

Interpretation of flow cytometry in primary immunodeficiency disorders

Vivian P. Hernandez-Trujillo, MD,* and Thomas A. Fleisher, MD†

INTRODUCTION

The laboratory evaluation of primary immunodeficiency disorders (PIDDs) has evolved with the development of new technologies, including flow cytometry, to characterize circulating lymphocytes.¹ This article is part of a series on the ambulatory diagnosis of immunodeficiency disorders,² and the focus of this discussion is on the application of flow cytometry in the diagnosis and/or evaluation of different groups of PIDDs, classified using the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification system³ (Table 1).

EVALUATION OF DISORDERS WITH DECREASED LYMPHOCYTE COUNTS

Different PIDDs present with decreased lymphocyte populations (Table 1). The starting point for this evaluation requires determination of the absolute lymphocyte count and comparison with age-matched reference (control) data.⁴ Classification of these disorders is based on the presence or absence of different lymphocyte populations. Owing to the fact that T cells constitute approximately three-fourths of the circulating lymphocytes in a newborn, a defect in T-cell development would be expected to result in lymphocytopenia. An example of a combined T- and B-cell disorder is severe combined immunodeficiency (SCID). This profound immune defect is associated with lymphocytopenia (an exception is significant maternal T-cell engraftment). SCID is characterized by the absence of T cells with a variable impact on B and NK cells. Using flow cytometry, it is possible to define 4 general categories of SCID: T⁻/B⁻/NK⁻, T⁻/B⁻/NK⁺, T⁻/B⁺/NK⁻, and T⁻/B⁺/NK⁺ (Table 1). Patients with adenosine deaminase SCID have been found to have the most profound lymphocytopenia among patients with SCID.⁵⁻¹¹ Diagnosing SCID is a medical emergency since, without appropriate therapy, the disease is fatal, and any blood product must be

irradiated before administration to prevent graft-vs-host disease. Curative therapy consists of bone marrow (stem cell) transplantation, and outcome is improved by therapy early in life before the appearance of significant infections.¹¹

Patients with markedly decreased to absent T-lymphocyte counts, but normal B and NK cell numbers, may have complete DiGeorge syndrome (DGS), a microdeletion syndrome associated with a thymic defect. Typical T-lymphocyte counts associated with a major thymic defect include a CD3⁺ cell count of 575/mm³ or less, a CD4⁺ count of less than 290/mm³, and a CD8⁺ count of less than 291/mm³.¹² Further workup would include evaluation for the presence of circulating naïve T cells by evaluating CD45RA expression and/or for the frequency of T-cell receptor excision circles. Mitogen proliferation studies are helpful in assessing the degree of T-lymphocyte dysfunction associated with DGS. Patients with complete or atypical DGS may also be candidates for thymic transplantation.¹² Partial DGS often presents with decreased total T lymphocytes at birth, but this may improve. A cohort of patients with partial DGS was studied, and decreased T-lymphocyte numbers with adequate ex vivo function were revealed.¹³ Typical median lymphocyte counts were as follows: CD3⁺ 1,485/mm³ (CD4⁺ 1,178/mm³) at 1 month, and CD3⁺ 857/mm³ (CD4⁺ 559/mm³) by 5 years.¹³ In these patients, the median number of T lymphocytes was low at birth but normalized by 24 months, although additional fluctuations in T-cell numbers occurred, suggesting that these patients should be followed up to ensure their immune function remains satisfactory.

Male patients with decreased immunoglobulin levels should be evaluated for B-cell numbers, and if these counts are low (<1%), X-linked agammaglobulinemia (XLA) should be suspected and mutation analysis for the gene encoding Bruton tyrosine kinase (BTK) undertaken.¹⁴ XLA was the first description of an antibody deficiency, and early identification of these patients is important to start immunoglobulin replacement therapy and prevent destructive lung changes associated with recurrent and/or chronic infection. In male patients without a BTK mutation, as well as agammaglobulinemic female patients without B cells, evaluation for autosomal recessive causes of congenital agammaglobulinemia should be considered (Table 1). Agammaglobulinemia, regardless of the defect, requires institution of immunoglob-

Affiliations: * Division of Allergy and Clinical Immunology, Miami Children's Hospital, Miami, Florida; † Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland.

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Table 1. Primary Immunodeficiency Disorders Evaluated Using Flow Cytometry

Disorders with decreased lymphocyte populations	
A. Combined T- and B-cell immunodeficiencies	
Severe combined immunodeficiency	
T ⁻ /B ⁻ /NK ⁻	
Adenosine deaminase deficiency	
Reticular dysgenesis (other hematologic abnormalities)	
T ⁻ /B ⁻ /NK ⁺	
RAG1 or RAG2 deficiency	
Artemis deficiency	
Ligase 4 deficiency	
Cernunnos deficiency	
T ⁻ /B ⁺ /NK ⁻	
Common γ -chain deficiency (X-linked)	
JAK3 deficiency	
CD45 deficiency	
T ⁻ /B ⁺ /NK ⁺	
Interleukin 7 receptor α -chain deficiency	
CD3 δ -chain/CD3 ϵ -chain deficiency	
B. Thymic defects	
DiGeorge syndrome: decreased or absent T cells	
C. Antibody deficiencies	
XLA: <1% B cells	
Autosomal recessive congenital agammaglobulinemia: <1% B cells	
λ 5 Deficiency	
μ -Heavy chain deficiency	
Ig α deficiency	
B-cell linker protein deficiency	
Disorders with a defect in expression of a specific cell surface protein detectable using flow cytometry	
A. Combined T- and B-cell immunodeficiencies	
X-linked hyper-IgM: CD40 ligand deficiency (CD154): activated CD4 T cells	
B. Congenital defects of phagocytes	
Leukocyte adhesion defect: CD18 deficiency (also results in failure of surface expression of CD11a, b, c) on neutrophils and lymphocytes	
C. Other defects	
Interferon- γ receptor 1 deficiency: monocytes	
Disorders with a defect in the expression of a specific intracellular protein detectable using flow cytometry	
A. Antibody deficiencies	
XLA: Bruton tyrosine kinase deficiency	
B. Other well-defined immunodeficiency syndromes	
Wiskott-Aldrich syndrome and X-linked thrombocytopenia: Wiskott-Aldrich syndrome protein deficiency	
Disorders with a functional abnormality detectable using flow cytometry	
A. Congenital defects of phagocytes	
CGD: Dihydrorhodamine 123 assay for oxidative burst	
B. Specific defects in cell activation	
Interferon- γ receptor 1 deficiency: STAT1 phosphorylation assay	
C. General evaluation of ex vivo lymphocyte function	
Carboxyfluorescein diacetate succinimidyl ester to analyze cell division after lymphocyte stimulation	
Determination of activation antigen expression following lymphocyte stimulation	
D. Other	
Assessment of apoptosis induced by various stimuli	

Abbreviations: CGD, chronic granulomatous disease; RAG, recombination-activating gene; XLA, X-linked agammaglobulinemia.

ulin replacement therapy to prevent end-organ damage from recurrent infection.¹⁵

EVALUATION OF DISORDERS WITH DEFECTS IN CELL SURFACE PROTEINS

Flow cytometry can be useful in the diagnosis of immunodeficiencies with defects in the expression of cell surface

proteins (Table 1). In patients with combined T- and B-cell disorders, such as X-linked hyper-IgM syndrome, activated CD4⁺ T lymphocytes can be studied for the expression of CD40 ligand (CD154).¹⁶ Flow cytometry is also useful in evaluating for the expression of interferon- γ receptor 1 on monocytes, since recurrent nontuberculous mycobacterial infections in a subgroup of patients are due to an autosomal

recessive deficiency of this receptor chain.¹⁷ Surface protein evaluation by flow cytometry serves as the optimal screening approach for the leukocyte adhesion deficiency type 1 evaluating for the presence of cell surface CD18 (and CD11a, b, c).¹⁸ In patients with leukocyte adhesion deficiency type 1, CD18 is usually less than 5% of normal.¹⁹ The caveat in all of these types of evaluation is that flow cytometric testing does not evaluate protein function, so protein absence is diagnostic but protein presence can be associated with disease due to a dysfunctional protein and representing a normal state.

EVALUATION OF DISORDERS WITH DEFECTS IN INTRACELLULAR PROTEINS

In certain PIDDs, defective expression of an intracellular protein can be screened by flow cytometry after cell fixation and permeabilization (Table 1). In Wiskott-Aldrich syndrome and X-linked thrombocytopenia, absence of the Wiskott-Aldrich protein can help establish the diagnosis; however, presence of intracellular Wiskott-Aldrich protein does not rule out the diagnosis since some patients (particularly those with X-linked thrombocytopenia) express a dysfunctional but detectable protein. In patients with XLA, a monoclonal antibody to detect BTK in monocytes can be used as a screening test with the same caveat that some patients with XLA have an abnormal BTK protein that can be detected but is not functional.²⁰

EVALUATION OF DISORDERS WITH FUNCTIONAL ABNORMALITY

More recently, flow cytometry has been used to evaluate specific functional features, and this has evolved into applications in the diagnosis of PIDDs (Table 1). Chronic granulomatous disease can be diagnosed through the use of flow cytometry. The most commonly used test for evaluation of oxidative burst after granulocyte activation is the dihydro-rhodamine 123 assay.²¹ In this assay, activation of granulocytes induces a fluorescent signal associated with oxidative burst, whereas cells from patients with chronic granulomatous disease fail to generate a fluorescent signal due to the defect in oxidative burst. In patients with interferon- γ receptor 1 deficiency, the normal phosphorylation of STAT1 induced in monocytes exposed to interferon- γ is absent, and this can be evaluated using a flow cytometer.²² Additional assays to evaluate the phosphorylation of other intracellular proteins have been described that may be useful in signaling defects associated with certain PIDDs. Multiple applications of flow exist to evaluate lymphocyte activation, including the use of carboxyfluorescein diacetate succinimidyl ester to analyze cell division and evaluation of CD69 expression to evaluate T-cell activation.^{23–24} In addition, flow cytometry can be used to quantify apoptosis.²⁵

EVALUATION OF DISORDERS WITH DECREASED LYMPHOCYTE COUNTS NOT DUE TO PIDDs

The unexpected finding of decreased absolute lymphocyte counts should provoke careful scrutiny of the complete blood

cell count, differential cell count, and cell morphologic findings. In previously healthy individuals, repeating the complete blood cell count and differential cell count would be the appropriate first step to ensure the finding is not related to a technical problem. If lymphocytopenia persists, further immune workup would be prudent, and lymphocytopenia associated with opportunistic infection should direct the evaluation to performing serologic tests and determining the viral load for human immunodeficiency virus.⁴ In general, when lymphocytopenia is present, one should consider potential causes, including decreased production (eg, primary or secondary immunodeficiency, early viral infection, premalignancy), increased destruction (eg, autoimmune disease), or increased loss (eg, lymphatic leak [intestinal lymphangiectasia]). When any immune evaluation involves flow cytometry, the data must include percentage and absolute numbers, and involve comparison with age-matched reference data (a future article in the series will address laboratories that perform flow cytometry). In addition, a simple validity check consists of assessing the sum of the percentage of T cells, B cells, and NK cells that should equal approximately 100% unless there are atypical lymphocytes (should be suggested by morphologic evaluation of the peripheral smear) or a technical problem. In addition, when control data are expressed as a 95% confidence interval, it means that 2.5% of “controls” fall below and above this interval, and this should be taken into consideration when interpreting the results.

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- Requests for reprints should be addressed to:
Vivian Hernandez-Trujillo, MD
Miami Children's Hospital
Division of Clinical Immunology
3100 SW 62 Ave
Miami, FL 33155
E-mail: vivian.hernandez-trujillo@mch.com
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