

Immunology

Last time we talked about asthma and the many allergens that are related to it. We mentioned that it is a type I hypersensitivity reaction.

- The third kind of allergen that we want to talk about is the skin allergen. We said that we might have an accumulation of mast cells directly under the skin and under the mucous membrane.

- **Eczema:** an allergic condition related to the skin.

The allergen is not well known but could be something related to food.

It's an inflammatory lesion that occurs in the skin. It sometimes involves the face, trunk, and other areas.

Usually appears early in life and affects the children in the first six months of life as infant type eczema, most of those affected may recover after a few years as they grow up but some of them unfortunately don't.

- **Manifestations of Eczema:**

Rash + intense itching (pruritus). This pruritus leads to exfoliations and if you continue itching this will lead to bleeding resulting in secondary bacterial infection / superimposed infection. When it becomes very chronic you may get a thickening and alkylation of the skin.

- **Gastrointestinal System:**

The GI is rarely involved but if it is you will get:

- Abdominal pain
- Diarrhea

Atopic diseases of the GI tract are really very rare but still can occur. Patients with atopy (presence of elevated levels of total and allergen-specific IgE in the serum) have a rise in the IgE and they have eosinophilia in addition to the manifestations of the disease itself.

- **IgE measuring:**

IgE is measured in international units (around 200) not in mg because the concentration of IgE is very low. There is elevation of IgE in atopic cases, it isn't very helpful since an elevation in IgE doesn't necessarily mean that there is atopy, some normal people may have high IgE. It does help when you have clinical manifestations to indicate atopy when the IgE levels are high but it is not diagnostic.

- **Allergy testing:**

We can measure IgE in the serum using 2 radioimmunoassay tests:
RIST and RAST.

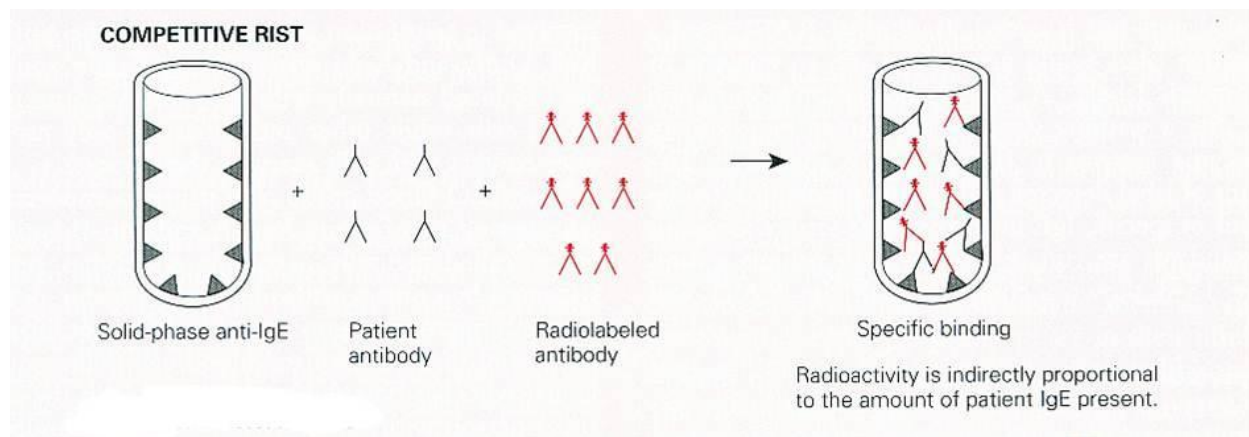
- **RIST:** RadioImmunoSorbent Test. A test used primarily for quantifying total serum immunoglobulin E (IgE) levels.

During experimental work in the lab there is a fluid phase where the reactant is found in the solution, and there is the solid phase where the reactant is fixed unto solid media.

1. We bring a test tube with anti-IgE fixed on the surface of the tube (this anti-IgE which is found on the surface is present in the solid phase.)

2. We mix the serum of the patient that we suspect contains IgE with a standard amount of radiolabeled (with radioactive Iodine) IgE. The mixture is then added to the antibody that is found on the tube's surface. This will result in the sharing of the fixed anti-IgE by both the serum IgE of the patient the radio labeled IgE. They will compete to bind with the antibody.

So the more IgE in the patient serum, the less radio labeled will be bound and visa versa.



- Imagine we have 8 molecules of IgE in the serum and 4 molecules of radio labeled IgE; we'll have 12 molecules of IgE in total. Notice that not all of them will bind to the anti-IgE.

They will bind in certain proportions: probably four of the anti-IgEs will be taken up by the IgE in the serum of the patient and will bind to them, and two of them will be taken up by the radiolabeled IgE (2 → Labeled, 4 → Serum). Therefore before measuring the radiation we wash the mixture to ensure that any unbound antibody is not there anymore.

- If it was the other way around: 8 radioactive molecules and 4 molecules in the serum:

Four will be taken up by the labeled IgE and two will be taken up by the patient's serum and the radiation will be more.

Therefore; the more the radiation, the less serum IgE and vice versa. (Since IgE in the serum will compete with the radiolabelled IgE for binding to the anti- IgE, the amount of radioactivity bound will be less than is bound in the absence of such competition. The reduction of binding provides a measure of the amount of IgE in the test sample.)

3. Wash after incubating to get rid of any unbound antibody

4. Measure the radiation

- RIST is a competition test, used to measure IgE specific for a particular antigen. Known amounts of radiolabeled IgE compete with the patient's (serum) unlabeled IgE to bind to a surface coated with anti-IgE. The reduction in radiolabeled IgE due to the presence of IgE in the patient's serum can be determined by comparison with known IgE standards; thus, the amount of the patient's total serum IgE can be determined.

- From the standard (antigen) we can plot a graph and this standard should be the same as the antigen that we want to measure and we can deduce the value from the graph.

- **Notes:**

- Antigen -antibody interaction is reversible and dynamic; as we mix the Ab and the Ag in an incubator, they will bind to each other and settle down after about 2 hours, and then we wash the mixture and measure the radiation.
- When labeling Ab or Ag, we should make sure that the labeling doesn't interfere with the affinity, they should have the same affinity.
- How do we measure the IgE?

We have to detect a signal, which in this case is the radiation. We said that we have 12 molecules, 2 of them are labeled so we will detect the radiation (using the gamma counter) emitted by those two immune complexes: original antibody + the radiolabeled IgE.

The concentration of this antigen is indirectly proportional to the amount of radiation. We plot a graph.

Conclusion:

Labeled IgE mixed with the patient's serum → bind with the anti-IgE (fixed) → incubate → wash → measure the concentration: plot a graph

- ✓ RIST is used to measure the amount of IgE in the patient serum
- ✓ The concentration of the antigen is indirectly proportional to the radiation
- ✓ We don't need to measure the entire serum and the labeled IgE ones since the concentration is proportional.

The end of RIST

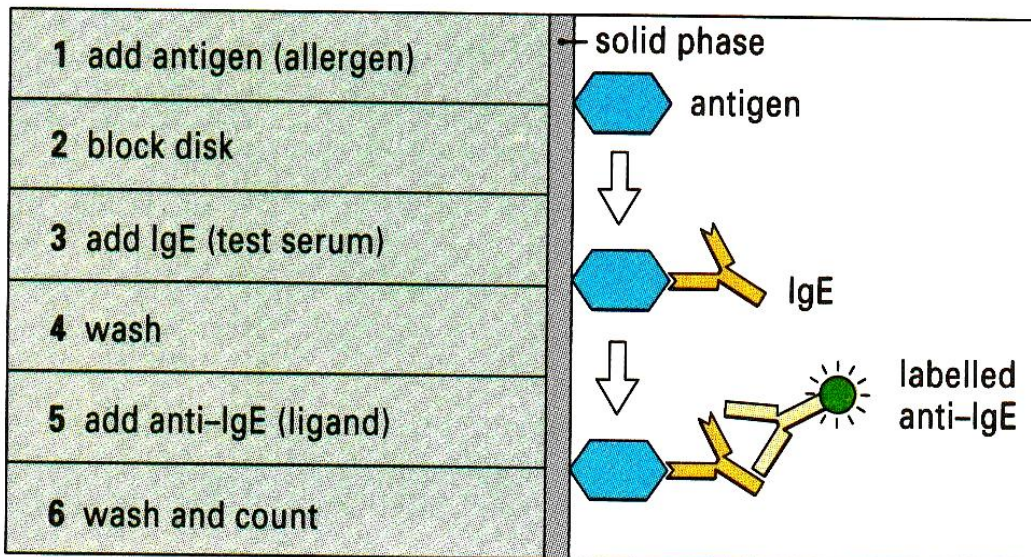
- **RAST**: RadioAllergoSorbent Test. It is a blood test used to determine the substances a subject is allergic to. There are paper discs with different antigens on them to test for allergy.

- Example: if the patient is allergic to X, a paper disc contains this antigen.
 We add the patient's serum to this disc. If the serum contains antibodies against X then they will bind to each other. These antibodies will of course be IgE (anti-X). To detect their presence we can bring anti-IgE that is labeled with radioactive material. We wash the complex first to make sure that anything other than the immune complex will go away, then we add the labeled anti-IgE, and then we can measure the radiation. This is a way of detecting WHAT allergen is causing the disease and the increase in IgE levels, where as with RIST we measured the AMOUNT of IgE present.

The Steps:

- 1) We take the serum of the patient
- 2) We add it to the paper disc that contains a certain antigen
- 3) If the patient has Abs against the antigen (IgE anti-x) then an immune complex will form (Ag-Ab)
- 4) Wash to get rid of extra reactants
- 5) Add labeled anti-IgE for detection
- 6) Measure the radiation

The radioallergosorbent test (RAST)



- If you detect radiation, this indicates that the person is allergic to X and has antibodies against it. We finished the type of hypersensitivity that is due to atopy.

- Some people might exhibit IgE without being atopic. They can exhibit elevated levels of IgE against the allergen but don't suffer from eczema or asthma.

The patients in this case will suffer from two conditions: **Urticaria** and **Anaphylaxis**

The allergens that usually cause these conditions are: food, drugs, and insect bites.

○ **Urticaria**

- An elevated, pinkish, erythematous rash that disappears after a few hours on its own.
- Patients are not atopic but somehow develop IgEs against certain foods, drugs or insect bites.
- Not serious, only irritating and annoying.
- This is due to the release of Histamine which causes the swelling, the redness and the itching.

Example: Some people may be allergic to

- Strawberries:
Once they eat it they will develop a rash (a wheal and flare reaction because it's histamine mediated) which is elevated and pinkish in color with different shapes all over the body, mainly the trunk. Also, the eyes might become poppy.
- A Drug:
Take the drug → develop IGE antibody → re-exposed to the same drug → rash formation.
- Insect Bites:
A very strong reaction that may spread all over the body.

○ **Anaphylaxis** (Anaphylactic Shock)

- A generalized systemic reaction to the allergens.
- Most of the mast cells in your body are involved, they will produce lots of histamine which leads to the following:
 1. *Vasodilatation*
 2. Extravasations of fluid from the inside the vascular compartment to the outside which results in *a drop in the blood pressure*. This leads to **hypovolemic shock**.
 3. *Constriction of the bronchial and airway passages*.
 4. *Swelling around the face*
 5. *Swelling of the larynx itself* (edema) that may shut the airways altogether.
- Those lead to not having enough blood circulating in the body and towards the brain and a reduction in the availability of the oxygen in the blood.
- This will result in anaphylactic shock which is a really serious condition that may lead to death if it's not treated.
- Anaphylactoid Reactions: (Complement involvement or Radioactive compounds mediated) can occur due to excessive complement activation because of the Anaphylatoxins that cause mast cells to degranulate (no allergic reaction, no IgE involved). Some iodine compounds can trigger this as well.

- Anaphylaxis can be induced by many things such as: penicillin (injections), strawberry, peanuts, fish, eggs...etc
- The antigens that lead to urticaria or anaphylaxis may be inhaled, eaten or injected.
- Since measuring IgE levels may not always be helpful and the tests are not diagnostic, you might have to resort to a clinical test if you really need to know the cause of the allergy.

○ **Skin Prick Test** (الوخز)

1. Bring a solution of the allergen that you want to test for and place in a small blob on the skin
 2. Bring a needle/pin and prick the skin through the solution very superficially
 3. *You must prepare two controls to add on the skin and perform the prick test:*
 - **The positive control:** Distilled water
 - **The negative control:** Histamine
 4. Wait for 10-15 minutes (it's an immediate type of hypersensitivity so the effects will appear after a few minutes).
 5. View what happened at the site where you pricked the skin to check for the wheal and flare effect (the wheal effect is the swelling, there is usually erythema around it)
 6. Compare this with the negative and positive controls
- The negative control can lead to an effect (the wheal and flare effect) due to mast cell injury and the release of histamine.
 - Histamine's effect lasts for minutes (immediate acute phase then disintegrates). The cumulative effects in an allergic condition are due to the prostaglandins and leukotrienes (the late phase).
 - After this procedure we can decide if the patient is allergic or not
 - If the person being tested displayed negative results but you still suspect an allergy to a specific allergen, you can do an **Intradermal Test**: take a small amount of a very, very dilute solution of the allergen and inject it intradermally. Then you repeat the +ve and the -ve controls and compare them with the experiment to see if there is an immune reaction or not.

You have to be very careful when you do the intradermal injection since it causes an allergic reaction and may lead to anaphylaxis if the person is very allergic.

And this is the skin test related to type I hypersensitivity reactions.

❖ Type II Hypersensitivity

- Antibody mediated, usually IgM or IgG. They will bind with antigens forming immune complexes
- The antigens in this case are usually a part of the tissue or organ affected.
- This is seen in most of the autoimmune diseases.
- Involves complement activation mainly, and it's the causative agent of the pathogenesis.
- Sometimes involves opsonisation.

For example:

- **Autoimmune Hemolytic Anemia**

Our body forms antibodies against antigens in the membrane of the RBCs. These antibodies will bind to the antigens and lead to:

- The activation of the complement system, this leads to the formation of the MAC which results in cell lysis.

This also applies to platelets as well in the case of **Autoimmune Thrombocytopenia** and in the case of:

- **Incompatible Blood Transfusion**

When we transfer blood from a person with the blood type A to a person with the blood type O: The person with the O blood type has antibodies against A, so once you transfer the type A blood, the anti-A will bind with A on the surface of the RBC and *activate the complement system*. This results in cell lysis, intravascular hemolysis and other complications. Anti-ABOs are usually IgMs.

- **Rhesus Incompatibility**

If we inject somebody with Rh +ve blood group and he is Rh -ve for the first time there are no antibodies present, but the cells will become sensitized. If you inject him with RBCs that are Rh +ve there will be a reaction: adherence of the Ab with the RBC. The antibodies in this case are IgG and not IgMs.

We will not get hemolysis because the Rh antigen is not very dense, so the antigens are far apart on the surface of the cell and there isn't enough of them to cross link with the adjacent Fc antibodies. Therefore, there is:

- No activation of the complement system
- No MAC formation
- No cell lysis

What happens here is the sensitization of these cells and hence they are *opsonized* and will be taken up by the *macrophages* and broken down. The net result is the destruction of the RBC. (For ABO incompatibility this was through lysis, but as for Rh incompatibility it's through opsonization and phagocytosis.)

- **Goodpasture's Syndrome**

- Anti- basement membrane antibodies present. The antigen is part of the basement membrane.

- **Thyrotoxicosis**

- Antibodies on the thyroid's surface

- **Vitamin D Deficiency**

- **Pernicious Anemia**

All of these are examples of autoimmune diseases where the pathology of the disease is caused by type II hypersensitivity.