The Sheet Team

Title of Lecture: Antigens

Date of Lecture: 14/10/2014

Sheet no: 3

Refer to slide no. : None

Written by: Dalin Jihad

Any material that can stimulate the immune system is called an antigen.

Today's objectives:

1. Antigens and their characteristics
2. Define immunogen, heptin, and antigen
3. Describe the factors that influence the immunogenicity, we'll see the chemical nature of these immunogens and compare what they activate (B and/or T cells).
4. The hapten carrier system characterized of antigen determined
5. Super antigen

The antigen is a molecule which can bind specifically to an immune response, that immune response could be an antibody or a T- cell receptor. In some conditions, we call this antigen allergen or tolerogen, depending on where that antigen is functioning. If it functions in allergy we'll call it allergen and if it is involved in suppressing the immune system we'll call it tolerogen, which mean the immune system will not be able to work again.

The immunogen is a molecule that can stimulate the immune system, so it will induce a response. An antigen does not necessarily induce a response, but it can react with a response.

Immunogen: a stimulus that produces a humoral or cell-mediated immune response

When we talk about antigen, we'll mean bacteria, for example, which is a foreign substance that entered the body causing infections. Now, the immune response generated could for the whole body or for a certain area where antibody or a T-cell can react.

Generally, there are small molecules found on the surface of antigen which can be seen by the antibody or the T- cell. Those small chemicals are called Epitopes, this is what is seen by the immune system, it will not see the whole antigen; just this small part.

> Epitope: the portion of an antigen that is recognized and bound by an Ab or TCR/MHC complex (aka antigenic determinant)

Hapten is a chemical, and when we inject it alone, it won't produce an immune response.

E.g. penicillin, it is a chemical molecule which is a foreign component. Since they are not immunogenic and not an antigen and so will not produce an immune response. But if it was bound to a molecule then an immune response will happen. Some people are allergic to penicillin, when penicillin is metabolized in the body it will produce penicillinic acid. Penicillinic acid is a chemically reactive molecule and can react with any molecule in the body, a cell or albumin or anything. When the penicillin binds to the albumin or the cell, the immune system will see it and produce antibody against it, thus allergy will be generated towards it.

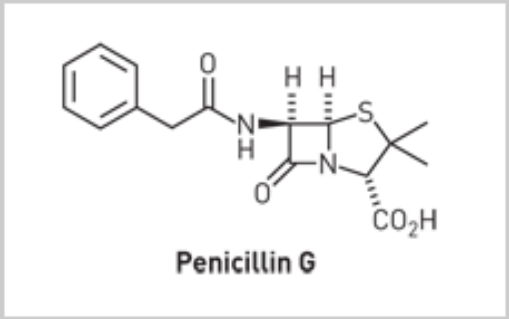

Figure 1: Penicillin Structure.

So, penicillin is a small molecule with a molecular weight not more than 300, changed to reactive molecule to react with self component and produce immune response.

A happen doesn't necessarily have to be a small chemical, it could be a very large chemical but not antigenic. Another e.g. bacillus anthraces, a gram positive microorganism and has a very large capsule. Capsule is very simple; it is composed of D- glutamic acid. Because the whole structure is made from one amino acid it is not an antigen, it is a hepten although the molecule is very large.

Hapten: a low molecular weight molecule that can be made immunogenic by conjugation to a suitable carrier

A paratope is the path of the antibody or the T-cell receptor which reacts with the epitope.

All immunogens are antigens but not all antigens are immunogens.

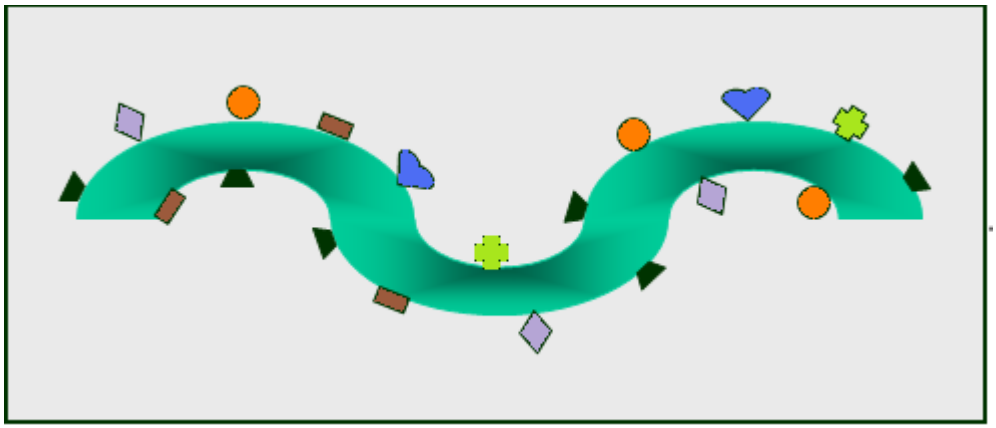

Figure 2 is just an example of the epitope, the small molecules on the surface of the antigen which reacts with the immune system. Or if it was bacteria, we'll find this epitope on the cell membrane.

Figure 2: Epitope

What are the factors affecting the immunogenicity? Why can a molecule produce an immune response and another cannot?

Some factors are due to the molecule itself, there should be certain characteristics to the molecule. The second factor is the biological system, the human body itself; maybe this body's immune response is suppressed. Also the method of administration how and where are we giving the material (Orally, I.V., Intradermal...etc)

The molecule's characteristics:

1. Should be foreign (if you take albumin from your body and re-inject in your body, you will get no response as this is recognized as self component. But, taking albumin from an animal and inject it in another you will get a response.) This is the main contributor for the immune system recognition.
2. The size (A small molecule like penicillin will not induce the immune response. Smallest known antigen is glucagon with a molecular weight of 3000. So the molecular weight is a good contributor.)
3. Chemical composition (if the material was very simple like polysaccharides, or starch which basically is a long chain of α-glucose, which is not an antigen. It is made of one molecule, therefore; the antigen must be complex. The chemicals should contain a primary, secondary, tertiary, and quaternary structure. The more the complexity of the structure the more the efficiency of the immunogen. The shape and structure are important for the molecule.)
4. The molecule itself (is it soluble material or a particle? Bacteria, an RBC, a WBC or globins? This is very important because the fate of the antigen is very important.
5. Physical nature (the monomer, polymer, cyclic or a linear molecule)

Antigenicity:

- Polymer> monomer
- Cyclic> linear
- Particulate> soluble

Although they're all antigens, but some are more antigenic, more active and more potent in producing this immune chemical.

6. Degradability (molecule should be metabolized in the body, otherwise it will not be an antigen even if it is a foreign, large, complex molecule)

What amino acids degraded in the body? The configuration of the amino acid?

The L configurations are metabolized in the body but not the D configuration. If the molecule is not metabolized it is not a good antigen that could produce an immune response. Antigen should be degraded by the phagocytic system, macrophages and the others. Cells that degrade the antigen and process it called Antigen Processing Cells. These are extremely important in the induction of the immune system.

Again, an antigen should be:

1. Foreign
2. Large
3. Complex
4. Degradable

Figure 3 shows the primary structure of a molecule then when a slight loop is visible it is called the secondary structure and in the tertiary structure there will be second structure. The quaternary structure is the most complex, effective, and most potent molecule that could induce a chemical response.

Figure 4 shows a molecule with an epitope, if this structure entered the body then the epitope will be recognized and the immune system will be activated. Now if the structure was metabolized outside the body yet we still have the same sequence, the body will not recognize it because they're simple chemicals. If this was degraded inside the body by macrophages, the epitope molecules will be the presented to the immune system not the whole molecules, the eptiope determined groups. These will be taken by the macrophages to the Antigen processing cells and then presented to the immune system.

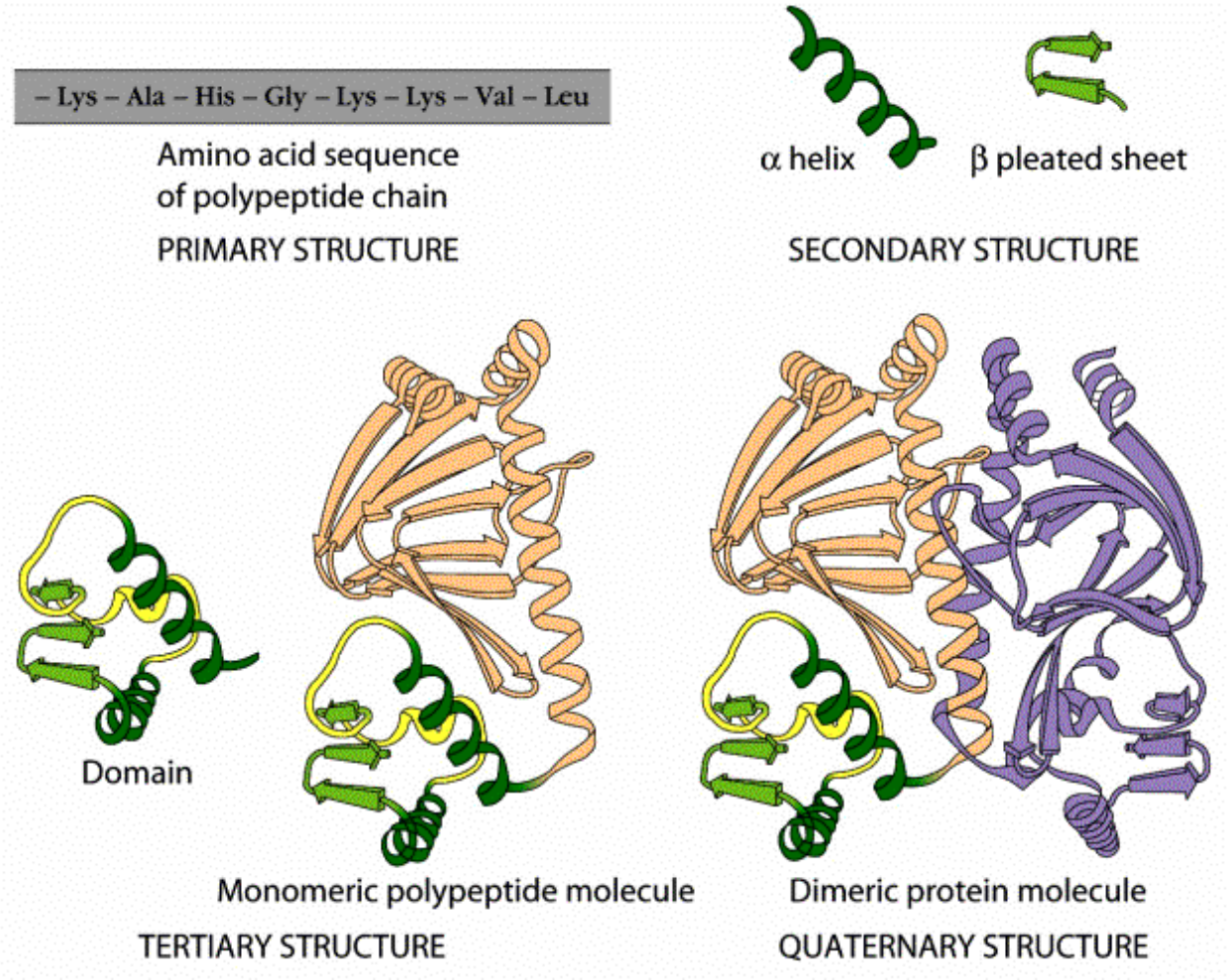

Figure 3: Chemical Composition and Heterogeneity

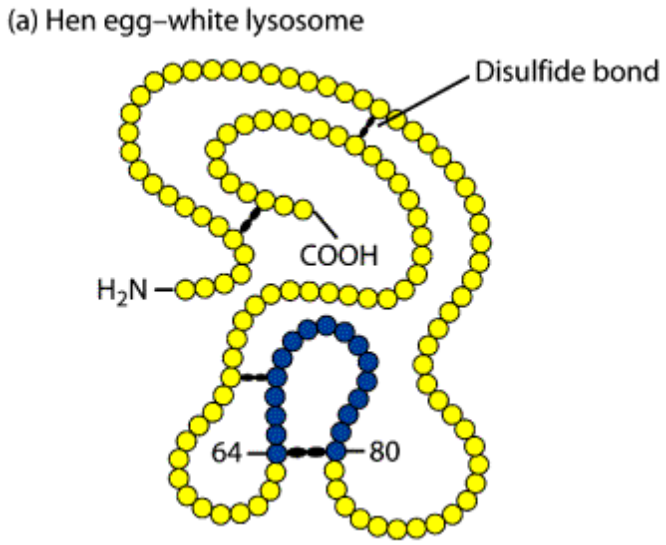

Figure 4: Epitop structure

The size of the epitope is ranging. In the peptide antigen from 5 to 23 amino acid residues, this is without the whole molecule. The molecule itself could be 100,000 amino acids and its epitope is 5. The polysaccharides are smaller, 5-7 amino acids. Nucleic acid antigen 6-8 amino acids nucleotides. The epitope seen by T-lymphocyte is larger than the one seen by the B-lymphocyte. So these sizes have decrease more when talking about the B-lymphocytes recognition on the cell surface.

The antigen is not composed of a single epitope. If the antigen has a single epitope it is not an antigen. To induce an immune response the antigen must have at least two different epitopes.

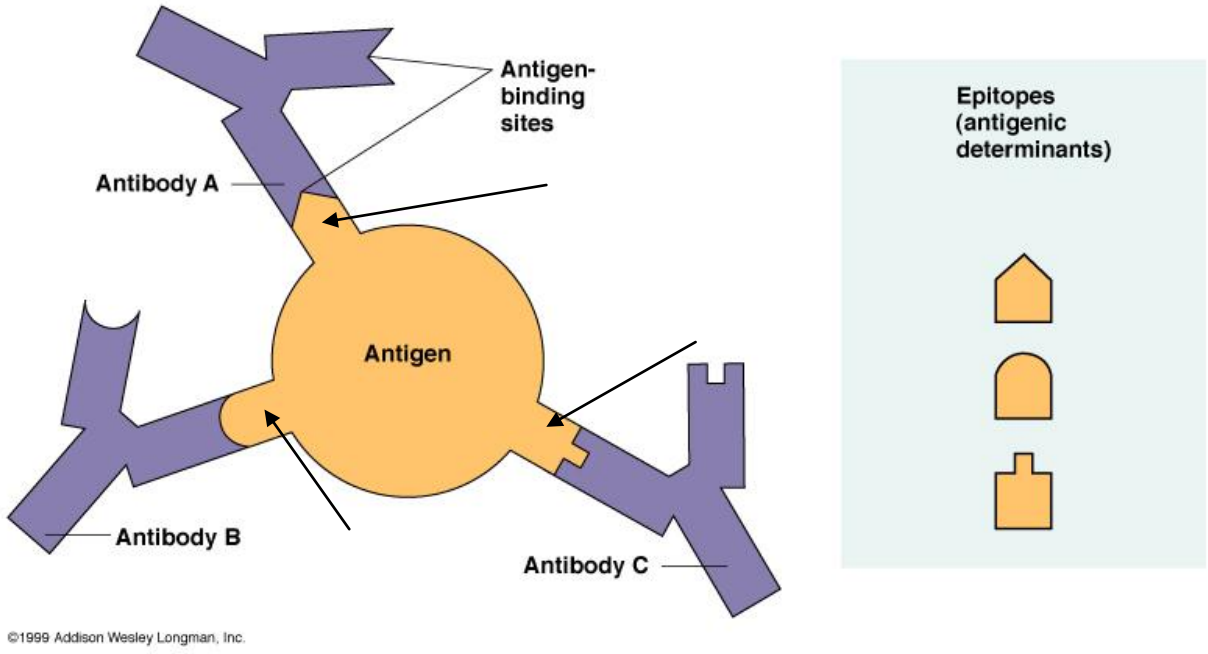

Figure 5: Epitopes: Antigen Regions that Interact with Antibodies

If this is an antigen (figure 5) and so, each one of the arrows represents a different epitope. Each one is seen and processed by a different antibody. If the antigen has 2 or more identical epitopes, then this is not an antigen, an antigen must have at least 2 different epitopes. This is what is meant by the complexity of the antigen.

Immunogenicity means the induction of the immune response and the reaction with the response. So, we produce an antibody then, when we add the antigen to the antibody, there will be a reaction between them.

We have some other characteristics, we already mentioned that a hepten alone will not be able to produce an immune reaction but if we bind the hapten to a carrier immunogen like albumin (like the example about the penicillin), a hapten carrier system then it will be immunogenic. The carrier always should be an immunogen but the hapten doesn't necessarily have to be.

[When the penicillin was injected into the body, it will not induce a response. But, when it binds to albumin, an antibody will be produced against the albumin and one against the penicillin.]

Immuno-reactivity: immunogen after the immune response, adding an antigen will produce a response. Say you injected antigen A and antibodies are produced, take those antibodies and mix them with the antigen outside the body and there will be a reaction between the two.

The antigen and the hapten can react with the immune response that is immune reactivity. They will react with the immune response but will not induce an immune response, only react with it. The hapten in this case itself is the epitope.

We should have 2 different epitopes on an antigen to produce an immune response, one of them is the hapten which is a monovalent, and when bound to a carrier it will be polyvalent. The more it becomes polyvalent the better. Another important thing is that we have two types of determinant epitopes. One is accessible which is found on the surface membrane and that will be used if we shock that chemically we'll have silent epitopes which are inside the molecules. By removing the crust or the cover, the silent epitopes will come out. These are normally not seen by the immune system.

If we treat the antigen outside and inject it, we'll expose the silent epitope to become dominant. So we have silent epitopes and dominant on the surface of the antigen. Back to figure 5, each and every epitope will have its own antibody produced against it, the specificity differs between them. However, this isn't the same case all the time. Sometimes if an antigen has 10 different epitopes, the immune system may not produce an antibody against every epitope. 1^{st} and 2^{nd} epitopes may have a high response, 3^{rd} and 4^{th} may show intermediate response, and 5^{th} and 6^{th} will show minimal response, this is for the immune system to decide which to make an antibody for. But for each epitope, antibody made has a different specificity from the other.

Another important thing, the bovine antigen, which is like our albumin, when injected into the body produces no response. When we bind it to penicillin it then produces a reaction. When two molecules bind to each other, the binding area will change; not only the epitope, since there are bonds made and charges have changed. So we'll find more than one antigen, because a chemical reaction had occurred. Later we'll talk about cross reactions. We already mentioned that the structure primary secondary and tertiary structure is important for the antigen to do this.

We have linear (continuous) antigen and we have the conformational (discontinuous) antigen. Not all the epitopes can bind with the antigen binding site, there a certain number of amino acids that can bind there. This is important when we talk about the antigen-antibody structure. Refer to figure 6 for clarification about the antigen structure binding. The linear determinant (epitope), in the end after denaturation, will still be there. While in the conformational

(discontinuous) the tertiary structure will still be bound to it. That means that this epitope is more potent in comparison compared to the other one. This is due to the complexity of the antigen.

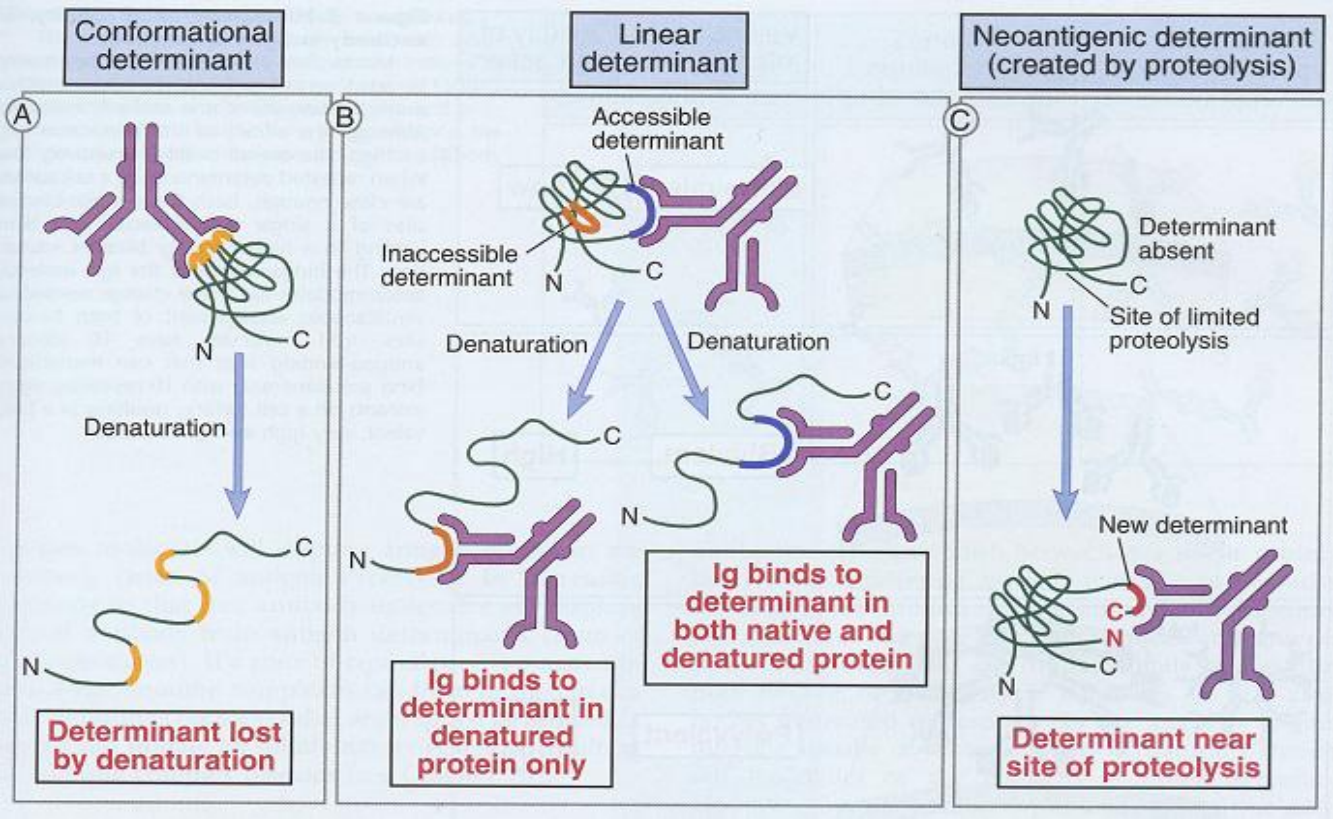

Figure 6: Antigenic epitopes

So conformational determinant form by amino acid residues but became specially exposed on the cell, the loop.

The B-lymphocyte will recognize the non-processed epitopes; it will recognize the epitope on the natural antigen without processing. That means that the antigen as it had entered freshly into the body will be recognized by the B-lymphocytes.

The T-Lymphocyte cannot recognize an antigen unless it had been processed.

It also depends on the response to those antigens; we have a T-dependant antigen and a T-independent antigen.

What does that mean?

In the immune response we have T-cells and B-cells, the B-cell producing antibodies while the T-cells produce the response. When we talk about an immunogen, it should induce both; the antibody and the response.

T-dependant antigens are antigens recognized by B-lymphocytes only, T-lymphocytes will not recognize them, and only antibodies will be produced. The only antibody which will be produced is the IgM.

In the T-dependant antigens, the antigens should be processed by the antigen processing cells, recognized by the T-lymphocytes and then the T-lymphocytes can help the B-lymphocytes in producing antibodies, which is because B-lymphocytes are dependent on T-lymphocytes to produce antibodies. So, if we have no T- lymphocytes then no antibody production.

If we take a mouse which had a thymectomy at birth and there had been no development to T-Lymphocytes, so it only has B-cells in the bone marrow. If this mouse was injected with a T-dependant antigen, it will not be able to produce antibodies. Only antibodies are produced are in the case of T-independent antigens.

Hidden or sequester determinants of the function.

During the development of the immune system, the T-lymphocytes and B-lymphocytes will be tolerated to the self components, they will recognize the self components and they'll die. During this development, any part of the body with no circulation will not be recognized as self. Body parts like:

1. Lens
2. Sperms
3. Thyroid

If you remove your lens and inject into your body, your body will recognize it as a foreign antigen and will produce antibodies to it.

Why?

Because this was kept outside the body during recognition of immune system to self. The same thing in most cases of infertility where men have antibodies against sperms could be due to a trauma to the testes or abnormal structure that activates the immune system to produce an antibody against it. So this is the meaning of sequester antigen, a hidden antigen. Like when we talked about the hidden epitopes which are not seen. This is important in the induction of the immune system.

Common antigen means it is present in more than one animal. If we take albumin from a human, from a bovine, from chicken...etc, we'll find a common structure among them. This is called a common antigen, the same antigen found in different species. This is important in cross reactions, as in immunization of TB by, not using the human bacilli but by using the bovine

bacilli, because the epitopes on the microorganism of those different species are almost the same. But the bovine TB will not cause as serious diseases in a human being so we use it as an antigen to produce immune reaction. So the bacteria used are from an animal not human because they have a common antigen, it has no pathogenicity but it has immunogenicity.

Mycoplasma are small microorganism that cause pneumonia, it is difficult to culture and difficult to produce an antibody against it. So we'll look for another microorganism with almost the same antigenicity, same structure.

All RBC's have similar antigen to those found on mycoplamsa which are called* *I can't hear it.* Mycoplasma produce antibodies which can react with the RBCs, and here we used RBCs as an antigen. This is called common antigen found in mediated blood. This determines the cross reaction between different organism and is extremely important in medicine.

Characteristics of T-dependant and T-independent:

These antigens are from two types, one is exogenous, injected from outside, and the other is endogenous which is within the body by infections, drugs, certain condition where the self component will be modified.

If we're talking about intracellular infection, the cell will be destroyed, and the components that were inside the cell like the DNA and so on are not recognized as self by the immune system since they were inside the cell. When the cell is destroyed the cell components will be see as foreign and will activate the immune system which will produce antibody against them.

Systemic arthritis, which is a very severe skin disease.

Chronic arthritis which is a deformity to the joints. These are examples where endogenous antigens will be activated and produce an immune response.

The exogenous antigen is any antigen coming from outside like chemicals, vaccines…etc.

The T-dependant antigens mostly are proteins which means they are complex antigens, have many determinants since they have many epitopes on their surface, can induce human immune response as cell mediated. E.g. microglial proteins

B-cells can produce all types of immunoglobulins, have immune memory, and the size of the residues of the epitope is from 8-15 amino acid residues.

The first antigen will be processed by the B-lymphocyte, but there is a direct relationship between the T and B cells to give a signal for starting the production of immunoglobulins. If

this relationship did not exist, we won't have a reaction from the B-cells. The T-dependant antigen will not have this reaction, only the T-independent.

The T-independent can activate the B-cells directly; they don't have to be processed by the antigen processing cells. Mainly, they are polysaccharides with polymeric structure which mean that the cell epitope is repeated on the surface. B- cell activation but with poor memory, i.e. if I inject a certain antigen and IgMs had been produced against it and then I re-inject the same antigen, it will not be recognized and the reaction will be as if it was the first time.

Any cell, a T or a B cell, to give a response needs two signals. One signal will not be able to produce a response.

In the T-dependant, a signal comes from the reaction of B and epitope and the other is from a T-lymphocyte. So the signal coming from the T- lymphocyte will affect the B- cells and the one coming from the epitope reaction.

In the T-independent, we don't have the T-lymphocyte and so, where does the second signal come from?

We already said that the molecules are close, foreign body, have the same determinants repeated and so the first signal comes from the reaction.

The cell is dynamic, not static, and since the T-dependant antigen is very large it will react with a large part of the cell membrane and stabilize it, do some sort of blockage to the cell to stabilize it. This will give the second signal to the T-lymphocyte.

If we talk about the lipopolysccharide which is part of the cell wall, a toxic material which can cause toxins in the body. This toxicity effect will give the second signal and, along with the epitope reaction and the B-lymphocytes will be produced. Since they are all the same epitope they will react with more than one place at the cell and stabilize it.

So the T-independent antigen has no repeated determinants, all the IgM's produced cannot induce cell mediated immunity, no immune memory and the size is small, because the B cell epitope is smaller than the T cell epitope.

Antigen can be defined according to species, xenoantigen is the same antigen found in different species, found in humans, rabbits…etc.

Alloantigen is the same antigen between two individuals from the same species. When they do transplantation, the do allotransplantation. And example is the blood groups, A/ B. The HLA system is antigens present on the nucleated cell body, those are verities that could have 700 types. Any person has at least 8 different types.

Auto antigen is the same antigen in me.

Heterophilic antigens are antigens found in different species. In medicine, if you want a diagnosis of certain disease, that this antigen can induce this disease.

How do you treat diphtheria?

We need an antitoxin that we'll produce in animals, rabbits, horses any animal.

When we produce antibody in rabbits for treating diphtheria, we'll just transfer the antibodies to the patient. However, at the same time we injected a strong antigen, animal proteins. From one side you're treating the patient and from another you're immunizing the patient with foreign proteins. Say the patient was treated from diphtheria.

After time had passed, the patient had tetanus and so we need to give him a titetaus which is prepared in the same way. Now, if we happen to give the patient the same animal protein, he might die from the reaction.

For bacteria an example, you can find many many antigens like the exotoxin, the capsular antigen, cell wall component, cytoplasm, the flagella all these they are antigens. These antigens have different uses from each other, like if you want to see if you're immune you'll use the flagella or for the wall antigen.

We have specific antigens are called tumor antigens. A tumor cell is a normal cell found in the body and due to infection, abnormalities, drugs or anything that will make these cells have antigens on their surfaces not normally found. We have tumor specific antigen and tumor associated antigen. The tumor associated antigens can only be seen in the presence of a tumor, like α-β proteins and CA antigen, and those are important in the development of the fetus. During pregnancy they'll be in higher blood concentration of the mother. We need them for the fetus development and called fetaloncogene. When a person becomes an adult, these proteins will not be seen or maybe seen in very small amounts. If they rise in concentration this is an indication for the presence of tumor.

If we have liver cancer, α-β proteins will rise in concentration.

In colon cancer CA will increase very much.

They are not for diagnostic purposes, but for indication that we have cancer.

If we a stomach cancer had occurred and they removed it, the concentration should drop. But if it rises then this is an indication for cancer. These associated antigens can be seen in the immune system.

How should we give the antigen?

1. Appropriate route, IV antigen will not be effective since it will be metabolized very rapidly.
2. Dose, low dose causes tolerance and high dose causes immune system paralysis.
3. How will it be injected?