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Editorial

Applications of Flow Cytometry in Inborn Errors of Immunity

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Flow cytometry, as a useful technology, has played an important role in the progress in basic and clinical research by measuring multiple characteristics of individual cells in a high-throughput manner. One of the most important applications of flow cytometry is the initial workup and subsequent management of inborn errors of immunity (IEI). IEIs, also known as primary immunodeficiencies, are a heterogeneous group of inherited disorders of immune system, presenting with heterogeneous clinical manifestations. Given that IEI patients present heterogeneous clinical and immunological features, diagnosing IEI using only clinical and conventional laboratory data is challenging. Genetic tests, including whole exome sequencing (WES) and whole genome sequencing (WGS), are involved in the identification of genetic defects in IEI; however, flow cytometry remains an important first step in defining the immunophenotype of the patient prior to genetic analysis. The applications of flow cytometry in the evaluation of IEI are diverse and include identifying distinct populations of immune cells, quantifying the expression of intracellular and intranuclear proteins, functionally validating genetic variants, monitoring treatment, defining the genotype-phenotype correlation, detecting carriers, and classifying disorders.

Immunophenotyping is considered a key part of the diagnostic work-up for patients suspected of presenting IEI, especially those with defective lymphoid cells. In this regard, measuring T, B, and NK cells is the initial screening approach for suspected IEI patients. This initial screening detects and classifies four categories of severe combined immunodeficiency (SCID): T-/B-/NK-, T-/B-/NK+, T-/B+/NK-, and T-/B+/NK+SCID. However, in cases of atypical SCID (Leaky SCID and Omenn Syndrome), it is necessary to measure naïve and memory T cells using CD45RA, CCR7, and CD45RO, as Leaky SCID and Omenn Syndrome present expanded CD45RO+ T cells. In some IEI categories, such as Common Variable Immunodeficiency (CVID), all T and B cell subsets might be affected (1, 2). CVID patients often demonstrate reduced memory B cells and plasma cells; however, some patients have disturbances in T cell subsets (3). Measuring lymphocyte subsets enables us to predict clinical progression or disease complications. Edwards et al. demonstrated that faults in Treg, Th17, and Tfh17 numbers predict disease progression in primary antibody

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deficiency patients with non-infectious complications (4). Advancements in flow cytometric methodologies have facilitated the measurement of lymphoid subsets, as all main lymphoid subpopulations can be now assessed in a single tube (5). Lymphoid immunophenotyping also contributes to treatment decisions and monitoring, such as in hematopoietic stem cell transplantation.

Flow cytometry is a powerful technique for measuring intracellular proteins and conducting functional assays. Assessing the expression of proteins such as DOCK8, BTK, WAS, SAP, XIAP, and FOXP3 by flow cytometry expedites the diagnosis of hyper IgE (autosomal recessive form), X-linked agammaglobulinemia (XLA), Wiskott Aldrich, X-linked lymphoproliferative disease (XLP) type 1, XLP type 2, and IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) diseases, respectively (6-10). NK and CTL cell functionally is determined by measuring intracellular perforin levels and degranulation assays (quantifying CD107a expression) via flow cytometry, which aids in diagnosing familial hemophagocytic lymphohistiocytosis (FHL) types 3-5 (11). WES and WGS identify many genetic variants in IEI patients, however, all of them are not a causative disease and need to be confirmed in terms of protein expression and functional assay by flow cytometry, particularly in cases involving hypomorphic defects (12). Genetic testing can be utilized by the results obtained from flow cytometry.

Flow cytometry can also be used to define the genotype-dependent variability and for carrier detection. For instance, the two genotypes of chronic granulomatous disease (CGD) can be distinguished by measuring Gp91-phox and p47-phox protein expression. Patients with p47phox deficiency exhibit higher fluorescence (SI value or broad CV) in dihydrorhodamin (DHR) assay compared to those with gp91phox deficiency, indicating that flow cytometry can recognize the genotype-dependent variability through the NADPH oxidase assay (13). Carrier cases can be detected by the bimodal distribution of protein expression, as has been reported in some IEI disorders, including XLA, XLP, CGD (6, 9, 14, 15).

Taken together, NGS sequencing facilitates the identification of IEI disorders; however, these methods are time-consuming and expensive. Given that early diagnosis is crucial for IEI patients, establishing a rapid diagnosis is imperative for effective management. Flow cytometry expedites the diagnosis of patients with suspected IEI, thereby improving patient care and management.

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