Practice parameters for the diagnosis and management of immunodeficiency

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PART ONE: SUMMARY STATEMENTS OF PRACTICE PARAMETERS FOR THE DIAGNOSIS AND MANAGEMENT OF HUMORAL IMMUNODEFICIENCY

CLASSIFICATION:	ICD-9 CODE NO.
Bruton X-linked agammaglobulinemia	279.04
Dysgammaglobulinemia	279.06
Gamma globulin deficiency in blood	279.00
Humoral deficiencies	279.00
IgA	279.01
IgM	279.02
IgG	279.03
congenital hypogammaglobulinemia	279.04
hyper-IgM (also increased IgM)	279.05
X-linked	279.05
autosomal recessive	279.05
non-sex linked congenital hypogammaglobulinemia	279.06
specified NEC	279.09
Immunodeficiency with:	279.30
defect predominant B cell defect	279.00
hyperimmunoglobulinemia	279.20
common variable immunodeficiency	279.06
X-linked, with increased IgM	279.05

I. Diagnosis and Evaluation

A. Clinical Evaluation of Immunodeficiency

It should be recognized that:

- Congenital immunodeficiency usually (but not always) has a characteristic appearance in infants, children, and adults according to the sex of the child, age of the patient, immunization history with live vaccines, and exposure to infections.
- Recurrent, serious infection, especially with encapsulated organisms is the hallmark of humoral immunodeficiency.
- Family history, particularly of affected males, is extremely important in the diagnosis of immunodeficiency.
- Physical examination of the patient is essential with attention to failure-to-thrive, weight loss, enlargement or absence of lymph nodes, organomegaly, dermatitis, oral candidiasis, short stature, clubbing, and listlessness.

B. Specific Diagnostic Techniques

It should be recognized that:

- Evaluation of humoral immunodeficiency requires age-matched laboratory controls.
- A complete blood count with morphologic review should be performed.
- Serum immunoglobulin measurements alone may not establish a diagnosis of immunodeficiency.
- Serum isohemagglutinin titer is a useful screening test for antibody function in infants and children even as early as 4 to 6 months of age.
- A sweat chloride determination should be performed in children with recurrent sinopulmonary infections.
- Consideration should be given to performing tests for HIV infection.
- Specific antibody deficiency is the sine qua non of diagnosing humoral immune deficiency as based upon pre- and post-immunization antibody responses.
- Measurement of IgG subclasses should not be used as a screening test and may not yield any more useful information than the total serum IgG level.

II. Management of Humoral Immune Deficiencies

It should be recognized that:

- Intravenous immunoglobulin (IVIG) replacement therapy should only be given to patients with specific antibody deficiency involving IgG.
- IVIG therapy for patients with normal humoral immunity but recurrent infections, particularly upper respiratory infections, has no scientific rationale.
- IVIG replacement therapy should be initiated with a dose of 200 to 400 mg/kg/3 to 4 wk in most circumstances; adjustment to higher doses and shorter intervals may benefit some patients.
- IVIG replacement therapy in humoral immunodeficient patients is usually life-long.

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- Concomitant therapy with systemic antibiotics is necessary in patients receiving IVIG who develop infections, such as chronic lung and sinus disease.
- Avoidance of live viral vaccines is mandatory in patients with complete absence of serum immunoglobulins.
- Patient/parent education and genetic counseling are essential for adjustment of the patient to a chronic disease.

III. Special Considerations

It should be recognized that:

- It is important to determine what type of agammaglobulinemia is present in a patient.
- The carrier state of females with some X-linked forms of humoral immunodeficiency can now be determined.
- Prenatal diagnosis of some forms of humoral immune deficiency can now be accomplished, giving parents information on reproductive choices.
- The advent of gene therapy for newly discovered molecular lesions in humoral immunodeficiencies may revolutionize patient management in the future.

IV. The Immunologist as Consultant

It should be realized that:

- Patients with recurrent infections should be considered for referral to an allergist/immunologist for evaluation of humoral immunodeficiency.
- The allergist/immunologist has special expertise in evaluating, diagnosing, and managing patients with humoral immune defects.
- Because humoral immune defects are a form of chronic disease, communication and follow-up consultation on a continuing basis between the referring physician and the allergist/immunologist is essential.
- In certain circumstances the allergist/immunologist and the primary care physician will need to consult specialists in otorhinolaryngology, infectious diseases, metabolism, hematology/oncology, pulmonology, rheumatology, gastroenterology, surgery, and other fields.

SUMMARY STATEMENTS OF PRACTICE PARAMETERS FOR THE DIAGNOSIS AND MANAGEMENT OF CELLULAR IMMUNODEFICIENCY

CLASSIFICATION:	ICD-9 CODE NO.
Immunodeficiency with:	279.30
predominant T cell defect	279.10
thrombocytopenia	279.12
Wiskott-Aldrich syndrome	279.12
adenosine-deaminase deficiency	279.20
lymphopenia, hereditary	279.20
thymic aplasia	279.20
thymic dysplasia	279.20
autosomal recessive, Swiss type	279.20
severe combined (SCID)	279.20
lymphocyte activation defects	279.30
ataxia telangiectasia	334.80

I. Diagnosis and Evaluation

A. Clinical Evaluation of Immunodeficiency

It should be recognized that:

- Congenital immunodeficiency usually (but not always) has a characteristic appearance in infants, children, and adults according to the sex of the child, age of the patient, immunization history with live vaccines, and exposure to infections.
- Recurrent serious infections, especially with opportunistic organisms, is the hallmark of cellular immunodeficiency.
- Family history, particularly of affected males, is extremely important in the diagnosis of immunodeficiency.
- Physical examination of the patient is essential with attention to failure-to-thrive, weight loss, enlargement or absence of lymph nodes, organomegaly, dermatitis, petechiae, facial abnormalities, cardiac abnormalities, oral candidiasis, dwarfism, short stature, digital clubbing, ataxia, telangiectasia, and listlessness.

B. Specific Diagnostic Techniques

It should be recognized that:

- A complete blood count, absolute lymphocyte count, and morphologic review should be performed.
- A chest radiograph, especially a lateral view, may delineate a small thymus gland and interstitial lung disease, hallmarks of congenital T cell deficiency.
- Delayed hypersensitivity skin tests to recall antigens can serve as a screening test for cellular immune defects.
- Evaluation of cellular immune deficiency requires specialized laboratory facilities and expertise not available in most clinical testing laboratories.
- To evaluate patient lymphocytes properly, age-matched laboratory controls may be required.
- Cellular immunodeficiency testing includes measurement of cell surface markers, termed clusters of differentiation (CD nomenclature), and cellular functional assays, such as lymphocyte proliferation assays to mitogens, antigens, and allogeneic cells, as well as natural killer (NK) cell function. In selected patients, the ability to secrete cytokines may need to be evaluated.

- Consideration should be given to performing tests for HIV infection.

II. Management of Cellular Immunodeficiencies

It should be recognized that:

- Only specialized treatment centers are capable of restoring cellular immunity in patients.
- The treatment of cellular immunodeficiencies depends upon the type of defect diagnosed.
- All blood products given to patients with suspected cellular immunodeficiency must be irradiated (3000 rads), leukocyte-poor, and virus-free.
- Potentially curative treatments of cellular immune deficiencies include: HLA-identical (sibling) bone marrow transplantation; HLA-haploidentical (parental), T cell-depleted bone marrow transplantation; HLA-matched, unrelated bone marrow transplantation; enzyme replacement therapy; stem cell (placental cord blood) replacement therapy; and gene replacement therapy.
- Engraftment of donor lymphocytes may occur with T cells or T cells plus B cells, which has an impact on the chronic care of the patient.
- Chronic IVIG therapy may be necessary in some patients reconstituted with T cells alone; antibiotic therapy may be an important adjunct as well.
- Avoidance of live viral vaccines is mandatory in patients with cellular immune defects.
- Patient education and genetic counseling are essential for adjustment of patient and family to therapeutic attempts and outcomes.

III. Special Considerations

It should be recognized that:

- The carrier state of females with some X-linked forms of cellular immunodeficiency can now be determined.
- Prenatal diagnosis of some forms of cellular immunodeficiency can now be accomplished, giving parents information on reproductive choices and early treatment.
- Molecular genetic testing is now available for some forms of cellular immunodeficiency.
- The advent of gene therapy and cord blood stem cell therapy for newly discovered molecular lesions in cellular immunodeficiency may revolutionize patient management in the future.

IV. The Immunologist as Consultant

It should be realized that:

- All infants with recurrent serious infection should be considered for referral to an allergist/immunologist for evaluation of cellular immunodeficiency.
- The allergist/immunologist has special expertise in evaluating, diagnosing, and managing patients with cellular immune defects.
- Because the period before engrafted lymphocyte function becomes normal can be prolonged (in some instances up to 2 years), the allergist/immunologist must remain involved in the primary care of these patients.
- Because even after lymphocyte engraftment, patients still need regular follow-up consultation with the allergist/immunologist, communication on a continuing basis between the referring physician and the clinical immunologist is essential.
- In certain circumstances the allergist/immunologist and the referring physician will need to consult specialists in histocompatibility typing, gene therapy, genetics, metabolic disorders, and other fields.

SUMMARY STATEMENTS OF PRACTICE PARAMETERS FOR THE DIAGNOSIS AND MANAGEMENT OF HEREDITARY AND ACQUIRED DEFICIENCIES OF COMPLEMENT COMPONENTS

CLASSIFICATION:	ICD-9 CODE NO.
Angioedema, acquired with malignancy	
Angioedema, hereditary (C1 inhibitor deficiency)	277.60
Angio (neurotic) edema (allergic) (any site) (with Urticaria)	995.1
Complement factor deficiency NEC	279.80
Complement factor deficiency specified:	
classical pathway components	279.80
C1q, C1r, C1s, C4, C2	
alternative pathway components	279.80
factor D, properdin	
C3 and its regulatory proteins	279.80
C3, factor I, factor H	
terminal complement pathway components	279.80
C5, C6, C7, C8, C9	

I. Diagnosis and Evaluation

A. Clinical Evaluation of Complement Component Deficiency

It should be recognized that:

- Primary deficiencies of complement components, aside from homozygous null C2 (1:10,000), are quite rare.
- Deficiencies of all complement components have been described.
- Deficiencies of the early components of complement (C1q,r,s C4, C2) are associated with systemic lupus erythematosus-like symptoms, glomerulonephritis and, less

frequently, with pyogenic infections.

- C3 deficiency is associated with severe pyogenic infections, glomerulonephritis, and systemic lupus erythematosus.
- Deficiencies of the terminal components (C5, C6, C7, C8, C9) are associated with recurrent disseminated neisserial infections.
- Factor D deficiency is associated with recurrent pyogenic infections.
- Properdin deficiency is associated with recurrent pyogenic infections and fulminant meningococcemia.
- Factor I deficiency is associated with recurrent pyogenic infections.
- Factor H deficiency is associated with pyogenic infections and glomerulonephritis.
- C1 inhibitor deficiency is associated with hereditary angioedema.
- Acquired C1 inhibitor deficiency has been seen in patients with different lymphoproliferative disorders.
- In acquired C1 inhibitor deficiency, the levels of C1 are reduced in contrast to the hereditary form in which C1 is normal.

B. Specific Diagnostic Techniques

It should be recognized that:

- Total hemolytic complement (CH_{50}) is a useful screening test for the majority of complement disorders.
- A CH_{50} value of 0 may be seen in deficiencies of C1 through C8.
- In C9 deficiency CH_{50} values are 25% to 50% of normal.
- CH_{50} will not detect deficiencies of properdin or factor D.
- Deficiencies of factor D and properdin can be detected by a different hemolytic assay (APH_{50}).
- Deficiency of factor I or factor H are associated with consumption of C3 and decrease in CH_{50}.
- C1 inhibitor deficiency is associated with decreased C4 levels and can be detected by a functional assay or quantitation of the specific protein.
- In C1 inhibitor deficiency, 85% of the patients have reduced levels of the protein, but 15% of the patients have normal or elevated levels of a dysfunctional protein requiring a functional assay for diagnosis.
- Normal C3 and C4 levels in the face of undetectable CH_{50} is strong evidence of congenital complement component deficiency, whereas a decrease in C4 and/or C3 with undetectable CH_{50} suggest complement consumption.
- The evaluation of specific component deficiency requires tests not available in the majority of clinical laboratories.

II. Management of Complement Deficiencies

It should be recognized that:

- There is no specific treatment for the congenital deficiency of complement components.
- In C1 inhibitor deficiency, the administration of semisynthetic androgens such as danazol and stanozolol is associated with a decrease in angioedema attacks and increased levels of C1 inhibitor. Because of possible androgenic effects, these drugs must be used with caution in children.
- If there is knowledge that a patient has a complement component deficiency, it is very important to alert the physician to the risk of severe infections that require more aggressive evaluations.
- Patients with deficiency of early complement components may develop autoimmune diseases particularly SLE or glomerulonephritis.
- Patients may be immunized with vaccines for pneumococci, *H. influenzae*, and *Neisseria meningitidis*.

III. Special Considerations

It should be recognized that:

- Obtaining a family history of complement component deficiency is extremely important in arriving at a proper diagnosis.
- Complement components lose activity very rapidly at room temperature and for this reason serum for functional assays requires special handling.
- A common cause of very low or absent complement activity (CH_{50}) is inappropriate handling of specimens; confirmation of results is indicated before more elaborate studies are performed.
- A purified C1 inhibitor preparation for treatment of hereditary angioedema attacks may be available in specialized medical centers.
- Gene therapy of complement component deficiency is a possible type of therapy under development.

IV. The Immunologist as Consultant

It should be recognized that:

- All patients with recurrent infections should be considered for referral to an allergist/immunologist for evaluation of complement component deficiency.
- Patients with recurrent bouts of angioedema should be considered for referral to an allergist/immunologist for evaluation of congenital or

acquired C1 inhibitor deficiency.
- The allergist/immunologist has special expertise in evaluating, diagnosing and managing patients with complement deficiencies.

SUMMARY STATEMENTS OF PRACTICE PARAMETERS FOR THE DIAGNOSIS AND MANAGEMENT OF PHAGOCYTIC CELL DISORDERS

CLASSIFICATION:	ICD-9 CODE NO.
Chediak-Higashi (Steinbrinck) anomaly, disease, or syndrome (congenital gigantism of peroxidase granules)	288.2
Chronic granulomatous disease (granuloma, infections NEC)	136.9
Granulocytopenia, granulocytopenic	
primary	288.0
malignant	288.0
Leukocyte adhesion deficiency	
Neutropenia, neutropenic (chronic, cyclic, drug induced, genetic, idiopathic, immune, infantile, malignant, periodic, pernicious, primary, splenic, splenomegaly, toxic)	288.0
chronic hypoplastic	288.0
congenital, nontransient	
neonatal, transitory (isoimmune),	776.7
(maternal transfer)	
Neutrophilia, hereditary	
giant	288.2

I. Diagnosis and Evaluation

A. Clinical Evaluation of Phagocytic Immunodeficiency

It should be recognized that:

- Diseases produced by various forms of neutropenia are relatively common, but inherited forms of neutrophil dysfunction are quite rare.
- Recurrent infection, especially with staphylococcal or gram negative bacterial organisms and aspergillus or other fungal organisms is the hallmark of phagocytic immunodeficiency.
- Physical examination of the patient is essential with attention to cutaneous or other deep seated abscesses and organomegaly.

B. Specific Diagnostic Techniques

It should be recognized that:

- An initial screen consists of performing a neutrophil count and occasionally serial neutrophil counts.
- Full evaluation requires specific and complex laboratory testing which may not be available in most laboratories.
- A decreased neutrophil count should be evaluated by examining morphology of the neutrophils and then assessing chemotaxis.
- Patients with histories of recurrent opportunistic bacterial and fungal infections but normal white blood cell counts when asymptomatic need to have a test of the neutrophil oxidative burst (chronic granulomatous disease).
- Similar patients with elevated neutrophil counts at all times need to have a test of neutrophil surface glycoproteins (leukocyte adhesion deficiency).

II. Management of Phagocytic Immune Defects

It should be recognized that:

- Patient/parent education and counseling is essential for adjustment of the patient and family to a chronic disease.
- With the exception of chronic granulomatous disease (CGD), there is a lack of specific therapies available to deal with phagocytic disorders.
- Some cases of CGD will respond to interferon gamma (γ) treatment.
- Most patients with phagocytic immune defects can only be treated with supportive care and appropriate antibiotics. Occasionally granulocyte transfusions can be used.

III. Special Considerations

It should be recognized that:

- Phagocytic disorders may be due to causes other than primary dysfunctions.
- The advent of gene therapy for newly discovered molecular lesions in phagocytic immunodeficiencies may revolutionize patient management in the future.

IV. The Immunologist as Consultant

- All patients with recurrent infections should be considered for referral to an allergist/immunologist for evaluation of phagocytic immunodeficiency.
- The allergist/immunologist has special expertise in evaluating, diagnosing, and managing patients with phagocytic immune defects.
- Because phagocytic immune defects are a form of chronic disease, communication on a continuing basis between the referring physician and the allergist/immunologist is essential.
- In certain circumstances the allergist/immunologist and the primary care physician will need to consult specialists in infectious diseases, metabolism, hematology/oncology, pulmonology, gastroenterology, surgery, and other fields.

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PART TWO: BACKGROUND INFORMATION FOR PRACTICE PARAMETERS FOR THE DIAGNOSIS AND MANAGEMENT OF IMMUNODEFICIENCY

Introduction and Definitions

Physicians often encounter patients who have had "too many infections." The immunodeficiency diseases are relatively rare disorders of the immune system that result in a predisposition for increased frequency of infections. Secondary immunodeficiencies can present anytime in that they are acquired disruptions in immune function that increase susceptibility to infection. Examples of secondary immunodeficiencies include those conditions that occur as a consequence of the exposure of the patient to immunosuppressive agents, infectious diseases, infiltrative diseases, and metabolic diseases. The best known among these is the secondary immunodeficiency resulting from infection by the human immunodeficiency virus type 1 (HIV) and its terminal consequence, the acquired immunodeficiency syndrome (AIDS). With a case acquisition rate of more than 2000/wk it is easy to see that, for the population in general, AIDS cases far exceed genetic defects in the immune system. Even in the pediatric age group that is true, as already more than 4,700 cases of AIDS have been reported in children born to parents with well-recognized risk factors. Acquired immunodeficiency syndrome is the ninth leading cause of death in children aged 1 to 4 years and the third leading cause of death among black children aged 1 to 4 years in urban Northeastern states. In contrast, primary immunodeficiencies are generally hereditary and congenital deficiencies of the immune system and are rare (1:333 to 1:500,000 in the general population). Thus, these deficiencies, such as severe combined immune deficiency, Wiskott-Aldrich syndrome, and congenital X-linked agammaglobulinemia are usually found in infants and children. This review presents an approach to the diagnosis and management of the immunodeficient patient.

Etiology of Immunodeficiencies

The etiologies of the primary and secondary immunodeficiency disorders are heterogeneous. Many immunodeficiencies have no elucidated etiology. With regard to the primary immunodeficiency disorders, exciting progress has been made in the recent past involving isolation of genes and gene products known to be deficient in certain immunodeficiency disorders.

Specific Gene Lesions

Three of the primary X-linked immunodeficiency disorders involving lymphocytes have had defective genes isolated that may result in disease. For congenital X-linked agammaglobulinemia, the defective gene has been identified as a member of the src family of proto-oncogenes which encode protein tyrosine kinases.[1,2] The defective gene results in the inability of a specific tyrosine kinase (named Bruton's tyrosine kinase) to function in normal intracellular signaling in the production of immunoglobulins by B cells. Also, immunodeficiency seen in the hyper-IGM syndrome, the X-linked variety, has, in some but not all cases, been linked to a faulty gene that normally produces the ligand present on activated T cells that binds to the surface molecule CD40 on B cells.[3,4] Lack of interaction of T cells and B cells is thought to lead to unregulated production of IgM and to the prevention of IgG switching. For the X-linked form of severe combined immunodeficiency (SCID), the abnormal gene produces a truncated gamma chain of the IL-2 receptor on T cells and NK cells[5] and also in the IL-4 and IL-7 receptors.

The primary biologic error is also known for leukocyte adhesion deficiency 1 (LAD-1 or CD11/CD 18 deficiency). Leukocyte adhesion deficiency-1 is due to a deficiency in CD18 which is a 95-kD molecular weight common beta-chain structure.[6,7] CD18 normally forms three distinct surface glycoproteins by binding with three distinct isoforms that form the alpha chain. Two of these glycoproteins, lymphocyte function-associated antigen-1 (LFA-1) and complement receptor type 3 (CR3) are known to be important in adhesion. Absence of these adhesion molecules on neutrophils is believed to prevent chemotaxis, aggregation, phagocytosis, and cytotoxicity.

The chronic granulomatous diseases of childhood are known to occur as a result of inability of granulocytes and monocytes to reduce oxygen (O_2) to superoxide anions (O_2) during phagocytosis. The X-linked form is known to be associated with an abnormal gene that codes for the 91-kD beta-chain of

NADPH-oxidase.[8] Two of the autosomal recessive forms of CGD are due to abnormalities in genes coding for two of the cytosolic components (47 kD and 67 kD) of this membrane bound electron transport chain which are essential for O_2 generation.[9,10]

Altered Protein Synthesis

Other immune deficiencies associated with altered synthesis of proteins include complement defects in which there is absence of a specific complement component such as with C2, C3, or C4 deficiency, or selective IgA deficiency in which immunity at mucosal surfaces is defective due to lack of secretory IgA (with or without absence of the IgG2 subclass). In complement protein C2 deficiency type I, no translation of complement protein is observed. A 28-base pair deletion has been found in patients with the C2 null allele which results in a premature termination codon and absent C2 protein.[11] In some cases of hereditary angioedema, the C1 esterase inhibitor is lacking functional activity.[12]

Enzyme Deficiencies

Enzyme defects have been identified in association with certain immune deficiencies. Adenosine deaminase (ADA) deficiency has been found in 15% of cases of SCID. The absence of this enzyme results in the accumulation of adenosine and deoxyadenosine which are toxic to the immune system.[13] A deficiency in purine nucleoside phosphorylase (PNP) is associated with some of the cases of cellular immunodeficiency with immunoglobulins or Nezelof syndrome.[14] NADPH-oxidase deficiency may be associated with deficient microbial killing in chronic granulomatous disease.[15]

Other Abnormalities

Other defects that can result in a poorly functioning immune system include metabolic deficiencies and nutrient deficiencies. For example, a syndrome similar to chronic mucocutaneous candidiasis is seen in biotin-dependent cocarboxylase deficiency in which cellular immunity to Candida is severely depressed.[16] Undernourished children may have poorly functioning immune systems due to the lack of vitamins, minerals, calories, or protein for proper immune functioning. Immunoglobulin production is suppressed due to lack of vitamin B12 for B cells in transcobalamin II deficiency.[17] Also, acrodermatitis enteropathica, which has been found in a reversible form of severe combined immunodeficiency, has been associated with zinc deficiency.[18]

Immunodeficiency can result from external influences such as drugs and infection. Epstein-Barr virus can produce a state of hypogammaglobulinemia, for example. The immunopathogenesis of AIDS is known to result from infection with HIV.[19] Infants with fetal alcohol syndrome may have depressed T cell function.[20] Anticonvulsants have been associated with selective and global deficiencies of serum immunoglobulins.[21,22] Malignancies, such as multiple myeloma can produce a functional hypogammaglobulinemia despite polyclonal gammopathy.

Clinical Features

Incidence

The incidence of congenital immunodeficiencies varies from the relatively common selective IgA deficiency (1:333 to 1:700) to the rare severe combined immunodeficiency (1:100,000 to 1:500,000). Chronic granulomatous disease occurs at a 1:200,000 incidence and common variable immunodeficiency occurs in 1:75,000.[23]

The hallmark of immunodeficiency is recurrent serious infection with frequent treatment failures. These disorders present in a male to female ratio of 5:1 in infants and children and 1:1.4 in adults.[23] The primary immunodeficiency disorders are more often seen in infants and children than in adults.

Inheritance Patterns

Inheritance patterns are important to establish when evaluating the immunodeficient patient. Many of the primary immunodeficiency disorders have X-linked inheritance patterns. Single recessive genes result in autosomal recessive inheritance in some forms of immunodeficiency disorders such as in some forms of SCID, CGD, and hyper-IgM syndrome. Other immunodeficiencies do not have a defined pattern of inheritance but clearly are found in families. Once the genetic pattern is established, genetic counselling can be considered, and early diagnosis or prenatal diagnosis can be entertained if feasible.

Detection of Carriers

Detection of heterozygote females who are carriers for certain X-linked inherited disorders is possible due to nonrandom inactivation of X chromosomes in those cells affected by the disorder. Women without disease will have a balanced pattern of X chromosome inactivation; therefore, half of their active chromosomes will be of maternal origin. B cell lines from female carriers of X-linked immunodeficiency disorders, however, demonstrate selective growth disadvantage in those cells containing the abnormal allele. Heterozygote detection is currently employed in Wiscott-Aldrich syndrome, X-linked agammaglobulinemia, and X-linked severe combined immunodeficiency.[24] Heterozygote detection by other methods can also be applied to chronic granulomatous disease, ADA and PNP deficiency with severe combined immunodeficiency, and with certain familial complement disorders.[18]

Prenatal Diagnosis

Prenatal diagnosis can be accomplished with several of the primary immunodeficiency disorders. Adenosine deaminase deficiency can be diagnosed by analysis of chorionic villous samples, cultured amniotic cells, and fetal cells.[25] Enzyme assay of cultured amniotic cells is also feasible for PNP deficiency or transcobalamin II deficiency.[18] Fetal blood can be examined for chronic granulomatous disease by an assay to test the granulocyte respiratory burst (eg, nitroblue tetrazolium dye test [NBT]). The NBT result will be abnormal for fetal blood cells in infants with CGD. X-linked agammaglobulinemia can be predicted by the

absence of B cells in the fetal blood and the virtual absence of T cells will help to establish the prenatal diagnosis of certain types of SCID.[18]

Evaluation of the Patient

Medical History

A thorough history of illness is necessary for a directed, concise evaluation for suspected immunodeficiency. The time of onset of symptoms can provide clues as to etiology. Infants who present with frequent infections developing from birth to 3 months of age are likely to have maternal immunoglobulin present. Deficiencies in the immune system at this age are likely due to severe deficiencies in other immune components such as neutrophil defects, complement defects, or T cell and combined immunodeficiencies. Aside from frequent infection, other important history implicating neutrophil defects includes the history of delayed cord separation, beyond 2 weeks, and poor wound healing. DiGeorge syndrome patients usually present very early due to hypocalcemic seizures/tetany associated with hypoparathyroidism or with cardiac difficulties rather than with recurrent infections. Due to the presence of protective maternal immunoglobulin, infants with primary immunoglobulin defects such as Bruton agammaglobulinemia usually do not present with serious infection until 3 months to 6 months of age when these antibodies are lost, or later if they have been treated with antibiotics for upper respiratory infections. Infants with Wiskott-Aldrich syndrome, or immunodeficiency with thrombocytopenia and eczema, also usually present prior to 18 months of age. In contrast, patients with selective IgA deficiency generally do not present until after 18 months of age. Common variable immunodeficiency typically presents in the second decade of life, but may present earlier or much later.

Immunization history is vital in the evaluation. A history of infection to a live viral vaccine is suspicious for immune deficiency since infants with T cell defects, T and B cell defects, and B cell defects may contract severe or fatal infections from live vaccines.

Historical information regarding the type and severity of illness and infection provides important clues to suggest immune deficiency. Children with immunodeficiency generally will have infections with an unusually prolonged course, infections of unusual severity, or infections as unexpected complications. Recurrent infections that involve more than one site are more suspicious for immunodeficiency than those involving a single site. A history of severe infections such as recurrent pneumonia, meningitis, sepsis, septic arthritis, osteomyelitis, or abscess would suggest possible immunodeficiency as does infection with organisms of low pathogenicity such as with *Candida albicans* or *Pneumocystis carinii*. Patients with antibody deficiency disorders tend to acquire infections with extracellular pyogenic organisms such as with Haemophilus, Pneumococcus, and Streptococcus. In contrast, patients with defects in cell-mediated immunity have recurrent infections with viruses, fungi, protozoa and mycobacteria. Furthermore, infections with unusual bacteria such as with *Serratia marcescens*, *Staphylococcus epidermidis*, and Pseudomonas may indicate a possible neutrophil defect. Disseminated neisserial infections may be found in individuals deficient in the terminal complement components.

Family history is vital in evaluation of suspected immunodeficiency. A history of early infant deaths should be sought. A clear pattern of inheritance may be found; information regarding possible consanguinity should be obtained. Family members of immunodeficient patients will often have histories of autoimmune disease or of connective tissue disease.

Physical Examination

As in any investigation of disease, the physical examination is of utmost importance in immunodeficiency diseases. Patients who are small for their age may have growth delay secondary to recurrent infections or may have short stature associated with certain T and B cell immunodeficiencies. A paucity of lymphoid tissue such as tonsils and lymph nodes suggests immunodeficiency and is especially seen in X-linked agammaglobulinemia. Hepatosplenomegaly and diffuse adenopathy might suggest HIV infection or common variable immunodeficiency. Certain specific physical findings are suggestive of specific disorders such as with telangiectasias over the bulbar conjunctivae, the bridge of the nose, and the ears and antecubital fossa with or without ataxia in ataxia telangiectasia, chronic dermatitis in hyper-IgE syndrome and eczema in Wiskott-Aldrich syndrome, chronic periodontitis in chemotactic defects of the neutrophils, and silvery hair, pale skin, and photophobia in Chediak Higashi syndrome. Children with LAD can present with severe gingivostomatitis and dental erosion as a consequence of abnormal leukocyte function.

Laboratory Investigation

After beginning the evaluation with a careful history and physical examination, laboratory evaluation can be tailored to detect specific suspected immunodeficiency. Most immunodeficiency disorders can be ruled out at little cost to the patient if the proper choice of screening tests is made. The initial screen should include a complete blood count and differential with platelet determination to evaluate the total white blood cell count and total numbers of neutrophils, lymphocytes, eosinophils, and platelets. Neutropenia, lymphopenia, and abnormalities in white blood cell morphology can be detected from this study. Anemia may be present in children with chronic disease. Examination of red blood cells for Howell-Jolly bodies will help exclude congenital asplenia. Platelets may be abnormally low in children with poor bone marrow function or autoimmune disease, and platelets will be low and morphologically small in children with Wiskott-Aldrich syndrome. Persistent elevation of the erythrocyte sedimentation rate may

be an indication of chronic infection due to immunodeficiency.

If an infant's neutrophil count is persistently high, even when the patient shows no sign of infection, a leukocyte adhesion deficiency should be suspected. If the absolute neutrophil count is normal, congenital and acquired neutropenia and severe chemotactic defects are eliminated. If the absolute lymphocyte count is normal, it is unlikely that the patient has a severe T lymphocyte defect. It is important to remember, however, that infant lymphocyte counts are normally very high. For example, at 9 months of age—an age when infants affected with severe cellular immunodeficiency are likely to present—the lower limit of normal is 4,500 lymphocytes/mm^3.

Immunoglobulin Levels

If an abnormality in humoral immunity is suspected, initial evaluation should include measurement of immunoglobulin levels including IgG, IgA, and IgM levels. The IgA level is especially helpful in that IgA levels are low in almost all permanent types of agammaglobulinemia and in selective IgA deficiency. IgE level measurement would be appropriate in patients with suspected atopy, Wiskott-Aldrich syndrome, or suspected hyperimmunoglobulin E syndrome. IgG subclass testing should not be used for screening patients for suspected immunodeficiency, but should be reserved for patients who lack specific antibody responses to antigenic challenge yet have slightly low or normal immunoglobulin serum concentrations. IgG_2 subclass deficiency may be associated with selective IgA deficiency.[26] Occasionally serum and urine protein electrophoresis may be helpful in evaluating patients with polyclonal or oligoclonal gammopathy and immunodeficiency. Analysis of urine for myeloma proteins also may be useful in the previous setting.

Multiple methods are available for the quantitation of immunoglobulin levels. These methods can be classified into two major groups. The first group involves measurement of physical properties (light scattering, precipitation in gels) from immune complexes generated by serum immunoglobulins and specific antibodies. The second group involves measurement of serum immunoglobulins using specifically labelled antibodies.[27] Three commonly used procedures include rate nephelometry, radial immunodiffusion in the first group[28], and solid-phase enzyme-linked immunosorbent assay (ELISA) in the second group.[29] The World Health Organization has prepared reference standards for serum immunoglobulin quantitation which are expressed in international units. Normal ranges of human serum immunoglobulins can vary with age, especially in children, environmental factors, and race.[30] It is extremely important to remember, however, that concentrations of IgG and IgA are both normally lower than adult levels until 6 to 7 years of age. Any values obtained other than those that are far below published normal ranges, should be investigated further in a laboratory which has its own age-appropriate normal values for infants and young children.

Specific Antibody Formation

For assessment of B cell function, antibody production must be measured. Patients with recurrent infection may warrant this measurement even if immunoglobulin levels are normal; cases of specific antibody deficiency have been documented in patients with normal immunoglobulin levels and immunoglobulin subclass levels.[23] An initial screen of antibody production may involve the measure of isohemagglutinins. Isohemagglutinins are IgM antibodies that are formed in all individuals after exposure to ubiquitous bacterial antigens that crossreact with polysaccharide blood group antigens A and/or B, which are not represented on the patient's red blood cells. For example, people with blood type AB will not form isohemagglutinins. Children less than 1 year of age will not reliably have isohemagglutinins. The patient with blood type A should have anti-B, blood type B patients should have anti-A, and blood type O patients should have anti-A and anti-B. These antibodies should normally be present in older children and adults in titers greater than 1:10; low or zero titers are found in patients with poor antibody production. Antibody production can also be measured following immunization with protein antigens, such as those derived from tetanus and diphtheria organisms, and polysaccharide antigens, such as those produced from the pneumococcus and unconjugated *Haemophilus influenzae* vaccines. Normal antibody production would include a rise in specific antibody levels within 2 weeks for protein antigens (eg, diphtheria and tetanus antigens) and within 3 weeks for polysaccharide antigens (eg, Pneumovax). Patients with agammaglobulinemia will have difficulty with all antibody production whereas those with IgG_2 subclass deficiency may only have difficulty with polysaccharide antibody production. Patients with selective IgA deficiency alone or with transient hypogammaglobulinemia of infancy should have normal antibody production. The pneumococcal vaccine is not recommended for children under 2 years of age because normal children do not respond to the pneumococcal antigen at this age. Not all pneumococcal antigens are equally immunogenic and normal patients may not respond to all pneumococcal vaccine antigens. Considerable care is therefore necessary in selecting patients for intravenous immunoglobulin replacement therapy based on lack of antibody responses to pneumococcal antigens.

Patients who receive immunoglobulin replacement therapy cannot be evaluated by measurement of the above antibody levels because the patients are receiving the antibodies exogenously. The bacteriophage, ΦX 174, can be used to evaluate such patients. The ΦX 174 antigen is a unique antigen not experienced by the general population, and challenge with ΦX 174 is considered an experimental procedure. Immunization with this antigen can allow measure of both the primary (IgM), and secondary (IgG), immune responses, and provides information

regarding immune memory, amplification, and ability to class switch.[31] Filing of a patient investigational new drug (IND) application with the Food and Drug Administration is required, however.

Lymphocyte Evaluation

Screening tests: Chest Radiograph and Delayed Hypersensitivity Skin Tests

If a cellular immunodeficiency is suspected, the initial evaluation should include posteroanterior and lateral chest radiographs to look for a thymic shadow, which is especially appropriate in the nonstressed infant. The physician should remember that the thymic size may shrink in response to such stresses as surgery and infection.

Cellular immune function can be screened with the use of the delayed hypersensitivity skin test. These delayed cutaneous tests, if negative, provide evidence of possible impaired cellular immunity and/or absence of prior sensitization. Histologic changes in delayed hypersensitivity initially involve the production of local edema and the dilatation of small arterioles, capillaries, venules, and lymphatics. By four to six hours, an infiltrate of lymphocytes and basophils is present in the perivascular areas. The perivascular changes are followed by a diffuse infiltrate of lymphocytes, basophils, and monocytes.[32] Marked dermal and epidermal edema appears due to the damage to the vascular endothelium; there is also red blood cell leakage and deposition of dermal fibrin at intravascular sites. With the tuberculin reaction, 75% to 90% of mononuclear cells in the perivascular aggregates are T lymphocytes or monocytes[33] and by 12 to 24 hours the dermal interstitium is infiltrated with T lymphocytes and monocytes. Maximal cell activation and migration with resulting induration does not occur until 48 hours after antigen exposure.[34] The purified protein derivative (PPD) of tuberculin provides a delayed cutaneous reaction in most but not all sensitized, but healthy, subjects, providing evidence of hypersensitivity rather than toxic reactions.

Delayed hypersensitivity skin tests are performed by using antigens of microbial origin to which the patient has had previous exposure. A 25 to 27-gauge needle is used for intracutaneous injection of 0.1 mL antigen volumes. Commonly used and accepted initial test antigens and doses include a 1:100 wt/vol dilution of *Candida albicans*, a 1:100 dilution of tetanus toxoid, a 1:30 wt/vol dilution of Trichophyton and the 5 TU bioequivalent dose of PPD-tuberculin.[18] PPD usually provides a negative control in infants and children, but can provoke a delayed cutaneous reaction in many adults. With a single test antigen, a 5 mm or larger reaction is usually considered necessary to identify intact cell-mediated immunity. When multiple test antigens are used, the detection of 2 mm or larger reactions at more than one test site can also be considered evidence of intact cell-mediated immunity. By convention a 10-mm reaction PPD is read as positive except in HIV-infected individuals (5 mm).

While a high level of prior sensitization is important in selecting recall test antigens for delayed cutaneous hypersensitivity testing, it is important that the particular antigen and dose elicit a reaction in some, but not all of the reference population used to define the prevalence of positive tests. A clinical diagnosis of anergy can be established with a chance of being wrong less than 1 time in 20 only when 95% or more of an appropriate reference population react to 2 or more of the antigens on the recall test antigen panel. A single uniformly reactive test antigen can not be used to establish a diagnosis of anergy because the implied 100% reactor rate in the reference population does not clearly distinguish between a toxic and hypersensitivity reaction.

Lymphocyte Enumeration

In vitro evaluation of lymphocytes first involves identification of B lymphocyte and T lymphocyte subsets. Both T and B cells can be identified and labeled with the use of the flow cytometer and fluorescent monoclonal antibodies. T cell enumeration involves the use of a pan T cell monoclonal antibody specific for CD3.[36–37] The CD4 marker serves as identification for T helper cells; the CD8 marker identifies cytotoxic T cells. B cells can be identified by using monoclonal antibodies against the cell surface markers CD19 or CD20. Natural killer cells can be identified by using monoclonal antibodies against the CD16 and CD56 surface markers.[37] A histogram of fluorescence intensity is obtained from which the percentage of each lymphocyte subset can be obtained. A reference range (95% confidence interval) is established for each subset by arranging values from the control population in order of magnitude and defining normals as those values which fall between the 2.5 and 97.5 percentiles for this population.[37] Separate ranges should be used for children because infants and children generally have higher absolute numbers of T-cell subsets and higher percentages of CD4 cells.[38,39] Other factors such as age,[40] gender,[41] and adrenocorticoid levels[42] can influence lymphocyte subset populations.

Lymphocyte Functional Analysis

To test lymphocyte function in the laboratory, lymphocyte proliferation or transformation studies are performed. For these studies, lymphocytes are stimulated to synthesize DNA, proliferate, and divide. Lymphocytes from immunized or previously exposed individuals will proliferate in response to antigens to which they are sensitized.[43] This response in vitro correlates with the in vivo delayed-type hypersensitivity response for specific immune recall. Histocompatibility antigens also react by specific immune mechanisms when leukocytes from two donors are mixed in culture. Mitogens such as concanavalin A, phytohemagglutinin, and pokeweed mitogen exert a nonspecific stimulation and proliferation of normal T (and B) cells. Proliferation of lymphocytes can be evaluated by the demonstration of transformed lympho-

cytes, which resemble blasts, or by increased DNA synthesis. Increased DNA synthesis is monitored by the use of radiolabeled nucleic acid, usually tritiated thymidine, in culture media. A measure of the amount of radiolabeled material incorporated into the cells would correlate with DNA synthesis. Functional analysis may also include assessment of the cytotoxic function of NK cells. This is accomplished by examining the ability of the patient's lymphocytes to kill the K562 tumor cell living in a 4-hour, ^{51}Cr-release assay.

Complement

For the diagnosis of inherited complement deficiency, the most useful screening test is the total hemolytic complement (CH_{50}). Hemolytic assays for alternate pathway function (APH_{50}) and assay for properdin levels should be obtained in patients with excessive infections or disseminated Neisseria infections if the initial screening test is normal. The evaluation of individual complement components can be performed by functional assays or by immunochemical methods. If the hemolytic activity (CH_{50}) is completely or partially absent, C3 and C4 levels should be determined; normal levels of C3 and C4 in the face of CH_{50} approaching zero suggests deficiency of one of the other complement components, and the serum should be tested at a reference or research laboratory for individual complement component determination. If the screening tests show partial lowering of C3 and C4, increased complement consumption is likely.

For the diagnosis of hereditary angioedema, a C4 level during an attack should always be significantly reduced. The diagnosis can be confirmed by determining C1 inhibitor levels. In 85% of patients, the C1 inhibitor protein will be significantly reduced, but 15% of patients have normal or elevated levels of a dysfunctional protein, and an assay for C1 inhibitor functional activity will be necessary. When acquired C1 inhibitor deficiency is suspected, measurement of C1 or functional C1 levels will distinguish acquired (low C1) from hereditary (normal C1) angioedema.

Phagocytes

The evaluation of a patient with a suspected phagocyte deficiency should always begin with a complete blood count. Granulocytopenia is likely the most frequently encountered disorder of the phagocytic system. With granulocytopenia, a qualitative neutrophil defect is unlikely and subsequent evaluation should investigate the etiology of the granulocytopenia. Isolation and separation of neutrophils permits the assessment of anti-neutrophil antibodies which can be associated with many of the diseases that are in the acquired neutropenia category, including drug-induced neutropenia, postinfectious neutropenia, autoimmune neutropenia, and any of the other disease in which excess destruction or limited production of neutrophils leads to the lowering of the absolute neutrophil count. Neutrophilia, although most commonly associated with acute infection, is a common finding in both forms of LAD. Sometimes, neutrophil counts can be in excess of 100,000 with these disorders.[44]

Abnormalities of white blood cell function may involve difficulty with adherence, locomotion, deformability, recognition, attachment, engulfment, phagocytosis, phagosome formation, degranulation, microbial killing, or elimination of engulfed material.[45] Standard tests of granulocyte function include the nitroblue terazolium test (NBT) and the chemiluminescence test. The NBT test is a test of oxidative metabolic response during phagocytosis in which NBT is reduced to formazan by the O_2^- produced by the stimulated phagocytes.[44] Isolated intact neutrophils are incubated with NBT, stimulated with phorbol ester, and examined under a microscope for the presence or absence of blue formazan on or in cells.[44] Controls have more than 90% of cells positive for formazan. Carriers of X-linked chronic granulomatous disease (CGD) have between 10% and 90% positive cells.[44] If flow cytometry is available, a fluorescent assay of oxidative burst using either 2′7′ dichlorofluoroscin diacetate or dihydrorhodamine 123 can be used as a measure of the respiratory burst and can detect carriers of X-linked CGD.

The chemiluminescence assay also provides a measure of the oxidative response of phagocytes to a variety of soluble and particulate stimuli. This assay is also abnormal in patients with CGD and is reduced in X-linked CGD carriers, in lactoferrin deficiency and in myeloperoxidase deficiency.[45] During chemiluminescence assays, a scintillation spectrometer is employed to measure light energy produced during microbial killing. During phagocytosis, free oxygen radicals are produced by neutrophils and monocytes which then react with oxidizable substrates on microbes including unsaturated lipids, peptides, and nucleic acids to form unstable intermediates which will release light energy when returning to their ground state.[45] Specific assays to measure neutrophil superoxide production are also available.

For the Chediak-Higashi syndrome (CHS), giant granules are seen in peripheral blood neutrophils and would be the best screening test. A confirmatory test that would significantly strengthen the morphologic diagnosis of CHS syndrome would be a test to measure neutrophil chemotaxis. For specific granule deficiency, abnormalities in chemotaxis should also be present as a screening test and an anti-lactoferrin stain of the neutrophil should demonstrate markedly reduced expression of lactoferrin, a specific granule constituent. More significant or confirmatory tests to identify specific granule deficiency would be electron microscopies of the neutrophils, and measurement and immunohistochemistry for B_{12} binding protein and lactoferrin contents within the granules. For actin dysfunction syndrome, abnormal chemotaxis and bacterial killing would be pertinent screening tests. Actual measurement of actin contents and the biochemical measure of the actin, looking for F-actin con-

tent, would be a more confirmatory level of testing. In addition to bacterial killing, there is failure to phagocytose. Finally, LAD is typically studied by evaluating for the cell surface expression (on granulocytes and lymphocytes) of CD11a,b,c/CD18. This may require evaluating cells both before and after activation (with for example, phorbol myristate acetate). In addition, Western immunoblot for either leukocyte function-associated protein-1 (CD18/CD11a), Mac-1 (CD18/CD11b) or CD18/CD11c can be used to examine the actual expression of these proteins in a suspected LAD patient.[46,47]

The clinical and laboratory evaluation of monocyte and macrophage function includes measuring the ability of monocytes and macrophages to mediate microbial killing and antimicrobial activity, to mediate tumor cytostasis, to act as antigen presenting cells, to produce secretory materials such as IL-1, TNF-alpha, and IL-6 or other monokines that may be most important in host defense and the ability to produce enzymes as well as complement proteins and bioactive lipids all of which are important in maintaining host defense. In addition to these secretory products macrophages also express a number of significant cell membrane proteins including Fc receptors for IgG and IgE, receptors for components of complements, fibronectin receptors, lipoprotein receptors, mannose binding proteins that are important for cell traffic, etc. All of these cell surface markers can be assessed with the appropriate antibodies and either immunofluorescence and/or fluorescence-based flow cytometric analysis.

SUMMARY

In this brief review, only the most useful immunologic tests available for defining host defects that lead to susceptibility to infection have been emphasized. It should be pointed out that those evaluations and tests ordered by the physician will rule out the vast majority of the currently recognized defects. Finally, it is important that any patients identified as abnormal by these screening tests be characterized as fully as possible in centers specializing in these diseases before therapy is initiated, since what may appear to be a simple diagnosis on the surface may be an indicator of more complex underlying problems.

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