

Applications of flow cytometry for the study of primary immune deficiencies

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Purpose of review

This review focuses on the current applications of flow cytometry for the diagnosis and evaluation of primary immune deficiencies (PIDs).

Recent findings

The immunophenotypic evaluation of selected PIDs provides diagnostic clues as well as information useful to classify patients and predict clinical outcome. In addition, the evaluation of intracellular proteins associated with selected PIDs has evolved as a useful diagnostic screening method. Finally, functional flow cytometry can now help to clarify possible sites of genetic defects associated with specific PIDs.

Summary

The range of PIDs in which flow cytometry has proven to be useful from a clinical and diagnostic purpose has significantly expanded. This now includes not only patients presenting with clinical histories consistent with classical antibody deficiencies and severe combined immune deficiency, but also patients with more limited infectious histories. Included among these are patients with genetic defects associated with Mendelian susceptibility to mycobacterial disease focusing the evaluation on specific surface protein expression and cell function analysis. In addition, flow cytometry appears to provide a useful screening approach to evaluate for possible toll-like receptor-pathway defects. Furthermore, immunophenotyping and intracellular flow cytometry have proven to be valuable discriminators in the evaluation of patients with immune dysregulation syndromes including immune dysregulation, polyendocrinopathy, enteropathy, X-linked, and autoimmune lymphoproliferative syndrome. Finally, flow cytometry has been shown to be useful to screen patients with possible X-linked lymphoproliferative syndrome and familial hemophagocytic lymphohistiocytosis.

Keywords

flow cytometry, immunophenotype, intracellular protein, primary immune deficiency, protein phosphorylation

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Introduction

The application of flow cytometry now plays a central role in the evaluation of patients with suspected primary immune deficiencies (PIDs). The initial use of this technology was for determining changes in lymphocyte populations or subpopulations associated with specific inherited immune disorders. Additional applications have emerged that have proven useful in clarifying the diagnosis of PIDs by focusing on the presence or absence of specific proteins that are associated with immune deficiency states. This now consists of not only evaluating cell surface proteins but also intracellular proteins. The latter approach requires cell fixation and permeabilization and has as its primary limitation the availability of appropriate monoclonal reagents

directed to the proteins of interest. In addition, this method has the same caveat as any immunoassay in which the absence of protein is generally diagnostic, whereas presence of protein can be associated with the production of a dysfunctional protein associated with disease or indicate nondisease. More recently, flow cytometry has evolved as a tool to evaluate specific aspects of immune cell function and these applications have also proven to be useful in establishing the diagnosis of certain PIDs. Finally, this method is proving to be useful in assessing immunologic recovery and monitoring immune status in immunodeficient patients following therapeutic hematopoietic stem cell and thymic transplantation. We provide below some examples of the applications of flow cytometry for the study of PID.

Congenital agammaglobulinemia

Congenital agammaglobulinemia, initially described in 1952 by Colonel Ogden Bruton, is characterized by recurrent bacterial infections involving the sinopulmonary tract that typically begin at 4–6 months of age after maternally transferred IgG has declined [1]. The diagnosis is dependent on establishing very low to undetectable levels of serum immunoglobulins (IgG, IgM, and IgA) and absent specific antibody responses. A key finding in congenital agammaglobulinemia is the absence (or marked decrease) of circulating B lymphocytes, easily identified by flow cytometry as the lack of CD20-expressing and/or CD19-expressing cells (Fig. 1). More recently, there have been reports of ‘leaky’ phenotypes in many of the PIDs including congenital agammaglobulinemia in which this phenomenon has been associated with less depression in immunoglobulin levels, but typically a significant decrease in the levels of circulating B cells (<2%) [2,3].

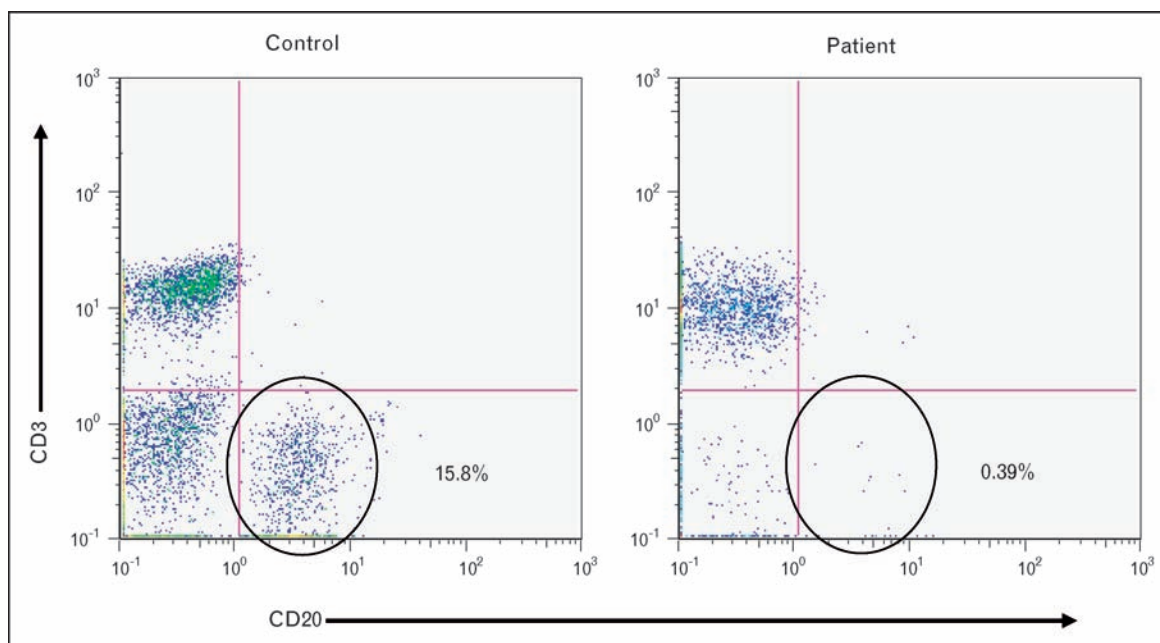
Approximately 80–85% of patients presenting with congenital agammaglobulinemia are male, and in many cases a careful family history will be consistent with an X-linked pattern of inheritance suggesting X-linked agammaglobulinemia (XLA). This diagnosis is associated with mutations in the gene encoding Bruton tyrosine kinase (*BTK*), and laboratory screening can be accomplished by evaluating for BTK expression using flow cytometry [4]. The approach is to test monocytes or

platelets (because the patient in question will have very low to absent B cells) for intracellular expression of BTK. The absence of BTK strongly suggests XLA, but establishing the definitive genetic cause requires mutation analysis [4,5]. The evaluation of intracellular BTK using flow cytometry can also be applied to evaluate for X-linked maternal carriers in situations in which an XLA patient demonstrates lack of BTK expression. In this setting, the potential maternal (or other female family member) carrier would demonstrate two populations of monocytes, one expressing normal BTK levels and the other showing no BTK expression based on the random inactivation of either the normal or the defective X chromosome [6].

Common variable immunodeficiency

Common variable immunodeficiency (CVID) typically presents in the second and third decades of life with symptoms similar to those described above. It is characterized by low immunoglobulin levels and absent antibody responses with typically normal numbers of circulating B cells (>2%). Flow cytometry can be used to classify CVID patients into subgroups according to B-cell subsets, and recent reports suggest that specific flow cytometry findings are associated with clinical features [7•]. For example, expansion of CD21^{low} B cells is reported as a marker for splenomegaly, while an increased population of transitional B cells has been associated with lymphadenopathy [7•]. Granulomatous disease has been linked to a

Figure 1 Lack of B cells in congenital agammaglobulinemia



CD3 and CD20 two color staining of lymphocytes from a control and a patient with XLA. The patient demonstrates virtual absence of B cells (CD20⁺CD3⁻) but normal T cells (CD3⁺CD20⁻). XLA, X-linked agammaglobulinemia.

marked reduction in switched memory B cells, and autoimmune cytopenia has been correlated with near absence in circulating plasmablasts [7^{••}].

In addition to classifying subgroups of patients with CVID, flow cytometry can be used to screen for three of the four genetic defects associated with CVID, namely deficiency of the inducible costimulator (ICOS), CD19, and BAFF-R [8[•]]. ICOS deficiency can be detected by the lack of ICOS upregulation on T cells following activation [9]. This has also been associated with virtual absence of memory switched B cells and a reduction of CXCR5⁺ CD4⁺ T cells [10[•]]. BAFF-R is readily detected on normal B cells, though BAFF-R-deficient patients with CVID have low B-cell numbers with an expanded proportion of transitional B cells [8[•]]. CD19 deficiency is characterized by the absence of CD19 but presence of other B-cell markers including CD20, CD21, and surface IgM [11].

Severe combined immune deficiency

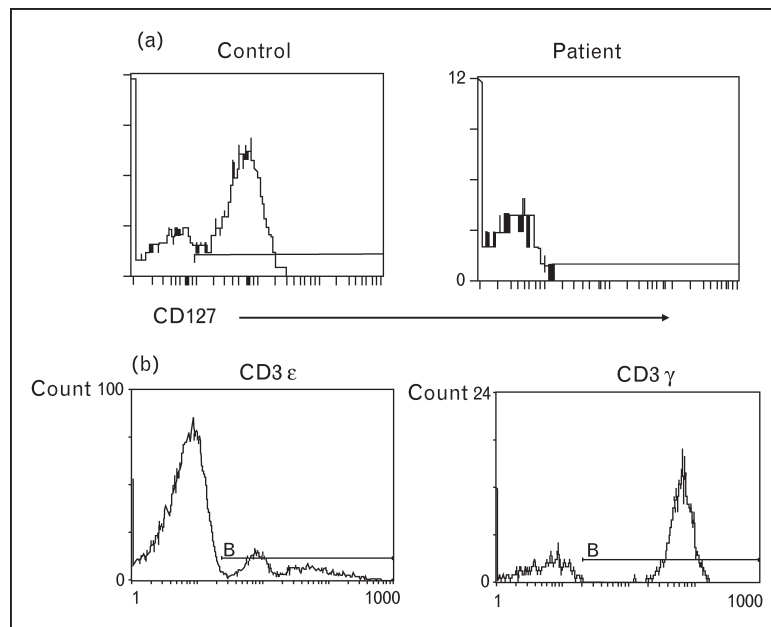
Severe combined immune deficiency (SCID) is a life-threatening condition presenting in infancy with recurrent opportunistic infections, chronic diarrhea, and failure to thrive. It is caused by a host of genetic defects affecting the development and/or function of T cells with variable impact on B and/or natural killer (NK) cells [12]. Flow cytometric classification based on presence/absence of

cell populations points to likely genetic causes: T⁺B⁻NK⁻ [e.g., adenosine deaminase (ADA) deficiency], T⁺B⁺NK⁻ [e.g., γ c, Janus kinase 3 (JAK3) deficiency], T⁺B⁻NK⁺ (e.g., RAG1/2, Artemis, ligase 4, Cernunnos deficiency), T⁺B⁺NK⁺ (e.g., IL-7R α , CD3 δ , ϵ , or ζ deficiency) [13^{••}]. Additionally, in specific circumstances, such as loss-of-expression mutations of γ c (CD132), CD127 (IL-7R α), or CD3 chains, flow cytometry can correctly clarify the specific genetic defect by evaluating for the specific cell surface protein (Fig. 2). However, with the wide range of genetic defects underlying SCID, flow cytometry should be viewed as a screening laboratory method providing information as to the potential underlying defect but a definitive diagnosis requires genetic studies.

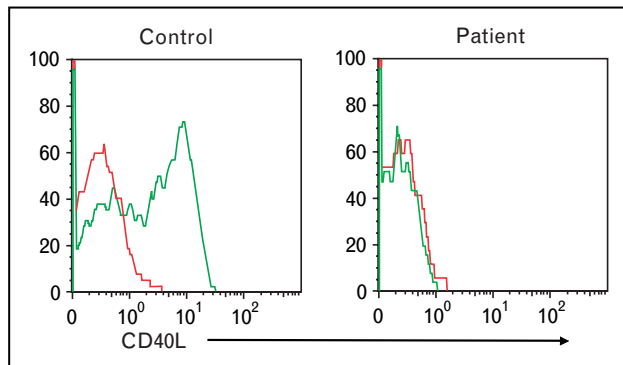
Hyper IgM syndromes

Hyper IgM syndromes (HIGM) encompass a group of genetic disorders affecting molecules involved in B-cell class switch recombination and somatic hypermutation [14]. Typically, the patients present with recurrent bacterial infections of the sinopulmonary tract, low serum levels of IgG and IgA and normal or increased IgM. Currently, mutations in six different genes are associated with HIGM: *CD40L*, *CD40*, *AID*, *UNG*, *NEMO*, and *IKBA*. The most frequent among these various defects is associated with an X-linked pattern of inheritance and also typically includes a history of opportunistic infections. The genetic basis of this specific disorder is a mutation

Figure 2 Specific severe combined immune deficiency diagnosis by flow cytometry



Lack of CD127 (IL-7R α) expression on the surface of the few circulating T cells in a T⁺/B⁺/NK⁺ SCID patient with a defect in the gene encoding CD127 (a); low CD3 ϵ and normal CD3 γ expression on the surface of CD3⁺ T cells from a T⁺B⁺NK⁺ SCID patient with a mutation in the gene encoding CD3 ϵ (b). SCID, severe combined immune deficiency.

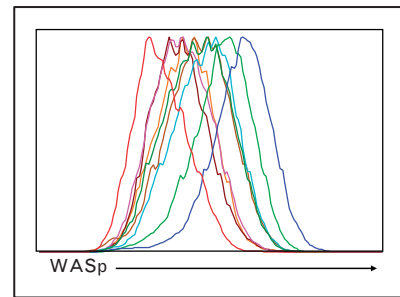
Figure 3 X-linked hyper immunoglobulin M syndrome

Absent CD40L (CD154) expression on the surface of activated CD4⁺ T cells of a HIGM1 patient, as compared with a control using PBMCs activated by PMA and ionomycin. ■, T-cell activation; ■, baseline. HIGM, hyper immunoglobulin M syndrome; PBMC, peripheral blood mononuclear cell; PMA, phorbol myristate acetate.

in *CD40L*. In a large cohort of these patients, Lee *et al.* [15] demonstrated that CD40 ligand (CD154) surface expression on activated CD4⁺ T cells (it is not expressed constitutively) was diminished or absent in 68% (41/61) of the patients with proven *CD40L* mutations (Fig. 3). Flow cytometric detection can be further improved by using a biotinylated CD40-Ig fusion protein to detect a functional CD40L receptor complex. The finding of absent or diminished binding of the fusion protein to activated CD4⁺ T cells correctly identified more than 90% of the patients with confirmed *CD40L* mutations, and a similar fusion protein is commercially available. Among the other genetic causes of HIGM, CD40 deficiency is clinically similar to CD40 ligand deficiency and can be screened for using flow cytometry to detect constitutive CD40 expression on B cells, monocytes, and/or dendritic cells [16]. Flow cytometry has not been demonstrated to be useful as a screening tool for the other genetic (*AID*, *UNG*, *NEMO*, and *IKBA*) defects associated with HIGM.

Wiskott–Aldrich syndrome

The Wiskott–Aldrich syndrome (WAS) is an X-linked PID associated with a defect in the gene encoding the WAS protein (WASp) [17]. The clinical phenotype includes the classical triad of immune deficiency, thrombocytopenia, and eczema typically associated with elevated IgE levels. The immune disorder is progressive with the initial defect involving antibody production particularly to polysaccharide antigens but evolving to more extensive antibody deficiency as well as an increasing defect in T-cell function. The flow cytometric findings include a decrease in CD8⁺ T cells as well as an increase in NK cells. Monoclonal antibodies to WASp can be used with an intracellular staining technique to screen for WASp deficiency recognizing that a number of patients

Figure 4 Variable Wiskott–Aldrich syndrome protein expression in cells from patients with Wiskott–Aldrich syndrome

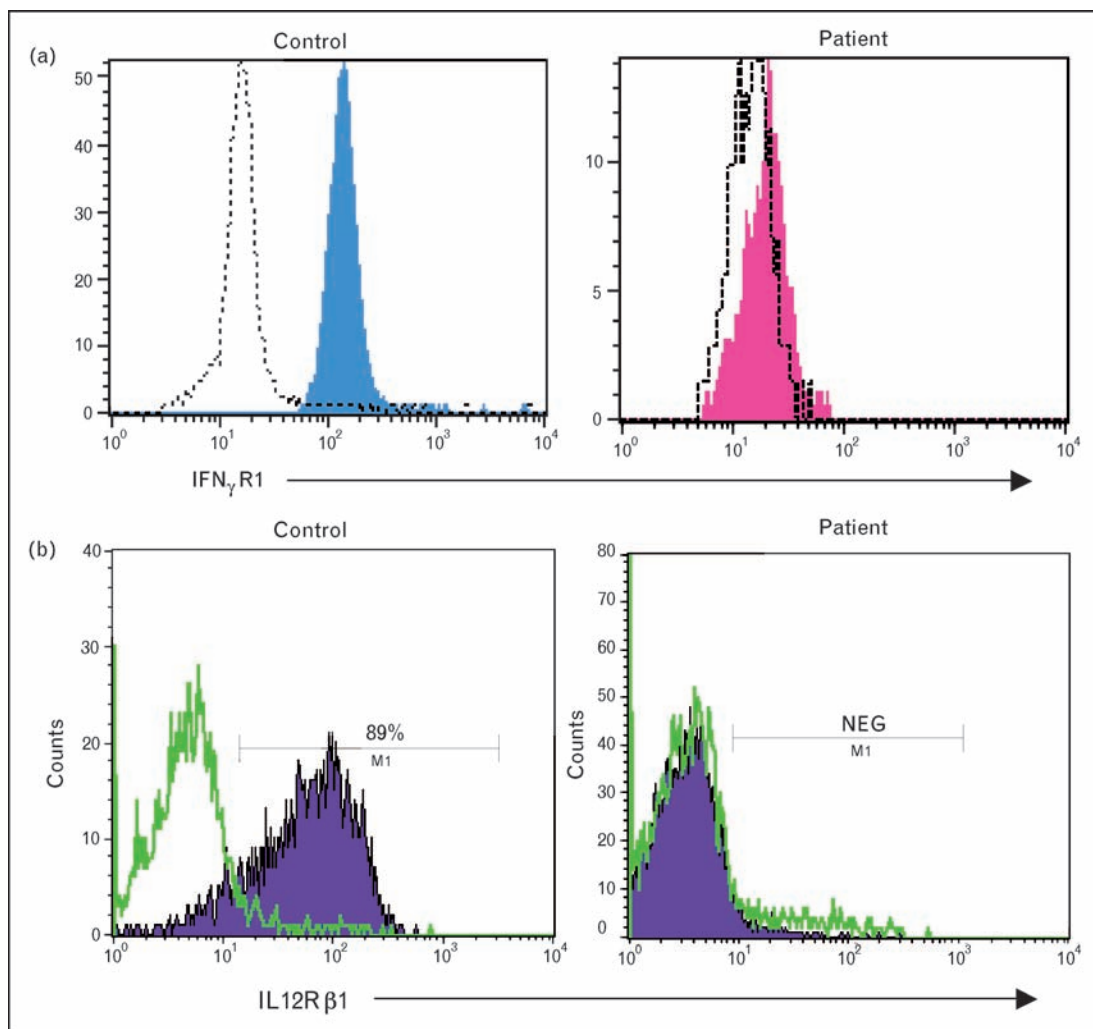
EBV-transformed B cell lines from patients with WAS were stained intracellularly with an antibody directed at WASp. Note the range of expression, with most mutations resulting in decreased WASp expression compared with a control (blue tracing, background staining shown by the red tracing). The codes reflect different *WASP* mutations. ■, negative control; ■, normal control; ■, P58R; ■, L39P; ■, D77H; ■, E31K; ■, D77G; ■, A236G; and ■, L105P. EBV, Epstein–Barr virus; WAS, Wiskott–Aldrich syndrome; WASp, Wiskott–Aldrich syndrome protein.

with WAS will demonstrate only decreased levels of WASp expression (Fig. 4). In addition, the more recently described disorder, X-linked thrombocytopenia (XLT), also associated with mutations in *WASP*, is more likely to have normal to decreased levels of WASp expression as measured by flow cytometry [18]. The evaluation of WASp expression by flow cytometry has also proven to be useful in the evaluation of chimerism posthematopoietic stem cell transplantation for WAS.

Defects in the IL-12/23–IFN- γ circuit

Mendelian susceptibility to mycobacterial disease (MSMD) 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* [19]. In some patients with MSMD this is linked to defects in several genes of the IL-12/23–IFN- γ axis, whereas in others the underlying genetic cause is unknown. Among those with defined defects, flow cytometry has proven to be a useful screening method for defects in IL-12R β 1 and IFN- γ R1 [20].

There is substantial allelic heterogeneity in IFN- γ R1 deficiency, resulting in variable receptor expression. Among described *IFNGR1* mutations, approximately 50% were associated with autosomal recessive inheritance and absence of surface protein expression (Fig. 5a) [20]. Additional patients include those with an autosomal recessive complete receptor defect associated with expression of a nonfunctional protein. The remaining patients demonstrate a partial receptor defect either due to an autosomal recessive or an autosomal dominant mutation. The latter is associated with increased expression of the

Figure 5 Identification of IL-12/23-IFN- γ axis defects by flow cytometry

Staining for IFN- γ R1 (a) and IL-12R β 1 (b) from controls and patients with nonsense mutations in either *IFNGR1* or *IL-12RB1*, respectively.

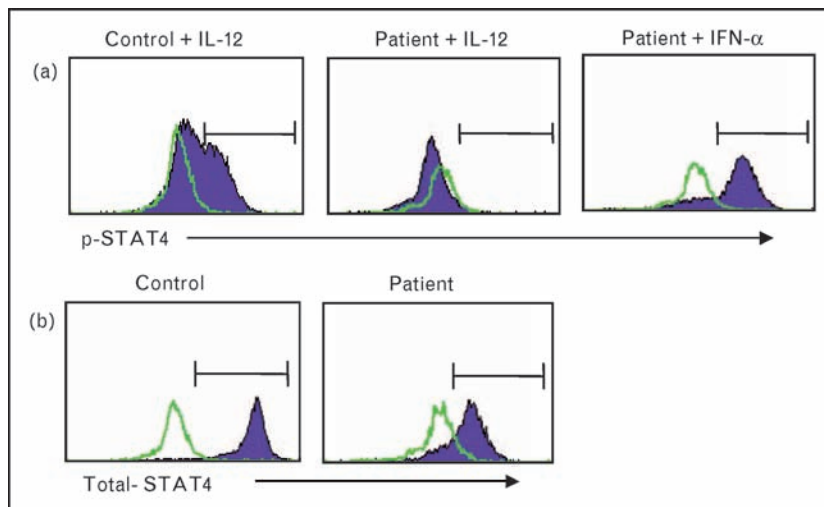
(abnormal) IFN- γ R1 chain and the increased level of expression can be demonstrated with flow cytometry. Most patients (~95%) with IL-12R β 1 deficiency harbor mutations that abolish receptor expression, allowing this to be readily detected using flow cytometry (Fig. 5b) [20]. STAT-1 defects are responsible for only approximately 5% of the genetically defined MSMD cases, and all three *STAT1* mutations described to date allow expression of STAT-1 protein. Flow-based screening is not effective for MSMD associated with NF- κ B essential modifier (NEMO), IFN- γ R2, and IL-12p40 defects. In summary, flow-based MSMD screening directed at evaluating expression of IL-12R β 1 and IFN- γ R1 should detect more than 50% of those patients with MSMD in whom a genetic defect can currently be defined.

An additional approach to evaluating patients with MSMD focuses on evaluating cellular signaling pathways. The flow cytometric assessment of the response to IFN- γ

can be performed by evaluating for the generation of phosphorylated STAT-1 and was initially demonstrated to be abnormal in patients with defects in IFN- γ R1 [21]. This screening approach evaluates intracellular STAT-1 phosphorylation in monocytes that should occur within minutes of the ex-vivo addition of IFN- γ . Identifying decreased STAT-1 phosphorylation in the monocytes from an MSMD patient suggests the defect involves IFN- γ R1, IFN- γ R2 or STAT-1. A parallel approach has been described for evaluating signaling through the IL-12 receptor based on detecting phosphorylation of STAT-4 by activated T cells in response to IL-12 and this can be used to screen for defects in this specific signaling pathway (Fig. 6) [22].

Toll-like receptor pathway defects

Genetic defects affecting toll-like receptor (TLR) pathways have been recently described in immunodeficient

Figure 6 Phospho-STAT-4 in IL-12R β 1 receptor deficiency

Functional assay evaluating for phosphorylation of STAT-4 (p-STAT-4) in response to IL-12 or IFN- α stimulation. PHA/IL-2 activated T cells from a patient with a mutation in IL-12R β 1 show no p-STAT-4 staining following IL-12 addition (a, middle plot) but do show STAT-4 phosphorylation (p-STAT-4 staining) in response to IFN- α (a, right plot). (b) This figure documents that the patient (and control) cells express native STAT-4.

patients with unique patterns of infection [19]. These include *IRAK4* mutations in patients with recurrent and/or severe pneumococcal infections; *NEMO* and *IKBA* defects in patients with atypical mycobacteriosis and other bacterial infections; and *UNC93B* and *TLR3* mutations in children with *Herpes simplex* encephalitis. However, testing toll-like receptor function by assessment of cytokine secretion in response to TLR ligand stimulation is presently only available in a few specialized centers. To overcome this difficulty and provide a rapid and reliable screening method, von Bernuth *et al.* [23^{••}] developed a flow cytometric assay based on L-selectin (CD62L) shedding following TLR stimulation. They demonstrate that incubation of whole blood with various TLR ligands induces a rapid shedding of CD62L from the surface of granulocytes. The absence of shedding reliably identified patients with *IRAK4* and *UNC93B* mutations. However, this procedure is not useful to identify patients with *NEMO* mutations and the same is likely to be the case for *IKBA* mutations. No patients with the very recently described *TLR3* mutations were included in this study. A caveat to this approach is that the blood has to be processed relatively rapidly because there is spontaneous shedding of CD62L that will compromise the test [23^{••}].

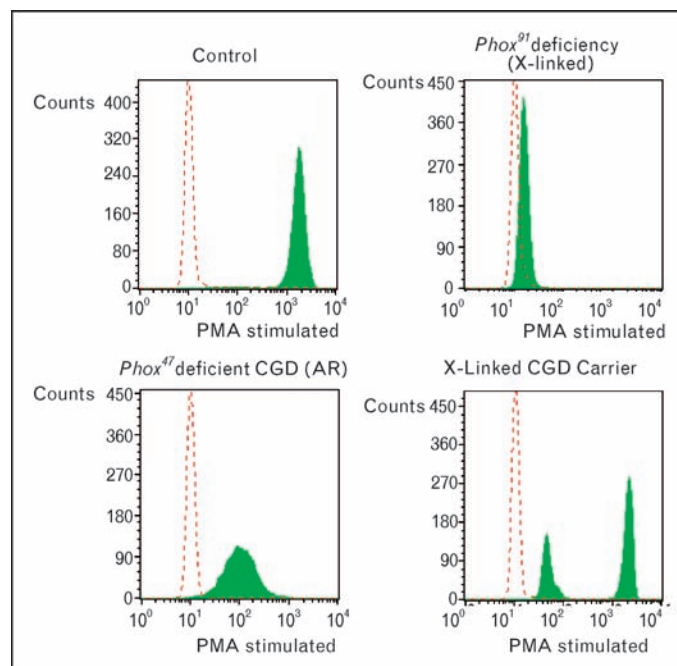
Chronic granulomatous disease

Chronic granulomatous disease (CGD) is a PID associated with defective phagocyte intracellular killing that presents clinically with recurrent severe bacterial and fungal infections [24]. CGD results from mutations in one of four different components of the nicotinamide adenine dinu-

cleotide phosphate (NADPH) oxidase system and is inherited in an X-linked recessive form (~65–70% of CGD patients) or one of three different autosomal recessive forms (~30–35% of patients). A clinical history of severe and/or recurrent deep-seated infections with *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia* complex, *Aspergillus* species or *Nocardia* species is strongly suggestive of CGD. The optimal screening test to establish the diagnosis of CGD is a flow cytometric assay of NADPH oxidase activity using the intracellular dye dihydrorhodamine 123 (DHR) that normally converts into a fluorescent compound following granulocyte activation (using PMA) [25]. In contrast, patients with CGD show markedly decreased or absent fluorescence in this assay and maternal carriers of X-linked CGD demonstrate two populations of cells corresponding to the random inactivation of either the normal or the defective X chromosome (Fig. 7). The DHR flow assay has proven to be sufficiently sensitive to establish the diagnosis of an X-linked carrier state associated with clinical disease consistent with CGD owing to a defect in X-chromosome Lyonization such that the frequency of normal granulocytes was less than 0.1% [26]. This finding confirms that the DHR flow assay is useful in monitoring myeloid chimerism posthematopoietic stem cell transplantation in CGD and it also has been used to monitor gene-corrected granulocytes following experimental gene therapy.

Leukocyte adhesion deficiency type 1

The leukocyte adhesion deficiency type 1 (LAD1) is a PID associated with recurrent bacterial and fungal infections based on a defect in phagocytic cell migration

Figure 7 Dihydrorhodamine 123 flow cytometry assay

Evaluation of granulocytes from a control, an X-linked chronic granulomatous disease (CGD) patient, an autosomal recessive CGD patient and an X-linked maternal carrier for CGD. PMA, phorbol myristate acetate.

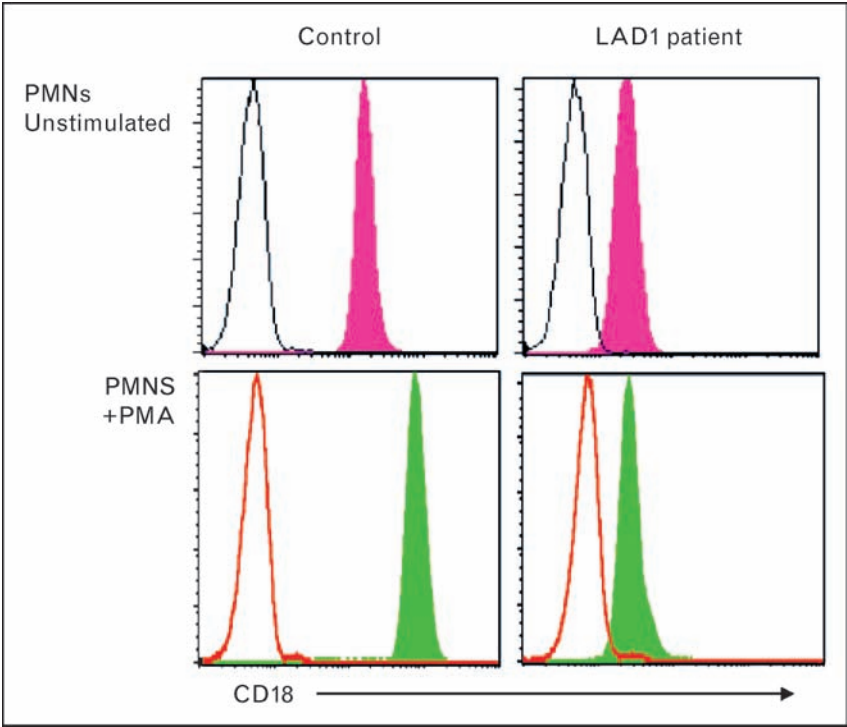
[24]. The clinical presentation includes recurrent serious infections in deep tissue sites and skin as well as significant periodontitis. The underlying defect involves the gene that encodes CD18 and this results in either decreased or absent expression of the three heterodimeric $\beta 2$ integrin receptors (CD11a/CD18, CD11b/CD18, CD11c/CD18). The optimal screening test to establish this diagnosis relies on flow cytometry to test for CD18 (and CD11a, CD11b, CD11c) expression on granulocytes [25]. The typical LAD1 patient has low (<10%) to absent CD18 expression (Fig. 8) along with a marked peripheral leukocytosis. The CD18 defect also precludes upregulation of the $\beta 2$ integrins that normally occurs following cell activation and this can be evaluated by stimulating granulocytes with phorbol myristate acetate (PMA) and testing for the normal rapid upregulation in the expression of CD18 that is absent in cells obtained from patients with LAD1 (Fig. 8).

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked inheritance syndrome

Mutations in *FOXP3*, the gene encoding a winged helix transcription factor forkhead box protein 3 (FOXP3), result in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome [27]. The

disease typically presents during infancy with the clinical triad of severe enteropathy, endocrinopathies (diabetes or thyroiditis), and eczematous dermatitis [28]. FOXP3 is important for the development and function of T regulatory (Treg) cells, which negatively control immune responses to self and foreign antigens. Patients with IPEX syndrome demonstrate defective Treg activity and many IPEX-related *FOXP3* mutations lead to diminished numbers of circulating Tregs. Detection of circulating FOXP3-expressing Treg cells can be performed using intracellular flow cytometry (Fig. 9) and approximately 80% of patients with IPEX syndrome demonstrate low levels of FOXP3+ Tregs compared with normal controls (T.R. Torgerson, personal communication). Given the severity of the disease and the benefits of early diagnosis, flow cytometric determination of intracellular FOXP3 expression using commercially available reagents serves as a simple screening test. One caveat is the timing between blood collection and analysis, which should not exceed 36 h, due to a significant time-dependent spontaneous loss of FOXP3+ Treg cells (T.R. Torgerson, personal communication). It is important to note that the percentage of CD3⁺CD4⁺CD25⁺ T cells in the peripheral blood of patients with IPEX syndrome is not decreased, making this not an appropriate marker for IPEX unless it is combined with intracellular FOXP3 evaluation.

Figure 8 CD18 staining of granulocytes



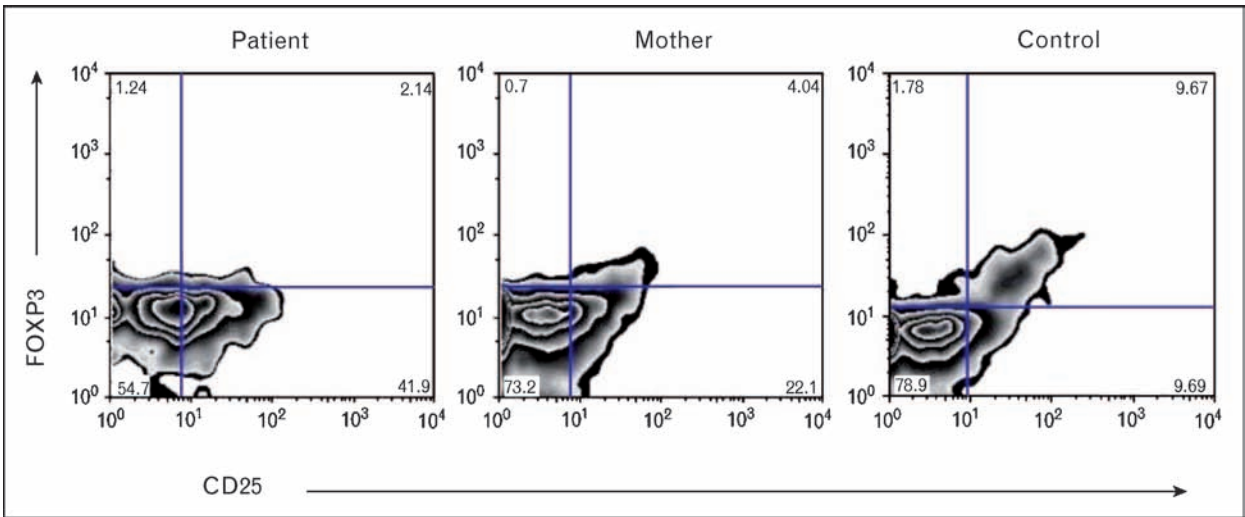
Evaluation of a control and a patient with leukocyte adhesion deficiency type 1 showing marked decrease in CD18 expression (<10% of control expression) and no upregulation in CD18 expression following cell stimulation with PMA. LAD1, leukocyte adhesion deficiency type 1; PMA, phorbol myristate acetate; PMN, polymorphonuclear neutrophil.

Autoimmune lymphoproliferative syndrome

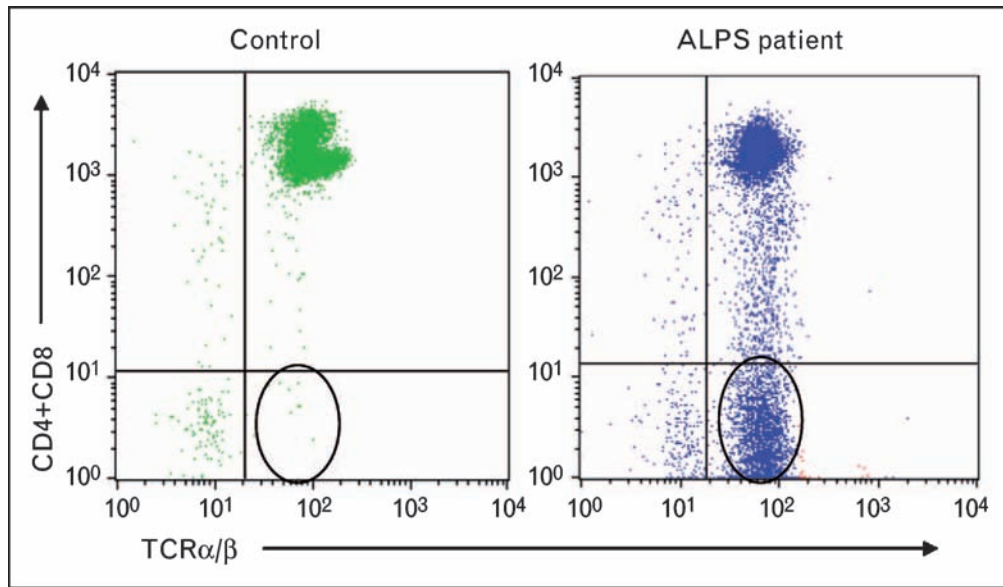
The autoimmune lymphoproliferative syndrome (ALPS) is a genetically heterogeneous disorder of lymphocyte apoptosis characterized by lymphocyte accumulation

(lymphadenopathy and/or splenomegaly), blood cell-directed autoimmunity and propensity to develop lymphoid tumors [29]. Most cases (70%) are caused by mutations in the gene encoding FAS (*TNFRSF6*), and a minority of patients have other genetic causes including

Figure 9 Immune dysregulation, polyendocrinopathy, enteropathy, X-linked screening using forkhead box protein 3 staining



Intracellular FOXP3 staining in an IPEX patient, the mother of the IPEX patient and a control. No CD4⁺CD25⁺FOXP3⁺ Treg cells were noted in the patient's peripheral blood and a diminished population of Tregs in the mother when compared with control lymphocytes. FOXP3, forkhead box protein 3; IPEX, immune dysregulation, polyendocrinopathy, enteropathy, X-linked.

Figure 10 Elevated double-negative T cells in autoimmune lymphoproliferative syndrome patients

Elevated numbers of $CD3^+TCR\alpha/\beta^+CD4^-CD8^-$ cells in an ALPS patient (right panel) compared with a control individual (left panel). CD3 T cells are evaluated using CD4 and CD8 antibodies conjugated to the same fluorochrome (Y axis) and a monoclonal antibody to a framework component of the $\alpha/\beta TcR$ (X axis). ALPS, autoimmune lymphoproliferative syndrome.

mutations in genes encoding Fas ligand (*FASL*), caspase-10 (*CASP10*), and neuroblastoma-RAS (*NRAS*) [29,30]. Mutations in the gene encoding caspase-8 (*CASP8*) cause a related but phenotypically distinct syndrome named caspase-8-deficiency syndrome (CED), with signs of T-cell and B-cell immunodeficiency in addition to impaired lymphocyte apoptosis. A unique immunophenotypic finding and required diagnostic criterion in ALPS is the presence of elevated levels of circulating alpha beta (α/β) T cell receptor (TcR) expressing $CD4^-CD8^-$ T cells, referred to as double-negative T cells (DNTs) (Fig. 10) [31]. In the authors' (T.A.F., J.B.O.) center, the presence of α/β TcR DNTs more than 1% or more than 19 cells/mm^3 is considered abnormal and with the appropriate clinical history would result in further studies directed at the evaluation of lymphocyte apoptosis and specific mutation analysis. Other helpful immunophenotypic findings in ALPS include low memory B cells ($CD20^+CD27^+$), increased $CD8^+CD57^+$ T cells, decreased $CD3^+$ T cell CD25 expression compared with HLA-DR expression and expression of the unusual CD45 isoform, B220, on DNT cells [31,32]. Evaluation of FAS receptor (CD95) on the cell surface is generally not of diagnostic utility, as most significant *FAS* mutations do not interfere with protein expression.

X-linked lymphoproliferative syndrome

The X-linked lymphoproliferative syndrome (XLP) is a rare and often fatal PID characterized by the hypogammaglobulinemia, lymphomas and the development of

hemophagocytosis usually after Epstein–Barr virus (EBV) infection. Approximately 60% of the familial cases (XLP1) are caused by mutations in *SH2D1A*, which encodes the signaling lymphocyte activation molecule (SLAM)-associated protein (SAP) [33]. More recently, mutations in the X-linked inhibitor of apoptosis (*XIAP* or *BIRC4*) gene were described in patients with XLP2 [34]. Intracellular flow cytometry for SAP and XIAP has been described as a sensitive screening tool for the identification of mutation-positive patients. In this recent work, Zhu *et al.* [35•] sequenced *SAP* and *XIAP* in 42 patients suspected of having XLP and detected 11 (26.2%) patients with mutations in *SAP* and one (2.4%) with a mutation in *XIAP*. Flow cytometry correctly identified all 12 patients with these mutations, similar to a previous report [36•] and, therefore, this approach appears to serve as a rapid and inexpensive screening tool for XLP. Moreover, in either XLP1 or XLP2, very low numbers of NKT ($CD3^+CD16^+56^+V\alpha 24^+V\beta 11^+$) cells are observed in the peripheral blood of patients with XLP, serving as a cue for this diagnosis [33].

Familial hemophagocytic lymphohistiocytosis

Familial hemophagocytic lymphohistiocytosis (FHL) refers to a group of genetic disorders resulting in prolonged fevers, cytopenias, and hepatosplenomegaly, typically occurring during infancy or early childhood. Defined causes include defects in proteins linked to the pathway of granule-mediated cytotoxicity: perforin (FHL2), MUNC 13-4 (FHL3), and syntaxin-11 (FHL4) [37].

Sensitive flow-based screening assays have been developed for all three subtypes. Intracellular staining for perforin focused on cytotoxic lymphocytes (CD8 T cells, NK and NKT cells) can accurately identify patients with *PRF1* (perforin) mutations as well as carriers [38]. In recent work, diminished expression of CD107a by NK cells following stimulation and degranulation has been shown to predict defects in the genes encoding MUNC13-4 or syntaxin 11, providing a useful and rapid screening tool for FHL3 and FHL4 [39,40].

Conclusion

Flow cytometry has emerged as a useful method in evaluating patients with PIDs based on evaluating cell populations and subpopulations, determining for the presence or absence of specific proteins and evaluating selected cell functions. This technology is particularly appealing because of its widespread availability and rapid turnaround time but it remains primarily a screening test that helps direct genetic studies, whereas in most cases it does not establish the underlying genetic basis of PID.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 591–592).

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