Flow Cytometry based Diagnosis of Primary Immunodeficiency Disorders

Flow cytometry provides a clinical tool for evaluating the immune system that can detect the absence of a specific cell population or subpopulation, screen for altered expression of a specific extracellular or intracellular protein, assess for biological changes associated with specific immune defects, and evaluate certain functional immune characteristics.

The design specifics of a flow cytometer are beyond the scope of this editorial, but briefly, the instrument has four major elements: optics, fluidics, electronics, and a computer (with specific software). The optical system uses monochromatic light sources (typically lasers) that provide the excitation energy. The optical bench collects light derived directly from each cell as it passes through the laser beam(s). Each cell emits nonfluorescent (forward and side scatter) as well as fluorescent signals if one or more fluorochrome conjugated monoclonal antibodies are bound to the cell. The two nonfluorescent parameters collected provide an index of cell size (forward light scatter) and a measure of cell granularity/regularity (side-angle light scatter).

Flow cytometry has emerged as a particularly useful approach to screen for the presence or absence of specific cell populations or subpopulations, the presence or absence of certain proteins that are associated with specific primary immunodeficiencies (PIDs), and biological consequences of specific immune deficiencies. Functional flow cytometry has evolved in the evaluation of specific PIDs with applications including evaluation of the phosphorylation of intracellular signaling proteins following cell stimulation and assessment of oxidative burst.

Common Variable Immunodeficiency
Common variable immunodeficiency (CVID) typically presents later in life (second and third decades) and is characterized by low immunoglobulin levels and absent antibody responses, usually with relatively normal numbers of circulating B cells (>2%). Flow cytometry can be used to classify CVID patients into subgroups according to B-cell subsets, which has clinical implications. CVID patients have low B-cell numbers with an expanded proportion of transitional B cells. CD19 deficiency is characterized by the absence of CD19 on B cells but the presence of other B-cell markers, including CD20, CD21, and surface IgM.

Hyper-IgM Syndromes
Hyper-IgM syndromes (HIGM) refers to a group of genetic disorders affecting molecules involved in B-cell class switch recombination and somatic hypermutation. Absent CD40L surface expression on activated CD4+ T cells can be demonstrated in up to 68% (41 of 61) of the patients with proven CD40L mutations.

Severe Combined Immune Deficiency
Severe combined immune deficiency (SCID), the most severe PID, presents in early infancy with recurrent opportunistic infections, chronic diarrhea, and failure to thrive. Flow cytometry can help in the classification of patients based on the presence/absence of the major lymphocyte populations that point to likely etiologies.

Wiskott-Aldrich Syndrome
Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder caused by defects in the gene encoding the WAS protein (WASp). The evaluation of WASp expression by flow cytometry has also proven useful in the evaluation of chimerism posthematopoietic stem cell transplantation for WAS.

Inherited Susceptibility to Infectious Disease
Mendelian susceptibility to mycobacterial disease refers to a group of disorders characterized by vulnerability to infections with weakly virulent Mycobacteria (environmental mycobacteria and Mycobacterium bovis, Bacillus Calmette-Guérin vaccine) and Salmonella. About 95% of patients with IL-12Rβ1 deficiency harbor mutations that abolish receptor expression, allowing easy detection by flow cytometry.
Chronic Granulomatous Disease
Chronic granulomatous disease (CGD) is a phagocytic disorder caused by defective intracellular killing of bacterial and fungal pathogens. Most patients (~65-70%) harbor X-linked recessive mutations in one component (gp91phox) of the NADPH oxidase system, while the remaining patients have an autosomal recessive defect affecting one of four additional components of this enzyme complex. The optimal screening test to clarify the diagnosis of CGD is a flow cytometric assay of NADPH oxidase activity using the intracellular dye dihydrorhodamine 123, which normally converts to a fluorescent compound following granulocyte activation (using phorbol myristate acetate)\(^7\).

Leukocyte Adhesion Deficiency Type 1
Leukocyte adhesion deficiency type 1 is caused by defects in the gene that encodes CD18 and results in decreased or absent expression of the three heterodimeric β2 integrin receptors (CD11a/CD18, CD11b/CD18, CD11c/CD18). The optimal screening test to diagnose this disorder relies on flow cytometry to test for CD18 (and CD11a, CD11b, and CD11c) expression on granulocytes\(^8\).

Conclusion
The application of flow cytometry provides rapid results and in many cases clarifies the likely diagnosis, directs further immunologic studies, and/or can be linked to clinical phenotype. Flow cytometry and functional testing will remain important adjuncts in the evaluation of patients suspected of having immune deficiencies.

References:

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