EDUCATIONAL COMMENTARY – IMMUNOLOGIC TESTING FOR AUTOIMMUNE DISEASES

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LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to:

• explain the relationship between self-tolerance and autoimmunity.
• choose laboratory tests to aid in the diagnosis and treatment of autoimmune diseases.
• discuss HLA antigen associations with autoimmune diseases.
• describe immune processes involved in protection of the host from autoimmunity.

Autoimmune Disease

The immune system is designed to protect the body from malignant cells, foreign organisms, and substances that may cause disease. Self-tolerance protects the body’s own cells and tissues from immune-mediated damage. Tolerance is defined as a state of immune unresponsiveness to an antigen that would normally evoke an immune response. Immune unresponsiveness to self-cells develop in the fetal and neonatal stages of life, when the host’s immune system is immature and unable to recognize antigens as foreign. The process that allows the host to differentiate between self and non-self was described by Nobel Prize–winning researchers Frank MacFarlane Burnet and Peter Brian Medawar, who theorized that clones of immune cells with the ability to respond to self-antigens are deleted during fetal and neonatal development. Many other theories related to acquired immune tolerance exist.

When tolerance fails, the autoimmune disease develops in the host. Autoimmunity is defined as the generation of an immune response against self-cells or tissues that involves self-reactive T lymphocytes, autoantibodies, and inflammation. Other immune mechanisms that protect the host from autoimmune disease are the actions of T-suppressor cells and anti-idiotype antibodies. An anti-idiotype antibody is one that is directed against the antigen-binding site of another antibody, thus, neutralizing it and preventing its attachment to another antigen. One theory of autoimmunity, called molecular mimicry, involves the production of antibodies generated against foreign substances that are similar to self-cells; these antibodies can later cross-react with self-cells.

Over 23.5 million people in the U.S. suffer from autoimmune disease.¹ Over 80 diseases have been identified, and most of those are rare.² Approximately 15 of these diseases are responsible for most cases of autoimmunity. Some of the most common include autoimmune thyroid diseases (Hashimoto thyroiditis and Graves disease), type 1 diabetes mellitus, rheumatoid arthritis (RA), Sjögren syndrome,
systemic lupus erythematosus (SLE), multiple sclerosis, and myasthenia gravis. Autoimmune diseases range from organ-specific diseases, such as Graves disease, to systemic diseases, such as SLE. These diseases occur with greater frequency in women. For example, more than 90% of cases of Sjögren syndrome and SLE occur in women. Estimates indicate that women constitute 78.8% of persons with autoimmune diseases. The increased incidence in women may be linked to the fact that estrogen increases the capacity to produce inflammatory cytokines after infection, resulting in increased antibody production. However, men with autoimmune disease often develop a more severe form.

The presence of certain HLA antigens increases the risk for contracting corresponding autoimmune diseases. The most dramatic example of this phenomenon occurs in persons who are HLA-B27 positive, who are 90 times more likely to develop ankylosing spondylitis than persons who are negative for HLA-B27. HLA-B27 also increases the risk for uveitis, juvenile arthritis, and reactive arthritis. A positive HLA result does not imply that the associated disease will definitely develop, but it reflects a greatly increased risk. HLA associations have also been recognized between Graves disease and HLA DR3; RA and HLA DR4; and Hashimoto thyroiditis and HLA DR3, HLA DR4, and HLA DR5. Autoimmune diseases often occur in members of the same family, often with different forms affecting family members. A person who has an autoimmune disease is also more likely to experience the development of a second autoimmune disease. In addition to genetic predisposition, environmental factors, such as exposure to viral and bacterial infections, radiation, drugs, toxins, and chemicals, are known to be involved in the development of autoimmunity.

**Laboratory Testing**

Assays for autoimmune diseases are not appropriate for screening. Most of the procedures are very sensitive but not very specific, (i.e., the tests produce many false-positive results). Diagnosis is often difficult, because symptoms of different autoimmune diseases may be similar and in many patients flares and remissions occur. Common symptoms include malaise, arthralgia, and fatigue. Only patients with the clinical history and symptoms of an autoimmune disease should undergo laboratory testing. In most cases, serologic testing is used to confirm the physician’s clinical diagnosis. Autoimmune disorders are not diagnosed using a single laboratory test. In most cases, several types of tests are involved. Complete blood cell counts, comprehensive metabolic panels, coagulation studies, acute phase proteins, HLA antigen typing, flow cytometry, and urinalysis, in addition to the more specific immunologic/serologic assays, are performed in the initial examination of the patient when autoimmunity is suspected.

The most common serologic laboratory assays used in the diagnosis and treatment of autoimmune diseases are for C-reactive protein or erythrocyte sedimentation rate, rheumatoid factor (RF), anticyclic citrullinated peptide, and antinuclear antibody (ANA). C-reactive protein or erythrocyte sedimentation rate
is used to detect the presence of inflammation, a common manifestation of autoimmune diseases. C-reactive protein is an acute-phase protein produced in the liver that rises very rapidly in concentration with the onset of inflammation and returns to normal quickly after the inflammation ceases. C-reactive protein is also useful for monitoring the effectiveness of treatment.

Commonly, patients in whom RA is suspected are screened using an RF assay. The assay detects immunoglobulin (Ig)M antibodies directed against the Fc fragments of IgG. Approximately 70% to 80% of patients with RA test positive for RF, but a negative result does not rule out RA. Patients with other autoimmune diseases, such as Sjögren syndrome (20%-30%), SLE (15%-35%), and systemic sclerosis (20%-30%), also test positive. Low-titer RF is often detected in persons with no apparent disease. Titers of RF also increase with age and are frequently elevated in the elderly. The RF assay is available in several technologic formats. It may be performed using an agglutination assay, immunoturbidimetric assay, or enzyme-linked immunosorbent assay (ELISA). Persistent high concentrations of RF predict more serious disease that leads to joint erosion. Anticitrullinated protein antibodies are a class of antibody assays used to predict aggressive disease. The most commonly performed anticitrullinated protein antibody assay is the anticyclic citrullinated peptide assay, which is used as a confirmatory test for RA. Its sensitivity is comparable to that of RF, but the second generation assay has a much higher specificity. A positive result predicts a more aggressive form of the disease, often long before the patient experiences symptoms. Patients with positive results are prescribed more aggressive therapy, such as disease-modifying antirheumatic drugs, in an effort to prevent severe joint damage.

Antinuclear antibody testing is used in the diagnosis of many autoimmune diseases, such as SLE, Sjögren syndrome, scleroderma, RA, and mixed connective tissue diseases. The criterion standard assay for ANA is the indirect immunofluorescent assay. Results of ANA testing are reported as 1 of 4 major patterns, homogeneous, rim, speckled, or nucleolar, along with the titer. The first laboratory test performed when SLE is suspected is an ANA. About 95% of patients with SLE test positive, but a negative result does not rule out SLE. With an ANA titer > 80, the most common pattern in patients with SLE is homogeneous although SLE may also show any of the 4 patterns. Homogeneous patterns are then tested for double-stranded DNA antibodies. This is the most specific test for SLE and is considered diagnostic when found in combination with low levels of complement C3. The most widely used method for detecting anti-ds-DNA is an enzyme immunoassay. An indirect immunofluorescent assay using *Crithidia luciliae* as the substrate can also be used to detect anti-ds-DNA. A speckled pattern may result when testing the serum of patients who have SLE, RA, Sjögren syndrome, progressive systemic sclerosis (scleroderma), or mixed connective tissue disease. Specimens from patients with scleroderma or Sjögren syndrome usually show a nucleolar pattern. Specimens with positive speckled or nucleolar patterns are tested for extractable nuclear antigens (ENA) to aid in the diagnosis of specific autoimmune diseases.
Anti-Sm is considered diagnostic for SLE. Anti Scl-70 is found in patients with scleroderma. Anti-Jo-1 is present in serum of patients with polymyositis. Anti-SS-A and anti-SS-B are found in SLE and Sjögren syndrome. Anti-RANA occurs in patients with RA.

Conclusion

Autoimmune diseases occur in nearly 8% of the US population, causing a large number of deaths and disabilities. Many of these diseases are treatable, but none are curable. Early diagnosis using laboratory assays is important for the timely initiation of treatment to prevent serious irreparable damage. Diagnosis is difficult, because patients experience remission and exacerbations, and symptoms are similar among the various autoimmune diseases. Laboratory testing alone is not diagnostic, but with careful physical examination and evaluation of the patient’s symptoms, results of assays can be important tools for the diagnosis and monitoring of disease progression.

References


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