Diagnosis of defects of antibody production

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Antibody function is a property of the immunoglobulins. Therefore antibody deficiency in its severest form implies immunoglobulin deficiency or agammaglobulinaemia. Since most patients with antibody deficiency produce at least some immunoglobulin, the term hypogammaglobulinaemia is to be preferred to the more absolute agammaglobulinaemia. Hypogammaglobulinaemia due to a failure of antibody synthesis may be congenital or acquired, primary or secondary. This failure of synthesis is usually associated with a lack of mature plasma cells and rather less commonly with a lack of B lymphocytes. Immunoglobulin deficiency may affect all classes but often may be restricted to only one or two classes or subclasses. Rare individuals show a functional antibody deficiency despite quantitatively normal amounts of all immunoglobulin classes. The spectrum of primary antibody deficiency syndromes is shown in Table 1. Secondary antibody deficiency due to immunoglobulin loss usually affects mainly IgG and is associated with a degree of hypoproteinaemia and hypoalbuminaemia.

Table 1 Primary antibody deficiency syndromes

Hypogammaglobulinaemia	Congenital X-linked Congenital non-X-linked Late onset With increased IgM Transient
IgG subclass deficiency	
Selective IgA deficiency	
Isolated IgM deficiency	
Qualitative antibody deficiency	

The diagnosis of antibody deficiency syndromes is a combined exercise of clinical evaluation and laboratory investigation. A history of multiple recurrent infections should always raise suspicion of an underlying antibody deficiency. Respiratory tract infections are particularly common, although anatomical abnormalities should be excluded as should also metabolic or systemic disease such as diabetes. Urinary tract infections, on the other hand, are rarely associated with antibody deficiency and usually result from anatomical defects. Recurrent or persistent infections at a single site also suggest an

anatomical or functional cause rather than a deficiency syndrome.

Clinical evaluation

The initial step must be a full clinical history. Laboratory investigations alone cannot make, or exclude, a diagnosis of antibody deficiency. The history will also distinguish the rare primary deficiencies from the more common secondary deficiencies.

Onset of symptoms in antibody deficiency syndromes is unusual before the age of 6 months because of partial protection by maternal IgG.

Maternal health in pregnancy may be of significance. Rubella infection with congenital infection of the fetus may result in IgA deficiency. Transplacental transfer of IgG from mother to fetus occurs maximally in the last four weeks of gestation. Gestational age is therefore of prime importance in assessing possible transient hypogammaglobulinaemia of infancy. Birth weight may also be of relevance in that small-for-dates babies tend to be more susceptible to infection, possibly owing to intrauterine malnutrition (Chandra, 1974). Drug treatment during pregnancy, particularly with corticosteroids or immunosuppressive agents, may also predispose the infant to immune deficiency.

Although some antibody deficiency syndromes have an X-linked recessive inheritance and others a slight male preponderance, a negative family history is of little value. With the other conditions exhibiting a more complex pattern of inheritance, relatives showing other forms of immunopathological disease may be of relevance.

While patients under investigation for possible immune deficiency syndromes should never receive live vaccines, their response to immunisations conducted before the possible diagnosis was suspected is relevant. A history of attempted immunisation will give a basis for specific antibody testing in the laboratory evaluation of the deficiency state.

PHYSICAL EXAMINATION

Some patients with antibody deficiency syndromes, notably the X-linked, have small or absent tonsils

and small adenoids (visualised by lateral pharyngeal radiography). Large hypertrophic tonsils, however, do not exclude the diagnosis. The size of peripheral lymph nodes is very variable and they may even be enlarged.

A common feature in infants is failure to thrive, and serial recording of height and weight may give useful information on the effectiveness of management (Thompson, 1978).

Physical and radiological examination of sites of previous infection may give evidence of the consequences of these infections, such as bronchiectasis or opaque maxillary antra due to chronic sinusitis.

PATHOGENS

The commonest organisms causing infections in antibody deficiency syndromes are staphylococci, streptococci, and *Haemophilus influenzae*. The common organ sites of infection are lung, upper respiratory tract, skin, and meninges. Viruses and fungi rarely cause significant clinical problems.

Laboratory investigation

The laboratory component of the full investigation of a suspected deficiency falls into two main categories: the initial screening tests done on all possible candidates (Table 2) and the more extensive or invasive procedures which are recruited as necessary (Table 3).

Table 2 Initial screening tests for antibody deficiency

Full blood count	Haemoglobin Differential WBC Platelets
T cells and B cells	
Zone electrophoresis	
Immunoglobulins, IgG, IgA, and IgM	
Natural antibodies	<i>Escherichia coli</i> Isohaemagglutinins Schick test if previously immunised

INITIAL SCREENING TESTS

Blood count

In addition to a full haematological profile and differential WBC lymphocytes should be examined for relative numbers of T cells and B cells. While the number of T cells is normal in most antibody deficiency syndromes, the number of B cells is reduced in the congenital X-linked and non-X-linked hypogammaglobulinaemias, but may be normal in some forms of variable hypogammaglobulinaemia. The number of B cells is normal in transient hypogammaglobulinaemia (Table 4).

Table 3 Additional investigations for further evaluation of antibody deficiency

Antibody function	Heterophile ASO
Natural antibodies	Typhoid Poliomyelitis Influenza Keyhole limpet hemocyanin Pneumococcal polysaccharide <i>Haemophilus influenzae</i> Bacteriophage Φ X174
Response to challenge	
IgG subclass distribution	
Biopsy	

Table 4 Cellular abnormalities in antibody deficiency syndromes

	T cells	B cells	Plasma cells
Congenital X-linked	N	↓	Absent
Congenital non-X-linked	N	↓	Absent
Late onset	N	N or ↓	Absent
With increased IgM	N	N	↓
Transient	N	N	N

N = normal.

Immunoglobulins

Hypogammaglobulinaemia may be suspected after zone electrophoresis but a quantitative assay of immunoglobulins G, A, and M is essential. IgD and IgE are not usually assayed and they have no relevance to the diagnosis of antibody deficiency.

Antibody function

The presence of immunoglobulin does not necessarily imply an ability to make adequate antibody. The initial evaluation of a patient with suspected antibody deficiency should include at least two tests of antibody activity against common or naturally occurring antigens. The detection and quantification of the naturally occurring *Escherichia coli* antibody (Webster *et al.*, 1974) is a useful test for IgG function and measurement of isohaemagglutinins presents a functional test for IgM antibody. This latter test does have some disadvantages: isohaemagglutinins are not present in patients who are of blood group AB, and they do not usually appear before the age of 6 months, reaching adult amounts by the age of 2 years.

In patients immunised against diphtheria the intradermal Schick test for antitoxin is a simple and reliable technique for evaluating IgG functional activity. Erythema and oedema at the inoculation site 96 hours after intradermal injection of diphtheria toxin indicates a lack of antibody. A positive result should always be repeated after three weeks, by which time the first test may have acted as a booster immunisation and converted a subsequent Schick test to a negative reaction. A positive result on

retesting indicates a significant functional IgG deficiency.

FURTHER INVESTIGATIONS

In the event of normal results from the initial screening tests a diagnosis of a significant antibody deficiency state is unlikely. But if the history is unusually convincing additional tests may be recruited as necessary to establish the diagnosis in the less common forms of antibody deficiency.

Antibody function

Two additional natural antibodies, the heterophile antibody to sheep erythrocytes and the anti-streptolysin O antibody, are present in almost all normal individuals. All other functional tests of antibody require prior challenge with the antigen.

Weekly subcutaneous injections of typhoid vaccine with serum sampled before and after challenge provides a test for both IgG and IgM antibody activity. IgG class antibody to the H antigen and IgM class antibody to the O antigen should achieve titres greater than 40 in the post-challenge samples. Similar antibody function tests on paired sera before and after antigen challenge use killed poliomyelitis and influenza vaccines, keyhole limpet hemocyanin, pneumococcal polysaccharide, and *Haemophilus influenzae* (Fudenberg *et al.*, 1971).

All investigations of suspected immunodeficiency by antigenic challenge must be with *killed* organisms or extracts. Under no circumstances should live or attenuated strains be used. Probably the most sensitive test for antibody formation, testing both IgM and IgG function, is the bacteriophage Φ X174 clearance test (Wedgewood *et al.*, 1975). In normal people the test stimulates an IgM primary response and IgG secondary response with clearance of the phage in five days. In antibody deficiency the phage may persist in the circulation for many weeks without ill effect.

IgG subclass distribution

Although anomalous subclass distribution is common in hypogammaglobulinaemia (Yount, 1975), the assay of individual subclasses adds little to the diagnosis except in the selective deficiency of IgG₂ and IgG₄ (Oxelius, 1974). Here the total IgG concentration is usually normal but the diagnosis may be suggested by the appearance of anodic truncation of the IgG arc on immunoelectrophoresis.

Secretory immunoglobulin

Immunoglobulin and antibody deficiency in the secretions invariably mirrors the abnormalities in the

serum. In selective IgA deficiency the absence of IgA in the secretions is usually compensated by secretion of IgM.

Biopsy

Recourse to biopsy examination in antibody deficiency is rarely indicated or necessary. The lack of mature plasma cells may be demonstrated by examining bone marrow aspirates or rectal biopsy (Davis *et al.*, 1971). In both instances immunofluorescent methods greatly enhance the sensitivity of the procedure. When the diagnosis or the results of antibody stimulation tests remain equivocal lymph node biopsy after antigenic challenge may provide additional information. Diphtheria, pertussis, tetanus (DPT) immunisation is given in the anterior thigh and biopsy of an inguinal lymph node carried out after 5-7 days. The lymph node is examined for thymic and bursa-dependent areas, plasma cell development, and germinal centres. In antibody deficiency there is a lack of primary lymphoid follicles and germinal centres, a thinning of the cortex, and a lack of plasma cells in the medullary cords. The thymic paracortical areas remain stable and well populated with lymphocytes.

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