Molecular mechanisms in allergy and clinical immunology

Series editors: WIliam T. Shearer, MD, PhD, Lanny J. Rosenwasser, MD, and Bruce S. Bochner, MD

Functional and molecular evaluation of lymphocytes

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The laboratory plays a central role in the evaluation of immune function and is critical in the diagnosis and treatment of immune deficiencies. The range of options available to evaluate lymphocyte function has expanded dramatically as our understanding of the immune system has expanded. As the choices of laboratory tests increase, so also does the need to choose testing in such a way as to appropriately direct the evaluation. Typically, this approach involves starting with screening tests and, on the basis of the results of these tests, deciding whether more sophisticated and expensive testing is warranted. The remarkable developments over the past decade leading to the identification of numerous gene defects underlying a variety of immune deficiencies has moved mutation analysis into the realm of the clinical laboratory. This information could be indispensable for immune deficiency diagnosis, prenatal screening, carrier detection, and family counseling. In this review a sequential approach to evaluating lymphocyte function is presented, starting with readily available screening tests and followed by more complex *in vitro* testing, including the application of newer assays. The various approaches are presented from the perspective of appropriate use and information garnered, whereas actual details of test procedures are not discussed but are referenced. The evolution of immune function testing suggests that it will continue to develop, and future assays are likely to provide even more insight into specific aspects of the immune response and be linked to immune deficiencies not yet defined. (J Allergy Clin Immunol 2004;114:227-34.)

Key words: *Lymphocytes, immune deficiency, diagnostic immunology*

Abbreviations used
DTH: Delayed-type hypersensitivity
mAbs: Monoclonal antibodies
NK: Natural killer
TCR: T-cell receptor
TREC: T-cell receptor excision circle

The capacity to assess the immune system has progressed in parallel with the advances in understanding the details of the immune response. This has been aided in great measure by the experiments of nature that present as immune deficiencies. The case report by Lynn et al[1] in this issue provides an example of a directed approach used to evaluate a patient presenting with a history suggestive of immune deficiency. This brief review will outline a coordinated approach to evaluating lymphocyte function. It is prudent to use a clinical history of infection, including type, site, frequency, and severity, as the guide to selectively evaluate the most appropriate components of either the acquired or innate immune system. The recommended approach to evaluating immune function begins with screening tests that are available in most clinical laboratories, and this typically includes one or more of the following: complete blood count with differential, immunoglobulin concentrations, specific antibody levels (eg, isohemagglutinins and anti-tetanus antibody), lymphocyte subset evaluation, and HIV testing. Testing beyond the screening level is generally available only at larger medical centers and typically involves a combination of *in vitro* lymphocyte functional testing and cytokine measurements. Mutation analysis and a host of new assays are available on an even more limited basis, generally in centers with research programs directed at investigating immune deficiencies. Barriers to in-depth clinical evaluation of immune deficiencies are the limited availability of many newer tests and the difficulty obtaining reimbursement for nonstandard laboratory testing. Additionally, the limited availability of testing often requires that patient samples are shipped to the testing facility, and this introduces sample integrity during transport as a variable. In our laboratory we address this by requiring that a normal sample is paired with the patient sample for all functional testing, but the extra testing will generally not be reimbursable. Finally, the Clinical Laboratory Improvement Amendment of 1988 established laboratory standards that apply to any laboratory reporting results that are used for diagnosis or treatment

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Disclosure of potential conflict of interest: T. A. Fleisher—none disclosed. J. B. Oliveira—none disclosed.

Received for publication May 6, 2004; revised May 28, 2004; accepted for publication June 1, 2004.

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0091-6749
doi:10.1016/j.jaci.2004.06.001

TABLE I. Major circulating B-cell subpopulations

Subpopulation	Cell-surface marker
Immature B cell	$CD19^+CD20^+sIgM^+$
Mature B cell	$CD19^+CD20^+sIgM^+sIgD^+$
Memory B cell	$CD19^+CD20^+sIgM^+sIgD^+CD27^+$
Isotype-switched memory B cell	$CD19^+CD20^+sIgG^+$ or $sIgA^+$ or $sIgE^+$
B1 B cell*	$CD19^+CD20^+CD5^+$

*B-cell subset responsible for the production of natural IgM antibodies. [20]

decisions. All of these factors emphasize the importance of actively involving clinical immunologists specialized in immune deficiencies during the evaluation of patients with complex immune disorders.

The immune system is composed of a network of cells and soluble components that cooperate to mount effective responses to microbes and other foreign substances. There are 2 major arms involved in host defense, the phylogenetically older innate system and the more recent adaptive system. The innate immune system includes natural killer (NK) cells and phagocytes together with antimicrobial peptides, complement, and cytokines. The adaptive immune system generates microbe-directed responses involving T and B lymphocytes together with specific antibodies and a range of cytokines and chemokines. It has become clear that these 2 systems function cooperatively to provide the optimal host defense, and a defect in either system can have a significant effect. This discussion focuses on methods useful for diagnosing specific immune deficiencies that involve lymphocytes, as well as evaluating the range of immunologic changes associated with these disorders. Throughout this presentation, each of the laboratory approaches will be discussed in brief, with references provided that include greater detail and specifics on test performance.

EVALUATION OF B-CELL FUNCTION

The laboratory analysis of B-cell function generally is prompted by a history of recurrent sinopulmonary infections with encapsulated bacteria.[2] Screening tests consisting of serum IgG, IgA, and IgM quantitation along with measurement of specific antibody responses identify the great majority of patients with defective humoral immunity.

Quantitative immunoglobulins

Evaluating serum immunoglobulins is the starting point for any humoral immune evaluation. Decreased levels might result from diminished production (eg, primary immunodeficiency and immunosuppressive therapy), excessive loss (eg, protein-losing enteropathy), or increased catabolism (eg, myotonic dystrophy). It is critical that serum immunoglobulin concentrations are interpreted by comparing the results with age-matched reference ranges because the levels change significantly during infancy and childhood.[3] The history and physical examination might provide clues to the diagnosis of underlying conditions causing excessive protein loss, although this is not always the case, as evidenced by the accompanying case report. Measurement of serum albumin concentration is often helpful to rule in or out excessive protein loss. Patients with increased protein loss affecting immunoglobulins generally also have a low concentration of albumin, whereas patients with decreased immunoglobulin production usually have normal albumin concentrations.

Rare patients with increased susceptibility to infection might have normal or borderline-low total serum IgG levels, with low levels of one or more IgG subclasses.[4] The interpretation of IgG subclass deficiency in the absence of specific antibody deficiency must be made with great caution, and it remains unclear whether IgG subclass measurements are relevant in the absence of evaluating specific antibody responses.[5-7]

Abnormalities of IgE concentration, including both increases and decreases, are found in humoral immunodeficiency syndromes.[8] IgE levels also are altered in other immunodeficiencies, with increases seen in Wiskott-Aldrich syndrome, Job syndrome, Nezelof syndrome, and Omenn syndrome and reductions found in ataxia-telangiectasia.[8]

Specific antibodies

Natural antibodies are those present without deliberate antigenic challenge and include isohemagglutinins, antibodies to the polysaccharide blood group antigens A or B.[9] Quantitation of these natural antibodies represents a simple screen for specific antibody (IgM) formation, assuming that the patient does not have blood type AB and is older than 1 to 2 years.

Specific antibody production in response to immunization is the most direct means to evaluate the B-cell response. This approach is particularly valuable in patients with recurrent bacterial infections and borderline-low immunoglobulin class or subclass levels. The results have to be interpreted in the context of the patient's age and preimmunization antibody concentration, which in some circumstances might affect the postimmunization response. Evaluation of antibody responses to tetanus toxoid and diphtheria toxoid is a standard approach to assess the response to T cell–dependent protein antigens. Live viral vaccines are absolutely contraindicated in patients with suspected immunodeficiency because of the risk of disseminated infection.[10]

A complete assessment of *in vivo* B-cell function also requires evaluation of antibody responses to carbohydrate antigens, such as pneumococcal and meningococcal polysaccharide vaccines.[11] These antigens activate B cells directly, without the absolute requirement of T-cell help. It is important to recognize that the newer carbohydrate vaccines (eg, Prevnar), which are conjugated to a protein antigen to increase immunogenicity, should not be used to establish a response to polysaccharide antigens. In addition, children younger than 2 years of age typically

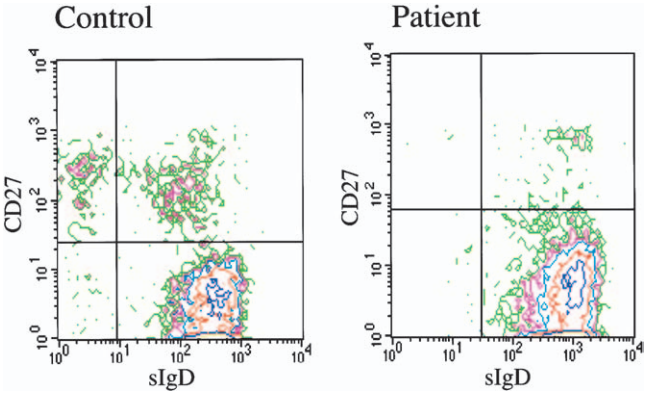

FIG 1. Immunophenotyping demonstrating normal B-cell isotype switching, with IgD–B cells, as well as $CD27^+$ memory B cells (IgD^+ and IgD^-), on the *left panel* and markedly diminished isotype-switched B cells and $CD27^+$ memory B cells in a patient with X-linked hyper-IgM syndrome on the *right panel*.

demonstrate little or no response to unconjugated carbohydrate antigens.[12]

The evaluation of specific antibody production in patients receiving immunoglobulin replacement can be accomplished with neoantigens, such as bacteriophage φX174 or keyhole limpet hemocyanin.[13,14] Both immunogens have been used safely in the setting of immune deficiency and allow both primary and secondary responses to be evaluated but are currently only available through research protocols in selected academic institutions.

Enumeration and characterization of B cells

B cells comprise 10% to 20% of the total lymphocytes circulating in peripheral blood, and immunophenotypic evaluation can be useful in establishing a specific diagnosis associated with hypogammaglobulinemia. Patients with X-linked and autosomal recessive forms of agammaglobulinemia have virtually no circulating B cells, whereas the majority of patients with common variable immune deficiency have normal or near-normal levels of circulating B cells.[15-19] Immunophenotyping is performed by means of flow cytometry with mAbs directed at cell-surface antigens. This approach yields information about B-cell number, maturation, differentiation, and isotype switching (Table I and Fig 1).[21] For example, activation of B cells can be evaluated on the basis of the expression of the marker CD23,[22] and memory status can be assessed by using antibodies to CD27 (Fig 1).[23] Immunophenotypic data should be compared with age-matched reference intervals on the basis of the significant changes observed during infancy and childhood, and appropriate interpretation requires both percentage and absolute cell numbers.[24]

In vitro studies of B-cell immune function are used primarily in the research setting to evaluate immunoglobulin secretion.[25] In these studies mitogens or antibodies directed against surface IgM, either with or without the addition of cytokines, are used as stimulants, and the concentrations of individual immunoglobulin isotypes in the culture supernatant are measured by means of immunoassay.[25] The ability of T cells to provide help for (or suppress) B-cell differentiation can be assessed with cell-mixing experiments. In addition, the ability of B cells to undergo isotype switching can be assessed by using purified B cells cultured with cytokines and stimulated with trimerized CD40L.[26] More recently, the use of single-cell PCR for heavy chain rearrangement has been applied to assess for immunoglobulin gene diversity and somatic hypermutation, a research technique that could prove useful in evaluating specific B-cell disorders.[27]

EVALUATION OF T-CELL FUNCTION

T cells are central to the adaptive immune response by providing helper activity, proinflammatory cytokine secretion (eg, IL-4 and IL-12), suppressor (regulatory) function through direct cellular interactions and/or inhibitory cytokine secretion (eg, IL-10 and TGF-β), and cytotoxic effector function. Assessment of T-cell function parallels the approach used to evaluate B cells: screening *in vivo* T-cell function followed by *in vitro* testing directed at cell characterization by using mAbs and cell-function assessment. This type of evaluation is usually prompted by a history of opportunistic infection, a family history of immune deficiency involving T cells, or both.[28] In this setting it is imperative to rule out secondary causes of immune deficiency, particularly HIV infection.

Screening

Delayed-type hypersensitivity (DTH) skin testing represents an *in vivo* screen of T-lymphocyte function. It consists of the intradermal injection of specific antigens, followed by evaluation of the injection site 48 and 72 hours later. Formation of an indurated area of 5 × 5 mm or larger at the site of injection characterizes a positive response.[29,30] The local inflammatory reaction indicates an intact recall response initiated by memory T cells that involves a coordinated process of cytokine-chemokine secretion and cellular infiltration. Injection of

TABLE II. Select T-cell cluster of designation molecules and their function

CD molecule	Function
Pan T cell	
CD2	Adhesion molecule, binds to LFA3
CD3	Part of the TCR signaling complex
CD5	Binds CD72; involved in T-cell activation
T-cell subsets	
CD4	MHC class II coreceptor
CD8	MHC class I coreceptor
Activation markers	
CD25	IL-2 receptor α chain
CD38	Binds to CD31
CD40L	Costimulatory molecule; binds CD40
CD69	Early activation antigen
CD71	Transferrin receptor
HLA-DR	MHC class II molecule

a standardized amount of at least 3 different antigens, such as tetanus toxoid, *Candida* antigen, and mumps antigen, should be used to increase the likelihood of a positive response.[30] Anergy is defined as the absence of response to all tested antigens. DTH skin test results have been reported to correlate well with other measures of T-cell function.[29] In summary, a positive DTH response indicates grossly intact cell-mediated immunity, but a negative test response might be the result of T-cell dysfunction, lack of prior exposure, or use of an improper technique.

Enumeration and characterization of T cells

T cells represent approximately 75% of the circulating lymphocytes, and thus a significant decrease in T cells might be first noted as lymphocytopenia on a complete blood count. This finding should prompt immunophenotyping to determine the total number of T cells on the basis of CD3 expression and the numbers of T-cell subsets on the basis of CD4 and CD8 expression.[21] Optimal immunophenotyping involves multicolor studies that enable better definition of subsets, and results should be compared with age-matched control data.[24] There are a host of additional surface antigens that can be evaluated to further define T-cell properties. Included among these are naive (CD45RA) versus memory (CD45RO) markers and activation (CD25, CD38, CD40L, CD69, CD71, and HLA-DR) markers (Table II). The accompanying case report identified the disproportionate loss of $CD4^+$/$CD45RA^+$ T cells, suggesting that naive T cells traffic primarily through the lymphatics.[31] Evaluation of the frequency of α/β and γ/δ T-cell receptor (TCR)–positive T cells and the distribution of TCR Vβ families can also be performed by means of flow cytometry with TCR-specific mAbs.[32] Thus immunophenotyping is useful not only to support specific diagnoses but also to explore biologic changes associated with specific immune disorders.

Standard laboratory methods for assessing *in vitro* T-lymphocyte function include measurement of lymphoproliferation, assessment of cytokine secretion after T-cell activation, and evaluation of cytotoxic T-cell function. Generally, this type of testing is performed in laboratories specialized for evaluating immune function.

T-cell proliferation *in vitro* can be induced with mitogens (eg, PHA, pokeweed mitogen, concanavalin A, and anti-CD3 antibodies), recall antigens (eg, tetanus toxoid and *Candida* antigen), or allogeneic cells. At the end of the incubation period, usually from 3 (mitogen) to 6 (antigen and allogeneic cells) days, the plates are pulsed with tritiated thymidine for 6 to 18 hours. The amount of tritiated thymidine incorporated into nuclear DNA is measured with a scintillation counter and reflects cell proliferation.[33] Results can be expressed as counts per minute or stimulation index (the ratio of counts per minute in stimulated cultures divided by counts per minute in unstimulated cultures). To interpret the test results, both counts per minute and stimulation index should be reviewed, and these must be compared with the laboratory-generated control data.

Flow cytometric evaluation of cell division and activation status can be used as an alternative to the radioactive test described above. Incorporation of the thymidine analogue bromodeoxyuridine into replicating nuclear DNA can be measured at the single-cell level.[34] Cell division can be assessed by evaluating the cell cycle with a DNA intercalating dye (eg, propidium iodide) and by using cell-tracking dyes that decrease by a factor of one half for each round of cell division (eg, carboxy-fluorescein diacetate succinimidyl ester).[35,36] Alternatively, evaluation of surface antigens, such as CD69, expressed on T-cell activation in response to mitogen stimulation can be performed by using flow cytometry, with the caveat that this does not formally evaluate cell proliferation.[37] The flow-based assays to evaluate either activation or proliferation can be combined with specific surface antigen assessment that allows directed evaluation of cell populations or subsets.

Many aspects of the immune response are initiated or regulated by networks of cytokines. As our understanding of these relationships expands, it is likely that an increasing number of undefined clinical phenotypes manifesting recurrent infections will be linked to defects in cytokine production, receptor function, or downstream intracellular signaling cascades. Cytokine levels in body fluids, such as serum or cerebrospinal fluid, can be quantitated; however, to date, this has not proved to be of major clinical utility.[38] In the setting of an immunologic evaluation, it appears more useful to stimulate T cells or mixed populations of mononuclear cells with specific stimulants and assay cytokine production in culture supernatants with commercially available immunoassays.[39] One can also assess cytokine-specific mRNA levels after *in vitro* activation by using quantitative RT-PCR assays.[40] Flow cytometry can be used to detect T cells containing specific intracellular cytokines after *in vitro* stimulation with either mitogen or antigen.[41,42] This method has the advantage of allowing the simultaneous assessment of cell-surface markers and one or more intracellular cytokines, an approach that has been used to

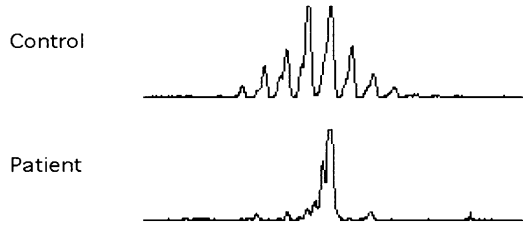

FIG 2. Spectratyping demonstrating a normal distribution for Vβ 6B PCR product *(top panel)* and a restricted distribution for the same Vβ PCR product in the setting of an immune deficiency demonstrating oligoclonal T cells *(lower panel)*.

assess T_H1 versus T_H2 status.[42] It can also be used to identify the frequency of antigen-specific T cells on the basis of the presence of specific intracellular cytokines after antigen exposure.[43] Alternatively, individual cytokine-secreting lymphocytes can be detected by using the enzyme-linked immunospot assay.[43]

The capacity to evaluate TCR diversity has proved to be particularly valuable in the characterization of certain immune deficiencies and to monitor T-cell reconstitution after stem cell transplantation. TCR Vβ diversity can be assessed by using a PCR-based assay referred to as T-cell spectratyping.[44,45] Normally, the majority of the 24 TCR Vβ families are expressed by circulating T cells, and each demonstrates a range of different-sized PCR products that are distributed in a Gaussian fashion (Fig 2). In immune disorders that involve abnormal T-cell development, such as Omenn syndrome, spectratyping demonstrates TCR Vβ skewing representative of an oligoclonal T-cell repertoire.[46]

During T-cell rearrangement, DNA segments between V(D)J gene elements are excised, generating circular T-cell receptor excision circles (TRECs). TRECs are not replicated during mitosis and consequently decrease in frequency on antigen encounter and cell division. This method has proved to be a reliable means of distinguishing recent naive thymic emigrants from memory T cells. The quantity of TRECs in the peripheral blood is detected by using a PCR assay and is expressed as the number of TREC-positive cells per 10^5 T cells.[47] This assay has proved to be particularly valuable in evaluating the status of thymic function after stem cell transplantation and in DiGeorge syndrome.[48,49] As with the majority of functional immunologic data, the results generated with TREC analysis and T-cell spectratyping must be compared with age-matched control data. In addition, at this time, these tests are only available in a limited number of medical centers.

Recently, technology to assess the frequency of antigen-specific T cells has been developed by using multimers of an antigenic peptide bound to an MHC molecule.[50] These multimers also contain a fluorochrome that allows antigen-specific cell frequency determination by means of flow cytometry. The first successful studies used peptide–MHC class I tetramers that enabled detection of antigen-specific CD8 T cells.[51] It has now been extended to MHC class II oligomers, allowing characterization of the frequency of antigen-specific CD4 T cells.[52] Tetramer technology has seen application in a variety of settings, including viral infections and autoimmunity.[53,54] The major caveats with this technology are the HLA restriction and the requirement for defined antigenic peptides.

The ability of T cells to kill allogeneic targets, virally infected autologous targets, or both can be evaluated by

TABLE III. Selected primary immunodeficiency diseases for which the underlying gene defects have been identified

Disease	Gene(s)
Antibody deficiencies	
• Agammaglobulinemias	
XL	*BTK*
AR	*IGHM*, *CD79A*, *CD179B*, *BLNK*
• Common variable immunodeficiency (AR)	*ICOS*
• Hyper-IgM syndrome (AR)	*AICDA*, *UNG*
Combined deficiencies	
• Severe combined immunodeficiency (SCID)	
Defective cytokine signaling	
XL	*IL2RG*
AR	*IL2RA*, *IL7RA*, *JAK3*
Defective TCR signaling (AR)	*PTPRC*, *CD3G*, *CD3E*, *ZAP70*
Defective TCR gene recombination (AR)	*RAG1*, *RAG2*
Defective nucleotide salvage pathway (AR)	*ADA*, *NP*
Defective MHC class I expression (AR)	*TAP1*, *TAP2*
Defective MHC class II expression (AR)	*MHC2TA*, *RFXANK*, *RFX5*, *RFXAP*
Defective TLR signaling (AR)	*IRAK4*
Other	*DCCRE1C*, *WHN*
• Wiskott-Aldrich syndrome (XL)	*WASP*
• Ataxia-telangiectasia group (AR)	*ATM*, *NBS1*, *MRE11A*
• DiGeorge syndrome	*del22q11*
• Hyper-IgM syndrome	
XL	*TNFSF5*, *IKBKG*
AR	*TNFRSF5*
• X-linked lymphoproliferative syndrome (XL)	*SH2D1A*
Other disorders	
• IFN-γ–IL-12 axis (AR)	*IFNGR1*, *IFNGR2*, *IL12B*, *IL12RB1*, *STAT1*
• Autoimmune polyglandular syndrome 1 (AR)	*AIRE*
• Defective NK function (CD16 deficiency) (AR)	*FCGR3A*

Adapted from Bonilla et al.[63]
XL, X-linked; *AR*, autosomal recessive; *SCID*, severe combined immunodeficiency; *TLR*, Toll-like receptor.

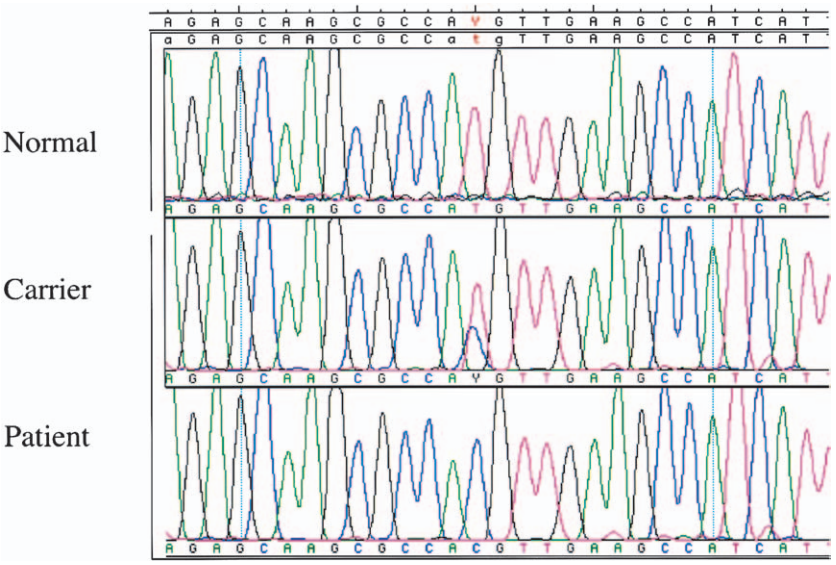

FIG 3. Sequencing of *IL2RG* demonstrating a single nucleotide (T to C) substitution in position 16 of exon 1 *(arrow)* in a patient with SCIDX1 *(lower tracing)* compared with normal sequence *(upper tracing)* and his carrier mother *(middle tracing)* with a normal and an abnormal allele.

using an *in vitro* radioactive chromium (^{51}Cr) release assay.[55] Mononuclear cells containing memory cytotoxic T cells are cultured with either the allogeneic or the virally infected target cells to generate effector cells. These effector cells are then incubated with ^{51}Cr-loaded target cells in varying ratios, and their cytolytic function correlates with the amount of ^{51}Cr released into the medium. The degree of lysis is based on background ^{51}Cr release (target cells cultured alone) and maximal ^{51}Cr release from target cells after exposure to a membrane-disrupting agent. Test results must be compared with reference values provided by the laboratory performing the test. Alternatives that use nonradionuclide indicator systems include flow cytometry–based assays[56,57] and a colorimetric assay on the basis of granzyme B activity.[58]

NK CELLS

NK cells are large granular lymphocytes that are recognized by their distinctive capacity to lyse target cells without the need for prior exposure.[59] In addition, NK cells are a rich source of cytokines for the initiation of an immune response. Their function is regulated by an array of surface receptors that send activating (eg, toll-like receptors, NKG2D, Nkp44, and Nkp30) and inhibitory (eg, killer inhibitory receptors 2DL1 and 3DL3) signals to the cell.[60] The natural ligands for these receptors include a number of microbial products (eg, LPS, teichoic acid, and CpG repeats), HLA molecules, and other yet unidentified ligands.[61] NK cells appear to serve as an important link between the innate and adaptive immune systems playing a central role in the host defense against intracellular pathogens and tumor cells. The current approach to evaluating NK cells includes enumeration by means of flow cytometry on the basis of expression of CD56 (NKH1), CD16 (low-affinity IgG Fc receptor), or both on CD3$^-$ cells.[21] NK cell activity has traditionally been measured with a chromium release assay that uses specific ^{51}Cr-loaded cell lines (eg, K-562) as the target.[55] Alternatively, nonradionuclide-based assays can be used to evaluated NK cell cytotoxicity.[56-58] Recently, patients with recurrent bacterial infections have been identified with defects in toll-like receptor signaling.[62-64] This represents a new area of investigation and suggests that evaluation of critical NK cell receptors and their intracellular signaling pathways will become more important in the future.

IDENTIFICATION OF KNOWN GENE DEFECTS

The gene defects responsible for many primary immunodeficiency diseases have been identified (Table III).[65] Traditionally, screening methods, including single-strand conformational polymorphism or dideoxy fingerprinting, have been used to initially evaluate for mutations associated with immune deficiencies.[66-68] However, the availability of fluorescence-based sequencing with automated multichannel capillary sequencers makes direct sequencing practical for mutation analysis in a number of immune deficiencies (Fig 3).[69] Mutation identification allows genetic counseling for prenatal diagnosis and carrier detection. These techniques are also being applied in the targeted evaluation of undefined immune disorders and have led to a host of newly defined immune deficiencies.[62-64,70] An example of the power of this technology is the finding that the hyper-IgM syndrome, historically categorized as a single disorder, is actually 5 distinct

genetic disorders affecting either B cells or T cells with varying clinical phenotypes, depending on the underlying genetic defect (Table III).[70,71]

SUMMARY

The capacity to evaluate the immune system has seen significant advances with the evolution of new methods to evaluate cell function and the definition of a number of newly identified gene defects associated with immune deficiencies. It is highly likely that the future will see additional test options that will provide even clearer definitions of immune dysfunction. However, a potential problem in this process relates to the difficulty in bringing highly complex laboratory tests from the research laboratory to the clinical setting, particularly when dealing with rare disorders, such as immune deficiencies. The recent identification of specific genetic defects associated with clinical phenotypes characterized by more limited recurrent infections suggests that there remain many undefined immunologic diseases. The case report in this issue also points out that not all patients with recurrent infection have a primary immunologic defect. This should serve as a reminder that a careful medical history followed by a directed immunologic evaluation and appropriate consultative input is the most effective means to establish the diagnosis of an immune deficiency and to define the optimal approach to therapy.

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