Next-Generation Inflammatory Response Assay: Advancing Cytokine and Immune Cell Characterization for Precision Bioanalysis

Corinne Ramos Ph.D., Melodie Boute Ph.D., Sandra Delebecq MS, Victor Senechal MS, Richard Ruez Ph.D.

Introduction

Inflammation plays a critical role in numerous disease processes, including autoimmune disorders, infectious diseases, and immune-related adverse effects in immunotherapy. The ability to accurately assess inflammation is critical for understanding disease mechanisms, monitoring treatment efficacy, and identifying novel therapeutic targets. Traditional cytokine assays often lack the cellular context necessary for fully understanding immune activation and regulation. Inflammatory responses are shaped by intricate cytokine networks and immune cell interactions, which demand a more integrated, high-resolution analytical approach to provide meaningful insights. Our next-generation inflammatory response assay bridges this gap by simultaneously measuring cytokines and immune cell phenotypes, delivering a multi-dimensional dataset to enhance translational research and clinical decision-making.

This approach allows to:

- Assess cytokine-driven immune regulation with unprecedented precision.
- Identify immune cell populations contributing to inflammatory or immunosuppressive states.
- Uncover novel biomarker signatures that correlate with disease progression or treatment response.

The insights gained from this platform have the potential to significantly impact fields such as immuno-oncology, autoimmune disease research, and vaccine development, facilitating the design of more effective therapeutic interventions tailored to individual immune profiles.

Method

Whole blood is directly stained, avoiding the bias introduced by PBMC isolation, ensuring a more accurate representation of in vivo immune dynamics (Fig.1).

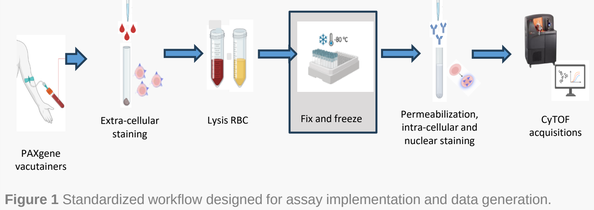
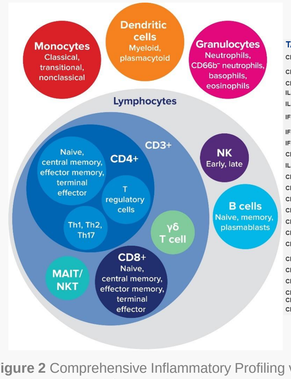


Figure 1 Standardized workflow designed for assay implementation and data generation.

The assay is based on CyTOF mass cytometry, utilizing a 46-marker panel to provide deep immune profiling. Whole blood is incubated with metal-tagged antibodies, allowing single-cell resolution analysis without the need for cell separation. Immune cell phenotyping is then performed by identifying major immune subsets, including T cells (CD4, CD8, Tregs), B cells, NK cells, and monocytes, as well as functional states such as naïve, central memory, effector memory, and exhausted T cells. Cytokine expression profiling is carried out to detect intracellular cytokines, including IL-6, IL-10, IL-2, IFN-γ, and TNF-α, providing a comprehensive assessment of immune activation and suppression (Fig.2).



TARGET	CELLS	TARGET	CELLS
CD45	Myeloid cells, Lymphoid cells	CD28	T cells
CD4	T cells	CD38	B cells and T cells activated, NK cells, DCs
CD8a	Cytotoxic T cells	CD56/NCAM	NK cells
IL-2	T cells, Treg cells induction	TCR gamma delta	Tgd cells
IL-6	T cells, Th17 cells induction	IL-10	Treg
IFN alpha	Th1 cells, Cytotoxic T cells, NK cells	CD294/CRTH2	Th2 cells
IFN beta	Fibroblasts, Macrophages, Dendritic cells (DCs)	CD197/CCR7	T cells, B cells, NK cells
IFN gamma	Th1 cells	CD14	Macrophages
CD196/CCR6	B cells, T cells (CD4 cells, Treg cells), Th17 cells	IL-13	Th2 cells
IL-4	Th2 cells	CD3	T cells
CD123/IL-3R	Stem cells, Plasmacytoid Dendritic cells	CD20	B cells
CD19	B cells	CD66b	Granulocytes
CD11c	DC, Macrophages, Monocytes	HLA-DR	APC cells
CD16	Macrophages	IgD	B cells
CD45RO	Memory cells	TNF alpha	Macrophages, Th1 cells and NK cells
CD45RA	Naive cells	CD127/IL-7R alpha	Lymphoid cells
CD161	CD4, CD8 T cells, NK cells, Th17 cells	IL-1b	Th17 induction cells
CD194/CCR4	Th2 cells, Treg cells	IL-12p70	DCs, Macrophages, Th1 induction cells
CD25	Treg cells	Tbet	Th1 (transcription factor)
CD27	T cells, B cells, NK cells and DCs	GATA3	Th2 (transcription factor)
CD57	NK cells	IL-17A	Th17 cells
CD183/CXCR3	Th1 cells	IL-22	Th17/Th22 cells
CD185/CXCR5	B cells and follicular T cells	FoxP3	Treg cells

Figure 2 Comprehensive Inflammatory Profiling via 46-Plex Immune Marker Assay. Simultaneous detection of cell surface markers, functional proteins, and intracellular cytokines.

Predefined gating strategies define immune subsets, enabling cutoff-based reporting for patient stratification and correlating immune phenotypes with disease states and treatment responses (Fig.3). Advanced bioinformatics tools are applied to cluster immune cell populations and extract clinically relevant immune signatures, offering valuable insights into immune modulation and therapeutic response prediction.

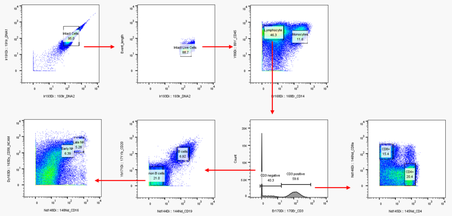


Figure 3 Gating Hierarchy - Major Immune Subtypes

Characterizing Immune States in Chronic Inflammation and Treatment Response

The immune profile reflects effective modulation following treatment, marked by a decline in T cells and monocytes, key mediators of inflammation. $CD4^+$ T cells notably decrease, while $CD8^+$ T cells, NK cells, and B cells remain stable indicating a balanced immune response. Within the NK compartment, early NK cells are more prevalent than late NK cells, suggesting a shift toward immune surveillance over cytotoxicity. Cytokine dynamics reinforce this trend: regulatory cytokine IL-2 decreases, and IL-10 increases, indicating reduced T cell activation and enhanced immune regulation. Meanwhile, pro-inflammatory cytokines IL-1β, IL-6, IL-17A, and IFN-γ decline progressively, consistent with dampened inflammation.

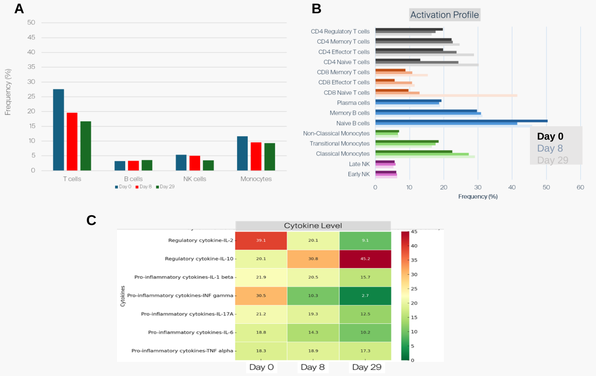


Figure 4 Deep Immune Phenotyping: Cell Subsets, Activation Profiles, and Inflammatory Markers. This figure presents a multi-dimensional analysis of immune responses across a population using 46-marker CyTOF profiling.
(**A**):Distribution of major immune cell categories (e.g., T cells, B cells, monocytes, NK cells) across patient groups.
(**B**):Activation profile of immune subsets, highlighting changes in effector and regulatory populations.
(**C**):Inflammatory cytokine signature, showing variation in IL-2, IL-6, IL-10, IL-17A, IFN-γ, and IL-1β expression levels.

These findings underscore the immunomodulatory effects of treatment interventions and their influence on disease trajectory. In oncology, the assay effectively distinguished immune profiles between cold and hot tumors. Cold tumors were characterized by an abundance of M2-like macrophages and regulatory T cells, whereas hot tumors showed elevated CD8+ cytotoxic T cell infiltration and IFN-γ expression, hallmarks of a more robust anti-tumor immune response. These immune signatures hold potential as predictive biomarkers for response to checkpoint inhibitor therapies.

High-Dimensional Immune Profiling Reveals Novel IL-10^+ B and IFN$^+$ NK Subsets in Viral Response

The high-dimensional single-cell analysis uncovered novel immune cell dynamics in viral infection models, including distinct subsets of IL-10^+ B cells and interferon-secreting NK cells. These insights highlight the assay's utility in infectious disease research and vaccine development, especially for profiling immune responses to emerging viral pathogens.

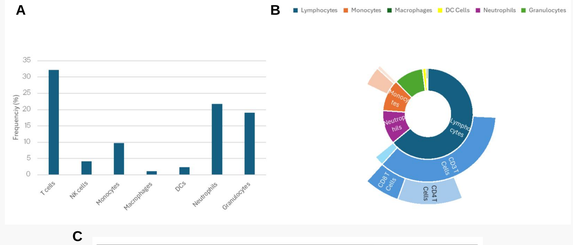


Figure 5 Comprehensive Immune Profiling in a Viral Infection Context
(A) Bar graph showing the frequency distribution of major immune cell types, with T cells, neutrophils, and granulocytes representing the most abundant populations.
(B) Sunburst chart visualizing hierarchical relationships among immune subsets, highlighting the distribution of $CD3^+$, $CD8^+$, and $CD4^+$ lymphocytes alongside monocytes and neutrophils.
(C) Heatmap summary of cytokine levels, revealing elevated pro-inflammatory cytokines (IL-12, IL-6, and IFN-α) and moderate regulatory cytokine responses (IL-10 and IL-4), indicative of a strong antiviral immune response with partial immune regulation.

Conclusion

This next-generation inflammatory response assay represents a transformative advancement in bioanalysis and precision medicine, offering a comprehensive, high-resolution, and multi-dimensional evaluation of immune dynamics. By incorporating cutting-edge spatial and quantitative technologies, it enables precise patient stratification based on immune response signatures, facilitating improved immunotherapy selection, disease monitoring, and vaccine efficacy assessments, ultimately enhancing clinical decision-making.