

Pathology in primary immunodeficiency diseases*

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INTRODUCTION

Immunodeficiency diseases are the conditions where the immune response fails to appear, and clinically characterized by recurrent infections extremely resistant to various medical treatments. They can be divided into primary and secondary forms.

Primary immunodeficiency diseases usually exclude hypercatabolic states of immunoglobulins such as nephrosis and protein-losing gastroenteropathy, immunodeficiency states due to immunosuppressive measures and those associated with reticular malignancies. Acquired immunodeficiency syndrome AIDS, used to be classified in primary immunodeficiency disease until the human immunodeficiency virus (HIV) was found to be the main cause of the disease. Primary immunodeficiency diseases are considered to be extremely important as "experiments of nature" in order to investigate and understand immune mechanisms in the human living body.

Immune responses are classically divided into those mediated by humoral antibody and by specific sensitized T lymphocytes. Therefore, laboratory investigations are performed on both B cell and T cell immune functions.

ASSESSMENT OF HUMORAL ANTIBODY RESPONSE

1. Quantitation of serum immunoglobulins

In order to investigate the humoral antibody response, first the serum immunoglobulin levels are quantitated. Serum protein electrophoretic fractionation is done first for screening abnormalities of the serum immunoglobulins. Most IgG is separated in the γ globulin fraction, and IgA and IgM and other minor immunoglobulins are separated in between the β and γ fractions. Therefore, IgG level could be easily estimated from the serum level of the γ globulin fraction.

In order to find out whether IgA, IgM and other immunoglobulins are abnormal qualitatively or quantitatively,

SUMMARY

Primary immunodeficiency is rare, but it is considered to be very important as "experiments of nature" in order to investigate immune mechanisms in human. Laboratory investigations are divided into those for assessment of humoral antibody response including measurements of the serum immunoglobulins and the serum specific antibody titration, and those for assessment of cell-mediated immune response including skin tests and analyses of peripheral lymphocytes. Immunopathological studies of various immune organs are occasionally performed to confirm abnormalities of the patient's immune system. Special attention is given to immunopathological changes in the palatine tonsil in the patients with primary immunodeficiency.

one must perform immunoelectrophoresis and eventually immunochemical quantitation of each class of immunoglobulins.

In primary immunodeficiency disease, various types of immunoglobulin deficiency is frequently encountered with or without T-cell immunodeficiency as shown in Table I.

Agammaglobulinemia and hypogammaglobulinemia:

Agammaglobulinemia is characterized by almost complete absence of the major immunoglobulin classes (IgG, IgA, IgM), demonstrating a complete absence of the γ globulin fraction and no precipitin lines for IgG, IgA and IgM on immunoelectrophoresis. Agammaglobulinemia is recognized in infantile sex-linked agammaglobulinemia, so-called Bruton's disease, severe combined immunodeficiency in some cases and common variable immunodeficiency syndrome. Hypogammaglobulinemia is considered to be a mild form of agammaglobulinemia.

Dysgammaglobulinemias:

Dysgammaglobulinemias are the conditions where one or two classes of the major immunoglobulins is markedly decreased. Among various forms of dysgam-

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Table-I: Various types of immunoglobulin abnormalities associated with primary immunodeficiency diseases.

1. Agammaglobulinemia
2. Hypogammaglobulinemia
3. Dysgammaglobulinemias
4. Dysproportionate κ / λ ratio
5. Oligoclonicity
6. Imbalance of immunoglobulin subclasses
7. Gene defects in immunoglobulin synthesis
8. Monoclonal gammopathy (M-proteinemia)

maglobulinemias, most commonly encountered is isolated IgA deficiency which may be encountered in association with ataxia-telangiectasia.

2. Quantitation of antibody activity

Even though the serum immunoglobulins are quantitatively within normal reference range, one may have deficiency of specific antibodies. Therefore in addition to serum immunoglobulin quantitation, one must screen whether or not any specific antibody deficiency exists. For example, in the patients with Wiskott-Aldrich syndrome, all the major immunoglobulins are within a normal range, occasionally the serum IgM may be slightly decreased because of its hypercatabolism. Yet ABO isoagglutinin is low, because the patients have a unique immunological abnormality, failing to respond to carbohydrate antigen stimulations. One may serially follow the specific antibody response after vaccination.

3. Analyses of B lymphocyte count and functions

Humoral antibody response or B-cell immune response is dependent on normal development of B cell immune system. Therefore, pathogenesis of antibody deficiency could be classified as shown in Table II.

Laboratory investigations for differentiating different types of antibody deficiencies include; 1) B cell identification and counting in peripheral blood, 2) immunoglobulin-synthesizing cell identification by immunofluorescent staining of lympho-plasmocytic cells, 3) immunoglobulin detection and measurement in culture supernatant.

ASSESSMENT OF CELL-MEDIATED IMMUNE RESPONSE

The cell-mediated of T cell immune response can be evaluated in three steps.

1. Peripheral lymphocyte count

The first step is a screening procedure by counting

Table-II: Pathogenesis of antibody deficiencies

1. Absence of pre-B cell
2. Absence of B cell
3. Dysfunction of B cell
 - a. Disorder in differentiation
 - b. Disorder in immunoglobulin secretion
4. Dysfunction of helper T cell
5. Dysfunction of suppressor T cell
6. Enhanced suppressor macrophage
7. Increased loss of immunoglobulins
 - a. Anti-immunoglobulins: anti-IgA
 - b. Increased catabolism: Wiskott-Aldrich syndrome
 - c. External loss: nephrosis, protein-losing gastroenteropathy, etc.

peripheral blood lymphocytes. Since about 60-70% of peripheral lymphocytes are mature T cells, a decreased number of lymphocytes in peripheral blood or absolute lymphopenia may indicate T cell deficiency.

2. Delayed hypersensitivity skin test

Various skin tests are done at bed side, since delayed type hypersensitivity reaction is entirely dependent on T cell immune response. Tuberculin test or PPD skin test used to be a useful screening test, but recently many young people have not exposed to the tubercle bacilli and BCG, so that most young normal individuals are PPD negative. Therefore, non-specific skin test using dinitro-chlorobenzene (DNCB) is usually done. Approximately 90% of normal individuals show a positive reaction, while the patients with T cell deficiency show a negative reaction.

3. Subsets of T cell

Subpopulations and subsets of peripheral lymphocytes used to be determined by various methods of determining surface markers of lymphocyte. Typically B cells have positive surface immunoglobulin, demonstrated by immuno-fluorescent staining. Typically T cells have sheep erythrocyte receptors, demonstrating a positive E-rosette formation.

In Japan, currently, peripheral lymphocyte surface antigens are analyzed by flow cytometry, using different monoclonal antibodies against various CD antigenic determinants. The lymphocytes reacted with fluorescent-labelled monoclonal antibody should fluoresce by laser beam, and thus each lymphocyte could be identified and counted by a flow cytometer.

If a patient is suspected to have functional disorders of lymphocytes, then blastoid transformation of lymphocytes is tested by stimulating with various mitogens or any specific antigen.

4. Histopathology of immune organs

Histopathological examinations of lymphoid tissues are sometimes necessary for the assessment of immune response, including peripheral lymph nodes, thymus and other gut-associated lymphoid tissues.

Peripheral lymph node:

Normal lymph node shows dense lymphocyte infiltration and follicles with germinal centers. The peripheral lymph node is a typical peripheral lymphoid organ controlled by the thymus. Almost characteristic hypoplastic changes are recognized, depending on the type of immunodeficiency.

Thymus:

A high magnification of the mature thymus shows normally Hassall's corpuscle, surrounded by epithelioid cells, dense lymphocyte infiltration. However, a normal fetus of 4 months in gestation still shows scarce lymphocyte infiltration, and Hassall's corpuscle has not been matured yet. The thymus is a central immune organ which controls T cell immune system, in both human and animals. T cell immunodeficiency is frequently associated with thymic hypoplasia, sometimes showing typical histological feature of fetal type.

Gut-associated lymphoid tissues:

In birds, the bursa of Fabricius is a central immune organ controlling B cell immune system. In human, however, no such organs corresponding to the Bursa of Fabricius in birds have found yet.

In human, gut-associated lymphoid tissues are prominent in Peyer's patches in the intestine and the appendix. However, the exact biological functions as a central organ have not been determined yet. In immune deficiency syndrome, gut-associated lymphoid tissues are also hypoplastic, characterized with deficient follicular structure and scanty lymphocyte infiltration.

HISTOPATHOLOGY OF PALATINE TONSILS

One of the most interesting lymphoid organs in human is the adenoid and palatine tonsils. Human has the largest tonsils, and pig has relatively large tonsils among animals. Therefore, we used miniature pigs for our experimental studies of tonsils.

1. Immunological characteristics of the tonsillar lymphocytes

Normal or hypertrophic tonsils removed by tonsillectomy show dense lymphocyte infiltration and follicular structure with germinal centers, as seen in a normal peripheral lymph node. Normally the palatine tonsil shows many lymphocytes migrating through the epithelial surface on both light and scanning electron microscopies. I believe that those lymphocytes exuding out of the tonsil may play an important role for immune resistance

against foreign antigens coming through oral and nasal routes.

Immunohistochemical estimation of lymphocyte subpopulations in the tonsil has proved that much more B cells are contained in the tonsil than in peripheral blood. It was estimated that T cells occupy approximately 20%(13-43%) of the lymphocytes eluted from the palatine tonsils, while about 60%(54-72%) are B cells. All classes of immunoglobulins could be identified on the lymphocyte surface. Also, by immunofluorescent staining we could demonstrate the secretory IgA in the submucosa and also through the epidermis, but no secretory component is identified with the surface IgA.

Another interesting finding in the adenoid and tonsils is an active local synthesis of IgE immunoglobulin. Abundant IgE producing cells are distributed only in the mucosa which are exposed to foreign antigens, and the fact may explain that the mucosa is actually the site where various allergens come in.

2. Site of immunoglobulin production in the palatine tonsils

The site of immunoglobulin production in the palatine tonsils includes the mantle zone of the follicular structure, the epithelial basement membrane and around the blood vessels of the medullary area. IgG and IgM are localized in the mantle zone, while IgG, IgA and IgE are localized in the epithelial basement membrane. All the classes of the immunoglobulins are localized in the medullary area. The IgA-producing cells are more abundant. The ratio between IgG and IgA levels in the tonsil tissue (IgG/IgA) is 3.5 to 4.0, being significantly lower than that in the serum. The tonsils removed from the patients with repeated tonsillitis were demonstrated to contain more IgA than normal tonsils.

3. Pathological findings for the tonsils in primary immunodeficiency

Pathological findings for the palatine tonsils were investigated among 14 cases of primary immunodeficiency, including 2 Bruton-type cases (congenital form and adult form), 1 primary hypogammaglobulinemia, 1 dysgammaglobulinemia, 1 DiGeorge type, 2 Nezelof type, 1 ataxia-telangiectasia and 5 severe combined immunodeficiency. In general, the following conclusions may be possible.

Hypoplastic findings in the lymphoid tissue of the tonsils are parallel to those seen in the peripheral lymph nodes, and the more deficient the cell-mediated immune system the smaller the size of the palatine tonsils. Considering that the tonsils are capable of producing humoral antibodies and of inducing cell-mediated immunity, as previously described, the tonsils certainly function as

one of the peripheral immune organs, but seem to be closely influenced by the thymus. The possibility that the tonsil may play a role as a central immune organ for humoral antibody response may exist. However, it is impossible to produce a condition which is entirely deficient in tonsil tissue either surgically or experimentally, since the adenoid tissue is widely distributed in the nasal and pha-

ryngeal regions. Therefore, it is still uncertain if one can assume that the tonsils play any role as a central immune organ.

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