Title of Lecture: Immunoglobulins

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Last lecture we started talking about antigens.

Now we'll talk about methods of administrations, we know the antigen; we know its characteristics and know the response of the organism within human or animal about this antigen.

Now how we can introduce this antigen in the human being?

- First we have to decide the dose / how much we can use, the dose is extremely important.

If you inject a very high dose, the immune system will be paralyzed, and it will not be able to produce an immune response to that antigen. It will produce some abnormality.

Low dose will generate tolerance (tolerance: it can see the antigen but will not respond to it). Sometimes tolerance is used in treatment and we'll talk about this later.

So there should a proper dose (optimum).

- Secondly, the route of administration. In order to get a good immune response, the antigen should stay in the body for a good period of time, at least several days if injected for the first time.

If metabolized very quickly and eliminated from the body in a rapid way, the we'll not be able to generate immune response.

If we inject it via:

- IV (intravenous) >> very fast metabolism we'll get nothing.
- IP(intraperitoneal)/IM(intramuscular >> here also we'll have some problems.

It depends also on what type of immune response we want, do we want humoral (here we need to produce proteins, Ig (immunoglobulins)), or do we want a cell mediated immunity.

E.g.

- Poliovirus vaccines, taken orally because the entrance of it was orally (here we get local immunity for the virus)
- Triple vaccines (tetanus/diphtheria/pertussis) vaccines were taker IM (important in producing antibodies).
- Smallpox vaccines were taken by scratches in the skin.

When we give vaccines, two things are important to be examined on animals:

- It shouldn't cause diseases
- If it's a protein it should be toxic
- The dose according to the animal's weight and reaction.

So the administration route is determined by what immune response is needed.

The best way is intracutaneous/ intradermal>subcutaneous>IV>IP>IM.

The last three (IV, IP, IM) are very difficult to get immune response from them.

The other thing is how we will give it, in a soluble form, with other microorganism, alone or with other material.

We can use a chemical which is called Adjuvant.

Adjuvant: a substance that enhance the immune response to the antigen, it will not change the response, it will increase it.

E.g. The triple vaccines (tetanus: it's a protein. Diphtheria: a soluble material. Partussis: it's a bacteria, game –ve; this mean the cell wall is mainly lipopolysccharides. Lipids in general are slowly absorbed by the body. Therefore, some additives, like emulsions or oils, are manufactured with the vaccine (the triple vaccines) in order to keep it in the injection area as much as possible.

This is an example of adjuvant, the bacteria are used as an adjuvant, and it will keep the microorganisms at the injection site longer, to enhance the immune system response.

The antigen also might be a particle or a soluble material.

If the antigen is soluble, when we inject it, at zero time, the concentration almost 100% at the injection site, after a while the concentration will go down, because there will be an equilibrium between the interstitial fluids. After a while, there will be metabolic activity of the antigen, so the concentration will go further down, till here we don't have antibodies.

Antibodies can be seen at the end of the first week, and when antibodies are produced the concentration orf antigens at that area will be almost zero. Then we will start to see the elevation in concentration of antibodies.

It the antigen is a particle material, bacteria for example. How are we going to determine the optimal dose for an infectious material? We should try the vaccines in animals to make sure that they are neither toxic nor infectious, and the optimum dose is determined in animals. Here we're going take in consideration two terms:

1. Infectious dose: the minimum dose of the infectious microorganism that will produce infection and it is variable from one microorganism to another. Tb for example, few are enough to cause infection, while in E. coli we need 100,000 cells to do infection.
2. Immune dose: the dose required for the immune system to produce an immune response, this dose might be below the infectious dose. Flue for example, if one of us has flue 5-6 will get the flue from him (the flu symptoms/infection) , but if we test the whole class , 80% will be forming antibodies for the antigen without infection (it is called subclinical infection). WHY? Because they took the virus in a dose less than the infectious dose, but it achieved the immunological dose, and so they get antibodies.

Another example, people who are allergic to penicillin, when the first are exposed to is they will show allergic reaction to it, even though it's the first time, how did they get the antibodies for it? It's due to several previous exposure to penicillin but in sub-infectious doses, this exposure could be from bread rotten, air particles or anyway that you're exposed to get an immune response (the only get the immune response).

Again, the adjuvant is a nonspecific materials we use them to enhance the immune response, and there is many types of them:

- Some stimulate the immune response like Alum (aluminum).
- Inflammatory like microbactria.
- Nonspecific like lippolysaccharides.

Classifications of adjuvant:

- Organic

- Inorganic
- Synthesized
- Complex adjuvant.

The age of the human being is also very important. If we give an antigen during the development of the immune system, we will get tolerance to that antigen and we will never be able to produce immune response to it.

Newborns get antibodies from their mother, their circulation will have an immunity that came from the mother, so the age of person is very important.

Some species can response to an antigen while others will not, so there is a response difference even from one individual to another, in the same family, a person could be a very good responder to an antigen and her brother could be a poor responder. This is dependent on the genetic makeup.

Last thing about the antigens is "superantigen". Remember that we said the the antigen should be metabolized then should be presented to the immune system, and that presentation is very specific, very critical.

Presenting cell will carry the epitope and present it to the immune cells, which will recognize it through receptors.

But in "superantigen" the way is different. There is no processing of that antigen, it will join the T-cell receptor with that molecule that joins from the outside (you stuck them together from outside and keep them like a clamp).

These types of antigen they are polyclonal T cell activator.

E.g staphylococcal intertoxins, toxic shock of other material like polysaccharide. So these are bacterial components that can do this.

There is a difference between superantigen from viral origin and bacterial origin. The bacterial origin will mix them together with the viral but at the same time we will find them in the cell because the virus itself can't stay unless it grow inside the cell so this is attached to the cell making it a little bit stronger because it will again mix them together after attaching from outside where we can see these types of characteristics.

Polyclonal: one epitope can be seen by one single cell.

Theoretically we have millions of antigens in the environment and millions of T lymphocytes and B lymphocyte in the body, each one will recognize different antigen. When we talk about

normal antigen, one cell will recognize and respond to it and this cell will divide and produce clones.

In the case of superabtigen, many cells will react at the same time that's why the superantigen could be dangerous and fatal. If they activate the immune system they can produce shock and the patient may die (so it activates more than one type of receptors "polyactivation of T lymphocytes)

In the body, there are about 4 or 5 cells to recognize one epitope, to pick the antigen and respond to it. In the superantigen, any T-lymphocyte with a receptor and antigen molecule can be connected together.

We are talking about the initial activation of the cells … we produce the antigen for the first time super antigen can activate a large number of cells non specifically, while a specific antigen will activate only one .

When we have a response of many cells the end result will be difficult because they will produce interleukins and chemokines and those will do the reaction which is harmful to the hemoglobin.

(superantigen is in the first exposure , no memory because there is no specific activation of T-cells, no specific materials will be produced.)

Super antigens generally are microbial products like staphylococcal inteertoxins these are fibrogenic materials produced by bacteria, this happens when the cells phagocytose the bacteria and the microbial products will be released and then they'll bind to the T-cells.

The response is huge because we are activating a large amount of the T lymphocytes which produces chemicals that have a lot of biological functions like blood vessels permeability and increase the temperature and many abnormalities we can see them in the body that's why u have shock.

Now we will talk about the immunoglobulin which will be produced in response to antigens

These antigens which are inducing the antibody production, some are T-dependent and some are T-independent. Itcould be infectious agent (bacteria or something) it could be blood product or land component (allergy) or molecules so we have millions of antigens from various sources

Since the immunoglobulins are proteins (globins) so we can separate serum proteins as we learned in biochemistry we will get albumin, $\alpha 1$, $\alpha 2$, β, and γ globins according to + and – charges.

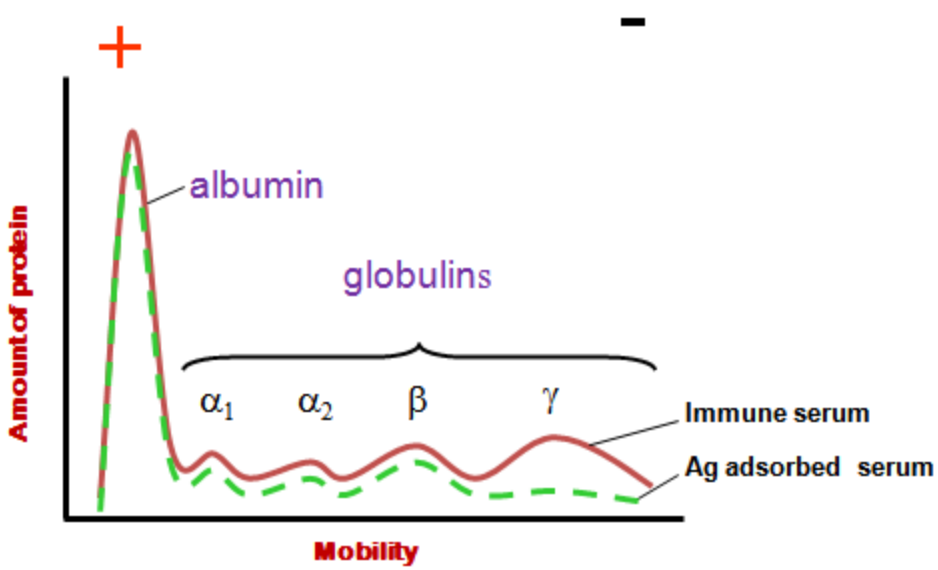

Figure 1: Seperation of Immunoglobulins

Most of immunoglobulins are in the γ globins.

Not all globins are antibodies. Only B lymphocyte in the body can produce immunoglobulins (the gene which is responsible of the production of these immunoglobulins is found in every nucleated cell in the body but they're not functioning , only B Lymphocytes can produce them).

The produced immunoglobulins are specific to their induced antigen.

One B lymphocyte can produce only one antibody to one epitop. If B-cell #100 had been exposed to epitope #100, they cell will only produce an antibody against this epitope.

Plasma cell is the end product of B lymphocyte (it will be generated after the recognition of the antigen).

Immunoglobulins are 2 types:

1. Receptor on the cell (stay on the B lymphocyte as a receptor)
2. Secreted outside the cell (produced by the plasma cell)

There's no difference in the specificity between them (they both have the same sequence of amino acids and the same specificity and the same characteristics, they are completely identical. Except that one will be bound to the tail stem of fc portion while the other is secreted.

The secreted antibody has functions, to neutralize the toxins, if it is specific to a toxin. It can recruit the effectiveness as a compensation of others. So it has different functions.

The structure of immunoglbulins structure is heterogeneous. If I take immunoglubins from one body, I'll find that they're not 100% identical. Even if I take both IgG types, they won't be identical, Heterogeneous immunoglobulin.

We have what we call myeloma proteins, which are homogenous. The plasma cell was mutated and became a cancerous cell which will only produce one homogenous immunoglobulin. All imuunoglubulins produced by a myeloma cell are exactly identical. And so, we have two types, heterogenous and homogenous immunoglobulins.

The immunoglobulin itself is composed of four side chains; 2 heavy and 2 light chains. If we look at the amino acid sequence starting from the N-terminal to the C-terminal, we can see that the first 100-110 amino acids are variable from one immunoglobulin to another. That's why we call this region the variable region, which is where the antigen's epitope binds. The other part is constant, since if we take two IgG's; one against antigen A and another against antigen B, we'll find this part a constant, non-variable.

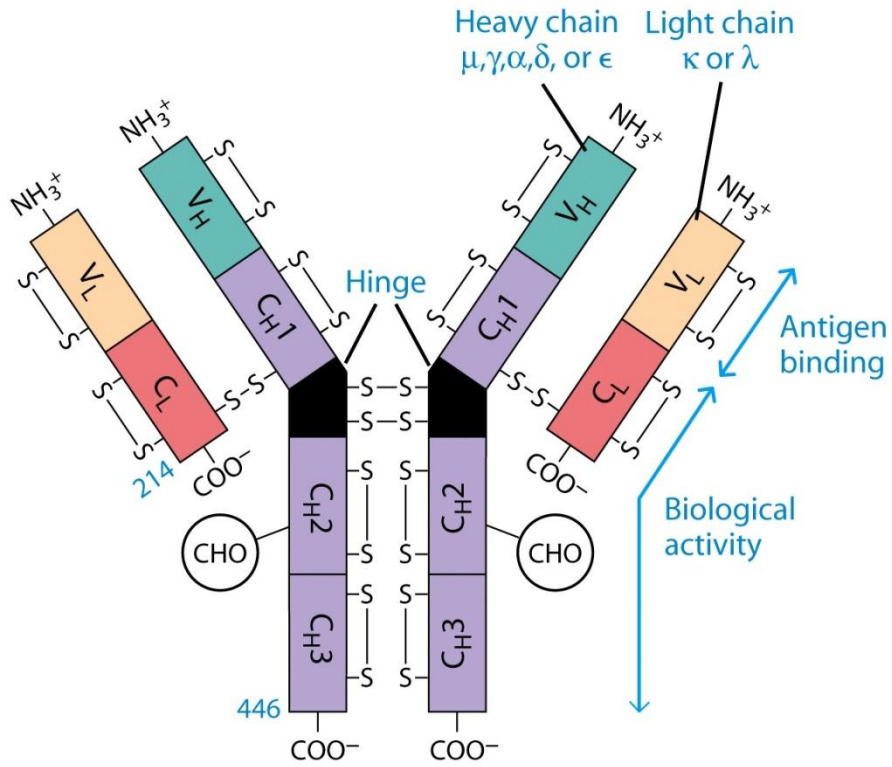

Figure 2: Typical side chains

Class	Heavy chain	Subclasses	Light chain	Molecular formula
IgG	γ	γ1, γ2, γ3, γ4	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n = 1$ or 5
IgA	α	α1, α2	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n = 1, 2, 3,$ or 4
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

Table 1: Ig configuration

These immunoglobulins are needed according to the configuration of the heavy chain.

If it was γ configured then it's called IgG

If it was α configured we'll call it IgA… and so on like the table.

Thus we have 5 different divisions. In the light chain there's only 2, either 2 Kappa (κ) or 2λ, but never 1 λ and 1κ.

Another thing, the IgG and the IgA have subtypes. IgA has 2 subtypes and the IgG has 4.

IgA1 and IgA 2, IgG1, IgG2, IgG3, IgG4.

If we look at the molecular structure of this, we can find that the molecules are bound to each other by disulfide bonds, between chains there are disulfide bonds and there are disulfide bonds within one chain between its molecules, intrachain. These intrachain disulfide bonds will change the aliphatic structure into domains.

The hinge region is also important in the specificity of the immunoglobulin. The amino acid at that region is mainly proline, which gives rigidity to the hinge.

Functionally, the variably part is the part where the antigen binds while the other parts have biological functions, not chemical.

We might have IgG κ or IgG λ, IgM κ or IgM λ but the IgM can be seen as a monomer, a one molecule, or as a pentameter, 5 molecules bound to each other.

IgA can be seen as a monomer, dimer, or trimere. 1,2, or 3. (Sometime we may see even 4, tetramer.)

IgE and IgG are always seen as monomers.

Those that can be seen as polymers (dimmers, trimers…etc) have an extra polypeptide called joining chain.

Within the first 100-110 amino acid which are variable, there are 3 areas are called hypervariable region. If the variable region changes in 10%, then the hypervariable region may change 60-70%. The hypervariable regions are about 15-20% of the variable region and are changes called framed region, complementary determined regions, since these regions make the configuration where the epitope will fit. (CBR1, CBR2, and CLDR3)

In the constant region, we already said it has a biological function. The intrachain will produce a domain.

So each immunologlubulin monomer has 4 different domains, 3 in the constant region and one in the variable region. The constant regions contain carbohydrates and the amount different from one immunoglobulin to another.

E.g IgE has higher carbohydrates in comparison to IgG.

IgM and IgE have extra domain in the constant region. Their molecular weight in the heavy chain is more than the others.

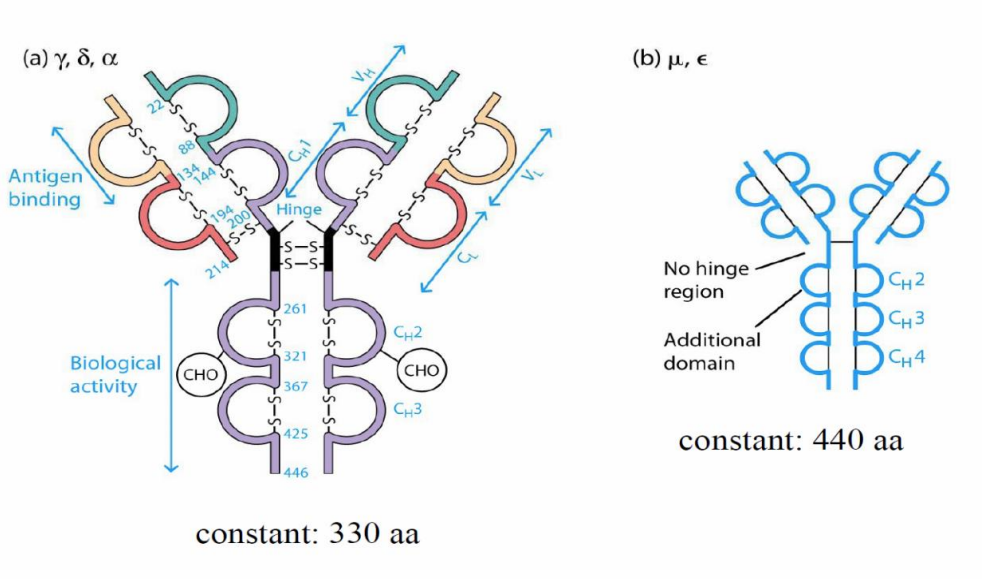

Figure 3

The function of the fab is:

1. The antigen binding site,
2. Neutralizing molecule
3. Crosslink and does some antigen-antibody reaction

The constant region which we call the Fc portion can:

1. Activate the complement
2. Do opsonization
3. Pass from mother to fetus through the placenta
4. Found in the mucosal membrane
5. Bound to mast cells

The Fc receptor can be found it in different calls.

Fc receptor 1 can be found on macrophages, neutrophile, eosinophile

Fc receptor 2 can bind macrophages, neutrophils, eosiophils, platelets, langerhans

So the non-specific cells in general have Fc receptor. The B- cells sometimes may have Fc receptor since they produce the immunoglobulin.

Molecules can be digested by different proteolytics enzymes, like any other proteins. Mainly we use two enzymes, pepsin and papain.

If by pepsin, the immunoglobulin will be degraded into 2 parts, Fc portion and Fab portion. Here the Fab portion is still bound to each other. The disulfide and the hinge remain present, and thus, this molecule could still bind to an antigen. This part is called F(ab)2 since the two antigen binding sides are together. There are lots of applications for this.

E.g. If we give an anti-toxin to a patient as a treatment for diphtheria, this is an antigen. The anteginicity of the molecule is mainly found on the Fc portion. If we produce antibodies to tetanus in animals and treat it with pepsin, this means we are chopping the Fc portion. When we give this to a patient, it is still functional with much less antigenecity, reducing the danger of it.

The F(ab)2 can still perform the following functions:

- Detect antigen
- Precipitate antigen
- Block the active sites of toxins or pathogen-associated molecules
- Block interactions between host and pathogen-associated molecules

But it cannot activate:

- Inflammatory and effector functions associated with cells
- Inflammatory and effector functions of complement
- The trafficking of antigens into the antigen processing pathways

Papain will degrade it into 3 fragments; an Fc fragment and 2 Fab fragments.

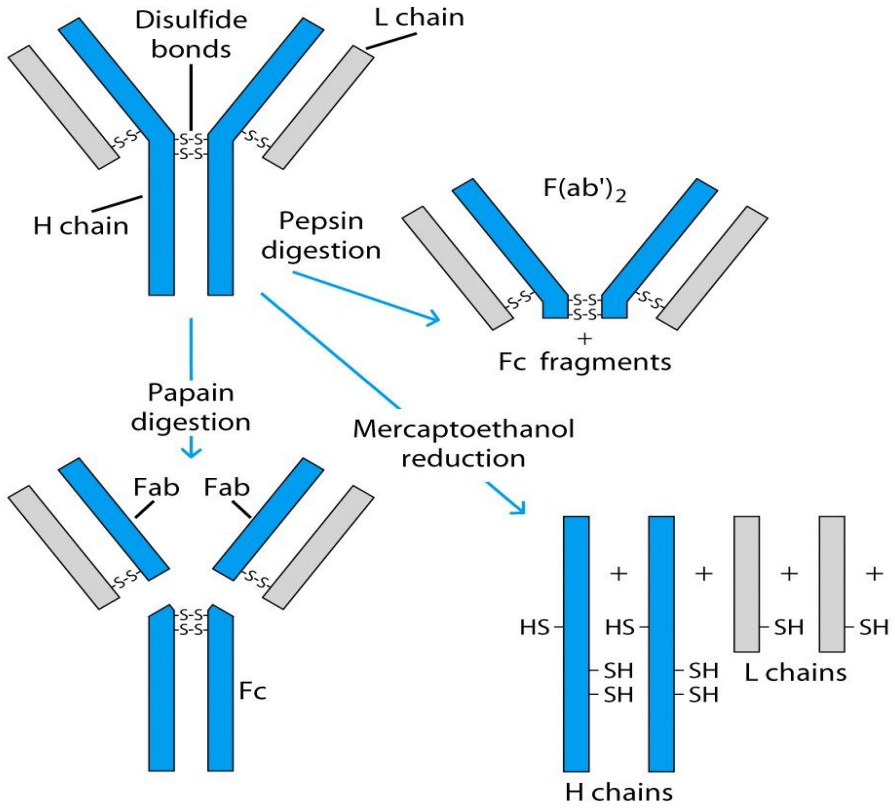

Figure 4: Immunoglobulin degradation