvanted influenza vaccination. By RNA sequencing of single cells (sc-RNAseq) in droplets along with DNA barcoded antibodies, CITEseq enables direct evaluation of transcriptome signatures at the single cell level together with cell surface phenotypes, thus describing the immune response at unprecedented resolution.

To validate our approach against cell phenotyping using flow cytometry, we investigated the concordance of peripheral blood cell type frequency detected with flow cytometry vs. CITEseq. Immune cells traditionally classified as a single “type” based on current limited phenotyping panels could be further delineated with CITEseq by the additional layer of information provided by transcriptomic state. We also describe an integrated demultiplexing procedure taking advantage of both sample “hashing” antibodies to resolve timepoints and genotypes to assign cells to the correct donor. Finally, we demonstrate the utility of improved experimental design and analytical considerations unique to CITEseq such as antibody titration and background count correction using unstained and isotype controls to remove unwanted experimental noise from downstream analysis.
Our work lays the groundwork for uncovering novel, predictive vaccination response signatures at the single cell, transcriptome-wide level and to define the cellular origin (and corresponding phenotypes)

underlying bulk immune response signatures from prior immune profiling studies. Our data can also serve as a rich resource to empower analysis of human immune states and responses, such as the analysis of a wide range of immune mediated diseases. This study was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, NIH.