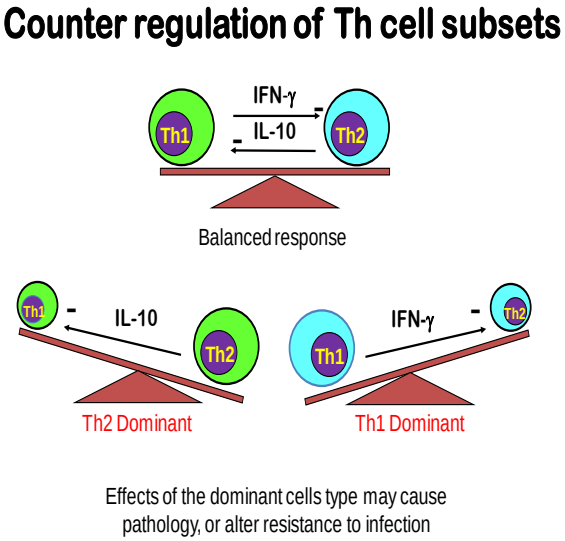
Sheet no. : 11

Refer to slide no. :

Written by: Areen Afghani & Brahim Khatib

Corrected by: Areen Afghani & Brahim Khatib

Last time we talked about the control of the immune system. As a reminder we talked about the immune regulation at the genetic level. When we talk about the T-cell receptor, B-cell receptor, MHC how can I control the immune response? We talked about the immune regulation using molecules like antigen, antibody, interleukins and some other molecules like the complement which can interact to control the immune system. We will talk about the immune regulation at a cellular level; the interaction between the cells themselves T-cells, B-cells, APC how they work together. And we will also talk about the immune regulation where the endocrine system also interacts.

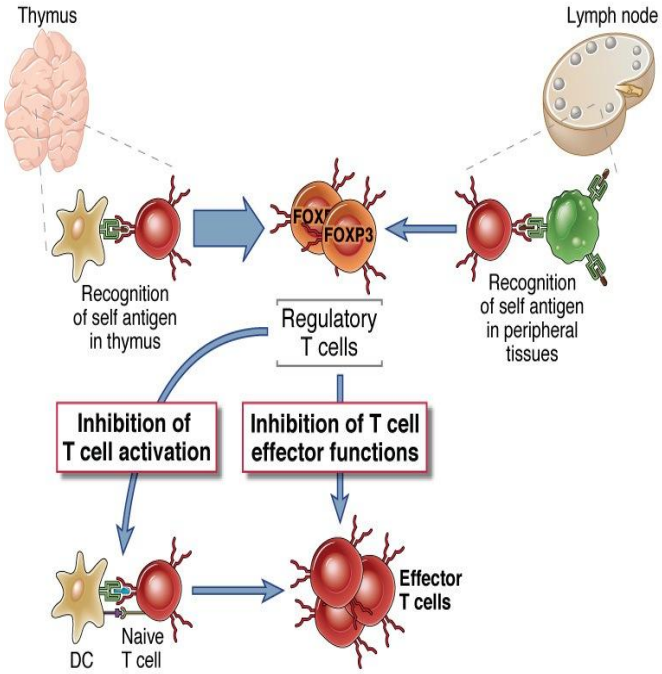


-We look to the cells, we can see that there's a balance between T-helper1 and T-helper2. T-helper1 is under the control of interleukin 10 which is secreted by T-helper2, so there is a negative feedback on the T-helper1 by the IL10. Again, Gama interferon which is secreted by T-helper 1 has a negative control on T-helper2 cells. If interleukin 10 increased then the T-helper 2 will be suppressed, and if gamma interferon increased T-helper1 will be suppressed.

-This interaction between the cells is controlled by the antigen which will tell the immune system what to do. If we look to the whole cells, you remember that the lymphocytes during their development (whether we talk about T-cells or B-cells), T-cells which will be developed in the thymus will generate 3 types of cells:

A) CD4+, CD25+,FOXp3+. This cell is generated in the cortex of the thymus in absence of any antigen stimulating or any other thing only can recognize the cell component elimination of that group (I have no idea what that means).

B) Suppressor T-lymphocytes but CD25- or CD4,25 -. Now the regulation of the immune system happens at two levels one is central which is right away from the beginning of the thymus (or BM) where cell deletion occurs when we delete cells that recognize self component it will be eliminated there or at later stage suppressor and helper T-lymphocytes they will induce a new type of cell which is a regulating cell but that's in the peripheral which will be produced in the lymph nodes or in the spleen.



- How will those interact, how will they control the immune system? There will be a recognition in the immune system where in the Thymus they will recognize the self antigen, all these cells will be eliminated through apoptosis (positive selection: when we get rid of the cells that will recognize the self component and keep those who don't). Again also the peripheral immune system where the regulatory cells are found in the lymph nodes and the spleen where there will be generation of other cells, now those regulatory cells will recognize certain cells. (FOXp3+ which is a growth factor which can be a transcribed factor under the function of the T-cell regulator cells those will produce regulatory T-lymphocytes which will inhibit the cell proliferation or they will inhibit the effecter cells, so when they inhibit the proliferation or the effector cells here we also will have a regulation mechanism.)

Regulatory T cells

- Regulatory T cells are CD4+ cells that express high levels of CD25 (IL-2 receptor a chain)
  - Generated by self antigen recognition in the thymus or peripheral tissues
  - Generation requires a transcription factor called Foxp3 (mutations in Foxp3 are the cause of a severe autoimmune disease in humans and mice)

FOXp3 is in the original cell, it is a transcription factor found in the regulatory T-lymphocyte, and it is controlled by the regulatory CD25+. We have regulatory T-cells found in the central

organs (Thymus and BM) those are CD25+ and FOXp+, now when the CD8 and CD4 go to the peripheral blood during their development there will be a generation of new regulatory cells those new regulatory cells are in the lymph nodes, those new regulatory cells will do those functions.

**FOXP3 appears to function as a master regulator (transcription factor) in the development and function of regulatory T cells.Regulatory T cells generally turn the immune response down.*

**The regulatory T cells (Tregs), formerly known as suppressor T cells, are a subpopulation of T cells which modulate the immune system, maintain tolerance to self-antigens, and abrogate autoimmune disease. These cells generally suppress or downregulate induction and proliferation of effector T cells.*

**Induced Regulatory T (iTreg) cells (CD4+ CD25+ Foxp3+) are suppressive cells involved in tolerance. iTreg cells have been shown to suppress T cell proliferation and experimental autoimmune diseases. These cells include Treg17 cells. iTreg cells develop from mature CD4+ conventional T cells outside of the thymus: a defining distinction between natural regulatory T (nTreg) cells and iTreg cells. Though iTreg and nTreg cells share a similar function iTreg cells have recently been shown to be "an essential non-redundant regulatory subset that supplements nTreg cells, in part by expanding TCR diversity within regulatory responses"*

-So the regulatory cells what are their functions what will they do? There are multiple functions that they can do but generally it's the secretion of immune suppressors cytokines which will stop the function of the T-Lymphocytes or there is a self tolerance maybe (remember when we talked about the need of two signals one of those signals will be stopped by that function so the cell will not respond). The FOXp3 mainly increases the CD25, it will increase the cytotoxic T-lymphocyte antigen, and it will increase the growth factor, decrease interleukin 2 and decrease the interferon so it has different functions.

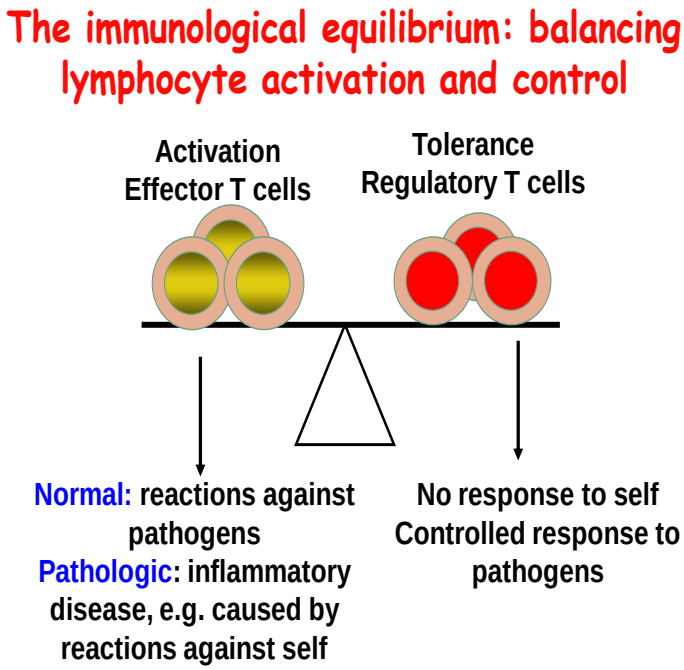
-In addition to the immune mechanism there's a certain hormone which again plays a role in the regulation of the immune system for example if we look to the hypothalamus where the corticosteroids are generated, the corticosteroids have a negative feedback on T-helper1 and Macrophages and at the same time, positive effect on interleukin 1& 6 and gamma interferon, this it has a positive function on the hypothalamus. So these interleukins that are produced by the T-lyphocytes and the macrophages they will increase the function of the hypothalamus which will generate the corticosteroids which activate the pituitary and the other organs. And again also that steroid (Estrogen) has a certain function on different type of T-

lymphocytes so there is a very close interaction between the neurotransmitters, hormones and the immune system.

-If we summarize all the regulation we can see it here (referring to a slide) -> he only pointed to the slide.

Now we will talk about Tolerance

-we will talk about the mechanism of tolerance, why is tolerance important? We will talk about the central and the peripheral tolerance in both B and T lymphocytes. We will talk about the tolerance in the periphery, the immunopathology and how the auto-immune diseases are generated.



-Again we said that the immune system works in balance if there is balance we have no problem. If there is an imbalance between the functional area and the non-functional area then we will start to see certain diseases which sometimes are very serious like for example if the normal reaction by one way or another, there is deficiency for example we will have a lot of serious diseases. If there is a mutation for example in the regulatory system then the tolerance will break and we will have auto-immune diseases. The surveillance of the immune system will be impaired.

- So when we talk about Tolerance means to avoid excessive lymphocyte activation and tissue damage during a normal protective response against infections (which is the main function of the immune system).

- To prevent inappropriate reaction we will have what is known as self tolerance. You can get self tolerance during development or by certain characteristics we will talk about.

-Failure of control mechanism underlying many causes like immune mediated inflammatory as well as infectious diseases.

-General principle of the control of the immune system we talked about them, **apoptosis** is very important, **memory cells** are very important for the surveillance of these abnormalities.

-So how do we define tolerance? We define it in **unresponsiveness (we inject the antigen and there will no response to that antigen).** Specific unresponsiveness to a certain antigen, this antigen is called either: tolergen or immunogen.

-Individuals are tolerant to their own antigens, therapeutic potential we can use tolerance we have many examples where we can use tolerance. For example when having **Penicillin Allergy** we can make the patient tolerant to Penicillin, how? By injecting the antigen in a very low dose daily for several or a long time what will happen is that as if you are closing ALL the antigen binding sites so even if I gave another dose there will be no binding sites so we will have tolerance, here the tolerance is a type of treatment. We prevented the allergy to Penicillin.

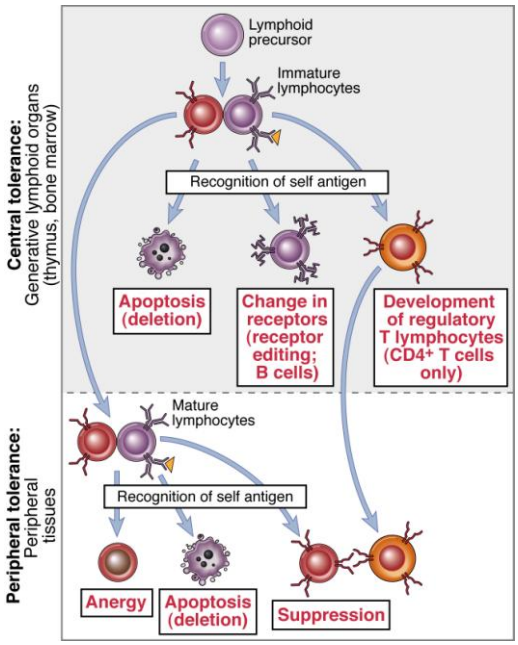
Now we will talk about the mechanisms.

-The food we eat contains proteins; the question is why don't we have antibodies to the protein we eat? Because we have oral mucosal tolerance to it.

BASIC FACTS ABOUT TOLERANCE-2

- Self tolerance – prevents the body to elicit an immune attack against its own tissues
- Mechanisms of active tolerance prevent inflammatory reactions to many innocuous airborne and food antigens found at mucosal surfaces

Central and peripheral tolerance



The principal fate of lymphocytes that recognize self antigens in the generative organs is death (deletion), BUT:

Some B cells may change their specificity (called "receptor editing")

Some T cells may differentiate into regulatory (suppressor) T lymphocytes

---

-So again we have a **central tolerance** and a **peripheral tolerance**.

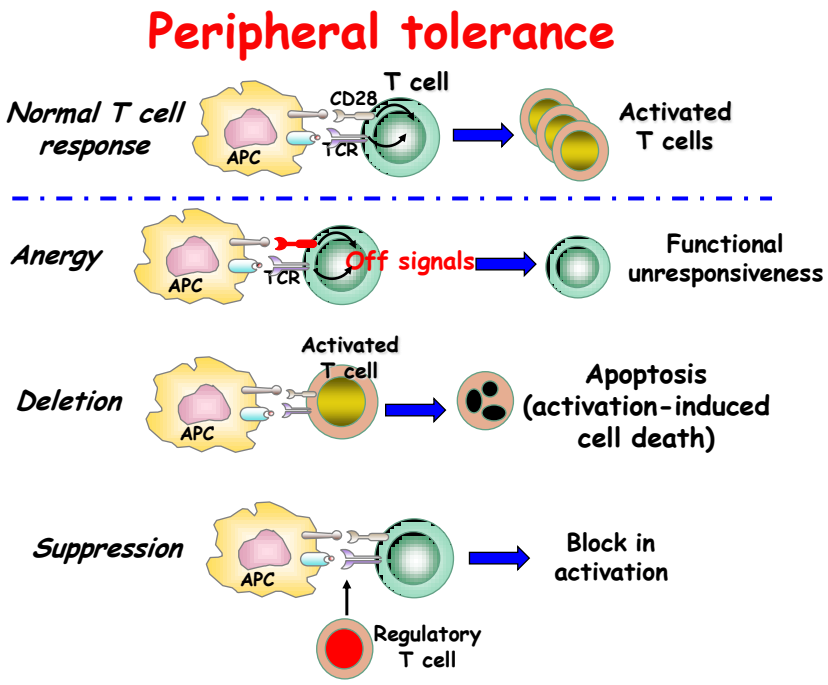
The central tolerance: When the T-lymphocytes develops in the cortex of the thymus, any antigen that contacts the T-lymphocytes at this stage will be treated as a self component so during this, those cells will be educated about the self component. So before they are done developing in at that area they will know the self component and they will stop the reaction against it. The cells which recognize the self component will either go for 1) **apoptosis** and they will be deleted or there will be a 2) **change in the receptors,** then the receptors are edited. Because the receptors are changed the B-lymphocytes will not recognize self components, this is called receptor editing. Or there is 3) **a regulatory T-lymphocyte which will stop the function**. So these **three conditions they will do the tolerance in the central area before the cells leave the thymus or the bone marrow** then the normal cell which can recognize the antigen will be transported from the thymus to the spleen and the lymph nodes.

Peripheral tolerance: At that area (peripheral tissues) the mature T-lymphocytes, those also can be tolerated BUT the tolerance here is different one it could be as anergy or again apoptosis or could be suppression.

POSSIBLE WAYS OF PREVENTION OF SELF-REACTIVITY

- *Clonal deletion* – physical elimination of cells from the repertoire during their lifespan
- *Clonal anergy* – downregulating the intrinsic mechanism of the immune response such as lack of costimulatory molecules or insufficient second signal for cell activation
- *Suppression* – inhibition of cellular activation by interaction with other cells:
  (Treg – CD4+, CD25+ T lymphocytes)

-so again this is what happens in the peripheral system: 1) those which recognize self component will be deleted (**Clonal deletion**: all clone of cells which can recognize self component will be deleted) OR there is a 2) **Clonal Anergy**. REMEMBER: we said that any cell T or B needs two signals for activation if only one cell is present this is called anergy,they can recognize the antigen but there will be no reaction between B7 and CD28 so the second signal is not present, so the recognition happens but there is no function no proliferating of these cells. OR there could be 3) **suppression** which comes from the T-regulatory cells which can suppress CD4+ or CD25+ T-lymphocytes.



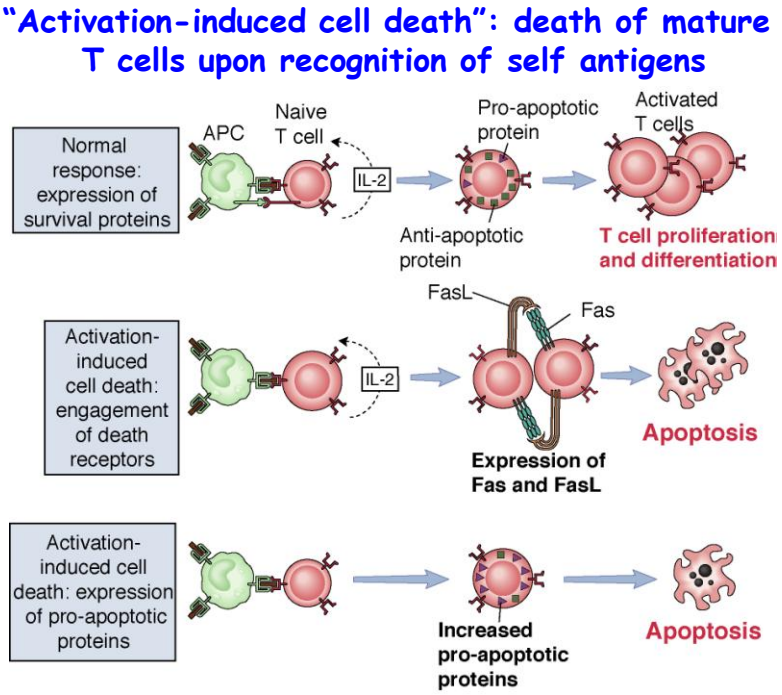
-So we either have a A) **normal response**: APC and T-lymphocytes with both signals present and we will get an activated T-lymphocyte.

B) When we talk about **Anergy**: there is a t-cell receptor recognition but between B7 and CD28 there is no recognition this cell will be functionally unresponsive.

C) When we talk about **apoptosis (deletion**) :there will be induction of cell death where there will be a fast function and there will be a fast production of ligands that will lead to apoptosis or there is

D**) Inhibition (suppression**): where the T-regulatory cells will destroy one of the two signals and there will be a block of the activation.

-The **T-cell Anergy:** only one signal will be present which is the one from the epitope of the antigen. This is not enough so this cell will not be able to proliferate and do its function.



-**Apoptosis** might happen in different ways, fas and anti-fasligands those when they react they will lead to cell apoptosis OR there is an increase in the pro-apoptic proteins inside the cell and the cell will go through apoptosis. OR there is a pro-apoptic protein that will stop the function of T-cells and their proliferation and differentiation. (He also mentioned that Interleukins (IL2) also leads to apoptosis)

-In the PERIPHERAL tolerance the reaction is a bit different as we said, so also in the peripheral it might happen using interleukins. Again also, the peripheral might happen using the interleukins not only the central. So we might have cytotoxic

processed T-lymphocytes, we have IL10 inhibiting the function of antigen processing cell, IL12 secretion and b7 expression. ("So when this there B7 will not be produced"<those are the doctor's words &I didn't understand them) or when we talk about the T-cell growth factor beta inhibit the T cell proliferation or we can talk about the **IL4 which will inhibit the gamma interferon production** or both they will inhibit the activation of the macrophages. So any of those or a combination of them will inhibit the action of the APC and there will be no signaling and this is seen in the peripheral not the central. We see it in the lymph mode or in the spleen.

Tolerance in B lymphocytes

- **Central tolerance:**
  - Deletion of immature cells by high-affinity antigen recognition in the bone marrow
  - Some immature cells may change their antigen receptors when they encounter antigens in the bone marrow ("receptor editing")
- **Peripheral tolerance:**
  - Anergy
  - Exclusion from lymphoid follicles, death because of loss of survival signals

Tolerance in B lymphocytes: We have both central and peripheral tolerance.

In the central we have receptor editing of the B-lymphocyte along with deletion, while in the peripheral we have anergy, or exclusion of certain follicles which results in death of the cell.

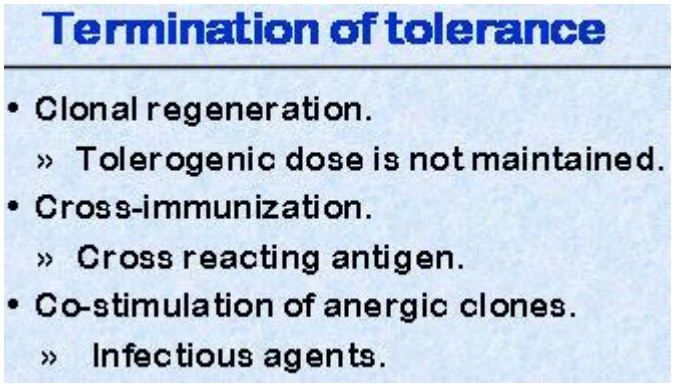
During receptor editing, the receptor's specifity may be changed, causing it to cease recognition of the antigen. This happens during the generation of the specifity of the B-lymphocyte.

Summary: B-lymphocyte will recognize the self antigen on the cell's membrane, the B-cell will either go through apoptosis / the receptor could be edited / we could get anergy / exclusion of the lymphoid follicles, resulting in apoptosis.

B-cell anergy is exactly the same as in the T-cell; however CD40 and the CD40 ligand will not be functioning (not B7 and CD28).

Autoimmune diseases:

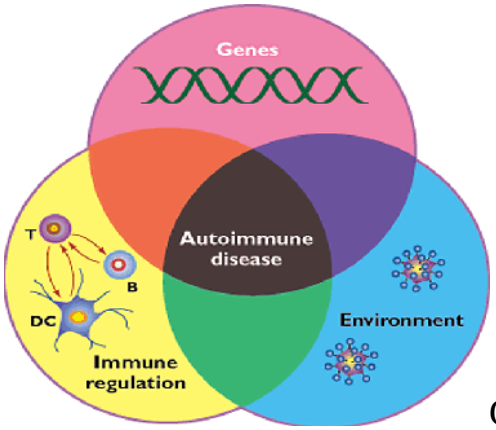
Failure of the immune system's tolerance.



Breaking the tolerance: the tolerance can be broken by different methods, such as **injecting a cross reacting antigen**, the new antigen will induce an immune response. A broken tolerance could be due to an **infection**, the initiated immune response may change the properties resulting in an alteration of the tolerated antigen producing a new reaction.

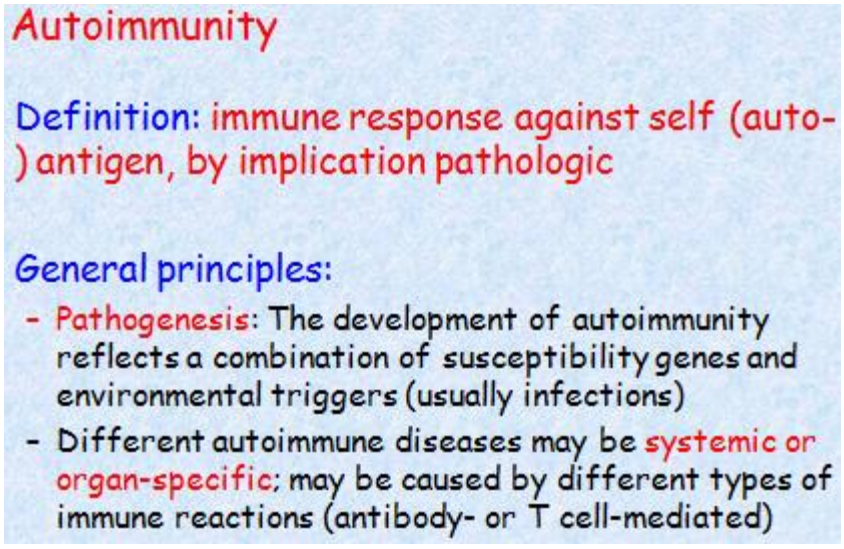
Autoimmune diseases are a direct result of the failure of tolerance.

Autoimmune diseases may be a result of a number of factors; including **genetic background** (Certain HLA types are more susceptible than others), or due to the **environmental factors** (Viral / bacterial infections), or due to a **problem in immune regulation**. Any abnormality in those three will result in an autoimmune disease.



Gender is also important; females in general have double the susceptibility to develop immune diseases (may be due to estrogen).

Autoimmune regulatory problems: Cells that recognize self antigens are not destructed nor dealt with.



Autoimmunity is usually classified under “Immune mediated inflammatory diseases”.

Autoimmune diseases could be localized (thyroglobulin & thyroditis), or generalized (rheumatoid arthritis / SLE-all nucleated cells in the body will be affected).

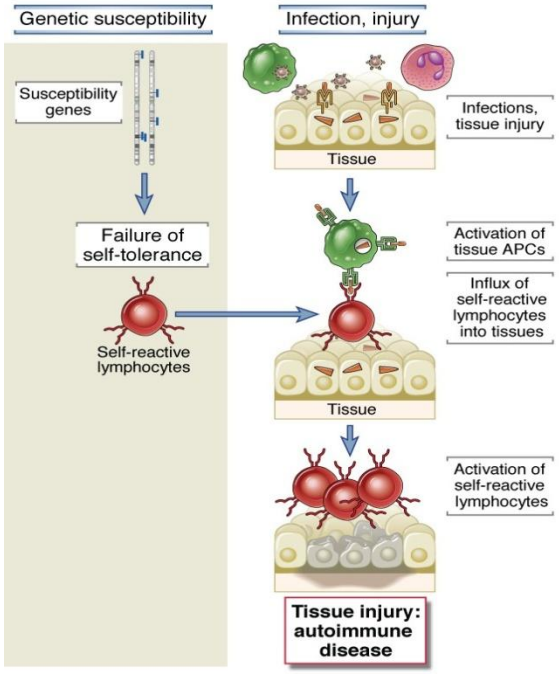
Termination of tolerance:

A) Clonal regeneration: cells which were already tolerated to certain antigens, by either cross antigens, infections, or environmental conditions…., become reactive against self antigens.

B) Cross immunization: Cross reacting antigen.

C) Co-stimulation of anergic clones: The second signal might come from another antigen, thereby overcoming anergy resulting in a reaction against a self antigen.

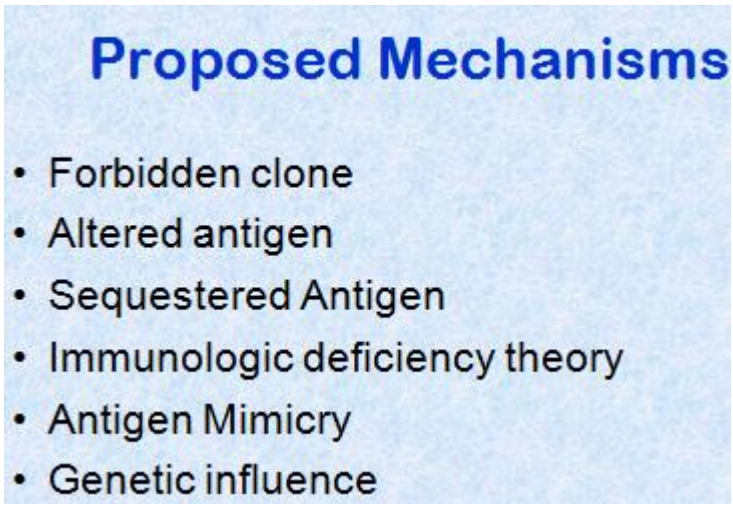
Pathogenesis of autoimmunity:



Genetic susceptibility → Failure of self-tolerance → persistent stimulation of lymphocytes.

Or it could be an environmental factor such as an infection → activation of self reactant lymphocyte.

Both will result in an autoimmune disease.



Proposed mechanisms by which autoimmune diseases arise:

1- The forbidden clone is the clone which doesn't have tolerance, if for any reason it was not dealt with and was allowed to persist; it can be activated by a viral infection or a metabolic change, resulting in an autoimmune disease.

2- Altered antigen: a self antigen that undergoes a mutation may be recognized by the host's cells as foreign.

3- Sequestered antigen: Parts of the body that never had any contact with the immune system (E.g. iris, sperm). If for any reason that part was to contact the immune system (because of a trauma for instance), the immune system will attack it.

4- Immunologic deficiency theory

5- Antigen Mimicry: An antigen which is similar to the original antigen.

6- Genetic influence: Male vs Female

T-cell Bypass: Under normal conditions, in order to get a response, the antigen must activate the T-helper cell, however in the T-cell bypass, in one way or another, the pathway will be changed resulting in **activation without the help of the T-helper cell**. This gives us an autoimmune disease.

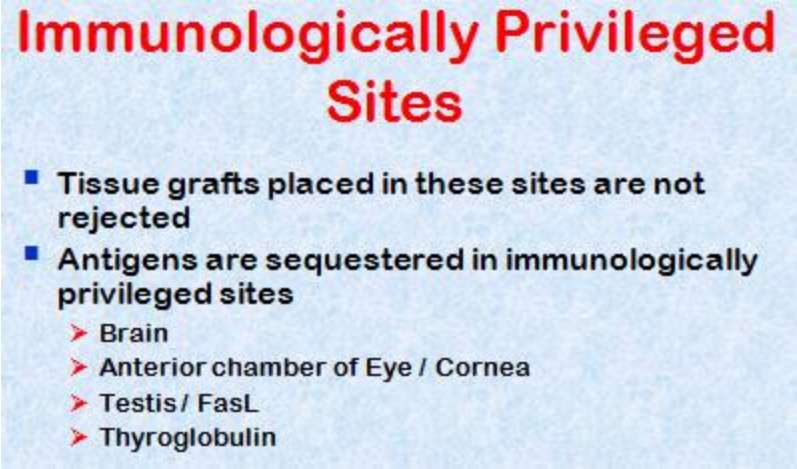
Role of infections in autoimmune diseases:

During an infection, the cell will be recognized and interleukins will be produced, along with the activation of macrophages. Those macrophages which are usually tolerated to the self-antigens will attack the host's tissues. (Some of the activated clones which have been infected will attack the self components).

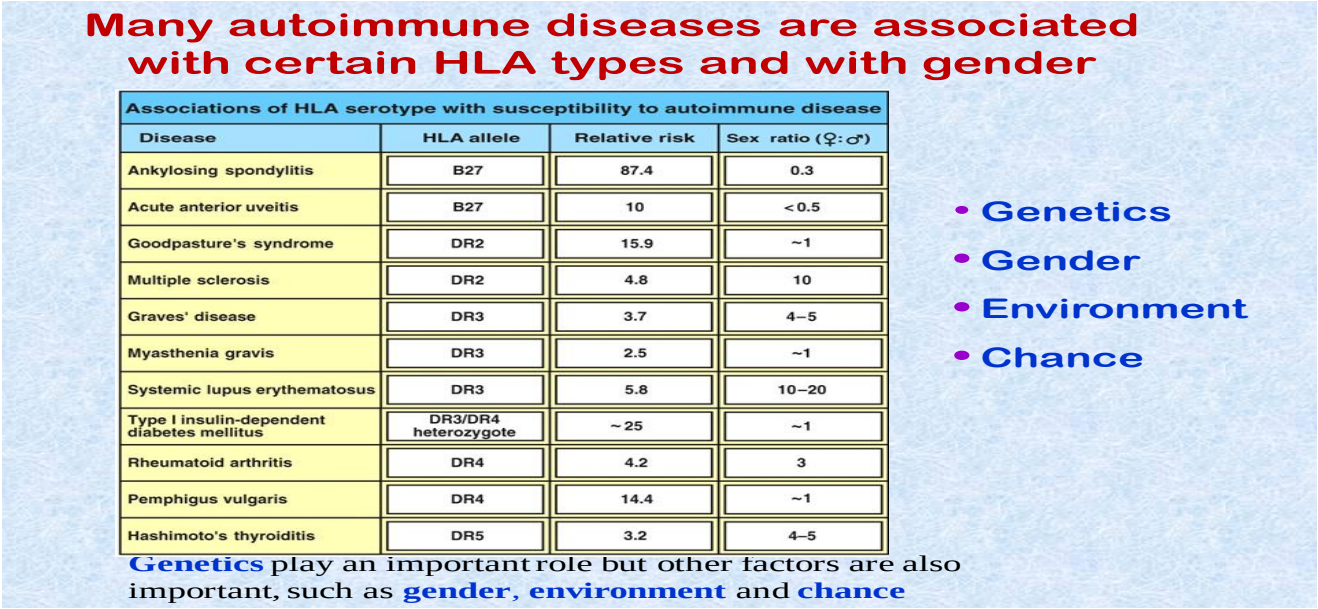
Any infection that results in tissue damage could cause an autoimmune disease. (E.g. tuberculosis)

The idiotype anti-idiotype can generate an autoimmune disease:

A lot of the idiotypes are autoantigens. In the case of an infection, the large number of idiotypes anti-idiotypes can generate an immune response. They produce an immune response to the autoantigen in the absence of the autoantigen if they can find the tolerated cell and activate it (similar to the action of a vaccine).



Immunologically privileged sites aka sequestered antigens.



There is an association between HLA types and certain diseases.

E.g. Ankylosing spondylitis, multiple sclerosis, Goodpasture's syndrome, and Rheumatoid arthritis have an association with certain HLA types.

Autoimmunity

Autoimmune diseases:
May affect 2-3% of population
Affect women disproportionately

Female: Male Ratios in Autoimmune Diseases

Multiple sclerosis	2:1
Myasthenia gravis	2:1
Diabetes	2:1
Rheumatoid arthritis	4:1
Graves' disease	7:1
SLE	9:1
Hashimoto's disease	50:1

As we can see, females are much more susceptible to autoimmune diseases than males (double).

This is mainly due to **estrogen.**

Genetic basis of autoimmunity -- 3

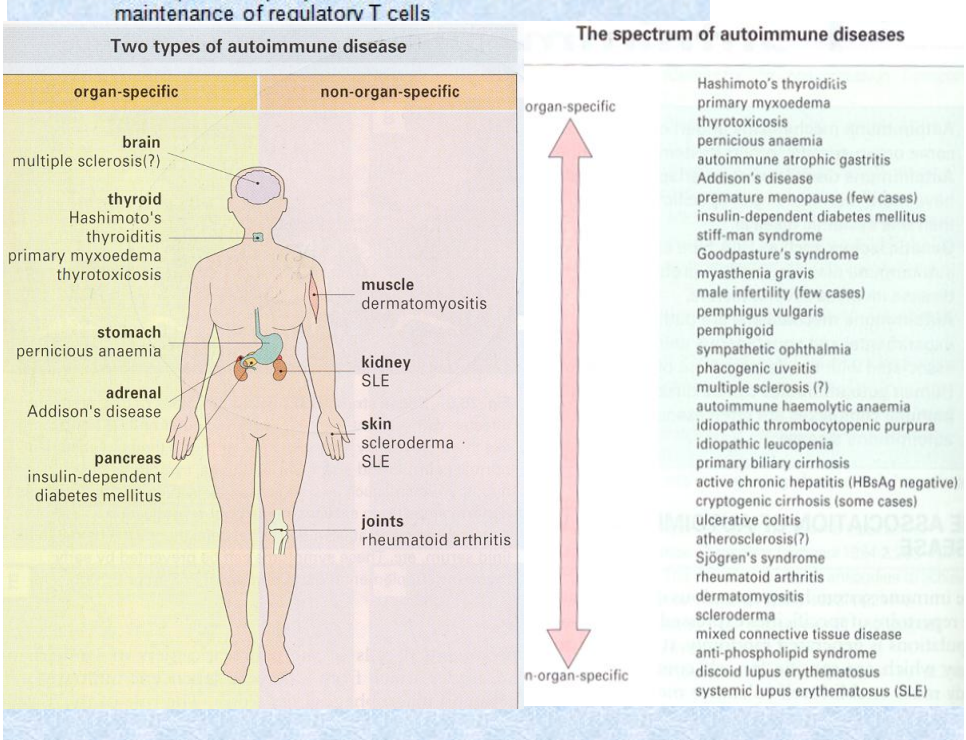
- Genome wide association studies are revealing genetic polymorphisms associated with autoimmune diseases
  - –Crohn's disease:
    - NOD-2: microbial sensor in intestinal epithelial and other cells
    - IL-23 receptor: involved in TH17 responses
  - –Rheumatoid arthritis, others:
    - PTPN-22 (tyrosine phosphatase): may control kinase-dependent lymphocyte activation
  - –Multiple sclerosis, others:
    - CD25 (IL-2 receptor): role in T cell activation and maintenance of regulatory T cells

<u>Genetic polymorphisms</u> associated with autoimmune diseases:

NOD-2 → Crohn's disease.

PTPN-22 → SLE and rheumatoid arthritis.

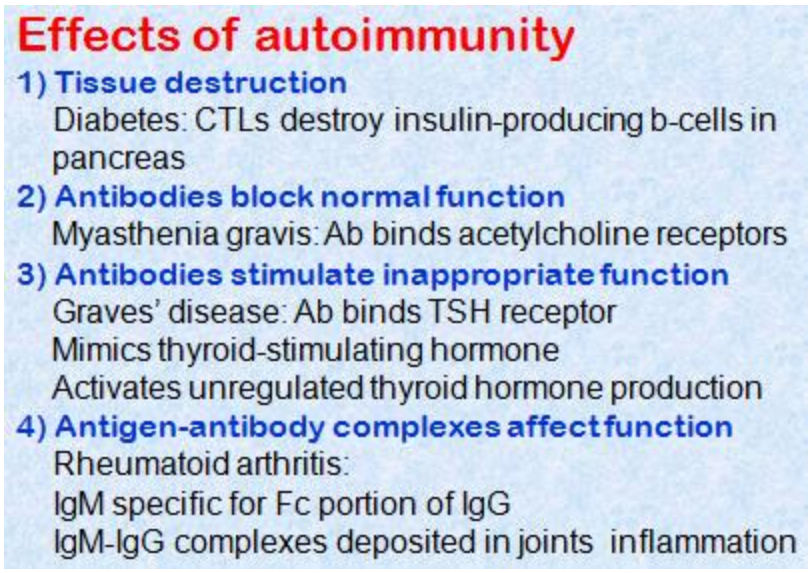
CD25 (IL-2 receptor) → multiple sclerosis.



Organ specific vs generalized.

Hashimoto's: very localized in the thyroid.

SLE: can be found in any part of the body.

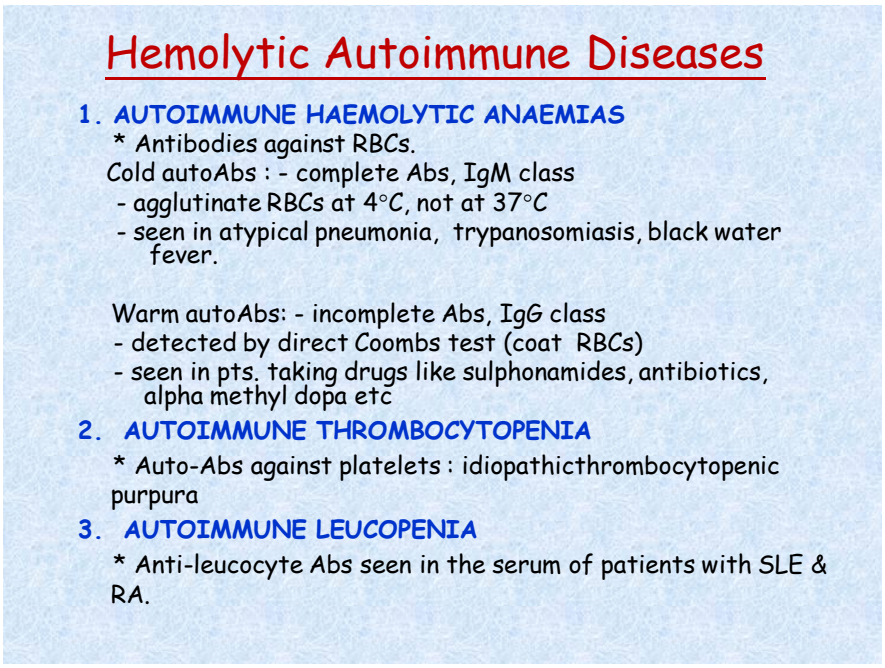


1- Tissue destruction: either through antibody or complement or cytotoxicity of T-lymphocyte.

2- Antibodies blocking the normal function.

3- Antibodies bind TSH receptor causing the release of thyroglobin. TSH receptor is regulated while antibodies are NOT regulated.

4-Antigen antibody complex deposits in joints (Rheumatoid arthritis).



Autoimmune leucopenia: antibodies against WBC.

Localized Autoimmune Diseases:

A- Insulin dependent DM –type 1 diabetes – autoimmunity against islets of Langerhans in Pancreas. (Islets of langherhans are insulin secretors).

B- Autoimmune diseases of the thyroid gland

A. Hashimoto's thyroiditis.

B. Thyrotoxicosis (Grave's disease) Anti-thyroglobulin

C- Addison's disease

Antibodies to the cells of Zona Glomerulosa layer of adrenal glands

D- Myasthenia gravis

E- Pernicious anemia

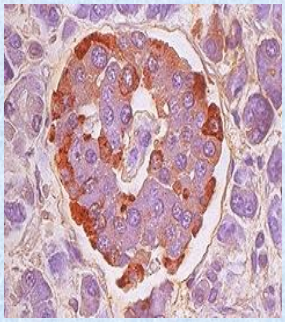
F- Autoimmune Liver and Gastointestinal Disease

Grave's disease: **Antibody to thyroglobin**. Normally, TSH would be secreted by the pituitary, the cells would be stimulated then produces thyroid hormones. If we have an antibody to the same receptor, they will react with the receptor, the cell will produce the hormone but without any control (**No feedback inhibition**).

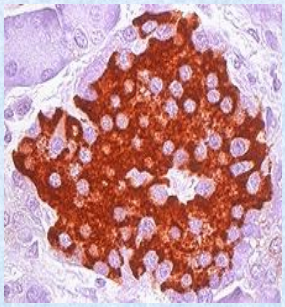
Type 1 diabetes: There are **autoantibodies to islets cells** (we have around 4 different types of cells). Mainly they will act on the Beta cells of the pancreas resulting in its destruction. When the number of Beta cells becomes around zero, we will start to see signs of type 1 diabetes. Patients must receive chronic administration of insulin.

Multiple sclerosis: **Antibodies attack the myelin sheath**. The sheath of the nerve will be destroyed causing persistent stimulation of that nerve. The patient will have trouble walking (They cannot control their movement).

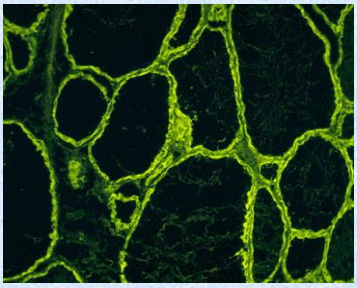
Myasthenia gravis: ACH will be generated by the neurons, it will go to the neuron gap between the neuron and the muscle, and it will react with the receptors on the muscle causing its contraction. If we have antibodies to the ACH receptors, the muscle will be activated completely and this results in a very serious disease. This will result in weakness of the muscle and some sort of abnormality.



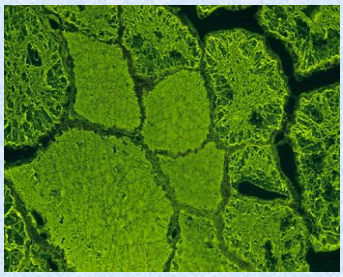
α cells Glucagon



β dells (Insulin)



Antimicrosomal Antibody



Anti-thyroglobulin Antibody

How do we diagnose these types of diseases? We look for antibodies in the target tissues (Using immunofluorescence or detection of antibodies mechanism).

Rheumatoid Arthritis: Immune Response to Joints

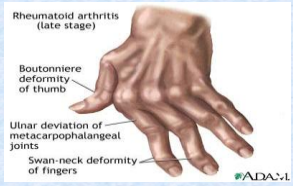
- **Rheumatoid factor:** IgM/G/A against the Fc region of IgG
- Leukocyte infiltration in the joint synovium: CD4 T, CD8 T, B cells, neutrophils and macrophages
- Plasma B cells produce rheumatoid factor
- Inflammatory cells produce prostaglandins and leukotrienes, lysosomal enzymes, proteinases and collagenases
- **Treatment:** anti-inflammatory and immunosuppressive drugs. e.g. anti-TNF-a

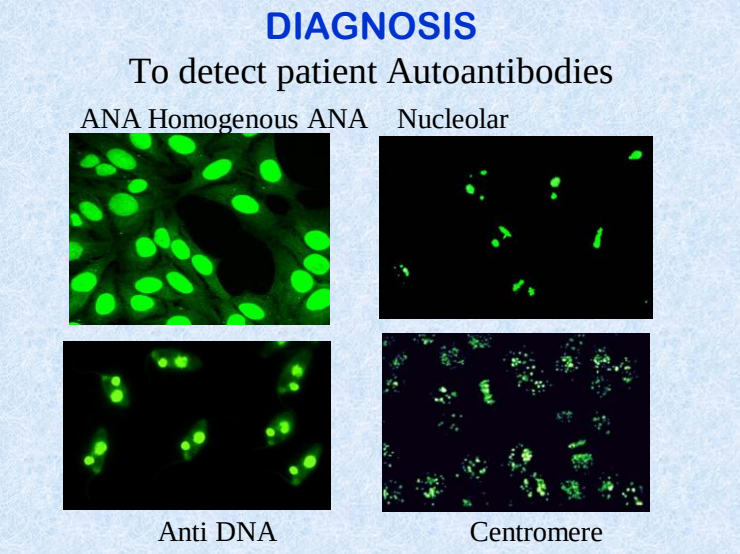
Complex deposition in the joints.

End result: Patient cannot move his fingers or his joints.

Rheumatoid Arthritis (RA)

- Symmetric polyarthritis with muscle wasting and subcutaneous nodules.
- Associated with myocarditis, vasculitis & other disseminated lesions.
- Presence of a circulating auto Ab called Rheumatoid Factor (RF)
- RF- IgM directed against one's own IgG
- Diagnosis : Latex agglutination test





Diagnosis of generalized diseases:

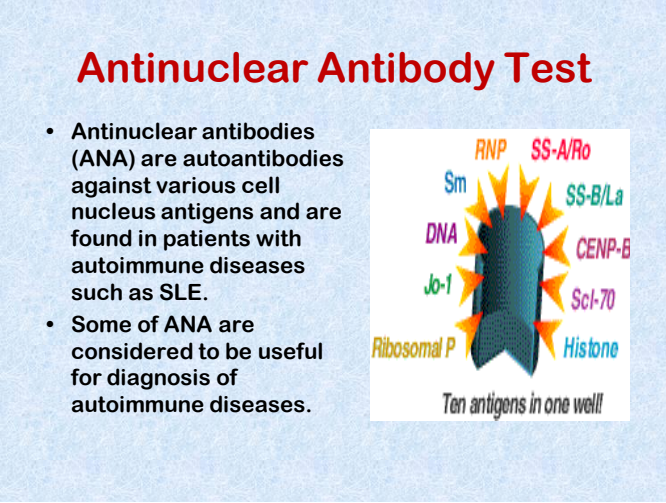
We can look for antibodies in the nucleus of any nucleated cell.

Percentage of people with antibodies to nucleated cells:

SLE 99%

Reumatoid arthritis 40%

Healthy control 5% (This means that 5% of the healthy population have antibodies against their nucleated cells).



Articles in italic were copied from the internet to compensate for the lack of explanation provided in the lecture.