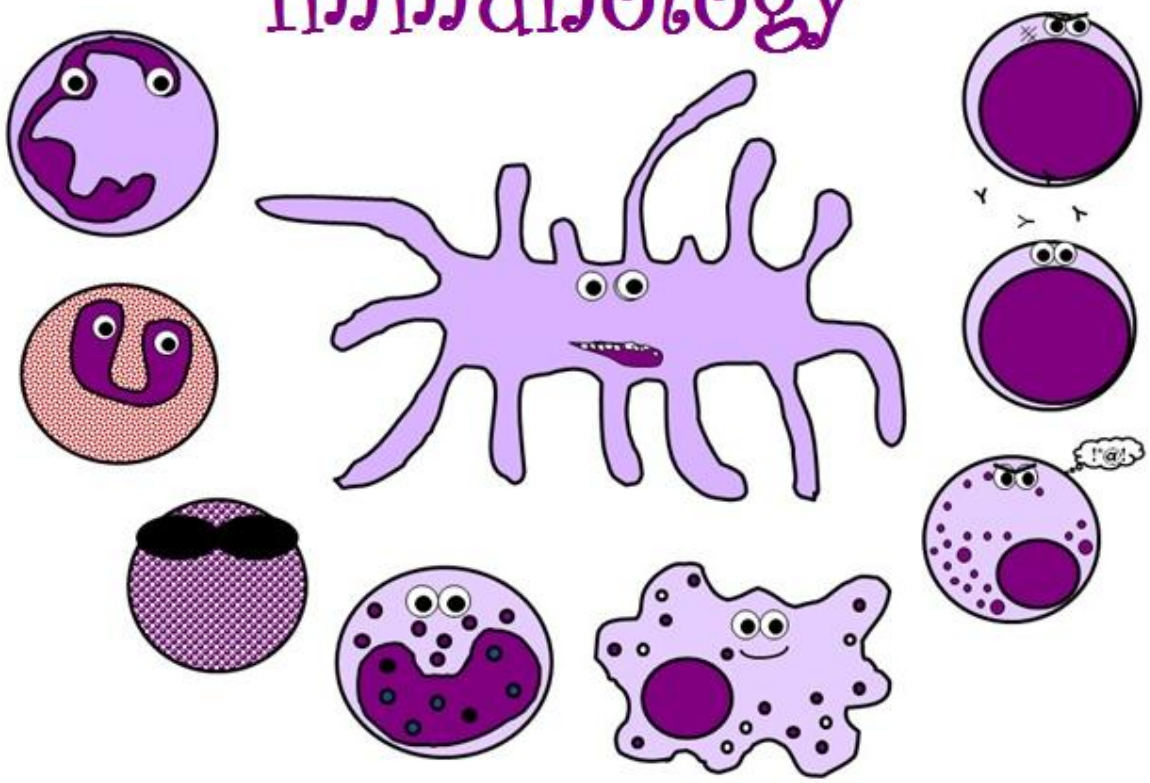
Immunology



Sheet Slides

Number: 16

Subject: the immune system going wrong

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Topics of the lecture:

- *Immune system going wrong:*
  - Pathological conditions caused by a normal immune response.
  - Diseases caused by defects in the Immune regulation.
  - Autoimmune diseases.
- NOTE: after each topic its related slides are attached.
- Some tests discussed in the lecture were explained by the doctor briefly, you need to understand the concept only.

❖ *Immune system going wrong (immune-pathology)*: When the immune system reacts in a wrong way with some conditions resulting in pathology in the human body.

→ Immune system can go wrong in one of the following cases:

1- Pathological conditions caused by a normal immune response:

→ When there is an infection in the body but the immune system respond in an inappropriate way -for one reason or another-. In other words, there is an infection but the triggering of the immune system is much more than it should be leading to **tissue damage**. It can occur in any infection such as tuberculosis.

2- Diseases caused by defects in the Immune regulation:

→ Instead of dealing with a specific antigen in a calm response depending on cellular immunity and producing IgG's, there is more forcing for humoral immunity and IgE's production resulting in **allergy**.

3- Autoimmune diseases: *they will be discussed in more details in the next lectures.*

4- Immune deficiencies: it is classified into;

► **Primary immune deficiency**:

→ The patient is born with such deficiency.

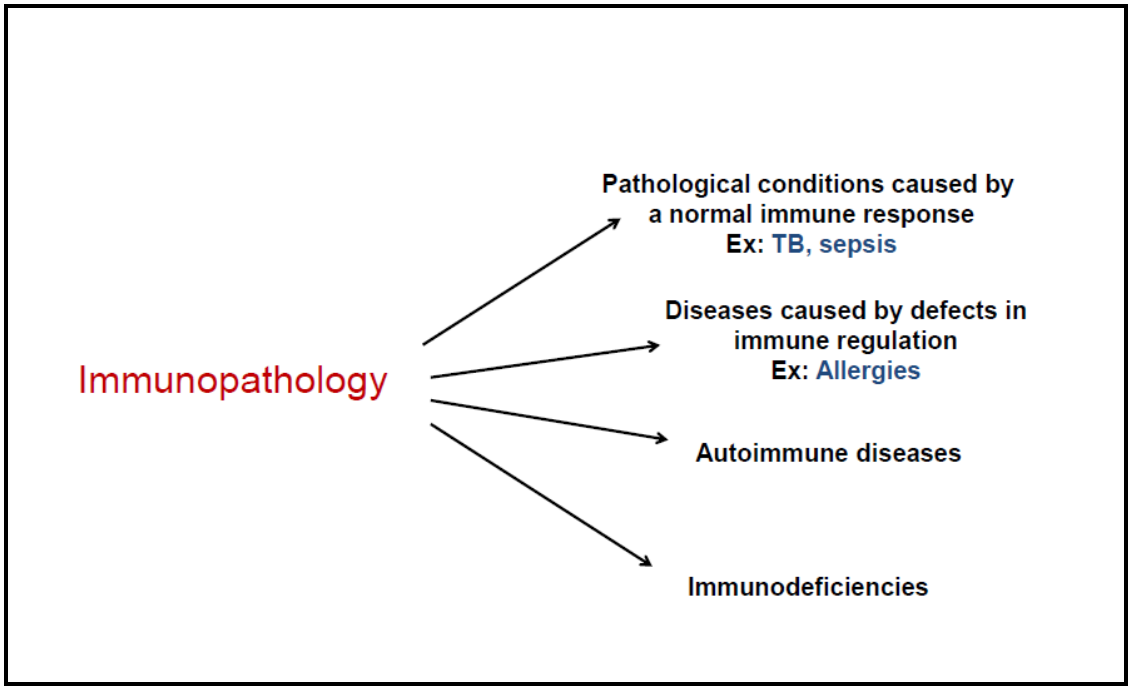
→ More prone to opportunistic infections

→ *Many of the case studies discussed in the previous lectures are examples on primary immune differences, such as Omenn syndrome.*

► **Secondary immune deficiency :**

→ The patient is born with normal immune system and at a certain point in his/her life he/she develops an immune deficiency caused by infection, treatment or any other reason.

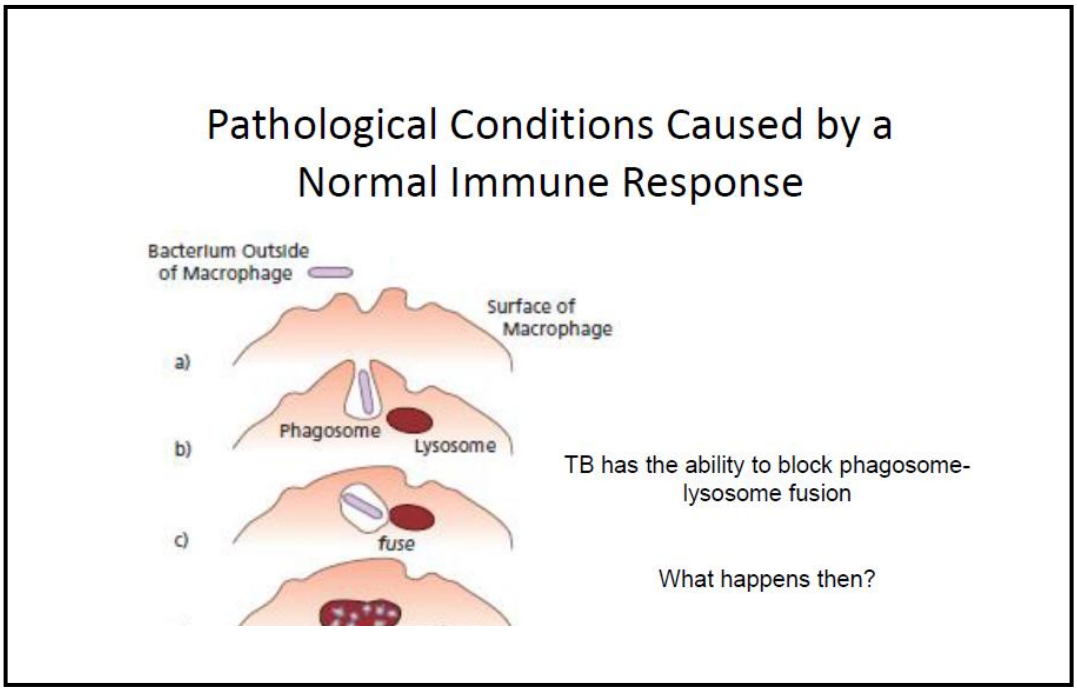
➢ ***NOTE:*** *Immune deficiencies are going to be discussed in more details in the upcoming lectures.*

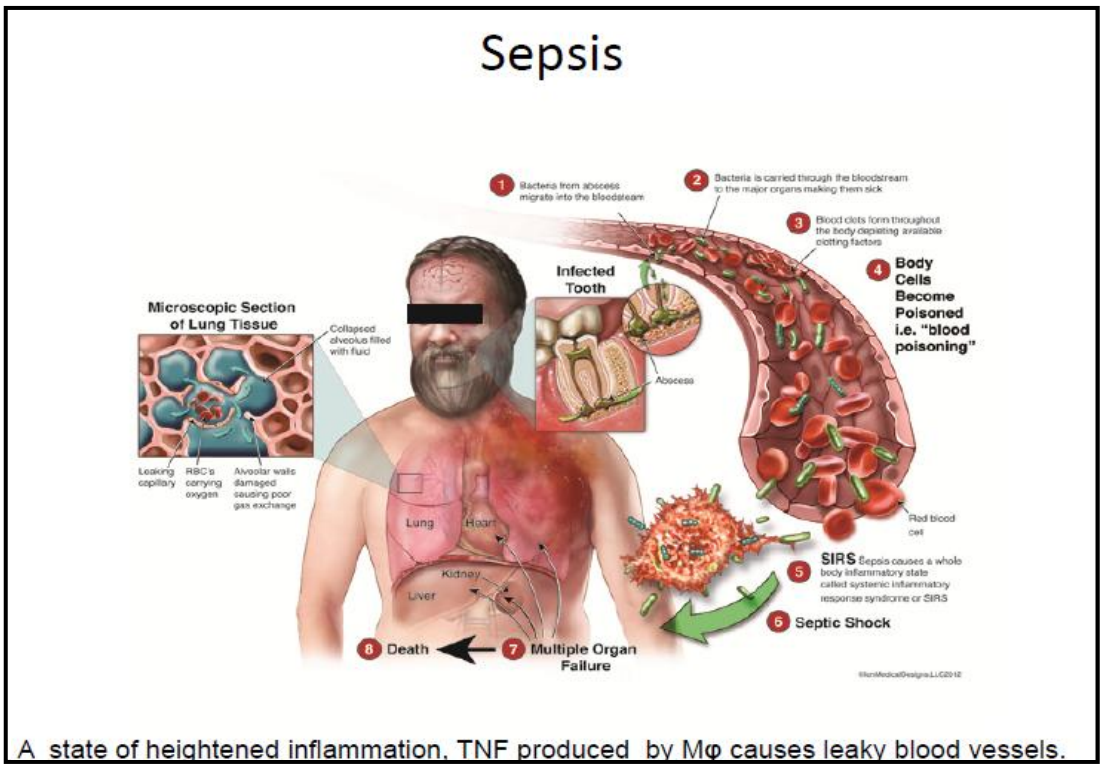


1. Pathological conditions caused by normal immune response:

- Normally,
  - → When a microorganism enters the body, one of the first cells to fight are macrophages that are able to detect antigens →phagocytose the microorganism carrying the antigen→ forming phagosome →fusion with lysosome → phagolysosme is formed that has low PH and many degradative enzymes that will destroy the engulfed microorganism and process the antigens to load them on MHC molecules introducing them to other immune cells.
- However;
  - → Some microorganisms have many mechanisms by which they can protect themselves from our immune system. One of these mechanisms that occurs in tuberculosis and leishmania infections is **blocking the fusion of the lysosome with the phagosome** →thus the phagolysosme ,that is able to destroy these organisms, **is NOT formed** → so these microorganisms will live within macrophages and divide freely until we reach a state at which the macrophage is totally filled with these abnormal phagosomes and they are overwhelming macrophages → macrophages will explode and die by necrosis -*not by apoptosis*- →inducing the release of many inflammatory cytokines that lead to **tissue damage.**

- **<u>Sepsis</u>** is another example on the immune system going wrong:
  → in some cases, bacteria reach the blood stream and for some reasons the spleen is not able to respond and fight the infection in a proper way →thus bacteria will start dividing in the blood ,many inflammatory responses are going to be there, also clotting cascade will be affected as well as depletion of clotting factors since there is blood clotting →this will lead to **systemic inflammatory response syndrome** (SIRS) which is a hyper-inflammatory state in which macrophages are producing a lot of TNF → eventually this will cause leakage of blood vessels , hypo-volemia and septic shock that may cause multiple organ failure -the heart ,lungs, liver and kidneys can be damaged- → if not controlled death may result.





A state of heightened inflammation, TNF produced by Mφ causes leaky blood vessels.

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2. Diseases caused by defects in immune regulation:

- Examples are allergy -like hay fever- and asthma.
- Allergy patients tend to produce **IgE antibodies** much more than normal people **(IgE based immune response).** On the other hand, normal people immune responses to same allergens are **IgG based response**.
- Remember that Mast cells (*الخلايا الصارية*) have IgE receptors on their surfaces. In the first exposure to allergen IgE antibodies are formed and they bind to their receptors on the mast cells - forming **armed mast cells**- . On the second exposure the allergen will bind to IgE that is already attached to a mast cell thus it activates mast cells and causes de-granulation releasing chemical mediators such as **histamine** and other enzymes.
- Similar scenario is seen in other cells such as **basophiles** that have an important role in allergies. And due to this importance; one of the important tests that are used to diagnose

allergies to test basophiles response to the allergen and it is known **as basophiles activation test -***further details are mentioned later in this sheet-.*

- These cells – basophiles and mast cells- are responsible for i**mmediate allergic reactions** which are the allergic reactions that take place directly after the exposure to the allergen.
- While eosinophils are involved in the **chronic allergic reaction** (like asthma) as they secrete IL-4 and IL-5. IL-5 is very important to recruit eosinophils from the bone marrow and this recruitment takes some time - i.e. it is NOT an immediate process- resulting in a **delayed** response in many cases of allergy. And these patients also will have high IgE levels in their blood. - *Note: other immune cells are involved in such reaction-.*
- **<u>The biological function for such cells:</u>**
  - → To fight **parasitic infections**; IgE is a potent antibody that can fight such infections and it can induce immune cells that release chemical mediators that are very effective in killing worms.
  - → And allergy is only an unfortunate side effect for these cells.
- **<u>NOTE:</u>** we need activation of Th-2 cells to drive an IgE class switching by secreting IL-4, 5 and 13. The decision to produce IgG or IgE Abs in response to an allergen will depend heavily on the type of Th cell present in the secondary lymphoid organ encountering the antigen.

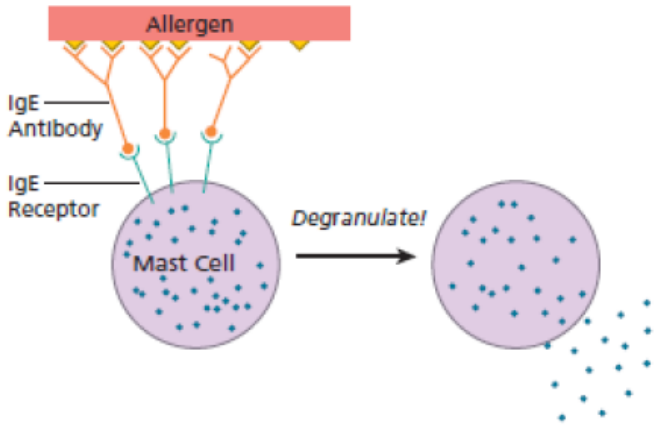
  (This is the reason behind why some people develop allergy while other do not).

Diseases Caused by Defects in Immune Regulation

Most common examples: Hay Fever, asthma.

Allergic people over-produce IgE antibodies. Non-allergic people usually respond weakly to allergens by producing IgG antibodies.

Mechanism of allergy



IgE antibodies become more long-lived once attached to surface of mast cells.

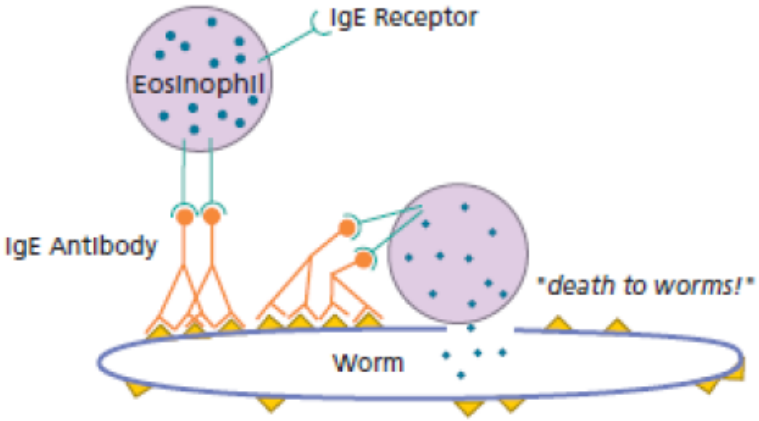
A similar scenario occurs with Basophils. (Immediate allergy).

Delayed Allergic reactions

These are chronic allergic reactions (Ex: Asthma); Eosinophils are involved.

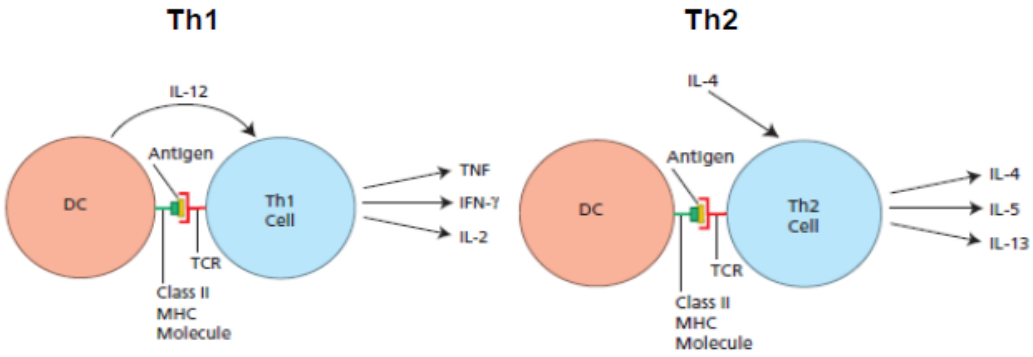
Allergic reactions cause Th cells to produce IL-5 which recruit eosinophils From the BM. = Takes time

What is the biological use of mast cells, basophils, and eosinophils??



Why do some people have allergies?

In order to produce IgE, Th2 cytokines are needed.



The decision to produce IgG or IgE Abs in response to an allergen will depend heavily on the type of Th cell present in the secondary lymphoid organ encountering The antigen.

➢ The hygiene hypothesis:

- The fetus can be considered as a foreign body to the mother's immune system, which means that the immune system can recognize it and attack it. This is the reason behind the presence of huge amounts of **anti-inflammatory** cytokines such as IL-4 and IL-10 in the **placenta**. So we have a Th-2 response in the placenta to prevent cytotoxic T cells and NK cells activation, make it possible for the mother's immune system to tolerate the presence of the fetus and guarantee the **survival of the fetus**.
- That explains why sometimes abortion occurs although everything is normal in the mother's body; it is caused by a highly active NK cells.
- **NOTE:** the fetus is semi-foreign to mother's body.
- Although this way is important to protect the fetus during embryonic life, when he is born he will have **T helper2 bias**; which means that he is much more prone to develop allergies and asthmas.

- Based on this;
  - **hygiene hypothesis** states that children who are born in the developed countries (USA, Europe for example) where they have a high standards of hygiene and they are not exposed to many pathogens and microorganisms, are more susceptible to develop allergies and asthmas than in the developing countries where children are more exposed to pathogens.
- And it has been found that when the child encounter pathogens (i.e. when the child plays with other children , goes to school and reacts with his environment) while he is young, he will be less prone to develop allergies, asthma and even cancers and lymphomas. While those who were raised in a highly clean environment and do not react with their surrounding environment are more prone to develop allergies and asthmas.

Thus the childhood infection rate is inversely related with the rate of developing allergies.

- Allergy in general is contributed to hereditary factors as well as environmental. The hygiene hypothesis deals with the environmental factors that are related to allergy.

- **<u>Allergy From a genetic perspective</u>**;
  - When one of identical twins has an allergy from something, the chance for the other to be allergic to the same thing is 50% (to be atopic); suggesting genetic factors that contribute to allergy.
  - Many forms of the MHC molecules have higher tendency to present allergens as antigens more than other forms, thus patients with specific forms of HLA are more prone to develop allergy than people with other forms.
  - **Mutations in the IgE receptors** that make the signaling of IgE much stronger, these people are also more prone to develop allergy.
  - Mutation of the **promoter region of IL-4** gene resulting in overproduction of IL-4 →more class switching to IgE →more prone to allergies.

<u>NOTES:</u>

- → ***It is very rare to find a disease that is purely caused by a genetic factor alone or environmental factor especially immunological ones.***
- → ***Many diseases in general have a genetic predisposition.***
- → ***Promoter region is an upstream region of the gene that triggers transcription of the gene when stimulated.***

Hygiene Hypothesis

Cells of the placenta produce large amounts of IL-4 and IL-10, why???

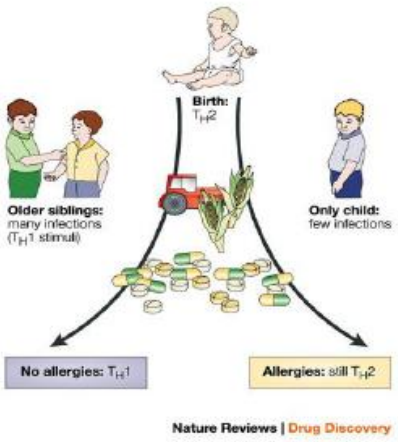
This drives a Th2 response, and blocks activation of CTLs and NK cells

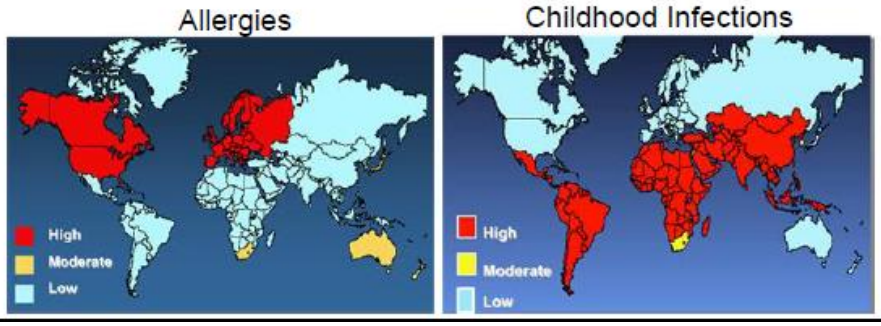
Advantage? Survival of the fetus...

But as a result, fetus has a Th2 bias when born!!!

What can balance the bias?

Infections with bacteria and viruses!
The earlier the better= allows a normal response to environmental allergens.





Environmental vs. Hereditary causes of allergy

Hygiene Hypothesis= Environmental causes

Hereditary causes exist. If one twin is atopic, other twin has 50% chance

Certain **MHC class II** molecules are more efficient at presenting antigens than others.

Some indiciduals possessing **mutated IgE receptors=** Send stronger signals.

Mutations in the **promoters of IL-4 genes**, can increase its production.

➢ Tests used to diagnose allergy:

1- Specific IgE testing:

- Generally, the test screens the patients IgE to see if the patient has specific IgE antibodies against one of the allergens that are found in the panel used in the lab.
- ***NOTE:*** *labs use two panels for allergens; one for allergens found in the food (around 32 allergens) and the other for the inhaled allergens (also around 32 allergens). These panels are specific for each country according to most common allergies found in it. Unfortunately, those which are used in Jordan are Turkish panels and Middle Eastern panels (i.e. they are not specific).*
- **NOTE:** the test is ELISA based.
- The procedure:
  - → Firstly, we add the patient's serum over the allergens we have → this will allow the binding of IgE antibodies to one of the allergens *-in case that the patient has an allergy-* → we wash the preparation we have to remove the unbounded antibodies →then a secondary antibodies are added , these antibodies will bind the Fc portion of the IgE antibodies and they are conjugated with an enzyme→ we wash again to remove unbounded secondary antibodies → finally we add a substrate that can bind the complex that is formed -which is the primary IgE bounded to the secondary

antibody- → this substrate will change the color upon binding→ each specific color indicates the type of the IgE found thus the type of allergy.

→ The intensity of the color produced is read by a computer to give the exact amount of the antibodies found on the patient's serum.

2- Skin prick test:

- It is done in dermatology clinics not in the labs.
- The skin of the patients is injected with different allergens and negative control (i.e. normal saline) and a positive control (i.e. histamine) in different areas of the skin.
- Allergens are injected either on the forearm or the back of the patient.
- The reaction is considered positive when the itching and redness result.
- This test needs high levels of accuracy when it is done; as if the injection was not deep enough a false negative may result and if the injection was too deep a false positive reaction may result.
- Results are detected within 15-30 minutes.

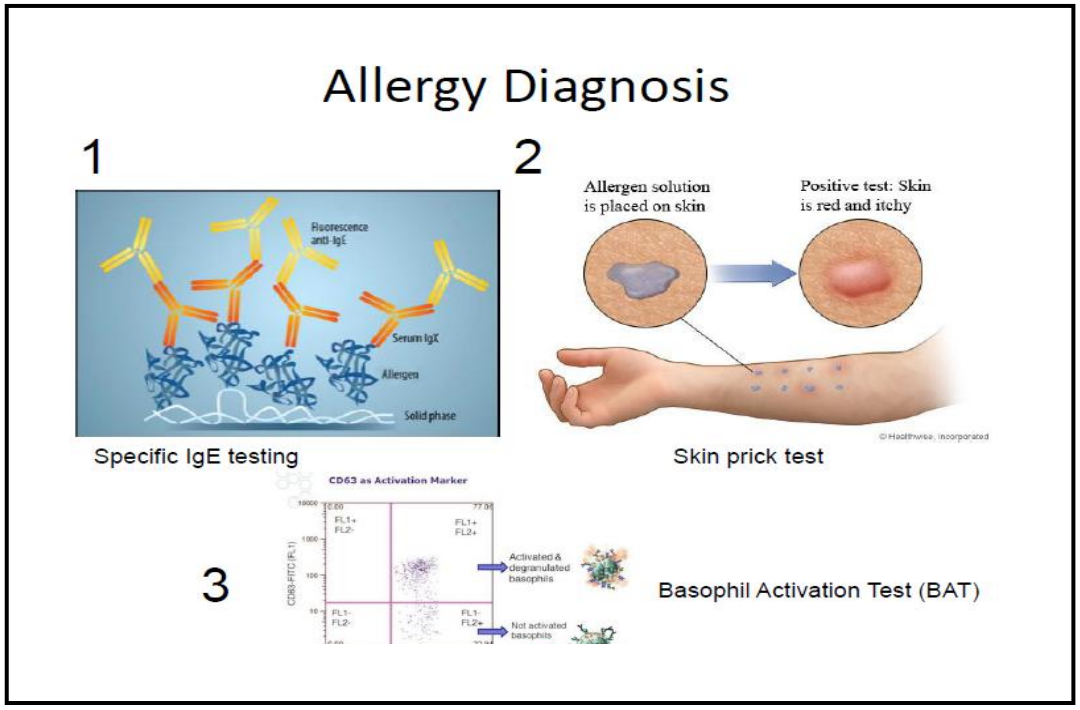
3- Basophile activation test (BAT):

- This test is based on the fact that basophiles are important cells in allergic reactions.
- All basophiles are detected by their marker which is **CCR-3** and activated basophiles are detected by **CCR-63. Any cells that express both markers is an activated basophile (upper right corner in the chart) and the ones which express only CCR-3 are inactive basophiles(lower right corner)**. *Cells that are found on the left side of the chart are not basophiles.*
- The test is performed by adding the allergen we suspect that the patient has allergy from to a blood sample drawn from the patient →if the patient is really allergic from the added allergen, basophiles will react with it → then a flow cytometry is done to detect the basophiles→If most of the detected basophiles are found in the upper right corner (**activated basophiles**) →this means a reaction has occurred →this confirm that the patient has an allergy from the added allergen.

➢ Comparison between the three methods :

- **Specific IgE test advantages**:
  - ❖ It can screen the patient serum reaction with 30 allergens **at the same time**. *Instead of trying the immune response of the patient against each allergen alone each time, in specific IgE test, you test the response against the whole panel at once.*
  - ❖ In case the skin prick test is weak for any reason, specific IgE test can give **more reliable results**.
  - ❖ It is a **non invasive test**.
  - ❖ It is **not affected by anti histamine therapy** which is an important point as most allergic patients use such drugs to decrease the severity of the symptoms. The reason behind this fact is that anti histamine drugs **do not affect B cells'** ability to produce IgE antibodies. On the other hand, skin prick test is affected by anti histamine drugs as it requires histamine to give the correct results, so in such case it will give false negatives.

- **Skin prick test advantage:**
  - ❖ In cases when the patient is not exposed to an allergen for very long time >> the serum IgE levels will decrease so much >> so testing the serum will not be effective as it depends on the IgE levels in the serum. While in skin prick test we **inject the allergen itself** in the patient's skin thus an immune response will take place for sure if he is allergic.
- **BAT advantages:**
  - ❖ Although it tests a single cell type reaction to an allergen, it is used to detect allergy from any substance (drugs, food, inhaled allergens…etc.) and this is very useful in testing the allergies caused by drugs(antibiotics like penicillin) instead of depending on error trails.
- **NOTES:**
  - → Wasp bites and jelly fish toxins (???) and many drug induced allergies are very dangerous allergies.
  - → All allergies can be life threatening if not treated as they cause **chocking**.
  - → Keep in mind that the immune system is very dynamic thus some cases of mild allergies may disappear with time and the patient will not be allergic any more, like egg allergies in children.
  - → The half life of the IgE is 3-5 days when it is **FREE**, while it becomes much longer lived when **bound to mast cells**.



➢ Allergy treatment:

1- **Anti-histamine drugs:**

- Prevent the release of histamine →prevent its actions→ they decrease the severity of allergy symptoms.

2- **Corticosteroids:**

- Block cytokine production by Th cells→ block the ability to produce IgE antibody→ prevent allergies.

3- **Omalizumab:**

- Used when the corticosteroids are not effective in some patients.
- It is an **antibody** that is able to bind to the Fc portion of the **free** IgE→ so IgE antibodies will not be able to bind to their receptors on mast cells any more →no allergic reaction.
- Very safe and effective drug but still expensive thus its use is limited.

these three drugs are to improve symptoms NOT to treat and cure allergy.

4- **Specific immune therapy:**

- → The ONLY **curative** treatment for allergies.
- → **The procedure:** the patient is given very minimal amounts of crude extract of the allergen at the beginning under very strict conditions *(given in the hospital)* →then the "doses" of the allergen is increased gradually until we reach a maintenance dose → this maintenance dose is given to the patient over the following 1 or 2 years.
- → **The immunological principle behind this way of treatment:** this mechanism induces certain T regulatory cells that **block IgE production** and drive the immune response more towards **IgG** production. And these patients after a while of the treatment they begin to produce minimal amounts of IgG against the allergen instead of IgE which means they are **cured** from allergy.
- → ***NOTE:*** *It is the same as desensitization therapy that was mentioned by the doctor once.*

Allergy treatment

Anti-histamines

Glucocorticoid steroids (block cytokine production by Th)

Omalizumab (Abs that bind to Fc portion of IgE) Safe, effective, but expensive

Specific Immunotherapy (Cure!): Injection of gradually increasing doses of crude extracts of allergens until a maintenance dose is achieved. (Several years)

Switch from IgE to IgG production. (Regulatory T cells involved?)

3- Autoimmune diseases:

- Remember that the immune system is in a state of tolerance and has many mechanisms by which it protects us from autoimmune diseases. However this tolerance is sometimes **broken** resulting in an autoimmune diseases!
- Autoimmune diseases have **genetic causes as well as non genetic causes**. For example ALPS is a disease caused by mutations in FAS or FASL (ALPS) that makes the patient more prone to develop autoimmune diseases *(genetic).* On the other hand; some diseases are not caused by genetic defects; instead they are caused by problems in the education of T cells, peripheral or central tolerance.
- 5% of the US population suffers from autoimmune diseases.
- **Three conditions** are required to develop an autoimmune disease:

Molecular mimicry means that the exogenous antigens share similarity with a self antigen.

<u>Extra note:</u> Antistreptolysin O (ASO) titer is a blood test to measure antibodies against streptolysin O, a substance produced by group A streptococcus bacteria.

1- **MHC molecules able to efficiently present auto-antigens**:
   → That is why some people are more prone to develop autoimmune diseases than others as their MHC are more able to present auto-antigens.
2- **Presence of T cells or/and B cells that recognize the auto-antigen**.
   → Which means that if there was an MHC molecule that present auto-antigen BUT there is NO auto reactive B or T cells , no immune response will be triggered >>thus no autoimmune disease.
3- **Environmental triggers:**

→ Most autoimmune diseases require environmental trigger and it is usually **viral or bacterial infection**.

→ Rheumatic fever for example occurs after a strep throat infection in which ASO titer is high and the infected baby is not treated. Due to antigenic similarity between streptococcal antigens and antigens found in the heart valves **(Molecular mimicry)** →the formed antibodies will attack heart valves causing rheumatic fever→valvular stenosis or regurgitation ensue.

Autoimmune Disease

Results when a breakdown occurs in the mechanisms meant to preserve tolerance of self that is severe enough to cause a pathological condition. (~5% of Americans)

Genetic Causes:

Autoimmune lymphoproliferative syndrome (Genetic defect in Fas or FasL)
(Canale-Smith Syndrome)

Non-genetic Causes (Loss of self tolerance):

Failure to eliminate self-reactive cells in genetically normal individuals.

Conditions Needed for Autoimmune Diseases

Three conditions need to be met:

Individual must express an MHC molecule that efficiently presents a self antigen.

Individual must produce T and/or B cells that recognize self antigens.

Environmental factor (trigger) that breaks self tolerance (Viral/bacterial infection).

(Molecular mimicry) ex: rheumatic heart disease

➢ Autoimmune diseases and inflammation:

→ Remember that if an auto reactive cell is present but no co-stimulation is found, no immune reaction will take place and cells will die by apoptosis.

→ In most cases of autoimmune diseases co stimulation is provided by inflammation. Inflammation triggers cytokines production >> cytokines activate APCs >> they will provide the co-stimulation signal wanted by auto reactive cells>> autoimmune disease is the result.

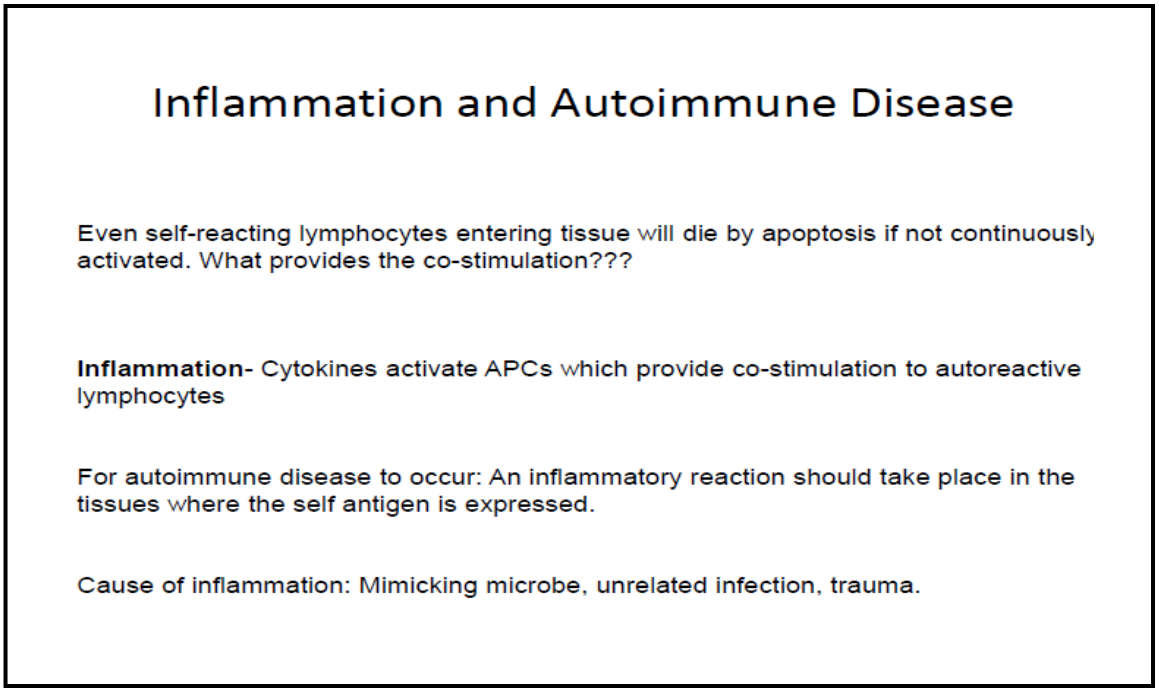
Inflammation is a key for the development of autoimmune diseases.

→ Inflammation should take place in the tissues where the auto-antigen is presented.

→ ESR and CRP are elevated in most autoimmune diseases indicating a presence of chronic inflammation.

→ The causes of the inflammation:

- Mimicking antigens of the pathogens (EBV or streptococcus for example).
- Unrelated infection.
- Trauma.



→ Examples on autoimmune diseases:

A. Type 1 diabetes:

- It is a pure autoimmune disease in which the body produces antibodies that attack the **beta cells** of the pancreas.
- There are genetic factors that increase the risk of developing diabetes type 1:
  - In identical twins, if one of them has diabetes type 1 then the other one will have a chance of 50% to develop it.
- Patients have less CTLA-4.
- Also defects in naturally occurring T-reg functions are suspected.
- Unfortunately this disease is not diagnosed until 90% of the beta cells are destroyed thus it is called a silent killer.
- If the patient was diagnosed early –i.e. before total damage of beta cells- we may suppress his immune cells to prevent further damage or at least prolong the time required for damage.
- Tests performed to type 1 diabetes patients:

  (a) **Antibodies against beta islets:**
  - On a slide we put the patient's serum on a pancreatic tissue we have and we see if there is ABs against beta islets or not.

  (b) **GAD-antibodies:** - glutamic acid decarboxylase antibodies-.
  - Glutamic acid decarboxylase is an enzyme found in the pancreas, and in case of type 1 diabetes antibodies against it are formed. *The doctor by mistake said that GAD is glutathione decarboxylase.*
- **NOTE:** Other cells in the pancreas secret glucagon and somatostatin and they are not affected in case of diabetes type 1.

Insulin- dependent diabetes mellitus

Organ-specific autoimmune disease. (β-islets of the pancreas)

Genetic component: 50% chance in identical twin

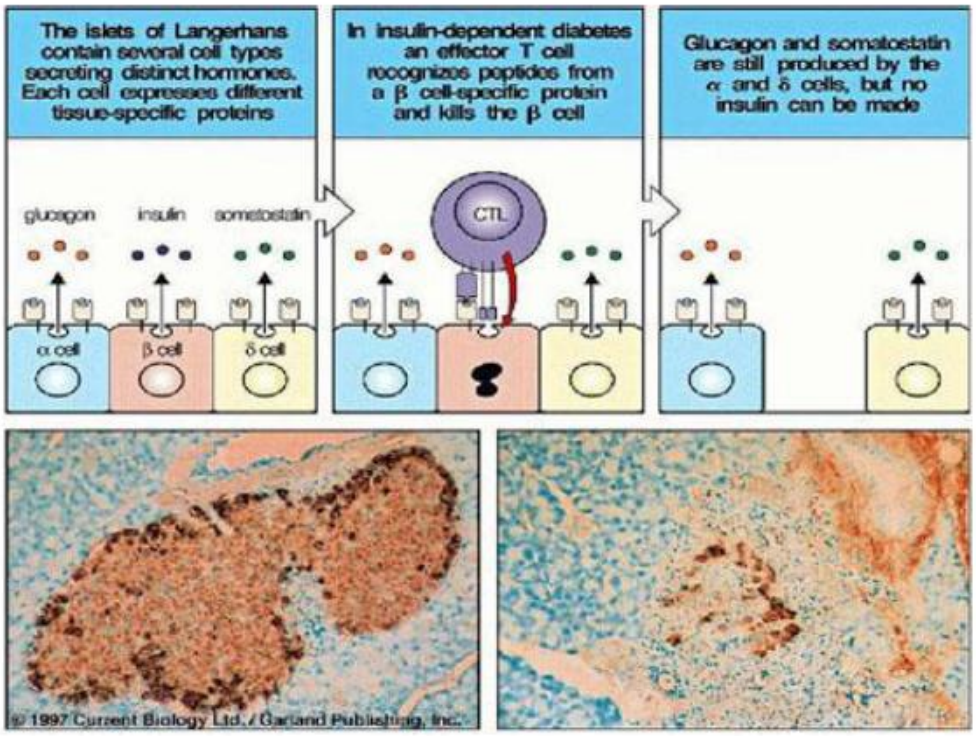
Patients make less CTLA-4 RNA- More activity for self reactive T cells

Defects in naturally occurring Treg functions suspected.

Silent Killer, symptoms appear when more than 90% of islets are destroyed.

Diagnosis: anti-GAD, anti- islet antibodies

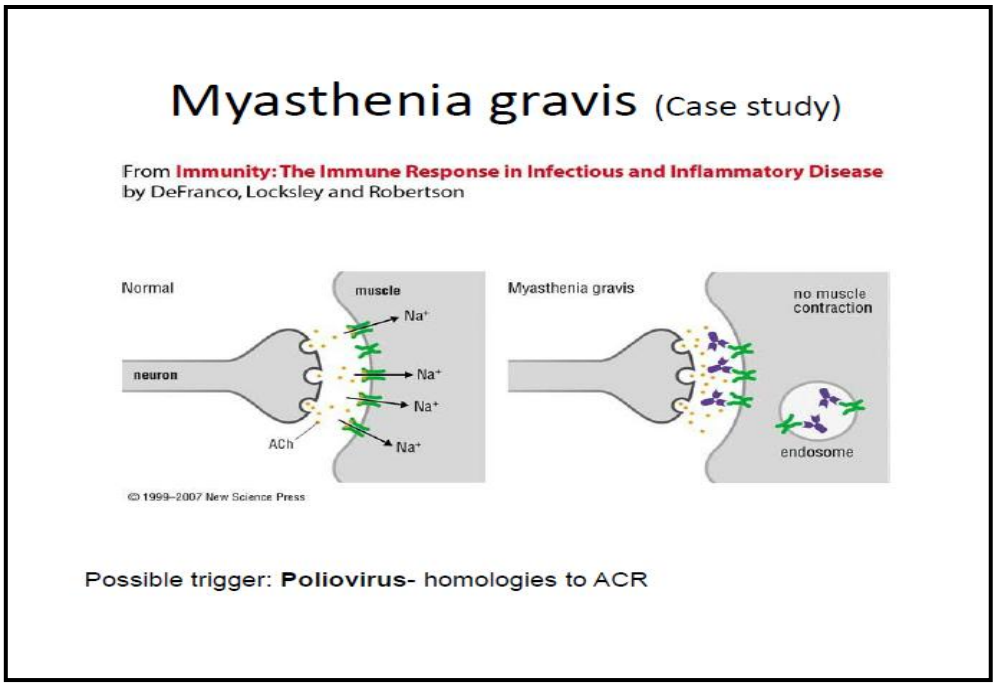
Diabetes- Type 1



B. Myasthenia gravis (الوهن العضلي الوبيل):

- **Auto antibodies against acetylcholine receptors**, which are found on the muscles, are formed. Thus no muscle contraction can take place.
- Characterized by eyelid dropping.

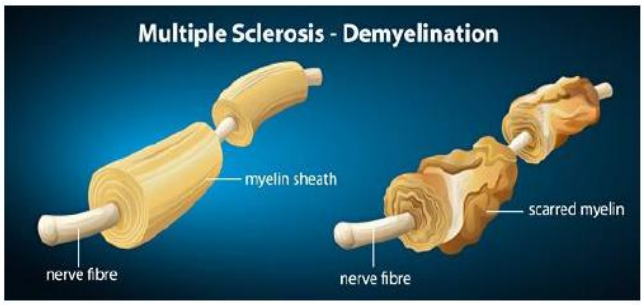
- Polio virus is a possible trigger for such disease, as its antigens have some homology with ACH receptors.
- *Further details are discussed in its case study.*



c. Multiple sclerosis(التصلّب المتعدد/التصلّب اللويحي):

- **Auto antibodies against basic protein found in the myelin sheath** of the neurons resulting in many defects.
- Possible triggers are **HSV** and **EBV** infections.
- The disease also has a genetic component as suggested by twin studies as well as race studies; Hispanic, Asian, Native Americans are less prone to develop multiple sclerosis than others.
- *Further details are discussed in its case study.*

Multiple Sclerosis (Case study)

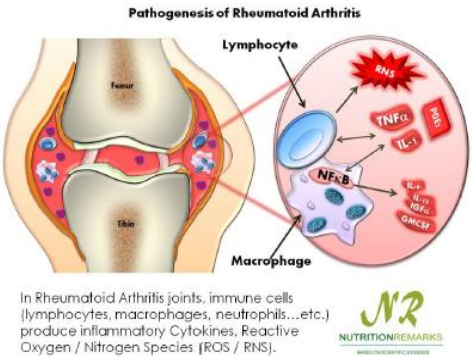


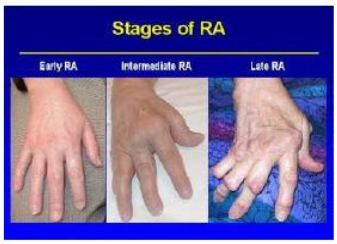
Defects in sensory inputs and paralysis.
Target: Myelin basic protein in myelin sheath.
Possible triggers: **HSV**, **EBV** infections
Genetic components: Twin studies, race studies (resistant groups: Hispanic, asian Native Americans).

D. Rheumatoid arthritis "RA"(اَلْتِهابُ المَفْصِلِ الرُّوماتزميّ) :

- ► It is different from **osteoarthritis** which is a degenerative disease of the joints, age related and mostly one joint is affected. *The doctor by mistake said osteoporosis instead of osteoarthritis.*
- ► On the other hand, Rheumatoid arthritis is an **auto immune diseases** in which ABs against the components of the joints are formed.
- ► There is inflammatory infiltration in the joint resulting in severe pain, and bone & joint erosion.
- ► More than one joint are affected as it is a **systemic disease.**
- ► Triggering factors are Mycobacterium tuberculosis, EBV and **smoking**.
- ► Genetic predisposition: HLA-DRB1.

Rheumatoid arthritis

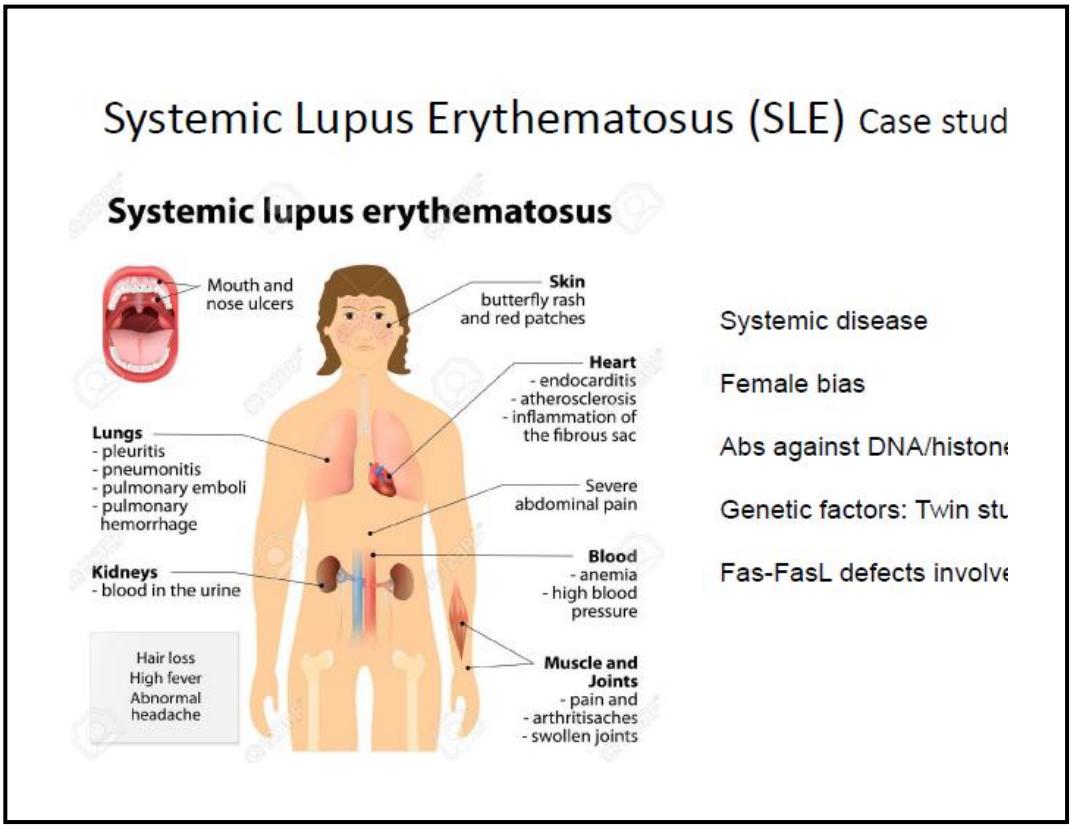




Systemic autoimmune disease: joint inflammation, lung, kidney, spleen, muscles, etc.
Autoimmune reaction against a **cartilage protein**
Genetic Predisposition: HLA-DRB1
Environmental triggers: *Mycobacterium tuberculosis*, EBV, smoking

E. Systemic Lupus Erythematosus (SLE): (الحمّى الذُئابيّة)

- More in females than in males (9:1).
- Complicated criteria around 11, 4 of which must be found in the patient to be diagnosed with SLE.
- Systemic disease that causes:
  - → Rash.
  - → May affect pericardium , kidneys, joints…
- Genetic factors: Twin studies suggest that.
- *Further details discussed in its case study.*



Sorry for any mistakes,

Wish you all best of luck ~.