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Review article

Flow cytometry-based diagnosis of primary immunodeficiency diseases

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Abbreviations:
ADA, adenosine deaminase;
ALPS, autoimmune lymphoproliferative syndrome;
APECED, autoimmune polyendocrinopathy-candidiasis–ectodermal dystrophy/dysplasia; BAFF-R, B-cell activating factor receptor; BTK, Burton's tyrosine kinase;
CD40L, CD40 ligand; CGD, chronic granulomatous disease; CMCD, chronic mucocutaneous candidiasis;
CTLA4, cytotoxic T-lymphocyte-associated protein 4; CTLs, cytotoxic T lymphocytes;
CVID, common variable immunodeficiency;
DHR, dihydrorhodamine; DNT, double-negative T; DOCK8, dedicator of cytokinesis

ABSTRACT

Primary immunodeficiencies (PIDs) are a heterogeneous group of inherited diseases of the immune system. The definite diagnosis of PID is ascertained by genetic analysis; however, this takes time and is costly. Flow cytometry provides a rapid and highly sensitive tool for diagnosis of PIDs.

Flow cytometry can evaluate specific cell populations and subpopulations, cell surface, intracellular and intranuclear proteins, biologic effects associated with specific immune defects, and certain functional immune characteristics, each being useful for the diagnosis and evaluation of PIDs. Flow cytometry effectively identifies major forms of PIDs, including severe combined immunodeficiency, X-linked agammaglobulinemia, hyper IgM syndromes, Wiskott-Aldrich syndrome, X-linked lymphoproliferative syndrome, familial hemophagocytic lymphohistiocytosis, autoimmune lymphoproliferative syndrome, IPEX syndrome, CTLA 4 haploinsufficiency and LRBA deficiency, IRAK4 and MyD88 deficiencies, Mendelian susceptibility to mycobacterial disease, chronic mucocuneous candidiasis, and chronic granulomatous disease. While genetic analysis is the definitive approach to establish specific diagnoses of PIDs, flow cytometry provides a tool to effectively evaluate patients with PIDs at relatively low cost.

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8; FHL, familial hemophagocytic lymphohistiocytosis; FOXP3, forkhead box P3; HIES, hyper IgE syndrome; HIGM, hyper IgM syndrome; ICOS, inducible co-stimulator; IFN, interferon; iNKT, invariant natural killer T; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked inheritance syndrome; IRAK4, IL-1 receptor-associated kinase 4; JAK3, Janus kinase 3; LAD, leukocyte adhesion deficiency; LPS, lipopolysaccharide; LRBA, lipopolysaccharide-responsive and beige-like anchor protein; mAb, monoclonal antibody; MSMD, Mendelian susceptibility to mycobacterial disease; MyD88, myeloid differentiation primary response gene 88; NOD, nucleotide-binding and oligomerization domain; PBMCs, peripheral blood mononuclear cells; PIDs, primary immunodeficiency diseases; PNP, purine nucleoside phosphorylase; RAG, recombination activating gene; SAP, SLAM-associated protein; SCID, severe combined immunodeficiency; STAT, signal transducer and activator of transcription; TCR, T-cell receptor; Th, T helper; TLR, Toll-like receptor; TNF, tumor necrosis factor; WAS, Wiskott-Aldrich syndrome; WASp, WAS protein; XIAP, X-linked inhibitor of apoptosis; XLA, X-linked agammaglobulinemia; XLP, X-linked lymphoproliferative syndrome; XLT, X-linked thrombocytopenia; X-SCID, X-linked severe combined immunodeficiency; ZAP70, ζ-chain-associated protein kinase of 70 kDa

Introduction

Primary immunodeficiency diseases (PIDs) are a heterogenous group of monogenetic disorders of the immune system, resulting in recurrent and/or severe infections, autoimmunity, auto-inflammation, or malignancies. A careful history focused on the types of infectious agents and other complications are important clues to suspect PID. Laboratory investigations including complete blood count, immunoglobulin levels, antibody titers, assessment of neutrophil function and complement components are also important tools to confirm the diagnosis of PID. As the spectrum of PIDs is expanding, it is often difficult to diagnose PIDs based on clinical and conventional laboratory findings alone. The more recently available genetic investigation is a definitive tool for diagnosing PIDs; however, DNA analysis takes time and is expensive. In contrast, technologies that use physical and chemical characteristics of fluorescent-labeled particles in fluid phase passed through lasers are cheeper than gene analysis, although they need experienced and skilled investigators. Thus, flow cytometry may serve as a bridge between conventional immunological testing and DNA sequencing, offering rapid and accurate results based on single cell analysis.[1]

Application of flow cytometry in the diagnosis of primary immunodeficiency diseases

Flow cytometry is a highly sensitive tool for evaluating the immune system and supporting the diagnosis of PID. The applications of flow cytometry in the evaluation of PIDs are multiplex and include the investigation of specific cell populations and subpopulations, specific cell membrane, intracellular and intranuclear proteins, biologic effects associated with immune defects, and functional immune abnormalities (Table 1).[2]

Quantitative assessment of cell populations and subpopulations is useful for the diagnosis of X-linked agammaglobulinemia (XLA) characterized by the absence of B cells in the peripheral blood. Patients with severe combined immunodeficiency (SCID) lack T cells, while the impact on B and NK cells is variable depending on the genetic defect. Patients with X-linked lymphoproliferative syndrome type 1 (XLP1) have a marked decrease in invariant natural killer T (iNKT) cells. Autoimmune lymphoproliferative syndrome (ALPS) is characterized by increased T-cell receptor (TCR)-α/β-positive double-negative T (DNT) cells. Patients with autosomal dominant hyper IgE syndrome (HIES), and those with chronic mucocutaneous candidiasis (CMCD) present with decreased number of circulating T helper (Th)17 cells.

As specific cell surface proteins are concerned, unique subsets of patients with common variable immunodeficiency (CVID) can be characterized by assessing $CD19^+$ B cells, B-cell activating factor receptor (BAFF-R) on B cells, and the inducible co-stimulator (ICOS) on activated T cells. Patients with X-linked hyper IgM syndrome (X-HIGM) fail to express CD40 ligand (CD40L) on activated T cells, and a group of patients with autosomal recessive hyper IgM syndrome lack CD40 expression on B cells. Mendelian susceptibility to mycobacterial disease (MSMD) has been associated with aberrant interferon (IFN)-γR1 expression on monocytes or deficient IL-12Rβ1 expression on activated T cells. Patients with leukocyte adhesion deficiency type 1 (LAD1) can be identified by absent expression of CD18 on granulocytes. Lymphocytes from patients with X-linked SCID (X-SCID) show deficient CD132 (common γ chain) expression. Patients suffering from gp91-phox- and p22-phox-deficient chronic granulomatous disease (CGD), lacking the

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