Immunologic assessment of the immunodeficient

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Although this article will deal primarily with assessment of deficiencies of the lymphoid system, it is important to remember that host defense is also dependent upon the phagocytic cells, the macrophages and polymorphonuclear leukocytes. Significant abnormalities in either of these systems can result in altered susceptibility to infection, which is of as much clinical moment as deficiencies of the lymphocytes themselves. Extensive literature has accumulated on the phagocytic cells, and the reader is referred to recent reviews for further information.

NORMAL LYMPHOCYTE PHYSIOLOGY

As the deficiency states result from failure of normal physiology, it is appropriate to consider the steps leading to competent immunity. The protective capability afforded by the lymphoid system results from normal maturation and development of pluripotential cells in appropriate sites of differentiation. The early differentiation steps take place in inductive organs that have been termed central or first-level lymphoid organs. Here the pluripotential cell undergoes its first major commitment along the pathway eventuating in T (thymus-derived) or B (bone marrow-derived) cells. The thymus gland subserves the function of a first-level lymphoid organ in man and the bursa of Fabricius a similar function for the B cell line in the chicken. The bursal equivalent in man appears now to be the fetal liver. Following an initial commitment to activities of the T cell series, a number of T cell subpopulations are generated which subserve specialized functions in the body (Table I). B cells also undergo further specialization so that they can produce major immunoglobulin classes. The normal function of the B cells in human biology is shown in Table II.

When an individual meets an antigen for the first time, receptors on T and B lymphocytes combine with the antigenic material. Aided by the macrophage population which may serve as an antigen-focusing mechanism, the T and B cell populations become "sensitized" and we say that the individual is immune. Subsequent exposure of these immune cells to antigens calls forth an immune response consisting of proliferation and the initiation of a number of intracellular steps. The end result of these steps is to provide the immunologic capability that allows the individual to resist an infectious agent or other unwanted foreign intruder.

The ultimate elimination of the agent requires the augmentation of body systems other than the lymphoid cells. The B cell system, following combination of the immunoglobulin and the antigen, employs the nine-component complement system for this purpose. An inflammatory response is generated as the various components of the complement cascade are sequentially activated. The terminal event of the complement attack sequence is lysis of the particle which combines with the immunoglobulin. In an analogous fashion, the union of the sensitized T lymphocyte with its antigen causes the release of a number of lymphocyte mediator substances known as lymphokines. These materials act in a manner similar to the complement system to create an inflammatory reaction characteristic of T cell killer activity and typified by the granuloma of a tuberculous infection.

The breakdown of the system just described results in syndromes of increased infectious susceptibility. The assessment of the immune patient can be conveniently divided into: (1) assessment of clinical features; (2) assessment of physical features; (3) morphology of immune apparatus including assessment of T and B cell lines (at the level of differentiation, at the level of the intracellular events, or at the level of secreted products); (4) biochemical analyses; and (5) assessment of the amplifier mechanisms. Assessment of amplifiers is beyond the scope of this discussion and will not be further considered. They are adequately covered elsewhere.

Assessment of clinical features

The T cells and the B cells subserve different roles in human biology (Tables I and II). It can be reasoned from this that abnormalities of only one cell line will
TABLE I. Role of T cells in human biology*

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<tr>
<th>Function</th>
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<tr>
<td>T helper function</td>
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<tr>
<td>T suppressor function</td>
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<td>T killer function</td>
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<tr>
<td>Containment of acid-fast bacteria</td>
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<tr>
<td>Containment of certain viral infections after</td>
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<tr>
<td>establishment (rubeola, varicella, herpes,</td>
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<td>cytomegalovirus)</td>
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<td>Containment of fungal infections (especially</td>
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<td>Candida)</td>
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<td>Containment of protozoan infections</td>
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<tr>
<td>Rejection of allografts</td>
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<td>Graft vs host reactions</td>
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<td>Contact dermatitis</td>
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TABLE II. Role of B cells*

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<th>Function</th>
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<tr>
<td>Prevention of viral infection (rubeola, varicella, infectious hepatitis)</td>
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<tr>
<td>Prevention of bacterial infection (staphylococcus, streptococcus, pneumococcus, Hemophilus influenzae)</td>
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<tr>
<td>Antibody-dependent cell-mediated cytotoxicity</td>
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<td>Allergic (IgE) reactions</td>
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<tr>
<td>Antigen-antibody complex disease</td>
</tr>
<tr>
<td>Direct cytolysis</td>
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<tr>
<td>Blocking factors in tumor immunology</td>
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<tr>
<td>Blocking antibodies in allergic desensitization</td>
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result in a unique type of infectious susceptibility. The clinical features which are suggestive of T or B cell deficiencies are shown in Table III.

Assessment of physical features

The major point which should be stressed in the physical assessment of the patient is the presence of lymphoid tissue. In a child, the presence of normal lymphoid tissue virtually rules out significant immunodeficiency. On a rare occasion enlarged lymph nodes are found in unusual forms of immunodeficiency (e.g., Omenn's disease). In adults small shotty nodes may be found in the face of immunodeficiency. Also, giant follicular lymphoma is frequently associated with adult-onset immunodeficiency, and in this case the lymph nodes are quite prominent. It has been observed that a number of children with bony abnormalities, especially short limb dwarfism, have associated serious immunodeficiencies. Characteristic facial features have been described in the DiGeorge syndrome and Weidemann-Beckwith syndrome. Abnormalities of the integument, including eczema, telangiectasia, sparse hair, and excessive seborrhea, are also associated in high incidence with immunodeficiency.

Morphology of the immune apparatus

The histology of the thymus, spleen, and lymphoid tissues in normals in various deficiency disorders has been well documented. Newer information should derive from assessment of the thymus by biopsy, as much of the histology based on autopsy examination represents terminal changes and the results of long-standing disease.

Assessment of B cells. An initial assessment of the B cell function begins with the detection of quantitative immunoglobulins. This technique is widely available throughout the country and, generally speaking, is fairly reliable. It is important that one not overemphasize the significance of low normal values of the Igs. Many laboratories are used to thinking of Ig levels in adult ranges. Extremely low levels can be quite normal in young infants, and comparisons should always be made with age-matched normals.

The finding of normal levels of IgA and IgM usually detracts greatly from the significance of a low IgG level. Most cases of significant B cell deficiency show panhypogammaglobulinemia. The exceptions to this are isolated IgM deficiency, a very rare disease, and "dysgammaglobulinemia" in which IgM levels are usually markedly elevated while IgA and IgG levels are markedly diminished. Ambiguous Ig levels can be resolved by functional analysis. It is easy to test for antibodies to antigens to which the patient has had exposure. It is customary to use isohemagglutinins or antibodies such as antistreptolysin-O for this purpose. In addition, one can measure antibody titers against antigens that the patient has received for routine immunization. When the degree of immunization is uncertain, one can purposefully administer an antigen to stimulate the immune response. Diphtheria and tetanus antigens are conveniently used for this purpose. It should be emphasized at this juncture that live virus vaccines should never be used in an individual in whom there is a question of competent immunity. The above analyses are readily available to most clinicians in this country and can form a sound basis upon which the clinician can confidently assign the patient to a significant immunodeficiency state and refer the patient for more definitive diagnosis.

Sophisticated analyses now permit more precise definitions of the defect. A number of surface markers for B cells have been described. Those most relevant for clinical assessments relate to the immunoglobulins. The mature B cell which is capable of secreting an immunoglobulin product has the typical morphology of a plasma cell or lymphocytoid plasma cell.
TABLE III. Clinical symptoms of immunodeficiency*

I. Suggest T cell defect
   A. Systemic illness following vaccination with any live virus or BCG; unusual life-threatening complication following infection with ordinarily benign virus (e.g., giant-cell pneumonia)
   B. Chronic oral candidiasis persisting after 6 months of age and resisting adequate chemotherapy
   C. Chronic mucocutaneous candidiasis
   D. Features of cartilage hair hypoplasia (fine thin hair, short-limbed dwarfism with characteristic X-ray features)
   E. Intrauterine graft-vs-host disease—most characteristic feature is scaling erythroderma and total alopecia (absence of eyebrows quite striking)
   F. Graft-vs-host disease after blood transfusion
   G. Hypocalcemia in newborn (DiGeorge syndrome, especially with characteristic facies, ears, and cardiac lesion)
   H. Small (less than 10 μm in diameter) lymphocyte count persistently less than 1,500/mm³; must rule out gastrointestinal loss, however

II. Suggest B cell defect
   A. Recurrent proved bacterial pneumonia, sepsis, or meningitis
   B. Nodular lymphoid hyperplasia

III. Suggest B and T cell (combined immunodeficiency disease, SCID)
   A. Features of I and II above, except IC and IIIB
   B. Features of wiskott-Aldrich syndrome (draining ears, thrombocytopenia, and eczema)
   C. Features of ataxia telangiectasia

IV. Suggest immunodeficiency without clearly implicating T or B cell defect
   A. Pneumocystis carinii pneumonia
   B. Intractable eczema
   C. Ulcerative colitis in infants (less than 1 yr of age)
   D. Intractable diarrhea
   E. Unexplained hematologic deficiency (erythrocyte, leukocyte, platelet)
   F. Severe generalized seborrhoeic dermatitis (Leiner’s disease) suggests C5 deficiency; seborrhea common in combined immunodeficiency disease
   G. Recurrent pyogenic infections seen in C3 deficiency

V. Suggest biochemical defect
   A. Features of combined immunodeficiency with characteristic bony lesions (adenosine deaminase deficiency)
   B. Features of Diamond-Blackfan aplastic anemia (nucleoside phosphorylase deficiency)

VI. Suggest leukocyte abnormality
   A. Primarily skin infections (if associated with asthma, eczema, coarse facies think of Buckley syndrome²)
   B. Chronic osteomyelitis with Klebsiella or Serratia species, draining lymph nodes (chronic granulomatous disease)

VII. Suggest that the deficiency is secondary
   A. Concomitant or preceding viral infection
   B. Lymphoid malignancy (chronic lymphatic leukemia, Hodgkin’s disease, myeloma)


Its actual ability to secrete immunoglobulin, however, is assessed by the circulating levels of immunoglobulin or by measuring the synthesis and secretion of the various immunoglobulin products after stimulation with a mitogen such as pokeweed.¹⁵ Cells which may have this potential but which do not necessarily undergo the terminal differentiation events bear immunoglobulins on their surface. They comprise approximately 15% of the circulating peripheral blood lymphocytes. Nearly all of these cells bear the IgM and IgD molecules and very few if any carry surface molecules of the other major classes (SLg cells). Many patients with the adult-onset type of hypogammaglobulinemia demonstrate SLg cells in their peripheral blood, suggesting that the problem is one of terminal differentiation. Cells earlier in development than these circulating B lymphocytes have been denoted pre-B cells by Vogler and co-workers.¹⁵ These cells do not have surface immunoglobulin molecules but contain cytoplasmic IgM in a characteristic distribution. Patients with congenital hypogammaglobulinemia seem to have normal numbers of
these cells but do not have surface Ig-bearing cells, suggesting an arrest at an earlier level of differentiation. Two other surface markers of the B lymphocyte series are the aggregated gamma globulin receptor17 and that which reacts with activated complement components C3b, C3d, and C4b.18 The relationship of these cells to the cells defined by ant-immunoglobulin antisera is presently unclear, but they may represent early B cells.

Rarely a subgroup deficiency of IgG can cause increased infectious susceptibility. In these cases, the total IgG level is normal but decreased amounts of one of the subgroups (IgG, 1-4) is found. Webster, Effer, and Asherson19 found that a group of such patients showed low titers of antibodies to a naturally occurring gastrointestinal antigen.

Assessment of T cells. One can get a useful estimate of T cell function by enumerating the number of small (<10 μ) lymphocytes in the peripheral smear; these should be more than 1,500/mm³. Further information readily available for the clinician can be obtained from skin tests using common antigens such as streptokinase-streptodornase, Candida, etc. In the latter instance, adequate antigenic exposure is necessary; this may not have occurred with young infants. To be sure of the degree of exposure, immunization with dinitrochlorobenzene can be performed.

In general, T cell assessment is more difficult than for B cells, and definitive evaluation is more appropriately within the province of the research laboratories.

Most detailed analysis of T cell function can assess the level of differentiation obtained, look for the presence of various subpopulations, measure ability to respond to antigens, and release mediators. These assessments are listed in Table IV.

The level of differentiation cannot as yet be precisely determined in man. In mice, a surface marker system has been well defined and correlates well with the acquisition of functional capability.20 Some hint of the developmental stage can be inferred from the nature of the conditions for sheep erythrocyte rosetting or character of the rosettes obtained. For example, large numbers of attached erythrocytes (>10), termed morula cells, are thought to be immature.21 Rosettes which still form after lymphocyte incubation at 37° are termed “active”; they apparently can be more closely related to those T lymphocytes capable of reacting in proliferative tests. Active rosette counts correlate more with the clinical status of the patient, whereas significant functional T cell deficiency can be found in the face of normal numbers of E rosettes.22, 23

Although as yet incompletely characterized, the study of various antigens found on malignant T cell populations can be expected to represent differentiation antigens by analogy to mouse studies.24, 25

Early studies suggest that the helper and suppressor T cell subpopulations may be characterized by receptors acting with IgM or IgG antibodies, respectively.26 These tests allow enumeration of the subpopulations in a way analogous to quantitative immunoglobulin studies. More direct assessment of the functional capability of these subpopulations is available. Helper T cell function can best be assessed by adding isolated T cell populations to isolated B cell populations or lymphocyte mixtures incapable of T cell functions because of genetic lack. The end point is immunoglobulin synthesis and secretion measured either by fluorescence studies, immunoglobulin quantitation, or plaque assay.15, 27, 28 Suppressor T cell function is measured by inhibition of immunoglobulin production when the study population is in the lymphocyte mixture.15 Excess numbers of suppressors may be intrinsically present in the mixture (e.g., in variable immunodeficiency) or can be generated by concanavalin A stimulation. Killer cell capability is evaluated in 2 ways: (1) Cell-mediated lympholysis (CML) measures a cytotoxic reaction against histocompatibility antigens after in vitro sensitization.29 It can be thought of as an in vitro transplant rejection. (2) Mediator release can be tested for after appropriate stimulation of T lymphocytes by relevant antigens. The most studied of these is migration inhibitory factor (MIF).6 It is possible for some or all of the above functions to be lacking in various diseases, so a complete assessment of T cell capability requires much experience, skill, and technology.

The thymic hormones are just beginning to be defined chemically.30 Some hint of the relative levels of hormone can be gotten from induction of rosette-
forming cells or differentiation antigens. At the moment, these tests are quite difficult to perform and therefore of limited use in large clinical studies. Hopefully, radioimmunoassay will become available soon.

Recently, thymus glands have been established as monolayers or organ cultures. It is now possible to induce thymic surface markers and generate subpopulations from undifferentiated T cell precursors. As these tests become more widely available, it is anticipated that further delineation of subpopulation defects and more rational therapy will be possible.

Biochemical defects

The high correlation of adenosine deaminase deficiency with severe combined immunodeficiency disease (SCID) and the finding that an enzyme deficiency at the next step of the purine salvage pathway was also associated with immunodeficiency have opened a new dimension in the study of host defense failure. It should soon be possible to describe a whole series of molecular lesions associated with disorders of the lymphocytes.

CONCLUSION

The first description of immunodeficiency was made 25 years ago. Laboratory studies available at that time seem incredibly unsophisticated and insensitive compared to the methods utilized today. During this period, simplistic chemical replacement therapy gave way to awesome experiments in cellular reconstitution. A most exciting era is at hand and intracellular correction of genetic errors seems around the corner. Toward that goal efforts to precisely assess the immune status must be directed.

REFERENCES

12. Petlet, J., and Hong, R. (Manuscript in preparation.)
32. Twomey, J. (Personal communication.)