Autoimmunity and Autoimmune Disorders

Reading: Stevens, pages 212-232

Computer-assisted instruction:
ANA Tutor – University of Washington cd-rom (or website)

I. The concept of autoimmunity
   A. Autoimmunity represents a state of immunologic self-reactivity, specificity of self-antigens or auto-antigens by immunoglobulins or T-cells. **There is a breakdown of the immune system's ability to discriminate between self and non-self.** Autoimmunity is a disruption of the normal tolerant state the body has to self-components.
   B. Classification of autoimmune diseases
      Autoimmune disease are grouped based on the type of antibody that is produced; antibodies can be produced to antigens specific for organs affected by the disease or antibodies can be produce to non-organ specific constituents such as nucleoproteins or connective tissue.
      1. Multi-system - react with nuclear or cytoplasmic antigens
         Examples: Systemic lupus erythematosus (SLE)
                   Scleroderma
                   Rheumatoid arthritis (RA)
         a. Antibodies and lesions are non-organ specific
         Rheumatic or connective tissue diseases are non-organ specific autoimmune diseases producing antibodies to nuclear antigen – ANA.
            When these antibodies react with their specific antigen, immune complexes are formed that may become deposited in various areas of the body. The immune complexes activate the complement system that results in inflammation of the area surrounding the complexes.
         b. Antigens are accessible at higher concentrations (as compared to organ specific disorders)
         c. Lesions caused by deposition of antigen-antibody (immune) complexes
      2. Organ-specific - resulting tissue damage and autoantibodies produced are directed at a single target organ
         Examples: Thyroiditis
                   Chronic liver diseases (primary biliary cirrhosis, chronic active hepatitis)
                   Pernicious anemia
                   Myasthenia gravis
         a. Antibodies and lesions are organ specific
         b. Antigens only available to lymphoid system in low concentrations
         c. Tendency to develop cancer in the organ
   C. Factors influencing the development of autoimmunity
      1. Genetic factors
      2. Age
      3. Exogenous factors
         a. Viral transformation
         b. Drug transformation
         c. Newly exposed antigens

II. Multi-system diseases (systemic)
   A. Systemic lupus erythematosus (SLE, Lupus)
      The prototype of immune complex disease is SLE
      1. Disease State
         a. Autoimmune systemic disease involving mostly skin, kidneys, joints and serous membranes
         b. Vasculitis (i.e., inflammation of a vessel) usually involving many organ systems
c. Skin lesions usually in the form of a red rash across the nose and upper cheeks - maculopapular (butterfly) rash or photosensitivity
d. Symptoms may include: Fever, weight loss, malaise, arthralgia (joint pain) arthritis, increased susceptibility to common opportunistic infections, glomerulonephritis, photo-induced skin lesions, anemia, migratory arthritis without joint destruction, myalgia, pleural and pericardial effusions, seizures, alopecia, hemolytic anemia, purpura, nephritis (50% have central nervous system involvement), and peripheral neuropathy. The symptom complex of SLE is inconsistent in duration, severity, and combination of individual symptoms.
e. SLE occurs more frequently in women than men. Non-white women in early and middle adulthood have the highest mortality rate. Prior to corticosteroid treatment, average life expectancy for SLE was two years. Also, only more obvious and advanced cases of SLE were recognized. With treatment a nearly normal life span is expected.

2. SLE Laboratory Diagnosis
a. ANA's (antinuclear antibodies) are present
Detection of ANA in patient’s serum by indirect fluorescence utilizing Hep-2 Cells (human epithelial cells) as a substrate

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<th>Major Patterns</th>
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<th>Characteristics</th>
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<td>Homogeneous (diffuse)</td>
<td>DNA, Histones</td>
<td>• Uniform fluorescence of entire nucleus</td>
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<td>Peripheral (rim)</td>
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<tr>
<td>Speckled (fine &amp;</td>
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<td>• Numerous small uniform points of fluorescence</td>
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b. LE cell: either a normal segmented neutrophil or other phagocyte cell with engulfed homogeneous and swollen nucleus of either a neutrophil or lymphocyte
In vitro test: expose PMN nucleus, LE factor (an antinuclear antibody) will attach to nucleus and cause the nucleus to undergo changes, it will round up and become homogeneous, neutrophils will then see the changed nucleus as foreign and will phagocytize it
c. Detection of autoantibodies of nDNA (dsDNA) via indirect fluorescence

Substrate - hemoflagellate: *Crithidia lucilliae*’s kinetoplast rich in nDNA

d. EIA tests developed for specific antibody detection
e. Double immunodiffusion used for detection of specific antibodies
f. Sensitivity & specificity of tests for SLE:

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
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<tbody>
<tr>
<td>ANA by IFA</td>
<td>Sensitive</td>
<td>Not specific</td>
</tr>
<tr>
<td>nDNA</td>
<td>Sensitive</td>
<td>Specific</td>
</tr>
<tr>
<td>LE cell prep</td>
<td>Not sensitive</td>
<td>Specific</td>
</tr>
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</table>

g. Other laboratory findings:
- Increased serum immunoglobulins
- Decreased levels of C3 and C4

B. Rheumatoid Arthritis (RA)
1. Chronic inflammatory disease primarily affecting the joints and periaricular tissues due to the formation of immune complexes. It is a debilitating disease affecting women two or three times more frequently than men. It can occur at all ages but most commonly in the fourth decade.
2. Symptoms may include: fatigue, weakness, anorexia, weight loss, and malaise with fever and joint pain. Stiffness often develops after periods of inactivity and eventual muscle atrophy near affected joints.
3. The inflammatory joint changes, which most often affect the small joints, may result in loss of function or permanent deformity.
4. **Rheumatoid Factor:** several abnormal proteins circulate in the blood of patients with RA. They are collectively known as “rheumatoid factor.” They are a group of immunoglobulins (IgG, IgM and rarely IgA) that interact specifically with the Fc portion of IgG.

   Rheumatoid Factor can occur in non-rheumatoid individuals with chronic infections (i.e., SLE, infectious hepatitis, chronic hepatic disease, syphilis)
5. Screening test for rheumatoid factor
   a. Latex agglutination: latex particles coated with IgG molecules
   b. If rheumatoid factor is present it attaches to the Fc portion of IgG and agglutinates latex particles

C. Scleroderma (SCL) or Progressive Systemic Sclerosis (PSS)
1. PSS is a generalized disorder of the connective tissue characterized by diffuse fibrosis involving the skin and several internal organs (lung, heart, kidney and GI tract). This collagen vascular disease affects women more often than men.
2. Degenerative changes of the skin (scleroderma)
3. Vascular abnormalities – **Raynaud’s phenomenon:** pain in the extremities when exposed to cold temperatures
4. **CREST syndrome:** is a milder form of scleroderma most often limited to the skin (often face and fingers only)
   - Calcinosis: abnormal calcium deposits in tissue
   - Raynaud’s phenomenon
   - Esophageal dysmotility: difficulty in swallowing
   - Sclerodactyly: scleroderma of fingers & toes
   - Telangiectasia: dilation of a group of small blood vessels
5. Laboratory findings:
   - Positive ANA with speckled or nucleolar pattern
   - With CREST see centromere pattern
D. Mixed Connective Tissue Disease (MCTD)
1. Combined clinical features of SLE, Scleroderma and RA with no kidney involvement
2. Laboratory findings:
   - Positive ANA with speckled pattern
   - Can have RF
   - Hypergammaglobulinemia
   - AntiDNA is absent

E. Sjögren’s Syndrome (SS)
1. SS is a chronic inflammatory rheumatic disease and often occurs secondary to RA, scleroderma, SLE or polymyositis (inflammation of numerous muscles at once)
2. Symptoms include dry eyes (lack of tears) and dry mouth with scanty, sticky saliva. The disease can occur at any age but is most often occurring in women between 30-60 years. Approximately 50% of patients have intermittent salivary gland enlargement, and approximately 50% of patients will also have rheumatoid arthritis.
3. Laboratory findings:
   - Positive ANA with speckled pattern
   - RA factor
   - Hypergammaglobulinemia

F. Review of ANA patterns by IFA with disease states
(See color plates posted on MT418 Blackboard course in Serology Folder)

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<td>Extractable nuclear antigens (ENA)</td>
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<td>CREST, SLE</td>
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III. Organ/Tissue Specific Diseases
A. Chronic Liver Disease
1. Primary biliary cirrhosis:
   a. Unknown etiology, inflammatory reaction of bile ducts, jaundice, putitis, malabsorption, may be self-limiting or ducts become more occluded leading to cirrhosis and end stage liver disease
   b. Serology: anti-mitochondrial antibody (AMA)
2. Chronic active hepatitis:
   a. Inflammation of liver with dysfunction
   b. Serology: anti-smooth muscle antibody (ASMA)

B. Pernicious anemia:
   1. Inflammation of gastric mucosa with inability to secrete HCL, intrinsic factor and pepsin followed by the development of macrocytic anemia
   2. Serology: anti-parietal cell and anti-intrinsic factor

C. Thyroid Diseases
   1. Hashimoto’s Thyroiditis (Autoimmune Thyroiditis):
      a. Enlargement of thyroid, hypothyroid
      b. Serology:
         Anti-thyroglobulin antibody
         Anti-microsomal antibody
   2. Grave’s Disease:
      a. Hyperthyroidism, 50% have long-acting thyroid stimulator (LATS), 90% have thyroid-stimulating immunoglobulins (TSI)
      b. Serology: Anti-TSH receptors

D. Other Examples of Organ or Tissue Specific
   1. Diabetes (Juvenile): anti-pancreatic cell
   2. Addison’s disease: anti-adrenal cortex
   3. Myasthenia gravis: anti-acetylcholine receptor
   4. Multiple sclerosis (MS): anti-myelin sheath
   5. Autoimmune hemolytic anemia: anti-red cell antigens
   6. Goodpasture’s disease: anti-glomerular basement membrane

Correlation of Specific Antinuclear Antibodies

1. Nuclear autoantibodies are common to many connective tissue diseases including SLE, RA, SS, SCL or PSS, and MCTD. Since the nuclear autoantibodies are directed against specific components of the nucleus, characteristic fluorescent patterns are demonstrated. However, a given autoantibody may be associated to more than one fluorescent pattern (i.e., homogeneous versus peripheral) and a given pattern may have several corresponding autoantibodies (i.e., speckled: SM, RNP, Scl-70, SSB, etc.). Consequently, a lucrative correlation exists between fluorescent patterns and specific connective tissue diseases.

NOTE: Patient sera containing more than one antinuclear antibody at different strengths may result in fluorescent patterns changing with progressive dilutions.

2. Often specific identification of nuclear autoantibodies or groups of autoantibodies correlates better to one or more connective tissue disease than the fluorescent pattern. Occasionally quantitation of these autoantibodies has been useful in differentiation among connective tissue diseases. Certain nuclear autoantibodies are highly specific and disease. Certain nuclear autoantibodies are highly specific and may considered markers for a given connective tissue disease.

3. When 3 or 4 different ANA patterns are observed simultaneously the probability of SLE is approximately 90%.

4. The normal population usually has negative results of autoantibodies. However, apparently healthy people over 50 years may have positive results without association to disease.

5. Characteristics associated with specific antinuclear antibodies:
   a. Deoxyribonucleic acid (DNA)
      At least 3 major classes of antibodies to DNA have been identified:
      Antibodies to double-stranded DNA (dsDNA)
      Antibodies to single-stranded DNA (ssDNA)
      Antibodies with specificity for both dsDNA and ssDNA
      Antibodies to dsDNA and antibodies with specificity for ssDNA and dsDNA are often referred to as antibodies to native DNA (nDNA).
Antibodies to only dsDNA are found in SLE but are relatively rare. These antibodies produce a rim and/or homogeneous IFA pattern.

Antibodies to ssDNA are found most frequently in SLE but can also be found in other rheumatic and non-rheumatic diseases. ssDNA antibodies are not detected by routine IFA.

The DNA antibodies with reactivity for ssDNA and dsDNA are the most frequent DNA antibodies in patients with SLE and produce a rim and/or homogeneous pattern. These antibodies can be found in other rheumatic diseases in low titer.

High levels of nDNA antibodies have a positive correlation with SLE and nephritis due to the presence of DNA-anti-nDNA immune complexes causing inflammation of the kidney. (Antibodies to dsDNA fix complement and form insoluble complexes that are deposited in the basement membrane of the glomerulus, resulting in glomerulonephritis and proteinuria.) Immune complexes may also lodge in the skin and vascular system.

d. Deoxyribonucleoprotein (DNP)
Antibodies to deoxyribonucleoprotein (DNP) have been called the LE cell antibodies, as they are responsible for the LE cell phenomenon that is positive in 60-70% of SLE cases. DNP antibodies are relatively specific for SLE and produce a rim and/or homogeneous pattern with IFA testing. Antibodies to DNP can be measured by RIA, EIA, immunodiffusion, CIE or passive agglutination. A latex agglutination test for DNP antibodies has been reported to lack sensitivity and is not recommended for screening.

c. Histone
Histone antibodies also produce a homogeneous immunofluorescent pattern. They are found in patients with drug induced lupus usually to the exclusion of other types of ANA characteristically found in SLE. Histone antibodies can also be detected in SLE patients along with other antibodies found in SLE. Histone antibodies can be identified with an immunofluorescent extraction procedure or more recently developed RIA and ELISA techniques.

d. Extractable Nuclear Antigens (ENA)
Extractable nuclear antigens (ENA) are non-histone antigens that are extractable form calf or rabbit thymus. A number of ENA antibodies have been identified.

i. Smith (Sm)
The Sm antibodies are considered highly specific marker antibodies for SLE and are found in 25-30% of these patients. Sm antibodies are rarely found in other connective tissue diseases. Sm antibody produces a speckled fluorescent pattern.

ii. Ribonucleoprotein (RNP)
The RNP antigen is closely associated with the Sm antigen. Anti-RNP can be found in a variety of rheumatic diseases such as SLE, RA, SS, PSS, and MCTD. Patients with MCTD have high titers of RNP antibodies to the exclusion of other types of ANA.

iii. Sjögren’s Syndrome
SSA and SSB antibodies are identical to the previously described Ro and La antibodies respectively.

SSA antibodies are found in 70% of patients with Sjögren’s Syndrome and 40% of SLE patients. SSB antibodies, although found in SLE, are primarily considered a marker antibody for Sjögren’s Syndrome and are found in 48% of these patients. Recently there has been a great deal of interest in the association of SSA antibodies, neonatal lupus and congenital heart block. The SSA antibodies are very significant to detect especially in pregnant women.

SSB antibodies produce a speckled fluorescent pattern. SSA antibodies are not detected in tissue substrates due to the low antigen concentration. Human cell lines such as the Hep-2 cell contain enough antigens to detect the SSA antibody when the substrate has been properly fixed. The immunofluorescent pattern is a speckled reaction.
e. Scleroderma-70 (Scl-70)
Scl-70 antibodies are very specific for PSS patients but are found only in 20-30% of these patients. Scl-70 antibodies produce a finely speckled staining with or without nucleolar staining. The Scl-70 antigen has been found to be chromosome associated and therefore will show a positive chromosome reaction with Hep-2 cells.

f. Nucleolar
Nucleolar antibodies can be detected in up to 90% of PSS patients, usually in high titers. These antibodies can be found in other rheumatic diseases such as SLE but generally in low titers.

Nucleolar antibodies produce the characteristic staining of nucleoli that may appear homogenous or speckled. Tissue substrates contain 1-3 nucleoli, while tissue culture cells such as Hep-2 cells may contain 2-7 irregular shaped nucleoli.

g. Centromere
Centromere antibody is found in a high percentage of patients with CREST syndrome, a less severe variant of PSS. Centromere antibody can be found in other rheumatic diseases and may predate a full-blown CREST syndrome by as early as 2 years.

Centromere antibody produces a discrete immunofluorescent speckled pattern on tissue or Hep-2, but can be positively identified on a Hep-2 cells by observing the chromosome staining of the mitotic cells where the speckles are clustered with the chromosomes.

5. Characteristics associated with other autoantibodies:
   a. Mitochondrial (AMA)
      A high AMA titer supports the diagnosis of primary biliary cirrhosis. Low titers of AMA may be detected in other liver disorders that include chronic active hepatitis and cryptogenic cirrhosis.

   b. Smooth Muscle (ASMA)
      ASMA is present in high titers in the serum of 70% of patients with chronic active hepatitis. In addition, 50% of these patients are positive for ANA, while 25% demonstrate low AMA titers. Low ASMA titers may be present in viral infections, malignancies, and good health. ASMA usually does not appear in SLE.

   c. Parietal Cell (APCA)
      APCA occurs in the serum of 90% of patients with pernicious anemia. With other clinical and laboratory data, a positive APCA result helps to distinguish autoimmune pernicious anemia from other megaloblastic anemias. Other disorders that may have APCA are gastric ulcers (33%), atrophic gastritis (up to 60%), thyroid diseases (33%), diabetes mellitus (12%), and iron deficiency anemia (24%). Although detected in less than 2% of the normal population under 20 years of age, the incidence of APCA increase in women over the age of 40 and may be present in up to 16% of the normal population over 60 years of age.
Profiles

Screening for antinuclear antibodies aids the physician in evaluating systemic rheumatic disease. Specific identification of antibodies aids in diagnosis as certain disease produce specific antibody profiles.

ANA Profiles:

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<tr>
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<tr>
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<td>nRNP</td>
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<tr>
<td>SLE</td>
<td>+</td>
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<tr>
<td>MCTD</td>
<td>+</td>
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<tr>
<td>Scleroderma</td>
<td>+</td>
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<tr>
<td>DM/PM</td>
<td>-</td>
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<tr>
<td>Sjögren's</td>
<td>+</td>
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<tr>
<td>RA</td>
<td>+</td>
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<tr>
<td>Drug induced lupus</td>
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* Low titers

- The presence of multiple antibodies suggests SLE. High titered nDNA antibodies are also characteristic of SLE and anti-Sm is a marker for SLE.
- Patients with MCTD usually have high titers of anti-RNP in the absence of other types of ANA.
- Drug induced lupus presents with histone antibodies without other ANA.
- In Sjögren’s Syndrome both SSA and SSB are likely to be present. These antibodies can also be found in SLE.
- Scl-70 and nucleolar antibodies are associated with PSS, and centromere antibodies are found in CREST syndrome.
DNA Testing

Principle:
The indirect fluorescent immunoassay principle has been discussed in earlier sections. Initially, serum is applied to the Crithidia lucilliae substrate. If there are anti-nDNA antibodies present they will attach to the kinetoplast forming an Ag-Ab complex. After removing the excess serum from the slide, a fluorescein tagged anti-human gamma globulin is applied that attaches to any Ag-Ab complexes already present. Once this material is removed, the slide is viewed under a fluorescent microscope. In a positive reaction, the kinetoplast will appear apple green while the negative will appear dull or dark. Since patients with SLE can have antibodies other than nDNA, the value of the test is in a positive result. While a positive is highly indicative of SLE, a negative result would not rule out SLE.

Clinical Value:
The anti-nDNA assay is an aid in the diagnosis and treatment of SLE by detecting and titering anti-double stranded (native) deoxyribonucleic acid (nDNA) antibodies in human serum. Many auto-antibodies have been associated with SLE. Some of these also share associations with other diseases as well. The group of DNA antibodies can be divided into three classes:

a. nDNA antibodies
b. ssDNA antibodies
c. Antibodies that react with both of the above classes

Of these, those directed against nDNA antibodies have gained most importance because of their diagnostic and therapeutic value. It is generally believed that high titers of nDNA antibodies occur only in SLE. The others can be found in SLE or other diseases as well. The titer of nDNA antibodies may also respond to therapy by decreasing with successful treatment and increasing in an acute disease state.

The anti-nDNA test is an indirect immunofluorescent test that utilizes the protozoan, Crithidia lucilliae, as the substrate. This method was chosen over others because the Crithidia possess a giant mitochondria called the kinetoplast that is composed of pure nDNA. Unlike some of the other methods that could easily become contaminated with ssDNA the composition of the Crithidia is consistent.

Results:
1. Positive
   In a positive reaction, the kinetoplast fluoresces greater than the negative as a 1:10 dilution. The following are all considered positive:
   a. Only the kinetoplast fluoresces
   b. The kinetoplast and nucleus fluoresce
   c. The kinetoplast and basal body fluoresce
   d. The kinetoplast, basal body and nucleus fluoresce

2. Negative
   In a negative reaction, the kinetoplast does not fluoresce at a 1:10 dilution. The following are all considered negative:
   a. No fluorescence
   b. The nucleus fluoresces
   c. The basal body fluoresces
   d. The basal body and nucleus fluoresce