

**California Association for
Medical Laboratory Technology
Distance Learning Program**

An Update On Autoimmune Diseases

by

**Paula J. D'Amore, Ph.D., D(AMBLI)
Assistant Professor, Division of Health Sciences
California State University, Dominguez Hills**

**Course DL-987
1.0 CE/Contact Hour
Level: Basic**

© California Association for Medical Laboratory Technology.
Permission to reprint any part of these materials, other than for credit from CAMLT, must
be obtained in writing from the CAMLT Executive Office.

CAMLT is approved by the California Department of Health Services as a
CA CLS Accrediting Agency (#0021)
and this course is approved by ASCLS for the P.A.C.E.® Program (#519)

1895 Mowry Ave, Suite 112
Fremont, CA 94538-

Phone: 510-792-4441
FAX: 510-792-

Notification of Distance Learning Deadline

All continuing education units required to renew your license must be earned no later than
the expiration date printed on your license. If some of your units are made up of Distance
Learning courses, please allow yourself enough time to retake the test in the event you do
not pass on the first attempt. CAMLT urges you to earn your CE units early!.

An Update on Autoimmune Diseases

OBJECTIVES

Upon completion of this course, the participant will be able to:

1. Define immune tolerance to self.
2. Identify and describe the two major phases of self tolerance induction.
3. Describe MHC and non-MHC genes linked to autoimmunity.
4. Discuss the role of environmental triggers in the development of autoimmunity.
5. Discuss the emerging technologies being used to detect autoantibodies in clinical laboratories.

ESTABLISHMENT OF IMMUNE TOLERANCE TO SELF

The most fundamental component of the immune system is its ability to discriminate between self (individual's own antigens) and non-self (antigens on invading pathogens). Normally, when lymphocytes are exposed to non-self, they become activated and mount an immune response against foreign antigens to eliminate them. However, when lymphocytes are exposed to self antigens on our own cells and tissues, they have the ability to remain unresponsive. This is called immune tolerance to self. In order for self tolerance to function, several mechanisms must be established and function properly. Initially, self tolerance is induced when immature lymphocytes encounter self antigens for the first time during their maturation process that occurs in the thymus for T cells and in the bone marrow for B cells. This first phase is called central tolerance. Briefly, lymphocytes with T-cell receptors (TcR) that have a strong reactivity for self antigens will be deleted by apoptosis (programmed cell death) or undergo a modification of their receptor by a process called "receptor editing." In addition, some of the developing T cells in the thymus may differentiate into regulatory cells that have the ability to prevent an immune response to self when they leave the thymus.

Although the induction of self tolerance in the thymus and bone marrow is highly effective, many autoreactive lymphocytes escape central tolerance. These cells will also encounter many self antigens in peripheral tissues that are not present in the bone marrow or thymus. In order to maintain self tolerance throughout the body, lymphocytes are subjected to additional selection mechanisms collectively called peripheral tolerance, including: (a) Mature B and T cells that are able to recognize autoantigens can be deleted by apoptosis or rendered anergic (unresponsive). (b) T regulatory cells (Tregs) that were generated in the thymus play a key role in maintaining self tolerance. Tregs suppress the activation and proliferation of autoreactive lymphocytes throughout the body through the elaboration of suppressive cytokines and possible direct cell to cell contact. Treg cells express CD3, CD4 and CD25. The survival of these cells is dependent on the cytokine, interleukin-2 (IL-2). (c) Additionally, some self antigens are sequestered from the immune system in privileged sites such as brain or cornea. Consequently, lymphocytes and antigen processing cells (APC) do not have access to these tissue antigens.

DEVELOPMENT OF AUTOIMMUNITY

When the mechanisms of self tolerance fail to function, alterations in the immune system occur, resulting in an immune response to autoantigens. This is defined as autoimmunity, which includes the production of autoantibodies and autoreactive T cells. What are the contributing factors that lead to a failure in the immune system's self tolerance mechanisms? It has been postulated that autoimmune diseases are multifactorial, suggesting that several factors appear to play a role in the breakdown or overriding of tolerance, resulting in the development of autoimmunity. The interaction between genetic susceptibility, gender, hormonal influence, and environmental triggers such as infections, chemicals, and smoking has been the primary focus of investigation.

Genetic Susceptibility:

Evidence indicates that there is a strong genetic component that predisposes individuals to autoimmunity. Autoimmune diseases are often described as polygenic (multiple genes influencing a specific outcome) and have a strong familial tendency. The concordance rate for disease among monozygotic (identical) twins is higher than the rate among siblings or even non-identical twins. This would indicate that the multiple genes shared by identical twins affect susceptibility. Frequently there is a familial clustering of an autoimmune disease among close relatives of an affected individual in several of the disorders. Moreover, a number of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), systemic sclerosis, and autoimmune thyroid diseases (Hashimoto's thyroiditis and Graves' disease) exist in families. Autoimmune thyroid diseases are the most commonly encountered among relatives of an affected individual. It has also been demonstrated that more than one disorder may occur in a single patient. Among organ-specific autoimmune diseases, type I diabetes and autoimmune thyroid diseases commonly occur together.

The primary genes linked to autoimmunity are those of the major histocompatibility complex (MHC). In humans, MHC is referred to as Human Leukocyte antigens (HLA). The primary function of the MHC is to present antigens in the context of Class I or Class II HLA molecules to T cells that control the development of cellular and humoral immunity. Class II HLA molecules present antigen to CD3+4+ T cells. CD3+8+ T cells recognize antigen that is presented by Class I HLA molecules. Some inherited MHC alleles are associated with autoimmune diseases because they do not present self antigens effectively to T cells. As a consequence, tolerance to these self antigens will not be generated. Among the strongest associations is the link between HLA-B27 and ankylosing spondylitis. Relative risk refers to the probability of an individual with the specific HLA allele inheriting a disease as opposed to an individual without the HLA allele. The relative risk for developing ankylosing spondylitis is 90 times higher in individuals who possess the HLA-B27 allele. Other autoimmune diseases linked to MHC alleles are SLE, rheumatoid arthritis (RA), type I diabetes, systemic sclerosis, autoimmune thyroid diseases, and pemphigus vulgaris. However, inheritance of a specific allele does not automatically bring about autoimmune disease.

Due to the recent advances in genome-wide linkage studies (identifying genomic areas where specific alleles are shared among family members with a disease) and candidate gene association analysis, other non-MHC susceptibility genes are being assessed. This is an exciting area of research that may lead to a better understanding of the mechanisms involved in the development of autoimmunity. However, many of these genetic polymorphisms (genetic variants) are located on large segments of chromosomes therefore their role in autoimmunity is being inferred.

A number of the newly-discovered candidate genes code for regulatory proteins (1) as follows:

1. The autoimmune regulator (AIRE) gene codes for a protein that facilitates the expression of self antigens in the thymus. If this protein is defective and there is no expression of some self antigens in the thymus, autoreactive T cells will escape central tolerance. Mutations in the AIRE gene have been found to be associated with the autoimmune polyendocrine syndrome (APS-1) causing autoimmune destruction of several endocrine glands and resulting in Addison's disease, type I diabetes, and hypoparathyroidism.
2. Another important susceptibility gene is the cytotoxic T lymphocyte antigen 4 (CTLA-4). The protein made is a receptor expressed by T cells that is inhibitory to T cell responses. Stimulatory molecules that bind to this receptor will shut off the T cell response and induce anergy (unresponsiveness), another component of tolerance. The CTLA-4 gene has been shown to be associated with several autoimmune diseases including type I diabetes and SLE.

3. The FOXP3 (forkhead box P3) gene codes for a transcription factor that is expressed in CD3+4+25+ Tregs and is essential for their development. There appears to be an association with a mutation in the gene coding for FOXP3 in humans and mice in a rare systemic autoimmune disease called IPEX (immune dysregulation, polyendocrinopathy, enteropathy, and an X-linked syndrome).

ENVIRONMENTAL FACTORS

The relationship between genetic susceptibility and environmental factors in the development of autoimmunity and autoimmune diseases is extremely complex. Many of the exogenous triggers appear years before the development of the disease. Consequently, knowing the exact effect of environmental agents is very difficult. Some environmental factors that have been linked to autoimmune disease include: bacterial and viral infections, exposure to chemical and physical agents, smoking, nutritional imbalance, and therapeutic drugs such as procainamide. Recently attention has been directed towards the cellular and tissue damage that is generated by environmental triggers that are present for a long period of time (2). Inevitably irreversible cellular damage, necrosis (cell death) and the formation of excessive amounts of cell debris will occur. The debris contains an abundance of intracellular antigens that have been physically damaged, chemically altered or were from privileged sites normally sequestered from the immune system. Monocyte and tissue macrophages are responsible for the uptake and removal of cellular debris. However, if there is too much to remove, a large quantity of debris will remain in circulation. Lymphocytes will react to the damaged intracellular antigens as “non-self,” mount an immune response and set the autoimmune process in motion.

The preponderance of autoimmune diseases in females is striking. It is apparent that gender and female hormones play a role in the induction of autoimmune diseases. The influence of reproductive hormones on systemic lupus erythematosus is noteworthy. The incidence of lupus is highest in women during their childbearing years with a female to male ratio of 9:1. Pregnancy may exacerbate the disease and contribute to periodic flare-ups characteristic of SLE. Female hormones have also been associated with myasthenia gravis, rheumatoid arthritis, and systemic sclerosis.

AUTOIMMUNE DISEASE

The presence of autoantibodies occurs to a certain degree in all normal individuals. Autoimmune disease is the pathological consequence of an ongoing autoimmune process. In a recent Danish study (3) 1 in 20 individuals in the general population were found to have autoimmune disease. Autoimmune diseases are generally classified into two different categories: organ specific and systemic. Organ specific autoimmune diseases generally develop as the result of an autoantibody being produced to a specific tissue or organ. Therefore, the disease process and/or tissue destruction remains limited to the specific organ. Some of the diseases included in this category are Hashimoto's thyroiditis, Graves' disease, myasthenia gravis, Addison's disease, pernicious anemia, and pemphigus vulgaris. Systemic autoimmune disorders are not organ specific and cause tissue injury and inflammation to several organs throughout the body. Systemic autoimmune rheumatic diseases (SARD) such as SLE, RA, Sjogren's syndrome, scleroderma, mixed connective tissue disease, and dermatomyositis are the primary disorders within this group.

DETECTION OF AUTOANTIBODIES IN THE CLINICAL LABORATORY

A key component in the clinical diagnosis of SARD is the detection of autoantibodies in the suspected patient's serum. Testing for anti-nuclear antibodies (ANA) is the traditional laboratory screening procedure used for diagnosis of a SARD. ANA are autoantibodies directed against nuclear antigens and cytoplasmic proteins located in cells. The indirect immunofluorescence (IIF) assay using

human epithelial cells (Hep-2) as the substrate is considered the gold standard for the detection of ANA. Commercially prepared microscopic slides with the Hep-2 cell substrate are now available. A consistent problem with the Hep-2 substrate has been the unreliable detection of the SSA/Ro antigen. Hep-2 cell lines can be transfected now with the insertion of the gene responsible for the expression of the 60kd native Ro peptide. Consequently, there are ample quantities of the autoantigen present in the Hep-2 cells. The patient's serum is incubated with the glass slide containing the Hep-2 substrate. If an ANA is present, it will bind to the specific antigen present on the substrate. A fluoresceinated anti-human antibody is added that will bind to the ANA-antigen complex. The slide is examined under a fluorescent microscope.

ANA react primarily to antigens present within the nucleus, nucleolus and various cytoplasmic organelles. Distinct staining patterns are seen. Those seen most commonly are homogeneous, rim, speckled, centromere, and nucleolar. Traditionally, the IIF staining pattern(s) identified reflected different antigenic specificities, thereby providing a guide to further testing. However, the pattern type does not always correlate reliably to specific antibodies present and cannot be regarded as definitive. It must also be pointed out that low titers of ANA do not always indicate autoimmune disease. They are normally found in healthy individuals and their presence can increase with age. ANA can also be detected in infectious diseases such as hepatitis, mononucleosis, tuberculosis, and subacute bacterial endocarditis. IIF ANA testing is highly sensitive and a titer of greater than 1:160 is usually considered diagnostic and more likely to be clinically significant. IIF ANA detection is still the most widely used method in clinical laboratories. However, the technique is laborious and pattern recognition can be subjective and is often dependent on the skill and experience of the Clinical Laboratory Scientist interpreting the slides.

Today, laboratories testing large numbers of patients' samples can use commercially-produced EIA-ANA kits as a screening procedure. In the kit, a Hep2 extract containing various ANA antigens is bound to the surface of each well on the microliter plate. A positive result from an EIA testing of ANA can be verified by IIF ANA.

Irrespective of which screening method is used for ANA detection, more definitive testing is needed to establish what specific autoantibody is present. Historically, laboratories have relied on a number of techniques including Ouchterlony diffusion, radiolabeled immunoassays, counterimmunoelectrophoresis and immunoblots to identify the antibody present. Today, EIA testing using recombinant or affinity-purified native autoantigens has replaced the older techniques in many laboratories. Many commercially prepared EIA kits contain panels of 5 or more autoantigens that can be run on each patient.

Emerging Technologies

Several new technologies, which are faster and not as labor intensive, have been developed to identify autoantibodies present in the patient's serum. Line immunoassay (LIA) is a technique that allows for simultaneous detection of multiple autoantibodies present in a patient's serum using nitrocellulose strips coated with highly purified recombinant autoantigens (4). Twenty autoantigens can be coated to each strip and each autoantigen corresponds to a fine line on the strip. Although the strips resemble those used in Western blots, there is no electrophoresis or blotting with LIA. A patient's serum is incubated with one strip. Visible inspection of the strip is used to determine the presence of autoantibodies. Computer software packages are also available for analysis of the strip.

Technologies based on multiplexing have emerged as well. Multiplex assays test a patient's serum sample for the simultaneous detection of multiple antibodies in one microtiter well (4). Instead of one autoantigen per well, several autoantigens are placed in one well. The technology is a fluorescein based microparticle assay. Individual beads (microspheres) are coated with 2 distinct fluorescent dyes to produce different levels of color intensity. Each set of microspheres, representing a specific level of color intensity, is conjugated by covalent binding to a particular autoantigen. All the bead sets are mixed

together and placed in each well of a microtiter plate permitting several different autoantigens to be present in one well. A patient's sample is added to one well and the autoantibodies, if present, will bind to the specific autoantigen-coated beads. A fluoresceinated anti-human IgG is added and binds to autoantibodies attached to antigen coated beads. The sample is analyzed in a flow cytometer (Luminex) and each bead passes through the flow cell. One laser will identify the color of an autoantigen-coded bead by discriminating the different excitation wavelengths caused by the intensity of the two different fluorescent dyes coating the beads. The second laser will measure the concentration of each autoantibody present by measuring the quantity of fluoresceinated anti-human IgG bound to each bead. Laboratories must abide by proper standardization and internal validation procedures with any of these new technologies.

CONCLUSION

Numerous studies have demonstrated that many autoimmune diseases are generally preceded by a period of time when only the autoantibodies are present (5). The actual disease process and clinical manifestations may not become apparent for months to years later. Therefore, can the identification of autoantibodies in high risk (genetically predisposed) individuals be used as a marker of future disease? The detection of strongly expressed patterns of autoantibodies by multiplex testing may identify future autoimmune disease and lead to the utilization of preventive measures. Additionally, the elucidation of the genetic markers involved in autoimmune disease will lead to a better understanding of this extremely complex group of diseases.

REFERENCES

1. Gregersen PK, Behrens TW. Genetics of autoimmune diseases-disorders of immune homeostasis. *Nature Rev Genetics*. 2006;9:917-928.
2. Mackay IR, Leskovsek NV, Rose NR. Cell damage and autoimmunity: A critical appraisal. *J Autoimmun*. 2008;30:5-11.
3. Eaton WW, Rose NR, Kalaydjian A, Pedersen MG, Mortensen PB. Epidemiology of autoimmune disease in Denmark. *J Autoimmun*. 2007;29:1-9.
4. Tozzoli R. Recent advances in diagnostic technologies and their impact in autoimmune diseases. *Autoimmunity Rev*. 2007;6:34-340.
5. Shepshelovich D, Shoenfeld Y. Prediction and prevention of autoimmune diseases: additional aspects of the mosaic of autoimmunity. *Lupus* 2006;15:183-190.

REVIEW QUESTIONS

Course #DL-987

Choose the **one** best answer.

1. The immune system has the ability to:
 - a. recognize self antigens only
 - b. recognize self antigens and non self antigens
 - c. distinguish regulatory cells only
 - d. distinguish foreign antigens only

2. Central tolerance is established in the:
 - a. lymph nodes
 - b. thymus only
 - c. spleen
 - d. bone marrow and thymus

3. The establishment of central tolerance is set in motion when:
 - a. immature T and B cells encounter self antigens in the thymus and bone marrow
 - b. mature T cells modify the T cell receptor in regional lymph nodes
 - c. mature T and B cells change their receptors in the lymph nodes
 - d. autoreactive lymphocytes are allowed to enter peripheral circulation

4. Mechanisms involved in peripheral tolerance occur:
 - a. during deletion of Tregs in the peripheral circulation
 - b. in the thymus and bone marrow
 - c. during inflammation
 - d. in the peripheral tissues

5. Two primary contributing factors for the development of autoimmunity are:
 - a. genetic susceptibility and thymus destruction
 - b. thymus growth and environmental triggers
 - c. genetic susceptibility and environmental triggers
 - d. genetic susceptibility and youth

6. An environmental factor associated with the development of autoimmune disease is:
 - a. allergies
 - b. HLA alleles
 - c. smoking
 - d. exercise

7. An HLA allele strongly linked to ankylosing spondylitis is:
 - a. HLA-B27
 - b. HLA-A27
 - c. HLA-DR4
 - d. HLA-DR5

8. Non HLA associated genes are now being studied for their association with autoimmune genes. One of the susceptibility genes under investigation is the FOXP3 gene. What is the role of FOXP3?
 - a. inhibit the expression of self antigens in the thymus
 - b. shut off T cell responses
 - c. essential to the development of CD3+4+25+ Tregs
 - d. inhibits the entrance of immature T cells into the thymus

9. Multiplex assays have the ability to measure for:
 - a. multiple autoantibodies in one microtiter well
 - b. a single autoantibody only in one microtiter well
 - c. several autoantigens attached to one microsphere
 - d. several beads attached to one microsphere

10. The line immunoassay (LIA) includes identification of autoantibodies by:
 - a. electrophoresis and immunoblotting of autoantigens
 - b. scanning electrophoresis patterns of autoantibodies
 - c. using strips coated with autoantigens without electrophoresis or immunoblotting
 - d. microscopic determination of fluorescinated patterns of antigen-antibody complexes

**Course #DL-987 – An Update on Autoimmune Diseases
Registration/Answersheet - 1.0 CE Credit**

Name _____ CLS Lic. # _____ Date _____

Signature (Required) _____

Address _____

Street _____ City _____ State/Zip _____
Payment Method ___ Check or ___ Credit Card # _____ Type -Visa / MC

1.0 CE Fee = \$15 (non-member) Exp. Date _____ Signature _____
\$12 (member)

Please circle the one best answer for each question.

- | | | | | | | | | | |
|----|---|---|---|---|----|---|---|---|---|
| 1. | a | b | c | d | 6 | a | b | c | d |
| 2. | a | b | c | d | 7 | a | b | c | d |
| 3. | a | b | c | d | 8 | a | b | c | d |
| 4. | a | b | c | d | 9 | a | b | c | d |
| 5. | a | b | c | d | 10 | a | b | c | d |

Distance Learning Evaluation Form

According to state regulations, this evaluation must be completed and returned in order to receive CE hours. Your comments help us to provide you with better continuing education materials in the distance learning format. Please circle the number that agrees with your assessment.

1. Overall, I was satisfied with the quality of this Distance Learning course.

(strongly agree) 5 4 3 2 1 (strongly disagree)

2. The objectives of this Distance Learning course were met.

(strongly agree) 5 4 3 2 1 (strongly disagree)

3. The difficulty of this Distance Learning course was consistent with the number of CE hours.

(strongly agree) 5 4 3 2 1 (strongly disagree)

4. I will use what I learned from this Distance Learning course.

(strongly agree) 5 4 3 2 1 (strongly disagree)

5. The time to complete this Distance Learning course was: _____ hours

6. What did you like or dislike about this Distance Learning course ?