Welcome to the November issue of Trending Now, a quarterly anthology of recent impactful publications by researchers using CyTOF technology.

This month’s issue is the first in a two-part series focusing on the recent application of mass cytometry to vaccine development.
WHAT’S NEW IN MASS CYTOMETRY

Uniquely enabled with CyTOF® technology, the ability to resolve diverse cell types and target specific and sometimes rare cell subsets is central to the analysis of a vaccine-triggered immune response.

The development and testing of new vaccines, and the optimization of current ones, are constantly evolving with advancing technology.

Vaccination continues to be the primary channel we rely on for infectious disease management and control. Because different infections initiate unique and complex immune reactions, the diversity of individual response and disease immunopathology has become a significant focus of infectious disease research. The more we learn about the interactions between pathogens and immune response, the better we can understand how to create effective vaccines.

A key consideration is having the right tools to achieve a comprehensive and rapid approach to identifying antigens that induce a protective, specific immune response or biomarkers that predict a desired reactive response. Mass cytometry advances the possibilities for systems immunology-based, highly multiplexed profiling of these cellular immune responses.

In this issue of Trending Now we highlight recent publications showing how researchers have used mass cytometry to detail the hallmarks of immune response that can support targeted vaccine development, investigate the impact of vaccine schedule on immune response and outline use of immune profiles to predict vaccine response.
CyTOF technology addresses challenges in vaccine development

A recent review (Brodin 2020) by Petter Brodin, MD, PhD, of the Science for Life Laboratory at the Karolinska Institute provides perspective on the utility of mass cytometry in vaccine studies. Brodin focuses on vaccine response in young children—a challenge because of small sample volumes and the variations found in a developing immune system, yet the stage when a majority of vaccinations are given. Systems immunology methods like mass cytometry that provide high-dimensional single-cell measurements make it an ideal technology for profiling the immune system in small-volume samples. Capturing multiple layers of information simultaneously can also reveal co-regulated features in the immune system and represent inter-individual variation better than traditional studies.

Brodin notes that defining the pathogen specificity of responding immune cells is essential, thus studies must distinguish vaccine-specific cells from other activated cells. For example, a recent publication (Schulien et al.) used MHC class I metal-labeled tetramer analyses to identify and analyze both pre-existing and newly induced SARS-CoV-2 specific CD8+ T cells and their responses, implicating them as important determinants of immune protection in mild SARS-CoV-2 infection and suggesting their possible role in the development of a vaccine.

Benaroya Research Institute at Virginia Mason Medical Center researchers used metal-labeled tetramers to investigate the origination and development of yellow fever virus (YFV)-specific cCXCR5+ T cell subsets, in which cCXCR5 provides a potential biomarker to deduce the activity of T follicular helper (TFH) cells within germinal centers (DeGottardi et al.). By comparing YFV-specific T cell activation to other pathogen-specific T cells after yellow fever vaccination (Figure 1), only measurable increases in the frequencies of YFV-specific T cells were elicited. The group further profiled these rare virus-specific CD4+ T cells based on expression of 21 distinct cell surface markers. The unique technical capabilities of CyTOF enabled a high level of detail on the subtypes of YFV-specific CD4+ T cells that allowed them to determine a hierarchy in the induction of cCXCR5+ T cells for different YFV antigens.

Figure 1. Surface marker expression of YFV epitope-specific CD4+ T cells pre and post vaccination (DeGottardi et al.)
Hallmarks of effective immune response

Much current COVID-19 research focuses on elucidating biomarkers of an effective immune response to SARS-CoV-2 infection, which is crucial to the development of vaccines and therapeutic interventions.

Work from the University of Zurich (Chevrier et al.) used 40-parameter mass cytometry and targeted proteomics of serum samples to assess phenotypic changes in the longitudinal innate immune response of 66 patients with mild to severe COVID-19 and 22 healthy controls (Figure 2). They noted profound differences between late-stage responses in mild disease, which resulted in a balanced innate immune signature, and severe disease, which presented an enduring chemokine-enriched inflammatory state.

The data revealed a dynamic pattern of inflammatory response to SARS-CoV-2 infection and led to an associated study (Adamo et al.) of the specific immune response in severe COVID-19 patients. The team used complementary technologies, including 42-marker mass cytometry, flow cytometry and targeted proteomics. These technologies confirmed that T cell activation, exhaustion and apoptosis are associated with disease severity. Results suggest decreased CD8+ T cells could serve as a hallmark of severe COVID-19, defining a damaging inflammatory environment that should trigger targeted and timely anti-inflammatory interventions to increase therapeutic efficacy.

Researchers from Stanford University (Arunachalam et al.) reported on a systems biology approach to assess the immune status of COVID-19 patients with mild to severe disease from two geographically independent cohorts. The team characterized immune cell phenotypes in peripheral blood mononuclear cells (PBMC) using a phosphoprotein CyTOF® panel that included 22 cell surface markers and 12 intracellular markers against several kinases and phospho-specific epitopes of signaling molecules and H3K27ac, a histone modification marker that drives epigenetic remodeling.

They observed distinct gene expression reduction, impaired signaling and enhanced levels of inflammatory mediators, correlating with disease severity. Most notable was an increase in plasmablast and effector CD8+ T cell frequency in all infected individuals and a prolonged CD8+ effector T cell response that increased well after symptom onset.

The application of mass cytometry to the development of new approaches such as that created by Nadia Roan, PhD, and her team at the Gladstone Institutes and University of California, San Francisco, facilitates high-dimensional phenotyping of multiple cell types as well as in-depth characterization of specific cell subsets. The team applied a customized ex vivo model to better define SARS-CoV-2 specific T cell

Figure 2. Experimental approach including cohort groups and full study design (Chevrier et al.)
Phenotypes in convalescing COVID-19 patients (Neidleman et al.) using a 38-marker CyTOF panel. The study identified common features of effective immunity against SARS-CoV-2 and suggests that inducing a similar long-lived CD4+ and CD8+ T cell response against the virus could serve as a vaccination strategy. Further study will aim to better understand what constitutes an effective versus an immunopathological T cell response against the virus and to assess the features of vaccine-induced SARS-CoV-2 specific T cell responses.

Impact of vaccine schedule on immune response

In the first reported assessment of vaccine schedule impact on development of innate and humoral responses, researchers from the France Vaccine Research Institute (Palgen et al.) sought to improve vaccine optimization by better understanding the mechanisms inducing immunity. The group compared short- and long-interval vaccination schedules on the modified vaccinia virus Ankara (MVA), a highly attenuated third-generation vaccinia-based smallpox vaccine, and vaccinia virus (VACV) in cynomolgus macaques, aiming to refine their use as both an effective smallpox vaccine and as a vector for new recombinant vaccines against other diseases.

A 35-marker mass cytometry panel enabled simultaneous assessment of cell identification, antigen presentation and modulation, Fc receptors, migration and adherence, and activation and cytokines. Timing of immunizations was found to be significant to priming the immune system for an effective response in longer-lasting protective immunity. Late phenotypic modifications correlated with more robust humoral and innate responses only in a delayed 2-month boost, while a 2-week boost resulted in an impaired secondary antibody response. Mass cytometry was key to the discovery of new cell populations, revealed new features of neutrophils and uncovered a relevant eosinophil population, determining the mechanisms of innate and adaptive immunity during prime-boost vaccination.

Figure 3. Examples of various cell populations that influence immune response
Assessing safety and efficacy based on immune response

A single mass cytometry panel can simultaneously evaluate multiple parameters related to vaccine efficacy. This broad data from one experiment can quantify changes in both innate and adaptive immune cell populations with vaccination and investigate functional status of all identified populations to support vaccine safety studies (Figure 3).

A team at the Vaccine & Immunotherapy Center at Massachusetts General Hospital led by Patrick Reeves, PhD, and Dr. Mark Poznansky, MD, PhD (advancingcures.org) performed longitudinal high-dimensional immune profiling of small samples from mice to identify novel correlates of effective vaccination and control of Coxiella burnetii (Cb) infection (Reeves et al.). To address an unmet need for a safe and effective vaccine for Q-fever, a flu-like illness caused by Cb, the team used mass cytometry to demonstrate significant alterations in circulating immune cell populations that distinguished vaccinated from naive mice within 10 days and persisted post-challenge. Uniquely enabled by CyTOF technology, use of small sample volumes of less than 200 µL and the ability to fix and store cells under biosafety level 3 before bulk staining facilitated detailed analysis of T cell, B cell and innate (NK cell, granulocyte and monocyte/macrophage) populations (Figure 4). This study refines the understanding of the integrated immune response to Cb vaccination, identified novel roles for key immune modulatory proteins, and informs the assessment of candidate vaccines for Cb while minimizing reactogenicity.

Based on evidence that vaccination for the 2009 Influenza pandemic led to an increased incidence in narcolepsy type 1 (NT1), a team at Lund University (Lind et al.) sought to understand the autoimmune pathogenesis behind the response. The group analyzed immunocyte signatures in Pandemrix® induced NT1, applying CyTOF analysis on cryopreserved PBMC of 50 patients compared to their first-degree relatives. Results showed a distinct decrease in specific T cell subsets among affected patients. The study signifies a new approach to dissecting immune processes using single-cell analysis to explain disease pathogenesis and validates the observation that CD8+ T cells could be further studied for potential future therapies.

Figure 4. Assessment of key marker expression following Cb vaccination and challenge (Reeves et al.)
The tangible impact of CyTOF

As evidenced by recent research, high-parameter mass cytometry in immune and vaccine studies can deliver a complete, contextual overview of innate and adaptive immune response to infectious agents, immunogens and vaccine formulation. The technology offers detailed immunologic information to inform vaccine safety and immunogenicity, efficacy and durability, and individual immune response. By understanding these induced perturbations in the human immune system, we can further uncover regulatory pathways that are important to better appreciate the immune system and its inner workings.

References


Learn more at fluidigm.com/covidresources

Listen to recorded seminars given by Patrick Reeves, Petter Brodin and others studying infectious disease at fluidigm.com/cytofseminars.

Learn more about the application of Fluidigm microfluidic, mass cytometry and Imaging Mass Cytometry™ platforms and analytic reagents to investigation of COVID-19.