Mass Cytometry Applications for Immunology Research

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The CyTOF® Mass Cytometer uniquely isolates signal from over 30 probes into single channels with minimal signal overlap, thereby enabling system-wide single-cell proteomic studies. Typical probes used are target-specific antibodies conjugated to rare earth metals and targets studied include cell surface markers, cytokines, signaling proteins and nucleic acids.

This document highlights applications uniquely enabled by mass cytometry in the field of immunology.

More information is available on the Fluidigm website: [www.fluidigm.com](www.fluidigm.com)

**Continuum of CD8⁺ T cell phenotypes revealed by deep profiling with mass cytometry**


**Uniquely CyTOF**

- Simultaneous measurement of 34 parameters per sample in healthy human blood including:
  - 17 cell surface markers
  - 6 peptide-MHC tetramers
  - 9 intracellular functional markers
  - DNA content and viability
- Identification of over 100 combinations of functional capacities in antigen-specific CD8⁺ T cells
High-dimensional mass cytometry analysis reveals that CD8⁺ T cells exist as a heterogeneous continuum rather than discreet subsets. Human PBMC were stimulated with PMA-ionomycin then stained with 16 phenotypic markers and 9 functional markers prior to analysis on a CyTOF. These 25 markers were used as inputs to derive three principle components (PC) summarizing the most informative variation in the data set. Marker analysis of gated populations revealed that PC1 distinguishes naïve vs. memory state of the cell, PC2 discriminates differentiation state, and PC3 identifies the central memory compartment. Spatial representation of the PC analysis (Fig. A) produced a Y-shaped continuum onto which overlaid gated populations reveal four clusters visualized from three different perspectives rotated around the PC2 axis: naïve (green), central memory (Tcm, yellow), effector memory (Tem, blue), and short-lived effector (Tslec, red). Memory cell progression analysis (Fig. B-C) highlights a gradual loss of markers related to central memory phenotypes with a concomitant induction of markers associated with later stage memory cell differentiation and senescence.

Simultaneous analysis of 34 parameters by mass cytometry allowed the complexity of CD8⁺ T cells to be characterized to an unprecedented degree. Additional data analysis of virus-specific tetramer positive T cells from this study reveals that immune responses to pathogens are not dominated by a limited number of distinct subsets, but rather shaped by hundreds of functionally distinguishable CD8⁺ T cell phenotypes each expressing unique combinations of cytokines.
The transcriptional landscape of αβ T cell differentiation


Uniquely CyTOF

- Simultaneous analysis of 22 parameters per sample in healthy and leukemic human bone marrow including
  - 15 cytoplasmic and nuclear signaling molecules
  - 7 cell surface phenotyping markers
- Use of mass cytometry phosphoproteomics to identify key mediators involved in the differentiation of T cells from thymocyte precursors

Definition of CD4+ and CD8+ transcriptional identities. The key signaling molecules involved in the differentiation of CD8+ (left) and CD4+ (right) T cell lineages were characterized by profiling the phosphorylation status of 15 intracellular targets in lymph node cells, following stimulation for the indicated time-points with anti-CD3 and anti-CD28. Potency of stimulation was visualized in Cytobank™ using a heat map displaying median fold-change in protein expression relative to the un-stimulated control (0).

Mass cytometry revealed marginally elevated levels of phosphorylation in CD4+ T cells, however similar dynamics of phosphorylation were revealed for key TCR-proximal (CD3ζ, Zap70, Lat, SLP-76) and downstream (Erk1/2, S6, MAPKAPKII, NF-κB) nodes. The detailed study of T cell differentiation provides a unique insight into the gradual nature of lineage commitment, providing an important reference for the study of T cell diseases such as leukemia, immunodeficiency and autoimmunity.
T cell epitope mapping by mass cytometry


Uniquely CyTOF

- Simultaneous analysis of 37 parameters per sample in human blood including
  - 109 combinatorial encoded peptide-MHC tetramers
  - 23 phenotypic and functional markers
  - DNA content and viability
- Identification of phenotypically distinct rotavirus-specific CD8\(^+\) T cells in 17 healthy donors

Combinatorial tetramer staining and high-dimensional mass cytometry analysis establish a relationship between antigen specificity and phenotypic diversity in CD8\(^+\) T cells. Peripheral blood CD8\(^+\) T cells from healthy donors were stained with peptide-loaded MHC tetramers and antibodies to surface and intracellular proteins prior to analysis on a CyTOF. Expression levels of 23 phenotypic markers by tetramer-stained CD8\(^+\) T cells relative to tetramer-negative cells (Fig. a, bottom) were analyzed in a clustered heat map for the indicated epitope specificities (Fig. a, right), or used as inputs to derive two principle components PC1 and PC2 analyzed for each single epitope-donor combination (Fig. b-d). Principle component analysis revealed that PC1 distinguishes naïve vs. effector state of the cell, whereas PC2 discriminates between central memory and late-stage effector states (Fig. e). Analysis of plotted normalized CD103 and integrin-β7 expression values by tetramer-stained cells highlight a unique antigen-dependent phenotype usage by rotavirus VP3-specific CD8\(^+\) T cells shared with gut-resident intraepithelial lymphocytes (Fig. f).
This study describes a new Mass Cytometry method for comprehensive T cell analysis that combines 23 marker immunophenotyping with combinatorial tetramer-based screening of over 100 TCR specificities in a single tube. The information-rich data obtainable by this technique enables potential discovery of immunodominant pathogen-derived peptides and holds tremendous promise for infectious disease and vaccine research studies.

**High-resolution profiling of the baseline diversity in human NK cells**


**Uniquely CyTOF**

- Simultaneous measurement of 38 parameters per cell in blood samples from monozygotic twins and unrelated human donors including:
  - 28 NK cell receptors
  - 8 cell surface phenotyping markers
  - DNA content and cell viability
- Use of mass cytometry to reveal a baseline repertoire of over 100,000 phenotypically distinct NK cells shaped by genetic and environmental determinants in humans
High-dimensional mass cytometry profiling of the NK cell repertoire uncovers a unique diversity of inhibitory and activating receptor expression patterns. Peripheral blood NK cells from monozygotic twins 5a and 5b (Fig. A) or two healthy unrelated individuals 003 and 007 (Fig. B) were analyzed by SPADE through clustering NK cell subsets into a minimum spanning tree according to the expression of 28 cell surface receptors. Node color is scaled to the median expression intensity of the activating NKG2D and KIR2SD4 receptors, or the inhibitory NKG2A and KIR2DL1 receptors. Nodes are sized according to the relative cell abundance within the entire population of NK cells.

High-resolution phenotyping by mass cytometry uncovered a remarkable breadth and diversity in human NK cells with over 100,000 phenotypically distinguishable subsets. By interrogating the diversity of this repertoire in unrelated healthy
individuals and monozygotic twins, inhibitory functions of NK cells were found to be tightly regulated by host genetics. In striking contrast, the expression and the combinatorial arrangement of activating receptors is shaped by environmental cues, thereby ensuring a highly diverse repertoire of NK cells in the face of infectious challenges and cancer.

Analyzing antigen-induced signal integration in human T cells by mass cytometry


Uniquely CyTOF

- Simultaneous analysis of 32 parameters per sample in human T cells including:
  - 10 phenotypic and functional markers
  - 21 signaling proteins
  - DNA content
- Single-cell analysis of the activating and inhibitory signaling nodes modulating the threshold of T-cell activation

High-dimensional functional profiling of the T-cell receptor signaling complex by mass cytometry revealed a mechanism through which extrinsic antigen availability is temporally integrated by opposing signaling modules in order to mute or license T cell responses. Leveraging the combinatorial knowledge uniquely produced by mass cytometry may help to identify signaling roadblocks and target defective pathways to restore immune functions.
High-throughput analysis of human T-cell signaling profiles by mass cytometry. Melanoma-specific cytotoxic T cells (CTLs) were cocultured for 2, 5 or 10 min with B lymphoblasts presenting predetermined surface amounts of MART I_{27-35} peptide-MHC-I complexes. Subsequently, cells were stained with 32 metal-conjugated probes identifying CTL surface markers and 21 signaling proteins prior to analysis on a CyTOF. Figure A summarizes the fold change increases in phosphorylation compared to unstimulated coculture conditions for 4 activating proteins (upper row), 3 inhibitory proteins (middle row) and 7 canonical pathway-signaling molecules (bottom row) in CTLs exposed to increasing amounts of peptide-MHC-I complexes presented by antigen-presenting cells. The data were further summarized in a heat map representation depicting fold change values relative to un-elicited cocultures (Fig. B). Red colors indicate increases in phosphorylation, whereas green colors indicate decreases in phosphorylation. Results indicate that increased phosphorylation of inhibitory proteins occurs when the activated T-cell receptor complex encounters a greater-than-optimal number of peptide-MHC-I complexes.

Bibliography


