Autoimmunity case*

L.R. is a 26-year-old woman who presents to the Emergency Room complaining of a 2 month history of increasing fatigue.

**History:** The patient was in good general health until 6 months ago when she noted the onset of arthralgias and swelling of the small joints of her hands and feet. Two months ago she noted that she was beginning to lose some hair with excessive hair coming out during combing. Four weeks ago she went to Hawaii on vacation and developed an erythematous rash over her face and upper body with sun exposure. Since returning 3 weeks ago, she has noted gradually progressive fatigue with some dyspnea on exertion. She now has swelling of her ankles. She denies chest pain, fever or chills.

**Family history** reveals that her sister also has arthritis and rash.

**Physical examination** a pale, thin white female, appears chronically ill. BP 140/100, P 120, RR 28, T 99°F. **Skin:** Erythematous rash over the cheeks and over the bridge of the nose but sparing the nasolabial fold. She has spotty alopecia throughout her scalp without evidence of scarring. **HEENT:** Pale conjunctivae but is otherwise within normal limits. **Lungs:** Clear to auscultation without pleural rubs. **Heart:** Sinus tachycardia. **Joint exam:** Synovitis in small joints of both hands. **Neurologic examination:** Normal. **Laboratory evaluation:** Hematocrit 19 (nl 40-45), WBC 2,400 (nl 5000-10,000), platelets 90,000 (nl 150,000-400,000), BUN 28 (nl <15), creatinine 2.4 (nl <1.2). Urinalysis: 10-20 RBC, 4+ protein. **Chest x-ray:** Normal. **EKG:** Normal. **EKG:** Sinus tachycardia.

**Questions:**

1. What disease do you think this patient has and why?

2. Discuss the potential bases of this patient's anemia and the mechanisms by which RBCs are destroyed in this disease. What is the basis of the low WBC and platelet count?

3. What are the major groups of autoantibodies produced in this disease?

4. Contrast the pattern of autoantibody production seen in this disease with the pattern seen in myasthenia gravis.

5. Describe the immunopathologic mechanisms involved in this patient's kidney disease. How would you compare this type of kidney disease with the disease seen in:
   a) Goodpasture's syndrome?  b) Serum sickness?

6. Is this primarily a B cell or T cell disease? Discuss.

7. Discuss the risk factors which L.R. has for developing this disease (environmental and genetic).

8. Discuss the various theories used to explain autoimmunity.
I. Definition and Terminology
   A. **Autoimmunity**: Autoimmunity is defined as the generation of an immune response directed against self. An autoimmune response results in the body's attempt to remove or destroy self-antigens which it recognizes erroneously as foreign. The result is an immune response directed against self-causing destruction of normal tissues. Clinically, autoimmunity can be divided into two categories, organ specific and systemic autoimmunity.

   1. **Organ specific autoimmunity** is defined as an immune response directed against a single autoantigen or a restricted group of autoantigens within a given organ. The result is autoimmune destruction of only those organs expressing the relevant autoantigens. Examples of organ-specific autoimmunity include myasthenia gravis (antibodies to acetylcholine receptors), Goodpasture's syndrome (antibodies to basement membrane type IV collagen of the kidney and lung), autoimmune thyroiditis, and type I diabetes mellitus.

   2. **Systemic autoimmunity** is defined as an immune response against multiple autoantigens rather than to autoantigens of a given organ. The resulting disease affects multiple organs both on the basis of circulating immune complexes and direct immune attack against organs. The prototype systemic autoimmune disease is systemic lupus erythematosus (SLE). Many of the rheumatic diseases have features of systemic autoimmunity, including Sjögren's syndrome and mixed connective tissue disease. Other rheumatic diseases are felt to be autoimmune in origin and have features of systemic autoimmune disease although they may focus on specific organs. Examples of this include polymyositis (muscle) and rheumatoid arthritis (synovium of the joints).

   B. **Systemic Lupus Erythematosus (SLE)**: SLE is a chronic, systemic autoimmune disease which affects multiple organ systems including the skin, joints, serosal surfaces (pleura and pericardium), kidneys, central nervous system, lungs, and hematologic system. For most of the disease manifestations of SLE, antibody-mediated effector mechanisms appear to be operative. Organ damage can result from either type II mediated immunologic damage (direct antibody binding to specific cells or tissues), or type III mediated immunologic damage (formation of immune complexes).

II. Clinical Features
   SLE is a chronic, systemic autoimmune disease which affects multiple organ systems including the skin, joints, serosal surfaces (pleura and pericardium), kidneys, central nervous system, lungs and hematologic system. There is no specific marker which is diagnostic for the disease, and in reality SLE is a syndrome composed of multiple manifestations. The clinical manifestations of SLE many vary over time within a given patient and may vary dramatically from patient to patient. Thus, one patient may have predominantly renal disease whereas another may have predominantly hematologic and/or CNS disease. Specific clinical features are described in detail in the pathology section.
III. Epidemiology
A. SLE is a disease primarily of the young women with a female to male ratio of 9:1, with onset after puberty reaching a peak during the childbearing years. The prevalence varies in different populations and varies from 0.5 to 5 per thousand and is more common in certain ethnic groups, particularly African Americans, Asians, and Hispanic Americans.

B. Predisposing Factors
1. Genetic: Although the etiology of SLE is unclear, there is overwhelming evidence for a genetic predisposition.
   a. Increased incidence of SLE among relatives of patients. (relative risk 2-3).
   b. Twin studies showing a concordance rate of about 35% in monozygotic twins compared to ≈ 2% in dizygotic twins.
   c. Association of SLE with HLA-DR3, and C4A null alleles (strongest association).
   d. Interferon (IFN)-α and IFN-β upregulate the expression of a variety of genes in lymphocytes. This “IFN signature” of gene expression is more prevalent in patients with active SLE.
   e. In the murine models of SLE, multiple gene loci appear to be involved in a complex fashion. In the NZB/NZW mouse model of lupus, one involved locus is linked to the MHC and another to an IFN inducible gene. Other involved loci are being studied.

2. Environmental: The expression of disease manifestations can be greatly affected by environmental factors.
   a. Sex hormones. There is a markedly increased incidence of SLE in women of childbearing age. The female to male ratio is approximately 9 to 1. The ratio is much closer to 1:1 if patients are pre-pubertal or postmenopausal, strongly suggesting that sex hormones affect the expression of disease. The disease-accelerating effect of estrogens and the protective effect of androgens have been elegantly demonstrated in the NZB/NZW murine lupus model.
   b. Sun exposure. SLE skin disease can be exacerbated by exposure to U.V. light (photosensitivity). Indeed, patients can sometimes have marked systemic or generalized flares of disease after excessive sun exposure.

IV. Pathology
A. For most of the disease manifestations of SLE, antibody-mediated effector mechanisms appear to be important. Organ damage can result from either type II mediated immunologic damage (direct antibody binding to specific cells or tissues), or type III mediated immunologic damage (formation of immune complexes). The pathology of disease reflects the following mechanisms:

B. Specific antibody-mediated disease (Type II):
1. Hemolytic anemia (Coombs' positive):
   Although most patients with SLE have a low red blood cell count (anemia of chronic inflammation), a minority (~ 10%) manifest clinically significant red blood cell destruction (hemolysis). These patients exhibit a positive direct Coombs' test, and most can be demonstrated to have both antibody (IgG) and complement on the red cell surface. The mechanism of red cell destruction is identical to that in other forms of autoimmune hemolytic anemia; the IgG and complement bound to the
RBC results in sequestration and destruction of these cells in the reticuloendothelial system of the liver and spleen (via Fc and complement receptors).

2. **Anti-Phospholipid Antibodies** (the lupus anticoagulant): Some patients with SLE produce antibodies to phospholipids, which can block prothrombin activation in the clotting cascade. This results in an elevated partial thromboplastin test (PTT), suggesting a clotting factor abnormality. However, this "anticoagulant" is associated with increased clotting. The exact mechanism by which antiphospholipid antibodies (aPL) cause clotting is still unknown. In animal models of experimentally-induced antiphospholipid syndrome, aPL appear to play a pathogenic role. Another serum cofactor (β₂-glycoprotein I), a powerful natural anticoagulant, is necessary to enhance the binding of aPL to phospholipids. In patients with autoimmune disorders, aPL are directed against a complex antigen of which β₂–glycoprotein I is an essential component. It is possible that β₂–glycoprotein I binds to platelets forming the epitope for aPL binding with resultant platelet aggregation and thrombotic events. In addition, several processes such as infection, trauma (including surgical procedures), pregnancy, withdrawal of anticoagulation, and drug administration (oral contraceptives, estrogens, and sulfur containing compounds) are additional risk factors or triggers for thrombosis in patients with aPL. Any process that causes endothelial cell activation (infection, trauma) could result in the binding of aPL to β₂–glycoprotein I. Antiphospholipid antibodies could neutralize the anticoagulant effects of β₂–glycoprotein I resulting in thrombosis. In mouse pregnancy experiments, complement activation, mediated by aPL binding to endothelial cell surfaces, may cause damage to endothelial cells resulting in thrombosis and fetal wastage. Thus, the beneficial effect of heparin in treating and preventing thrombosis in the aPL syndrome may in part act through its inhibition of complement activation.

3. **Central Nervous System Manifestations**: Neuropsychiatric manifestations (depression, cognitive dysfunction, psychosis, organic brain syndromes, and seizures) occur in up to 66% of patients with SLE. Autoantibodies that bind to neurons (anti-neuronal antibodies) may account for some of these CNS manifestations. Other forms of CNS involvement such as stroke and movement disorders (chorea) are due to vascular ischemia from vasculitis, thrombosis due to aPL, or embolic disease.

C. **Immune complex mediated disease (Type III):**

1. **Lupus nephritis**

   The most important determinant of prognosis in SLE is the presence and degree of kidney involvement (especially glomerular involvement). Nearly all patients with SLE will have abnormalities on renal biopsy, and over 50% will have clinical evidence of renal disease. However, the extent of renal damage and the clinical course vary considerably. In almost all SLE patients, histologic evaluation reveals immune complex and complement deposition in the glomerulus, as demonstrated by electron microscopy and immunofluorescence microscopy. This is the result of deposition of circulating immune complexes or binding of antibodies directly to glomerular antigens.

   Antibodies to double-stranded or native DNA (dsDNA) of the IgG class and DNA-anti-DNA immune complexes may be involved in the pathogenesis.

   Once complexes are formed or deposited in the glomerulus, complement activation...
is important for pathologic damage to occur. Thus, complement-fixing anti-DNA antibodies are important causes of damage in lupus glomerulonephritis.

D. Antinuclear Antibodies (ANA):
Antinuclear antibodies are the hallmark of abnormal antibody production in SLE. Over 95% of patients with SLE have evidence of excessive ANA production, as evidenced by elevated serum levels. Antibodies are directed to multiple nuclear antigens, including DNA, RNA, histone, and others. This antibody response is a classic example of systemic (as opposed to organ specific) autoimmunity. ANAs are not specific for SLE as they can occur in other autoimmune disorders.

1. ANA detection. ANA are detected in most laboratories by an indirect immunofluorescence assay. The substrate (human epithelial cell tumor line) is "fixed" onto a slide and thus is permeable to antibodies. Diluted serum from a patient is then placed onto the tissue. After washing, any antibodies that have bound to nuclei are detected by adding a fluorescein-conjugated anti-human Ig antisera.

2. Specific antinuclear autoantibody systems. The above test will detect most antibodies to nuclear antigens. Laboratory tests have also been developed to measure ANA with particular specificities. Some of these antibodies are now routinely measured by radioimmunoassay (RIA), enzyme-linked immunoassay (ELISA), and immunoprecipitation.
   a) Antibodies to DNA: Antibodies to double-stranded or native DNA (anti-dsDNA) are highly specific for SLE. As discussed above, they appear to be especially important in renal disease. Antibodies against single stranded or denatured DNA have much less specificity for SLE.
   b) Antibodies to histones: These antibodies are frequently present in both SLE and in drug-induced lupus.
   c) Antibodies to non-histone, non-DNA nuclear antigens: Some of these antibodies have been associated with specific disease manifestations. For example, antibodies to SS-A antigen have been associated with neonatal lupus as well as photosensitivity. The importance of ANA in tissue damage other than those that form immune complexes (i.e. anti-dsDNA) is presently unclear.

V. Pathophysiology
A. The fundamental defect in autoimmunity is the misdirected recognition of self as foreign, resulting in an autoimmune response. The etiology of autoimmune disease is not known, and probably autoimmunity can arise by several pathways. The following theories have been put forth to explain autoimmunity.

1. Loss of T cell tolerance: Normal individuals possess autoreactive B cells which continuously produce low levels of "natural" autoantibodies. These autoantibodies are usually IgM and bind with low avidity to their antigens. T cell help is required to stimulate these cells and induce somatic mutation to allow them to produce high avidity pathogenic IgG antibodies. Autoreactive T cells are controlled in two ways. The major mechanism appears to be deletion of autoreactive T cells by programmed cell death (apoptosis) during thymic development ("central tolerance"). In addition, peripheral tolerance occurs either by deletion of activated cells (by apoptosis) or by induction of a state of unresponsiveness called anergy. In most animal models of SLE, central tolerance is intact. However, in some
mouse models of SLE, peripheral tolerance is "broken", resulting in emergence of autoreactive T cells.

2. Polyclonal B cell activation: Since we all possess autoreactive B cells, agents such as LPS (endotoxin) or unknown factors may polyclonally stimulate B cells resulting in increased production of all antibodies.

3. Molecular mimicry: An exogenous antigen with molecular similarities to autoantigens may be introduced to the immune system, resulting in an appropriate immune response which then cross-reacts with self-antigens. The classical example of this is rheumatic heart disease, but this model may also apply to rheumatoid arthritis and other autoimmune diseases.

4. "Illicit help" - A foreign antigen may be combined with an autoantigen such that the foreign antigen may be processed and presented to the T cell, thereby allowing the T cell to provide help. Because of the autoantigen component, the combined antigen may also be capable of binding directly to B cells and receiving the "illicit" T cell help provided by the foreign antigen.

5. Sequestered antigen: This hypothesis states that certain autoantigens are kept in sequestered compartments such as in the eye, and are never seen by the immune system. If tissue damage occurs, these antigens may be released, thereby eliciting an immune response. Although this mechanism may apply in autoimmune eye disease, such as some forms of uveitis, most autoantigens appear to be ubiquitous and this mechanism probably does not apply to most forms of autoimmunity.

6. Immunodeficiency: Individuals with complement deficiencies of C1q, C4 and C2 have a very high incidence of an SLE-like disease. Fc receptor deficiencies are also associated with SLE. Some individuals believe that SLE is a manifestation of immunodeficiency leading to ineffective clearance of immune complexes and that loss of tolerance is also related.

B. Cellular Immune Mechanisms Involved in the Excessive Production of Autoantibodies:

The best study systems to investigate the cellular immune mechanisms involved in ANA production have been murine models of lupus. SLE patients are nearly always studied after they present with clinical disease. Thus, immunologic abnormalities that are "causal" are difficult to separate from those that are secondary to the disease.

Some inbred strains of mice, including the New Zealand Black x New Zealand White hybrid (NZB/NZW F1), MRL-\(lpr/lpr\), and BXSB strains, spontaneously develop a syndrome characterized by high levels of ANA and a fatal lupus-like glomerulonephritis. Lupus-like disease and ANA production can also be induced in certain strains by creating graft-versus-host disease.

Although multiple autoantibodies (including ANA) are produced in SLE and murine models of SLE, antibodies are not produced to all autoantigens. Therefore, autoantibody production does not appear to be simply due to polyclonal activation of autoreactive B cells. There is also evidence that T cell help is necessary for the production of pathogenic antibodies and clinical disease. In the MRL-\(lpr/lpr\) mouse, a defect in the induction of lymphocyte apoptosis by Fas results in the development of SLE-like disease. Altered apoptosis in human SLE has been demonstrated.
VI. Treatment as it Relates to Pathophysiology

Treatment of SLE is directed at decreasing exposure to disease triggers (sun blocks), decreasing the inflammatory response [NSAIDs, and corticosteroids (both topical and systemic)], and decreasing the cellular immune response (anti-malarials and immunosuppressive drugs including azathioprine, mycophelolate, and cyclophosphamide). Other treatment modalities such as intravenous immunoglobulin (IVIG) have been shown to work in certain autoimmune disease states but there is no clear mechanism for their efficacy.

VII. Key Points

1. SLE is a multigenic disease with a major MHC contribution.
2. The pathophysiology of SLE involves both Type II and III antibody-mediated mechanisms.
3. Both T and B cells are necessary for SLE to develop.
4. SLE flares can be triggered by environmental exposures such as the sun.
5. The kidney is a major target organ in SLE, and the extent of damage is directly correlated with overall patient outcomes.
6. The presence of high-titer ANA in SLE patients is a manifestation of loss of tolerance to self antigen as well as a useful diagnostic marker.

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