Combining high-plex proteomics with single-cell transcriptomics for deep characterization of antigen-specific T cell responses

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POSTER NUMBER: 131

CellCarta

BACKGROUND

T cells are the primary mediators of cellular immunity and provide co-stimulation for humoral immunity as well as durable memory against encountered pathogens. Many immunotherapies, such as immune checkpoint inhibitors, CAR-T cells, TILs, cancer vaccines, and T cell engagers, work by inducing a robust T cell response targeting tumor-associated antigens. Laboratory tests that provide detailed T cell activation profiles may, therefore, have predictive value as biomarkers of clinical response, and can reveal mechanistic information for these drug classes. Here we show how high-plex proteomics alongside single-cell transcriptomics enables deep characterization of antigen-specific T cell responses.

METHODS

Peripheral blood mononuclear cells (PBMC) from five COVID-19 infected subjects were cultured overnight with a pool of 315 peptides derived from Spike protein (JPT) or with CytoStim polyclonal stimulator (Miltenyi) (**Figure 1**). After stimulation, cell culture supernatants were harvested for high-plex proteomic analysis by Olink Reveal. In parallel, the cells were stained with a panel of antibodies and sorted by FACS to isolate activated CD4 or CD8 T cells defined as double- or triple-positive for CD69, CD134 (OX40), and CD137 (4-1BB). Single-cell transcriptomics was performed on the sorted cells using the 10x Genomics 5' HT v2 workflow. Olink Reveal and 10x Genomics 5' libraries were constructed using SOPs established at CellCarta and sequenced on an Illumina NovaSeq to each specified depth. Sequencing files were processed using respective pipelines from Olink (ngs2counts and NPX Map) and 10x Genomics (Cell Ranger), followed by analysis with custom CellCarta pipelines.

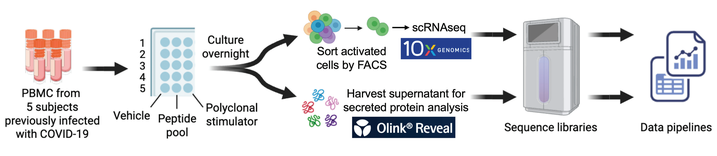


Figure 1. Workflow for secreted protein profiling and single cell transcriptomic characterization of model antigen-specific and polyclonally stimulated T cells.

RESULTS

Secreted protein profiles showed hallmarks of T cell activation

To characterize antigen-specific T cell response, we used peptide pools derived from the Spike protein of COVID-19 as a model antigen to stimulate cells from 5 subjects previously infected with COVID-19. CytoStim, a bispecific stimulator that bridges TCR to MHC, was included as a polyclonal (i.e., non-antigen-specific) comparator.

Following overnight stimulation, we evaluated 1,034 protein readouts provided by Olink Reveal. Polyclonal stimulation induced secretion of a broader set of proteins as well as higher levels of most proteins compared to specific peptide stimulation (**Figure 2**). Hallmarks of T cell activation were present under both conditions, including upregulation of IFN-γ, IL-2, IL-4, IL-17, TNF, and Granzyme B. Factors known to be secreted by antigen presenting cells during T cell stimulation, such as IL-1α/β, IL-6, IL-12, and CXCL12 (SDF-1) were also detected in the supernatant.

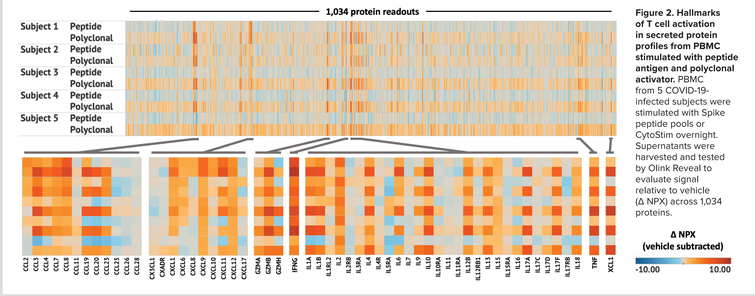


Figure 2. Hallmarks of T cell activation in secreted protein profiles from PBMC stimulated with peptide antigen and polyclonal activator. PBMC from 5 COVID-19-infected subjects were stimulated with Spike peptide pools or CytoStim overnight. Supernatants were harvested and tested by Olink Reveal to evaluate signal relative to vehicle (Δ NPX) across 1,034 proteins.

Antigen-specific activation profiles differed from polyclonal T cell activation

The difference between secreted protein profiles of antigen-specific versus polyclonal activation was rank-ordered to identify proteins preferentially produced under each condition (**Figure 3**). Consistent with Figure 2, many proteins showed higher secretion under polyclonal stimulation. However, IL-2 was induced more strongly under antigen-specific stimulation across all 5 subjects.

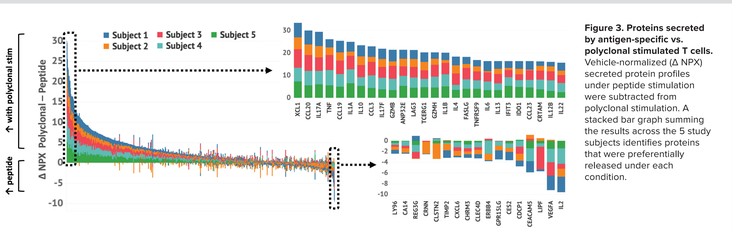


Figure 3. Proteins secreted by antigen-specific vs. polyclonal stimulated T cells. Vehicle-normalized (Δ NPX) secreted protein profiles under peptide stimulation were subtracted from polyclonal stimulation. A stacked bar graph summing the results across the 5 study subjects identifies proteins that were preferentially released under each condition.

Individuals exhibited different T cell response profiles to stimulation

The five subjects showed differences in their secreted protein profiles that can be used to understand the quality of T cell response on a personalized basis. To visualize how the profiles differed across conditions and individuals, we used principal component analysis (**Figure 4A**) and hierarchical clustering (**Figure 4B**). The PCA plot showed that subjects generally grouped by the type of stimulation, with some exceptions like the response to peptide stimulation by subject 2, which looked similar to polyclonal stimulation. Clustering showed that polyclonal responses across all five subjects grouped together, with a subcluster of strong responders formed by subjects 2 and 5. The unstimulated (vehicle) profiles of subjects 2 and 5 also clustered together. As shown by PCA, clustering revealed that the response to peptide by subject 2 was different from, and stronger than, the responses of the other four subjects.

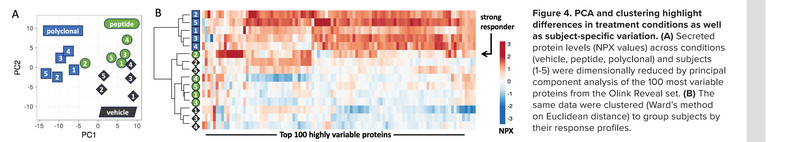


Figure 4. PCA and clustering highlight differences in treatment conditions as well as subject-specific variation. (A) Secreted protein levels (NPX values) across conditions (vehicle, peptide, polyclonal) and subjects (1-5) were dimensionally reduced by principal component analysis of the 100 most variable proteins from the Olink Reveal set. **(B)** The same data were clustered (Ward's method on Euclidean distance) to group subjects by their response profiles.

Single cell transcriptomics contextualizes secreted protein profiles by defining which cells express genes for any proteins of interest

To better understand the secreted protein patterns detected by Olink Reveal, we used single-cell transcriptomics to investigate which cell types expressed genes for important immune factors (**Figure 5**). All sorted cells showed an activated T cell transcriptional profile, while only a small number of CD4 T cells expressed proliferation genes. Genes corresponding to cytotoxicity were strongly expressed by CD8 T cells. Genes like IL-2, IFN-γ, and IL-17 were expressed by a small subset of CD4 effector T cells. TNF was expressed more broadly, including by CD4 memory, Treg, and CD8 cells. Expression of CCL5 was biased towards CD8 T cells. CXCL12 is an example of a chemokine known to be secreted by antigen presenting cells, not T cells, explaining its detection by Olink Reveal but not by RNA sequencing. To further integrate these two omics data sets in the future, we are developing tools that link subject-specific differences in protein secretion with individualized transcriptional profiles.

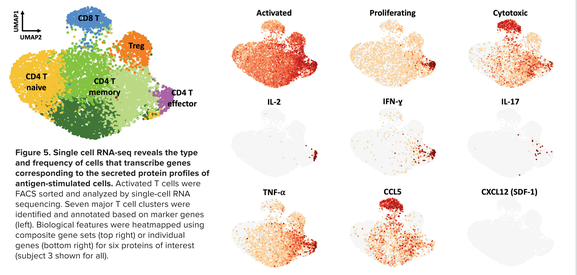


Figure 5. Single cell RNA-seq reveals the type and frequency of cells that transcribe genes corresponding to the secreted protein profiles of antigen-stimulated cells. Activated T cells were FACS sorted and analyzed by single-cell RNA sequencing. Seven major T cell clusters were identified and annotated based on marker genes (left). Biological features were heatmapped using composite gene sets (top right) or individual genes (bottom right) for six proteins of interest (subject 3 shown for all).

KEY TAKEAWAYS

- Secreted protein profiling offers deep, functional characterization of antigen-specific T cell response and illuminates personalized differences across subjects.
- Combining high-plex proteomics with single-cell transcriptomics provides a comprehensive picture of T cell activation that can extend clinical biomarker strategies in oncology and beyond.

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