

## 38\_00\_Evaluation of Immunity

Ricardo U. Sorensen, M.D  
Professor and Chairman  
Department of Pediatrics  
LSU Health Sciences Center  
1542 Tulane Ave  
New Orleans, LA 70112-2822  
e-mail: [Rsoren@lsuhsc.edu](mailto:Rsoren@lsuhsc.edu)

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### GENERAL INDICATIONS FOR THE EVALUATION OF IMMUNITY

The evaluation process of one or more aspects of the immunity is quite varied and covers primary immunodeficiencies (PID), secondary immunodeficiencies, autoimmunity, hypersensitivity, and inflammation. In this review, only the indications for evaluating for PID will be discussed.

The main indication to evaluate for a primary immunodeficiency is the presence of recurrent or unusually severe infections. However, many other historical, physical examination or laboratory signs are also important clues to a possible immunodeficiency. Appropriate use of such clues may allow the diagnosis and management of immunodeficiencies prior to the development of infections, malnutrition, bleeding episodes or malignancies that frequently complicate the course of PIDs and worsen their prognosis.

A partial list of findings suggestive of PID is offered below:

	<b>Associated PID</b>
Recurrent or unusual infections	Any
Positive family history	Any
Delayed separation of umbilical cord	Leukocyte adherence deficiency
Systemic reaction to blood products `	IgA deficiency
Angioedema	C1 esterase inhibitor deficiency
Failure to thrive	CMI* deficiencies
Short stature	Cartilage hair hypoplasia
Severe eczema, hyper IgE	Hyper IgE syndrome
Eczema, hemorrhagic purpura	Wiskott Aldrich syndrome
Partial albinism	Chediak Hygashi syndrome
	Griscelly syndrome
Abnormal facies, conotruncal abnormalities, hypoparathyroidism	Di George/C22q11 microdeletions
Graft versus host disease	CMI deficiencies
Persistent decidual teeth	Hyper IgE syndrome
Ectodermal dysplasia	NEMO mutations
Hypoplastic lymphoid tissue	XLA*, CMI deficiencies
Hyperplastic lymphoid tissue	CGD*, some HIGM*
Ocular apraxia, ataxia	Ataxia telangiectasia
Persistent thrombocytopenia	Wiskott Aldrich syndrome
Neutropenia	Neutropenia, XLA, HIGM
Neutrophilia	Leukocyte adherence deficiency
Giant lysosomes	Chediak Hygashi syndrome
Iron resistant anemia	Chronic mucocutaneous candidiasis
Endocrinopathies	Chronic mucocutaneous candidiasis

\* CMI = cell mediated immunity; NEMO = NFkB essential modulator; XLA = X-linked agammaglobulinemia; CGD = chronic granulomatous disease; HIGM = hyper IgM syndrome

## **INFECTIONS**

Infections are the main presentation for most PIDs. Infections that suggest an immunodeficiency have been extensively reviewed under **26\_00, "Infections That Suggest An Immunodeficiency."**

## **FAMILY HISTORY**

The inheritance of PIDs is quite variable, including several X-linked immunodeficiencies, and both autosomal recessive and autosomal dominant forms.

The following information obtained from the family history is relevant:

- Unusual infections or unexplained early death in family members
- Consanguinity. Many autosomal recessive defects are expressed when the parents are first cousins or otherwise related.
- History of immunodeficiency in males on the maternal side of the family.

## **PHYSICAL ABNORMALITIES**

The table lists a number of physical examination findings that suggest specific PIDs and that are an integral part of these syndromes. Many other conditions, e.g. Down syndrome, Ring chromosome 18, muscular dystrophy, etc, may have infections suggestive of a PID that need to be investigated because PIDs may coexist with these conditions although they are not part of the primary syndrome or disease.

**Further information about primary immunodeficiencies can be found under 40\_00 (pending).**

## **GENERAL APPROACH TO EVALUATION OF IMMUNITY**

The type of evaluation to be performed depends on:

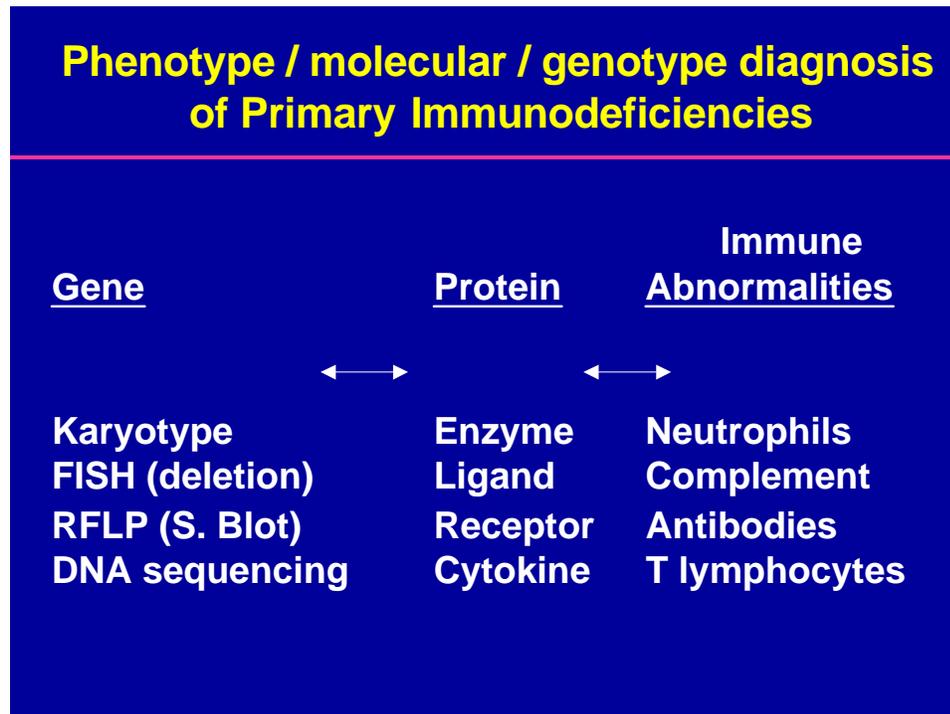
- type of infections
- age of the patient
- family history
- presence of the signs and laboratory findings listed above

In addition, the frequency of various PIDs also significantly influences the evaluation to be performed.

The **frequency** with which evaluations of the various components of the host defense mechanisms need to be performed varies significantly. Antibody deficiencies account for over 50% of all diagnosed immunodeficiencies [Javier, 2000 #67]. This high frequency combined with the fact that infections suggestive of antibody deficiencies are quite common in children (see also Table 4) explains that evaluations of antibody mediated immunity are quite common. Antibody deficiencies are followed in frequency by deficiencies in cell mediated immunity. Defects of phagocytosis and complement deficiencies are much less frequent. Since these deficiencies are characterized by infections that are not frequently seen in normal populations, evaluation of these two host defense mechanisms should be reserved for patients with infections listed in Tables 2 and 3.

**Immunologic methods** can be organized into those identifying phenotypic abnormalities, those identifying molecular defects, or those identifying gene defects and errors in gene transcription. These methods cannot always be clearly separated, as on some occasions the same general method, e.g., flow cytometry, is used to describe a phenotype of an immunodeficiency with

decreased lymphocyte subpopulations and also to identify a molecular defect responsible for a disease phenotype.



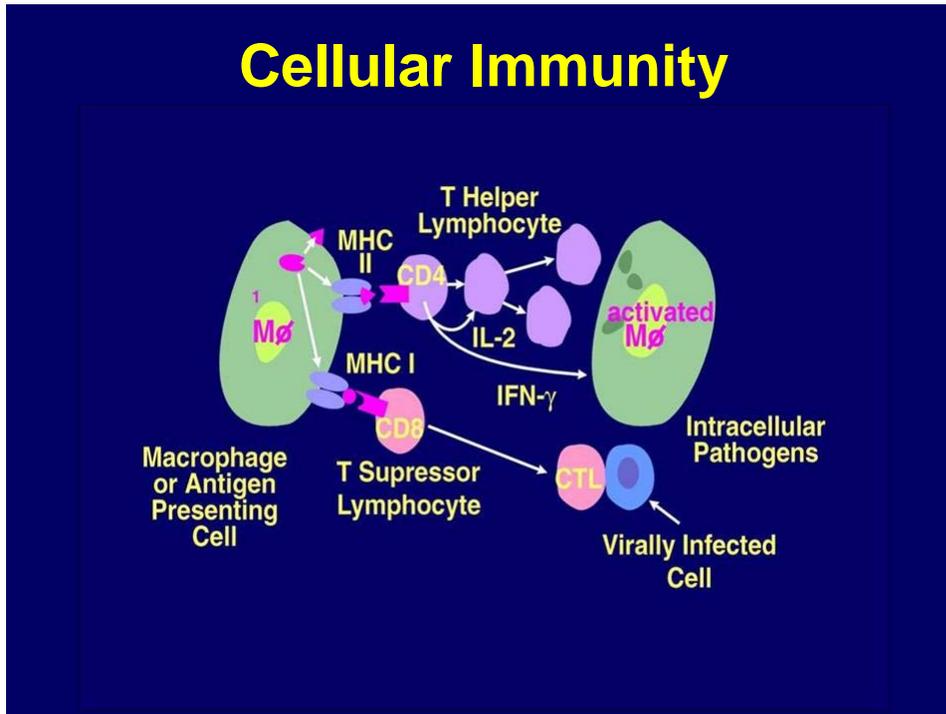
As knowledge of functional defects in presence of normal proteins or cells has increased, it has become important to consider that it is no longer sufficient to measure only circulating immunoglobulin concentrations or lymphocyte subpopulations to assess antibody or cell-mediated immunity. Evaluation for functional antibody or lymphocyte deficiencies is part of a state of the art workup of the immune system. Without the evaluation of the functional component of host defenses, some forms of immunodeficiencies can't be diagnosed or ruled out.

In this review, we will discuss only the evaluation of the main aspects of each component of the immune system. These tests are sufficient in general to determine if the tested aspect of immunity is normal or if there is an abnormality that needs to further investigated.

**Our strong recommendation is to always consult an Allergist/Immunologist before performing an immune evaluation. The complexity of PIDs and secondary immunodeficiencies is such that expert advice is necessary to perform the best evaluation possible and avoid repetition of tests when the initial results are incomplete or inconclusive for the specific challenge posed by each patient. Furthermore, interpretation of test results is also affected by many aspects that are almost impossible for the primary physician to predict.**

**If the primary physician would prefer to initiate an evaluation, we recommend performing the test listed under SCREENING EVALUATION.**

## EVALUATION OF CELL-MEDIATED IMMUNITY



### SCREENING EVALUATION

CBC, lymphocyte count  
Frontal and lateral chest X-ray for thymic IMAGE

Lymphocyte subpopulations  
Delayed hypersensitivity (above 2 years of age)

### ADVANCED EVALUATION

#### Lymphocyte numbers

Lymphocyte subpopulations with special phenotype identification

#### Functional assays

Blastogenic responses to antigens and mitogens  
Cytokine production

#### Molecular defects

Red cell enzymes: adenosine deaminase, nucleoside phosphorylase  
HLA testing for bare lymphocyte syndromes  
Cytokines and lymphokines  
Intracellular kinases  
Signal reception and transduction  
Cytokine receptors

Secreted cytokines

## **CRITERIA FOR NORMAL CELLULAR IMMUNITY**

### **Under two years of age**

- Well developed lymphoid tissue
- Thymus gland present
- Normal circulating lymphocytes

This does not rule out functional defects

### **Over two years of age**

- Normal circulating lymphocyte numbers on CBC
- Present delayed hypersensitivity
  - Tuberculin
  - Diphtheria toxoid
  - Tetanus toxoid
  - Candida antigen

## **EVALUATION OF ANTIBODY-MEDIATED IMMUNITY**

Deficiencies of antibody-mediated immunity include:

- Immunoglobulin deficiencies
- IgG subclass deficiencies
- Specific antibody deficiencies (with normal or abnormal immunoglobulins)

Immunoglobulin deficiencies and IgG subclass deficiencies are easily ruled out or diagnosed by measuring circulating immunoglobulins and IgG subclasses.

We use a history of recurrent or severe infections and/or chronic or recurrent antibiotic use as an indication to evaluate antibody mediated immunity. A relatively low threshold for an immunologic evaluation is justified for the following reasons:

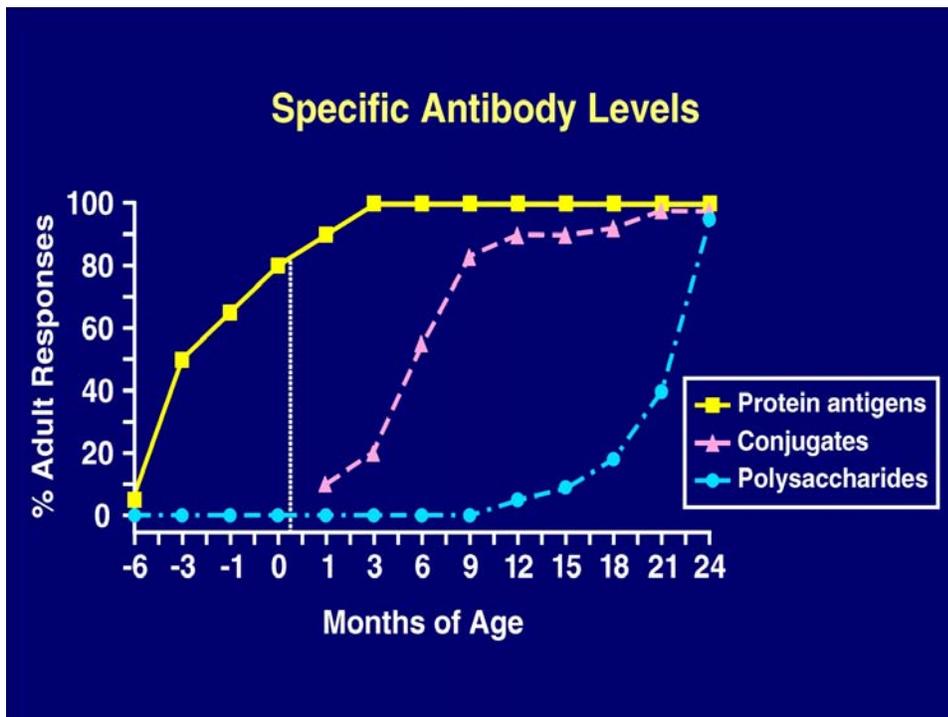
- It is not possible to clinically differentiate between patients with infections due to an antibody deficiency, and patients with normal antibody mediated immunity
- The use of vaccines as part of the evaluation of antibody mediated immunity benefits many patients

Excessive use of antibiotics should be prevented to avoid further development of antibiotic resistance.

Evaluation of antibody-mediated immunity is based on the measurement of immunoglobulin concentrations and of specific antibodies developed in response to immunizations

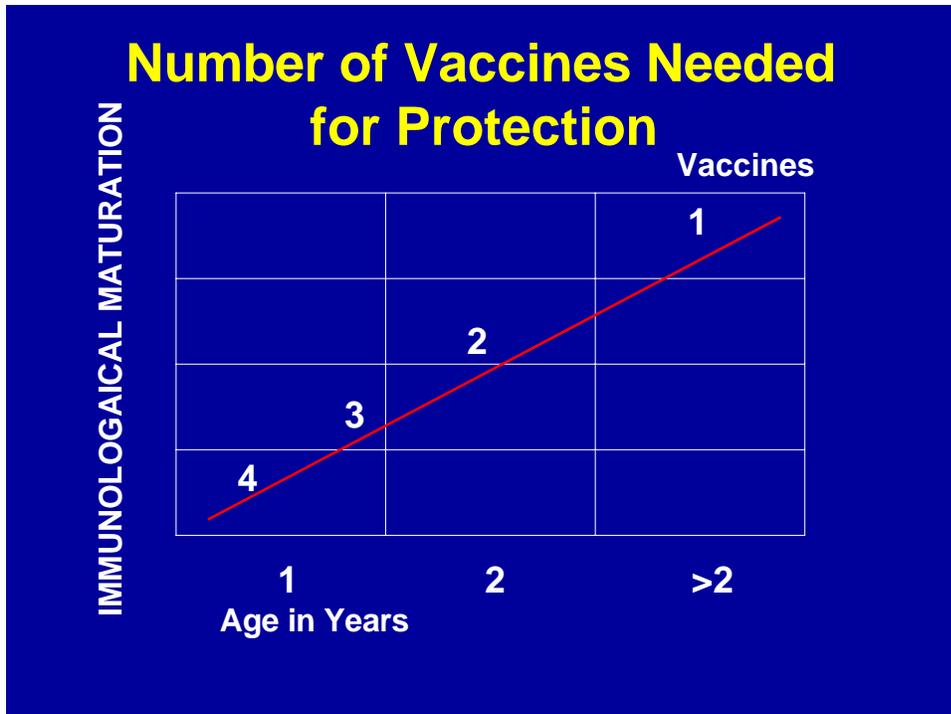
The assessment of specific antibodies requires not only the measurement of specific antibodies but also the assessment of the immunization status and, sometimes, the use of vaccines to evaluate the response to immunization.

The most important specific antibodies to measure are antibodies to pneumococcal polysaccharides developed in response to immunization. In the ontogeny of the antibody response in humans, the ability to develop antibodies to proteins is present already in fetal life while the ability to develop antibodies against bacterial polysaccharides matures only at about two years of life. Therefore, if antibodies against polysaccharides are normal, antibodies against proteins are also normal. In most patients, the measurement of anti-polysaccharide antibodies is sufficient to assess specific antibody formation.



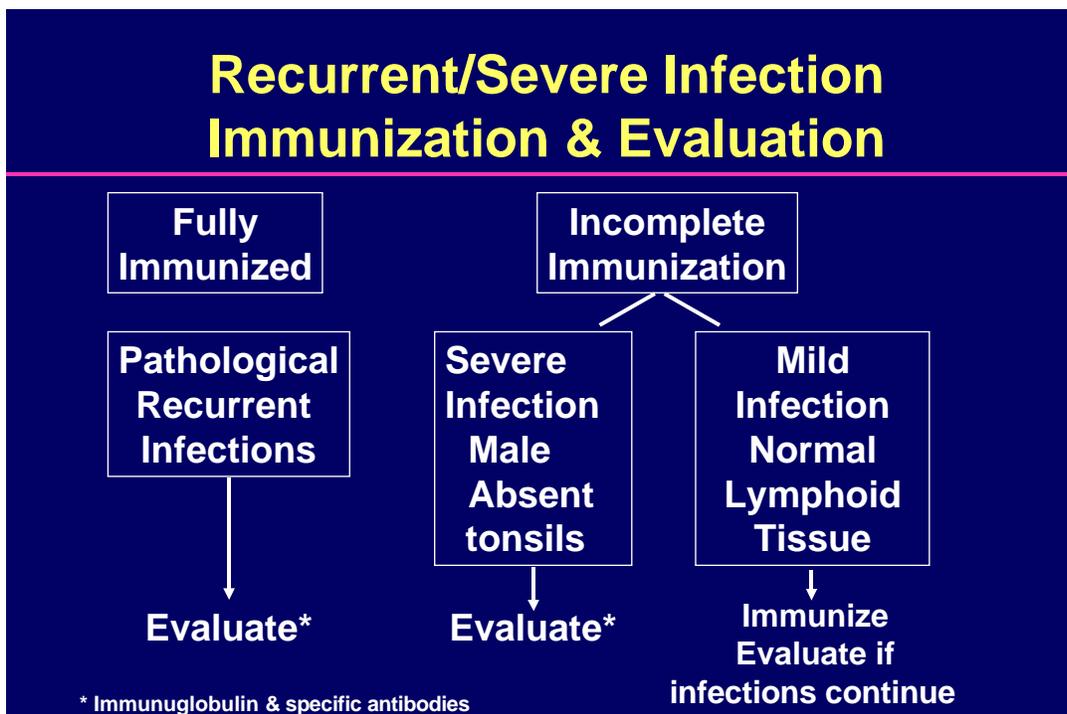
The recent introduction of a heptavalent pneumococcal conjugate vaccine (PCV-7, Prevnar R) has led to the need for a careful evaluation of the pneumococcal immunization history to determine the significance of measured anti-pneumococcal antibodies. Knowledge about the impact of infant immunization on the evaluation of responses to polysaccharides is still evolving and at the present time requires consultation with a clinical immunologist before definitive diagnoses about normal or abnormal antibody function can be formulated.

Present recommendations for the use of PCV-7 take into account the age at which immunization is initiated, with a decreasing number of doses needed the later the immunizations is started. In order to determine if immunization with PCV-7 is complete, one needs to take into account the number of vaccines specified in the figure shown below:



Recurrent respiratory infections are the main presentation for antibody deficiency syndromes, and many of these infections are caused by *Streptococcus pneumoniae* serotypes. Appropriate immunization and adequate immunity tend to protect against these infections. When infections continue to occur despite full immunization, a form of immunodeficiency is more likely.

On a practical basis, we recommend following the actions suggested in the algorithm shown below:



## **SCREENING EVALUATION.**

- IgM, IgG, IgA and IgE concentrations
- IgG subclass concentrations
- Specific antibodies against
  - Protein antigens. Tetanus and diphtheria toxoids
  - conjugate polysaccharide antigens. Hib and PCV-7 serotypes
  - pure polysaccharide antigens. Pneumococcal serotypes not included in PCV-7 and iso-hemagglutinin

Anti-protein antibodies generally include anti-tetanus and anti-diphtheria toxoid antibodies. Patients  $\geq 2$  years of age with normal immunoglobulin concentrations rarely have a deficient response to proteins. In adults, low anti-protein antibody concentrations are likely to be due to the time that has elapsed since the last immunization.

In children under two years of age, the presence of antibodies against protein antigens is important to differentiate transient hypogammaglobulinemias of infancy from other rare forms of antibody deficiency that have an onset early in life and where low IgG concentrations are accompanied by the inability to produce anti-protein antibodies.

The measurement of anti-conjugate Haemophilus influenza and pneumococcal polysaccharide antibodies may help to define unresponsiveness to conjugate polysaccharides. We are in the early stages of understanding the spectrum and implications of these abnormalities.

The anti-polysaccharide antibody detection methods generally refer to the measurement of IgG anti-pneumococcal polysaccharide antibodies. The methods to measure anti-conjugate polysaccharide antibodies are the same as for IgG responses to purified polysaccharides. The difference between anti-pure and anti-conjugate polysaccharide antibodies is distinguished by the immunization used and by the pneumococcal serotype antibodies measured.

The measurement of anti-A and B isoagglutinins is not useful in the diagnosis of the polysaccharide antibody deficiency. Blood groups A and B are galactosamines on red cells cross-reactive with galactosamines on the capsule of gut E coli bacteria. The method currently in use does not differentiate between IgM and IgG responses.

## **ADVANCED EVALUATION**

B lymphocyte measurement

Identification of memory B cell phenotypes

Identification of molecular defect and gene mutation in patient and family members

## **CRITERIA OF NORMALITY FOR ANTIBODY-MEDIATED IMMUNITY**

### **NORMAL IMMUNOGLOBULINS**

Normal immunoglobulin concentrations are defined according to normal values provided by the performing laboratory. It is important to make sure the laboratory is using the right normal values for each age group.

IgG4 subclass may be undetectable in normal children up to 10 years of age. If all other immunoglobulins and subclasses are normal, absent IgG4 is not an abnormal finding.

### **NORMAL SPECIFIC ANTIBODIES**

#### **Protection vs. immunocompetence**

An unresolved issue in the interpretation of specific antibody results is the difference between the minimum antibody concentration that may protect against invasive infections and the antibody concentration necessary to document immunocompetence and protection against recurrent mucosal infections.

Specific antibody concentrations have to be evaluated in the context of the patient's, immunization history, and the time elapsed since the last immunization

Very low antibodies in a patient where the last immunization was given > 5 or 10 year ago may need to be checked after a booster immunization

#### **Protein antibodies**

Tetanus and diphtheria antibodies:

Absent and non-protective: < 0.1 IU/ml

Low or non-responder: 0.1 to 1.0 IU/ml

Normal:  $\geq$  1.0 IU/ml

**Note:** concentrations  $\geq$  0.1 IU/ml are considered protective

#### **Conjugate polysaccharide antibodies**

Hib antibodies:  $\leq$  1 ug/ml: non responder, non-protective

$\geq$  1ug/ml: protective Other sources may consider  $\geq$  0.15 protective)

#### **Pneumococcal antibodies (conjugate and pure polysaccharide):**

The interpretation of pneumococcal antibodies is different for PCV-7 serotypes (developed in response to PCV-7 vaccination) and antibodies to non-PCV-7 serotypes (developed in response to pneumococcal polysaccharide immunization, PPV).

In each case, one has to evaluate immunity to each single serotype and collective immunity to all serotypes measured.

## PCV-7

Adequate response to single serotype  $> 0.6 \text{ ug/ml}^*$

Collective antibodies: adequate response to at least 4 of the 7 PCV-7 serotypes.

\*Immunocompetence and protection against mucosal infections may require higher concentrations.

## PPV

Adequate immunity to single serotype:  $\geq 1.3 \text{ ug/ml}$  or four-fold increase

Adequate response to several serotypes::  $\geq 50\%$  of serotypes 2-5 years of age

$\geq 70\text{-}80\%$  of serotypes  $\geq 5$  years of age

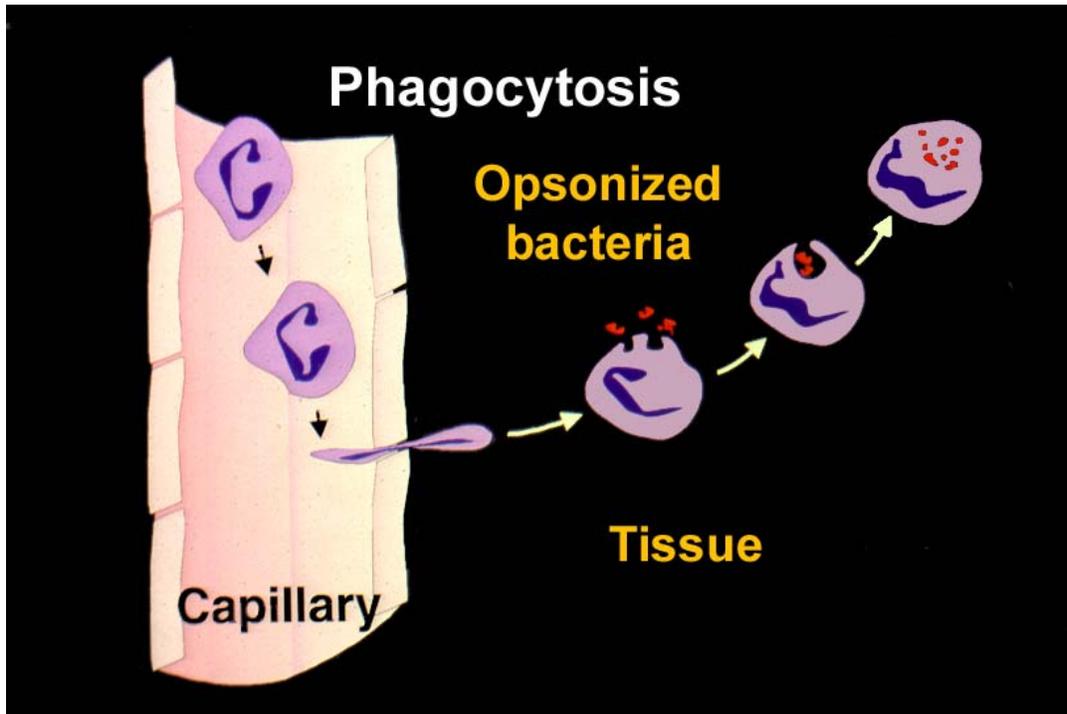
## SUMMARY

Antibody concentrations  $> 1.3 \text{ ug/ml}$  to 50% of serotypes tested in patients 2-5 years and to 80% or more serotypes in older patients documents normal antibody-mediated immunity, although poor immunological memory with rapid loss of protective antibody concentrations still needs to be considered.

**Patients with any form of immunoglobulin deficiency and/or abnormal specific antibodies and patients with normal antibody-mediated immunity that continue to have infections need to be referred for further evaluation and management.**

## EVALUATION OF PHAGOCYTOSIS

Normal phagocytosis depends on normal circulating neutrophils, on normal adherence and chemotaxis to the site of infection, and on normal ingestion and intracellular killing.



### SCREENING EVALUATION

Neutrophil count and smear

### ADVANCED EVALUATION

Tests to evaluate functional aspects of phagocytosis are all advanced tests that are infrequently indicated and that need to be requested by subspecialist expert in immunodeficiency diseases.

- Chemotaxis (Rebuck window, Boyden chamber)
- Detection of adhesion molecules (CD18 measurement by flow cytometry)
- Superoxide generation (NBT test, chemiluminescence test, respiratory burst measurement by flow cytometry)

**Indirect evaluation of abnormalities associated with some neutrophil abnormalities (Hyper IgE syndrome)**

Eosinophil count  
IgE concentration

## **CRITERIA FOR NORMAL PHAGOCYTTIC FUNCTION**

- Circulating neutrophils appropriate for clinical circumstance
  - increased during infections
  - normal during well-being
- Normal chemotaxis
- Normal CD11/CD18 surface expression
- Normal superoxide generation
- Normal or moderately elevated IgE

## **EVALUATION OF SPLEEN AND SPLEEN FUNCTION**

### **Presence, size and number of spleen(s)**

Ultrasound

### **Spleen function**

Howell-Jolly bodies in red cells

## **EVALUATION OF COMPLEMENT**

Most complement PIDs affect the classic pathway of complement activation. Deficiencies of alternate pathway components are rare and its evaluation is rarely indicated.

### **SCREENING TESTS**

- Serum hemolytic complement (CH50)\*
- Quantitation of C3 and C4
  
- C1 esterase inhibitor

### **ADVANCED TESTS**

- Individual classic pathway complement components\*

\*If CH50 is low but hemolytic activity is present, repeat test after infection is controlled making sure that the sample is handled properly to avoid low CH50 activity due to in vitro degradation of C3.

If CH50 is very low or absent and C4 and C3 are normal:  
Measurement of remaining classic pathway components

- Alternative pathway (AP50)

If AP50 is low:  
Measurement of alternative pathway

( If AP50 determinations are not readily accessible, determination of factors B, P, and D can be done directly).

## **CRITERIA FOR NORMAL COMPLEMENT FUNCTION**

- Normal CH50
- Normal C4 and C3 (C4 deficiencies may exist with normal CH50)
- Normal C1Esterase inhibitor (protein and function)
- Normal AP50

## **IMPORTANCE OF DOCUMENTING NORMAL IMMUNITY**

A properly indicated and well designed immune evaluation can be a very important part of the management of patients at risk for an immunodeficiency disease. When the appropriate test results are normal, the following benefits should be achieved:

- Reassurance and decreased demand for treatment if immunity is normal
- Identification non-immunodeficiency causes of infection
- Risk-free use of anti-inflammatory agents to treat inflammation predisposing to infections and inflammatory complications of infections, such as:
  - Allergic bronchopulmonary aspergillosis
  - Fungal sinusitis
  - Pseudomonas bronchitis in cystic fibrosis
  - Candida vaginitis

When an abnormality is identified, early institution of prevention and treatment significantly improves the prognosis for all forms of immunodeficiency.

## **INDICATIONS FOR REFERRAL**

Presence of an abnormality in any of the screening tests listed above  
Recurrent or severe infections with normal screening tests

Recurrent infections precluding immunization  
Recurrent infections requiring antibiotic use  
Allergy to antibiotics

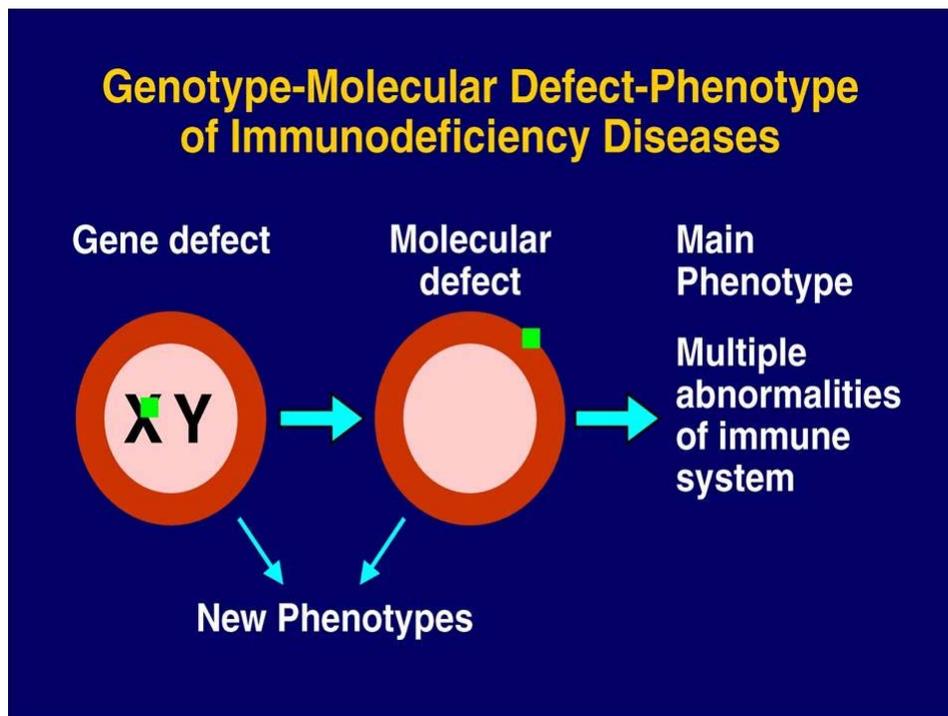
## **SUBSPECIALTY SERVICE**

Identification of immunologic phenotype  
Identification of molecular abnormality  
Genetic counseling and family evaluation  
Assesment of risk for infections and other complications  
Management advise:

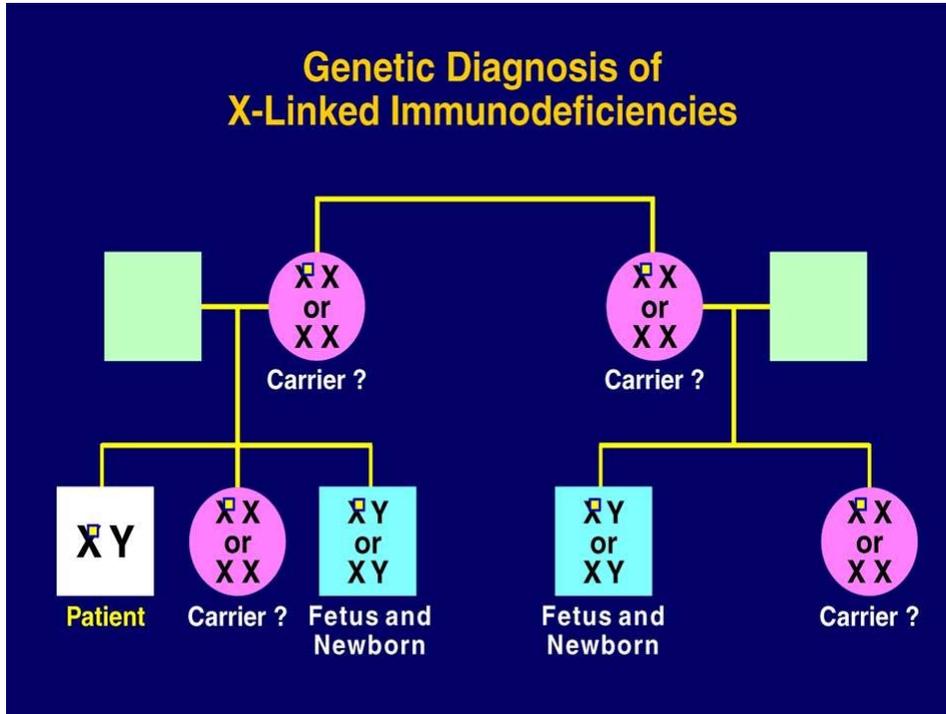
Infection prevention: avoidance, antibiotics, vaccines  
Immunological substitution: IgG, cytokines

Immunological reconstitution  
Monitoring and long-term follow-up

The diagram shown below shows how it is frequently possible to identify a molecular abnormality and/or a gene mutation from a phenotypic abnormality detected by the testing of immunity outlined in this review. In turn, identification of such molecular and gene abnormalities allows us to identify different or milder phenotypes in other individuals and family members before the onset of clinical problems



An example of the importance of the identification of molecular and gene defects in a patient with an X-linked PID is shown below. If the molecular or gene defect is identified in the patient, the search for carriers and for other affected males still asymptomatic is greatly facilitated.



## REFERENCES

This review is based on the author's long-term experience in the evaluation of patients suspected to have an immunodeficiency.

References for specific points made in this review are available upon request. Furthermore, in this rapidly evolving field, recent references can be readily identified through appropriate searches.