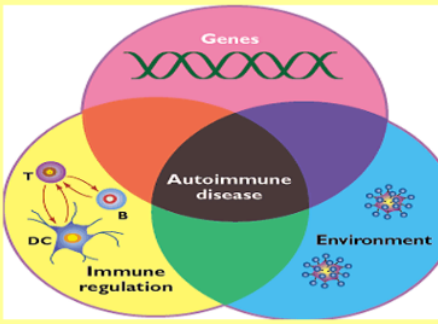
Immunology practical – week 12

Autoantibodies. Immunofluorescence.

Tolerance is the failure of the immune system to respond to an epitope in an aggressive way. Most **self-tolerance** results from the deliberate inactivation or destruction of lymphocytes bearing BCRs or TCRs that recognize and binds self epitopes. Inactivation or destruction may occur during early development (central tolerance) or may be imposed on lymphocytes in the periphery (peripheral tolerance). An understanding of how the immune system naturally imposes self-tolerance can provide critical clues for the development of therapeutic strategies for autoimmune diseases caused by the loss of self-tolerance.
Central tolerance occurs during the early differentiation of B cells in the bone marrow and T cells in the thymus. Normally, both B and T cells that bind self-epitopes at distinct early stages of development meet an apoptotic death, thus eliminating large numbers of potentially self-reactive cells before they enter the circulation

- One common theory across all of the autoimmune diseases is:
- some outside agent is required to start the process.
- Even with a genetic tendency, a person may not develop an autoimmune disease without an environmental influence or physical trauma to set it off.

- List of possibly identified suspects for some common autoimmune conditions:
- **Lupus:** hair dye and certain drugs, smoking
- **Scleroderma:** silica exposure
- **Diabetes:** gluten, coxsackie virus
- **Rheumatoid Arthritis:** mycoplasmas, smoking
- **Thyroid:** smoking
- **Multiple Sclerosis:** hepatitis B infection



Autoimmune diseases involve numerous different molecules, cells, and tissues that are targeted by the autoimmune responses. Some autoimmune diseases are systemic or diffuse, because of the distribution of the target antigens. For example, SLE and rheumatoid arthritis affect a variety of joints and other body tissues. Other diseases affect specific organs and tissues

AUTOIMMUNE DISEASES

Humoral-associated autoimmune diseases

Some autoimmune diseases result from the binding of self-reactive antibodies, leading to Type II and Type III hypersensitivity responses. The antibodies responsible for initiating the diseases are usually of the IgG isotype, although IgM antibodies can contribute as well. The activation of complement and the opsonization of injured cells promote inflammatory responses that increase the damage inflicted on the targeted cells and tissues. Autoreactive T cells are typically present as well, but their role is primarily the activation of the autoreactive B cells rather than directly attacking host cells. Examples of these autoimmune diseases include:
- Autoimmune hemolytic anemia: type II hypersensitivity
- Goodpasture's syndrome: type II hypersensitivity
- Hashimoto's thyroiditis: type II hypersensitivity
- Rheumatic fever: type II hypersensitivity
- Rheumatoid arthritis: type III hypersensitivity
- Systemic lupus erythematosus: type II and type III hypersensitivity

Cell-mediated autoimmune diseases

Type IV hypersensitivity responses involve cell-mediated injury leading to autoimmune disease. These may include cytotoxic T cell responses or macrophages driven by DTH responses. The inflammation that is generated

can eventually involve numerous simultaneously ongoing responses. In some diseases, particular antibodies may also be characteristically present, but they have not been demonstrated to contribute to the disease pathologies. The following are examples of autoimmune diseases involving type IV hypersensitivity responses. Rheumatoid arthritis provides an example of an autoimmune disease that involves both humoral and cell-mediated injury.

- Insulin-dependent diabetes mellitus (type 1)
- Multiple sclerosis
- Reactive arthritis
- Rheumatoid arthritis

HLA Association with Autoimmune Diseases

The risks for many autoimmune diseases appear to be associated with the presence of particular HLA . In some cases (e.g., HLA-B27 and HLA-DR3), a single HLA gene is associated with increased risk for multiple autoimmune diseases. The molecular mechanisms underlying these statistical associations are still uncertain but presumably involve some influence on processing and presentation of self epitopes to self-reactive T cells. The strength of the statistical association between a particular HLA gene and a particular autoimmune disease is expressed as the **relative risk**. The relative risk compares the frequency of the particular disease among carriers of a particular HLA gene with the frequency among noncarriers. For example, the relative risk of 6 for the association of SLE with HLA-DR3 means that SLE occurs approximately three times more frequently among DR3+ individuals than among DR3– individuals. Relative risk calculations are made within defined populations, and results may vary among groups of different ethnic or geographic origin.

Affected Tissue	Disease	Target Antigen
Anterior parts of the eye	Uveitis (anterior)	Beta B1-crystalin, other proteins of the ciliary body epithelium
Connective tissue	Scleroderma	Scl-70, PM-Scl antigens
Erythrocytes	Autoimmune hemolytic anemia	Erythrocyte surface molecules
Heart valves and sarcolemmal membranes	Rheumatic fever	Streptococcal M protein, cardiac muscle antigens
Joints of lower extremities; sometimes eyes and genital, urinary, or GI systems	Reiter's disease (reactive arthritis)	Possible association with infectious agents
Kidneys, lungs	Goodpasture's syndrome	Type IV collagen of basement membranes
Large intestine	Ulcerative colitis	Unknown
Lower spine	Ankylosing spondylitis	Unknown
Myelin of the central nervous system	Multiple sclerosis	Myelin proteins (several)
Pancreatic islet β cells	Type I insulin-dependent diabetes mellitus (IDDM)	Glutamate decarboxylase, preproinsulin, other β cell products
Platelets	Thrombocytic purpura	Platelet integrin molecules
Skeletal muscle	Myasthenia gravis	Acetylcholine receptor
Skeletal muscle	Polymyositis	Jo-1, PM-Scl antigens
Skin	Pemphigus vulgaris	Desmoglein-3
Skin	Psoriasis	Unknown, but there is some association with streptococcal infections
Skin, vasculature, muscle, joints, kidney	Systemic lupus erythematosus (SLE)	Nucleic acids, chromosomal proteins
Small intestine	Crohn's disease	Unknown
Spermatogonia, sperm	Male sterility (??)	Unknown
Synovial membranes, joints	Rheumatoid arthritis	Unknown
Tear ducts	Sjögren's syndrome	Ro/SS-A antigens
Thyroid gland	Graves' disease	TSH receptor
Thyroid gland	Hashimoto's thyroiditis	Thyroglobulin

- Diagnosing an autoimmune disease involves identifying which antibodies body is producing.
- **Antinuclear antibody tests (ANA) -** a type of autoantibody test that looks for antinuclear antibodies, which attack the nuclei of cells in the body
- **Autoantibody tests** - any of several tests that look for specific antibodies to own tissues
- **Complete blood count** (CBC) - measures the numbers of red and white cells in blood
- **Erythrocyte sedimentation rate (ESR) -** this test indirectly measures how much inflammation is in body

Detection of autoantibodies

anti nuclear antibodies ANA – homogenous, specled, rought specled, centromer.....

- - extracted nuclear antibodies ENA
- - anti ds DNA antibodies dsDNA
- - anti ss DNA antibodies ssDNA
- - anti myeloperoxidase antibodies – anti MPO
- ELISA test

Antinuclear antibodies (ANA) are a group of autoantibodies produced by a person's immune system when it fails to adequately distinguish between "self" and "nonself." The ANA test detects these autoantibodies in the blood. The ANA test may be positive with several autoimmune disorders. Patients with the autoimmune disorder systemic lupus erythematosus (SLE) are almost always positive for ANA, but the percentage of patients with other autoimmune disorders who have positive ANA results varies.

Depending on a person's signs and symptoms and the suspected disorder, ANA testing may be followed by additional tests for specific autoantibodies. Some of these tests are considered subsets of the general ANA test and detect the presence of autoantibodies that target specific substances within cell nuclei, including:

- anti-dsDNA,

- anti-centromere,

- anti-nucleolar,

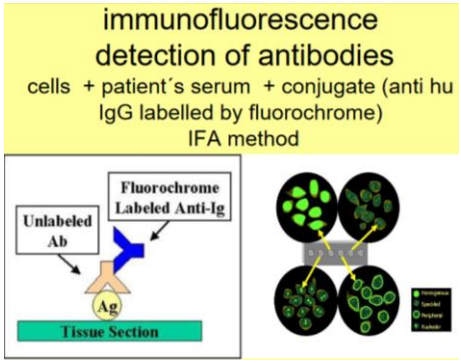
- anti-histone

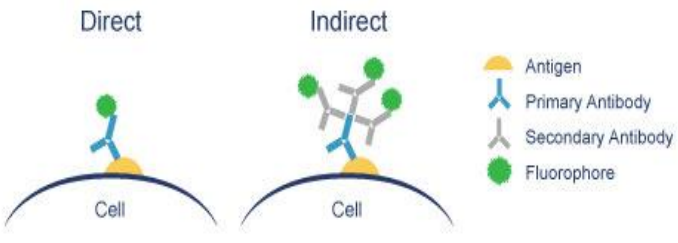
- anti-RNA antibodies

Different laboratories may use different test methods to detect ANA.

- Indirect fluorescent antibody (IFA)—this method is the traditional approach. A person's blood sample is mixed with cells that are affixed to a slide. Autoantibodies that may be present in the blood react with the cells. The slide is treated with a fluorescent antibody reagent and examined under a microscope. The presence (or absence) and pattern of fluorescence is noted.

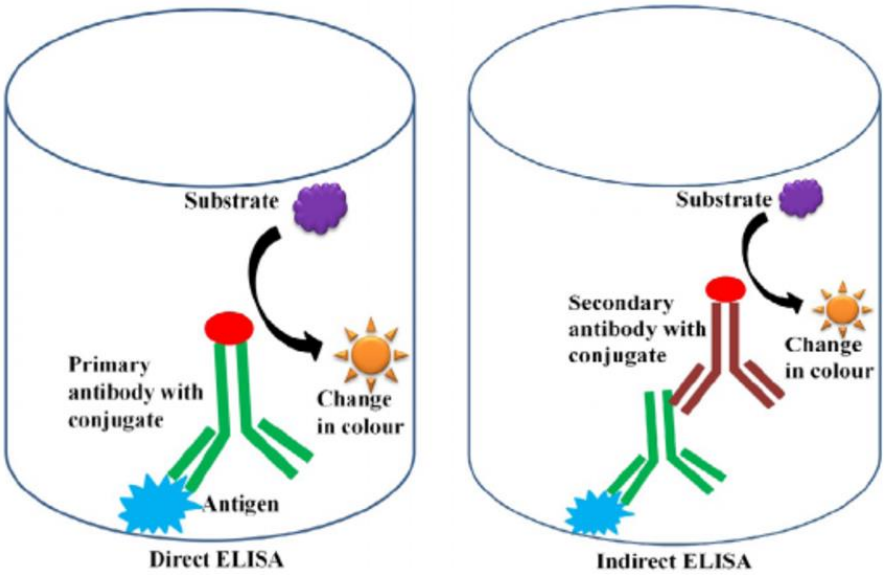
IMMUNOFLUORESCENE PRINCIPLE





- Immunoassays—laboratories may also use immunoassay to screen for ANA and may only use IFA to confirm positive results or results that are not clearly positive or negative. These methods are usually performed on automated instrumentation. They may be less sensitive than IFA in detecting ANA but may be more specific for autoimmune disorders

ELISA PRINCIPLE



SOURCES:

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