Single cell transcriptomics reveals memory B cell activation and antibody selection

Researchers at Stanford University (Stanford, CA, USA) characterized a distinct transcriptional profile for B cells that had been activated by influenza vaccine and undergone clonal expansion. Tracking the changing immune repertoire over time, they identified five vaccination-responsive clones. Fewer than half of the antibodies produced by these clones were specific to influenza, suggesting vaccination can cause clonal expansion of bystander antibodies. F Horns, CL Dekker, SR Quake, *Cell Reports. (2020).*

Snapshot	10x Genomics product
Research area: Vaccines & Immunotherapies **Organism:** Human **Sample type:** PBMCs isolated from blood **Research question:** How does immunization enable immune protection? What is the relationship between transcriptional response, activation of memory B cells, and clonal expansion?	**Chromium Single Cell Immune Profiling Solution** • Chromium Single Cell 5' Library and Gel Bead Kit • Chromium Single Cell V(D)J Enrichment Kit, Human B Cell • Chromium Single Cell A Chip Kit • Chromium i7 Multiplex Kit • Cell Ranger Analysis Pipelines

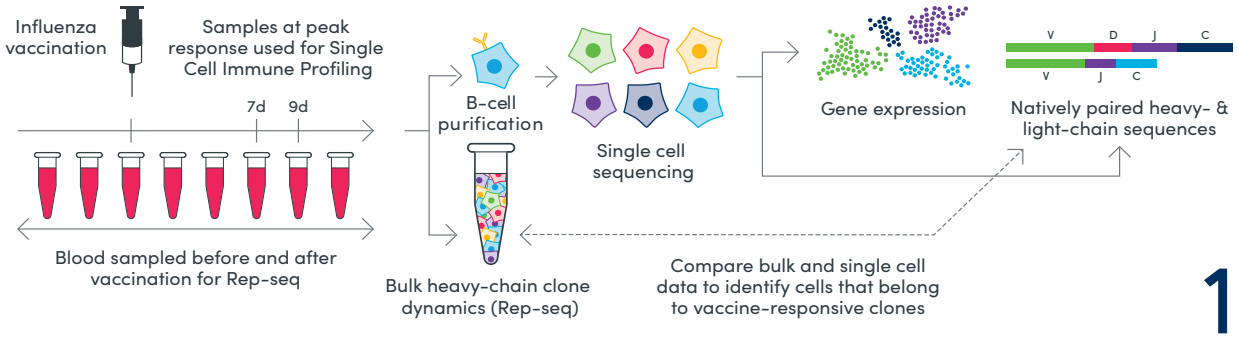
Experiment overview

Track changing immune repertoire response to influenza vaccination

- Draw blood from single healthy adult at multiple timepoints before and after influenza vaccination
- Bulk sequencing of peripheral blood antibody repertoire (Rep-seq) at all timepoints
- 131,585 cells were sequenced for full-length, paired antibodies, which resulted in 94,259 singlets with a productive heavy and light chain
- Single cell V(D)J libraries sequenced on Illumina NextSeq 500 with 2x150 bp chemistry

Transcriptional profiling of B cells during immune response

- Total B cells magnetically enriched from PBMCs using B Cell Isolation Kit II from Miltenyi
- Single cell RNA-seq of 35,631 cells using the Chromium Single Cell Immune Profiling Solution
- Single cell gene expression libraries sequenced on Illumina NextSeq 500 with 26x98 bp chemistry
- Comparison of clustering by RNA-seq to clone dynamics from Immune Profiling and Rep-seq



Why single cell?

The adaptive immune system, through gene recombination, junctional diversity, and heavy- and light-chain pairing, creates enormous diversity that can mount a response to millions of foreign microbes. Techniques like repertoire sequencing lose the resolution provided by pairing needed to understand immune response mechanisms. Single cell analysis helps solve the problem by providing paired sequences of antibody receptors for individual cells and enabling large-scale experiments, where tens of thousands of cells can be efficiently processed.

Computational analysis

Mapping single cells to clone dynamics

From bulk sequencing of B-cell heavy chain, clones were identified based on V and J gene usage, and length and sequence composition of HCDR3. For single cell sequencing, heavy and light chains were assembled by Cell Ranger and only cells with productive heavy- and light-chain sequences were kept. Using a custom algorithm, heavy chains from single cell data were matched back to clones from bulk analysis, filtered initially by V and J gene usage and CDR3 length. Sequences with >90% nucleotide identity within and outside the HCDR3 region were considered a match. 8,377 single B cells matched clones identified in bulk.

Results

Characterization of vaccine-responsive clones

Comparison of clone dynamics to paired B-cell receptor sequences before and after vaccination revealed that 8% of cells expressed heavy-chain sequences identified in bulk. Of these, 5 clones were vaccine-responsive. Cells in vaccine-responsive clones had distinct transcriptomic signatures and clustered together within the broader memory B-cell cluster (Figure 1). These cells, designated activated memory B cells, expressed genes associated with B-cell activation and cell migration to inflammatory regions. Activated memory B cells share several markers with age- or autoimmune-associated B cells (ABCs) that have been linked to viral infection, autoimmunity, and aging.

Vaccine-responsive clones are not always vaccine-specific

Unlike bulk methods, paired receptor sequencing results in actionable data, enabling activated memory B-cell receptors to be expressed and functionally tested. Of 21 antibodies expressed by vaccine-responsive clones, only 12 (57%) bound vaccine. Although other common viral and bacterial antigens were tested, none bound these antibodies, suggesting bystander activation of non-specific B-cell clones may be common.

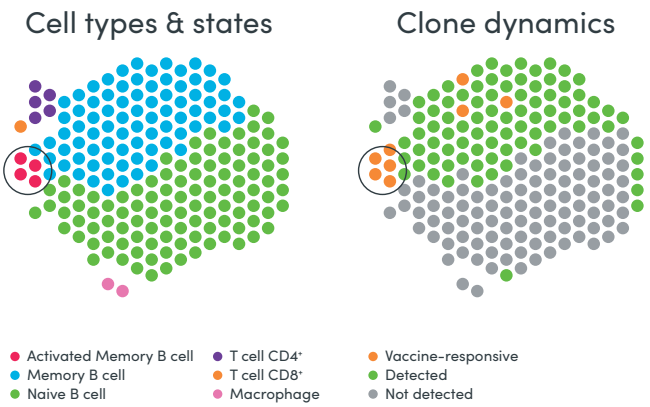


Figure 1. tSNE representation of sorted B cells. Clusters on left are color-coded by cell type annotation based on whole transcriptome data; cells on right are colored based on presence of clones found with Rep-seq. Memory and naïve B cells separate clearly. Within the larger memory B-cells cluster are the activated memory B cells (circled), which contain most vaccine-responsive clones.

References

1. F Horns, CL Dekker, SR Quake, Memory B cell activation, broad anti-influenza antibodies, and bystander activation revealed by single-cell transciptomics. *Cell Rep.* 30, 905-913e6 (2020).

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