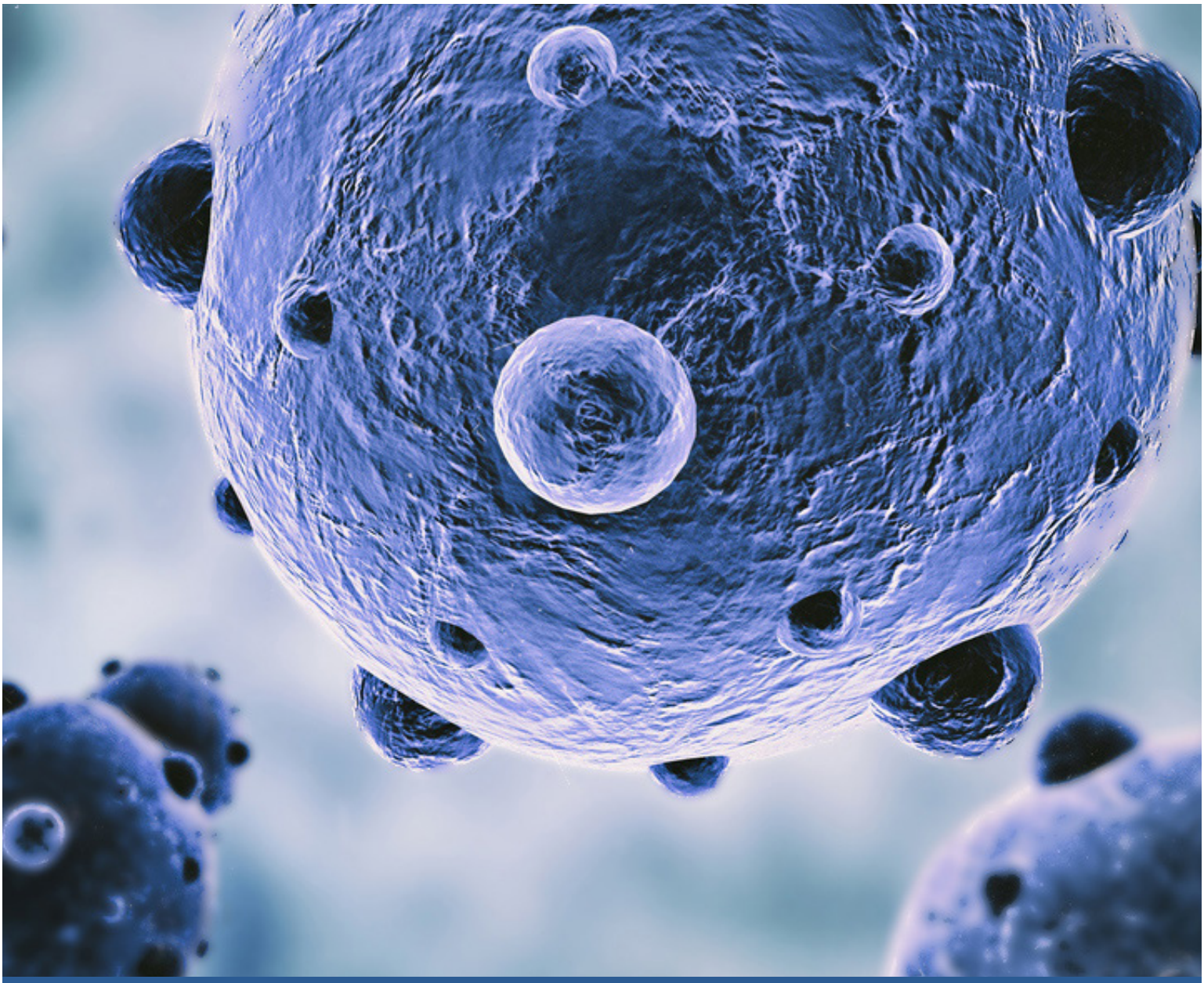

FLOW CYTOMETRY AND IMMUNOLOGY RESEARCH

A Powerful Tool For Cutting Edge Basic And Translational Research

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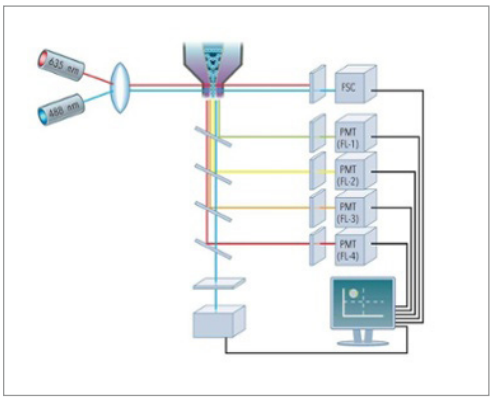
Flow cytometry is a technique that is essential to immunology research. Most immunologists have used flow cytometry on a regular basis because it provides critical data for characterizing and measuring different types of immune cells simultaneously. Flow cytometry can be used to analyze cells from in vitro experiments or from animal models or human blood and tissue, and protocols can be customized to measure specific cell types on different flow cytometers. Flow cytometry applications span the immunology field and have been used to advance our understanding of immunological mechanisms and develop novel treatments for cancer, infectious diseases, and autoimmune diseases.



This white paper highlights novel uses of flow cytometry in different immunology subfields and how this tool has advanced our scientific knowledge.

Flow Cytometry Toolkit

Flow cytometry is a semi-quantitative technique in which the frequency and phenotypic characteristics of cells stained with fluorochrome-conjugated antibodies can be measured.

Flow cytometry analysis can measure millions of cells in a single experiment to determine frequency of multiple cell types, such as T cell subsets, macrophages, dendritic cells, and neutrophils. This ability to measure different cell subsets concurrently gives scientists ever greater insight into how the immune system works and how different conditions, such as infection, cancer or chronic inflammation, can skew the immune system toward destructive or protective responses.

Flow cytometry is not only used for phenotypic analysis of immune cells, but can be used to measure intracellular molecules, such as cytokines, signaling molecules, and transcription factors. Measuring these molecules can provide critical mechanistic insight how different immune cells develop, differentiate and execute their effector functions. Flow cytometry protocols can be customized to measure any cell type or intracellular molecule and can be adapted to work within the parameters of a particular flow cytometer. In addition, flow cytometry assays can be validated and performed under good laboratory practices (GLP) conditions for use for preclinical or clinical studies.

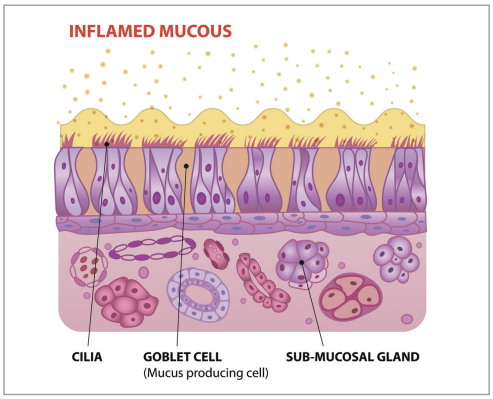
Innate Immunity

The innate immune system is considered a first line of defense against pathogens and is characterized by cells with innate immune receptors or sensors, including Toll-like receptors (TLRs) that can detect foreign entities or danger signals and activate rapid defensive responses. Innate immune responses involve different immune cells including macrophages, dendritic cells, neutrophils and mast cells, but these responses are not antigen specific nor do they generate memory cells as seen with T cell responses. Flow cytometry can measure the expression of different TLRs on the surface of cells or within subcellular compartments, and components of signaling pathways activated by TLR engagement can also be measured, including the presence

of phosphorylated signaling molecules like phospho-IRF3[1]. Measuring innate immunity pathways by flow cytometry can provide insight into mechanisms of a novel vaccine adjuvant or pathogenesis of a viral infection.

Mucosal Immunology

Mucosal surfaces, which include the gut, skin, female reproductive tract, and oral and nasal mucus membranes, are sites that are considered susceptible to infection. But the mucosal immune system is now appreciated as a critical arm of the immune system that can defend against pathogens but support the presence of beneficial commensal bacterial[2]. Dysregulation of mucosal immunity has been associated with chronic diseases, like Crohn's Disease and Ulcerative Colitis, but recent studies have provided critical insights into the mechanisms behind this dysregulation.



Flow cytometry has been an integral tool for these studies by characterizing biomarkers expressed by immune cell subsets unique to the mucosa, including innate lymphoid cells and intestinal effector T cells[3]. Flow cytometry studies have been critical to understanding how changes in a host's microbiome, or resident microbial population, alters mucosal immune responses and can lead to excess inflammation and mucosal damage. Flow cytometry studies have also contributed to our understanding of food allergies and the mucosal immune system, particularly how commensal bacteria are critical to protecting against

sensitization to food allergens[4]. Flow cytometry analysis of intracellular cytokines has also been critical to understanding the underlying mechanisms of chronic inflammation in conditions like Crohn's Disease and has been critical to identifying novel targets for development into therapeutics[5].

Viral Immunology

The immune system is essential to combating acute and chronic viral infections, and adaptive immune responses by B and T cells generate immune memory to viral pathogens for long-term protection. Flow cytometry studies have been instrumental to understanding chronic viral infections like HIV and many flow cytometry reagents and resources have been developed for HIV diagnostics and vaccine development[6,7]. Flow cytometry analysis has also been critical for understanding immune responses to viruses that cause acute lethal infections, like Ebola[8] and Zika, and for developing and assessing vaccine candidates for such infections[9]. Basic immunology has also been advanced by using different viruses, like lymphocytic choriomeningitis (LCMV) and influenza, as tools to characterize different immunological phenomena like MHC restriction, T cell activation and exhaustion, and memory formation[10,11]. The widespread use of flow cytometry for studying viral immunology has improved our understanding of how the immune system works and how we can defend against or develop vaccines for specific viruses.

Transplantation and Tolerance

Transplantation of solid organs or bone marrow has been significantly improved by advances in our understanding of how the immune system distinguishes between self and non-self, and how it can be induced or altered to accept seemingly foreign tissues. The study of immune tolerance aims to understand mechanisms used by the immune system to tolerate the presence of non-self-antigens, such as commensal bacteria, food components, or transplanted tissue. Breakdown of tolerance can lead to excessive and destructive immune responses and inflammation and organ rejection. Flow cytometry has greatly advanced our understanding of tolerance and aids in making better predictions of how well a graft may be accepted by a transplant recipient[12]. Flow cytometry is an ideal tool for monitoring tolerance and rejection because these responses can be monitored to some degree by looking at cell subsets in peripheral blood[13]. Flow cytometry will continue to be an integral tool for studying and monitoring transplant tolerance.

Tumor Immunology

Cancer research is now driven by significant advances in using immune-based therapies for treatment. New cancer therapies are targeted to activate specific anti-tumor responses or are customized to attack a patient's unique tumor. Flow cytometry assays have facilitated the movement of these immunotherapies from the research bench to the clinic[14]. Flow cytometry-based monitoring of experimental therapies has been instrumental in identifying viable immuno-oncology candidates that induce specific and effective immune responses against a tumor without causing undesirable off-target responses. Cancer researchers now recognize that flow cytometry is an integral tool in developing novel therapies and monitoring the effectiveness of candidates that alter the immune response.

Conclusion

Flow cytometry has emerged as an integral technique for immunology research and has been adapted for use into numerous immunology subfields. As flow cytometry technology advances to be able to measure more parameters and handle larger samples, this technique will continue to be critical to promoting immunology breakthroughs.

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