Host-Pathogen Interaction (Immunology)
Prof. Himanshu Kumar
Laboratory of Immunology and Infectious Disease Biology
Department of Biological Sciences
Indian Institute of Science Education and Research (IISER) - Bhopal

Lecture: 53
Adaptive Immunity-Antibodies

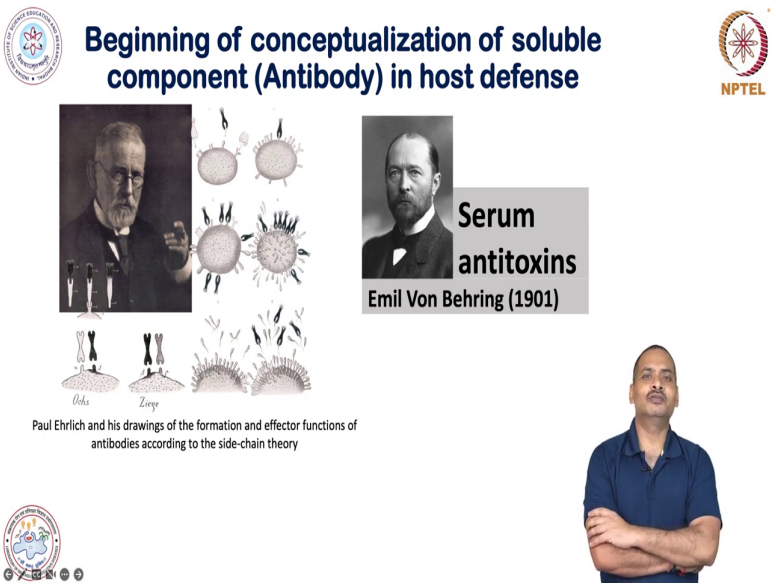
Hi so, in the previous session we talked about antigen properties of antigen and what kind of this antigen is can induce the immune responses in terms of B cell and T cells and how these B cells basically recognize these antigen and T cells also how they recognize what kind of interactions are there. So, now let us move on to the antibody in previous session I have discussed about how to generate the antibody.

That is two different kinds of antibody the polyclonal antibody which is extremely simple it is very cheap but generation of monoclonal antibodies manifold expensive very difficult and it is a technically very challenging in order to make the monoclonal antibody right. Now let us move to the antibody here I would like to discuss about after the after the conceptualization of that there is a some humeral component.

You remember the history of Immunology there are two line of or two school of thoughts one is that one school of thought is that some cell is involved in defense and another school of thought is a some soluble molecules are in a soluble substances involved in defense. And if you remember you probably remember that Metchnikoff is the key person who is who is basically initiated this concept.

There is some phagocytic cells which can phagocytose the antigen or microbial pathogen and there is a school of thought where they have explained you probably remember that name Paul ehrlich~~Acrylic~~ he is a key person in developing that concept and these school of thoughts are known as a cellularist and humoralist.

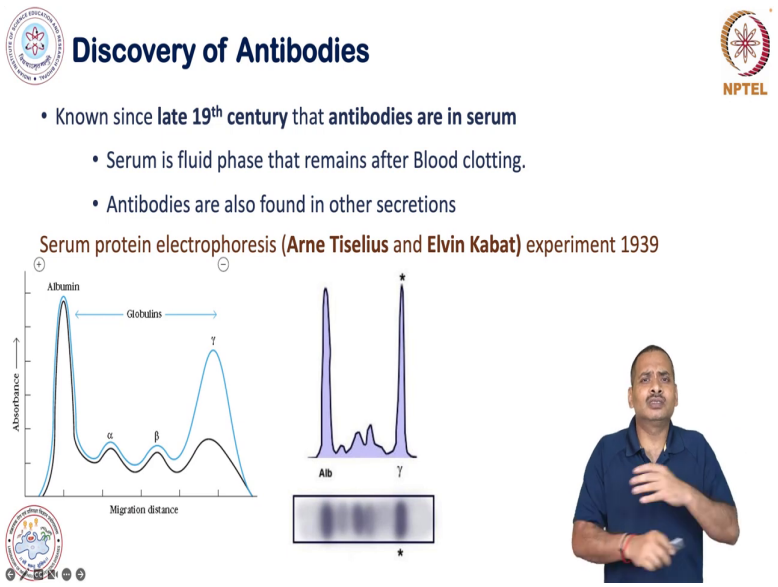
(Refer Slide Time: 02:47)



So, let us begin with that thing. So, so he is a Paul ehrlich ~~Acrylic~~ and ~~one bearing~~ von Behring these are the key person who are who are postulating this or showed by experiment that there is a some soluble factor and these soluble factors are involved in defense. You may remember that his work Paul ehrlich~~acrylic~~ his side chain Theory cell cell makes some side chain and then this basically bind with the antigen and this is present in soluble form and blood.

So, this side chain theory and this serum toxin work by Emil Von Behring both of these worker received the Nobel Prize for their contribution. Now there was a after this work now people wanted to know what kind of molecule it is how this molecule work. So, in order to understand how this molecule work and how this function you need to understand first you need to find out whether this molecule is present or not.

(Refer Slide Time: 04:25)



And this work was a very elegantly presented by one or two people this these people basically very nicely showed that there is a some molecule or some protein is there which is giving this kind of protection. And these works was initiated after 1901 and people were giving several theories but no one able to convey and no one are able to ~~convincially~~convincedly show this thing.

So, the serum you know that since late 19th century the antibodies are in serum this was kind of established because of the work of a previous worker like Paul ehrlich~~Acrylic~~ and Emil Von Behring. So, they understand that this is present in the serum but how to how to find out and what to do it. So, that was that work was further done by another people which I am going to show you in a short while.

So, serum is a fluid phase that remain after the blood clotting. So, here I just want to tell what is serum? Serum is just simply you take out the blood allow it to clot at room temperature and the fluid which is remain in the clotted blood over the clotted blood we call it as a serum. There is a one more contrasting term just for your information I am telling what is plasma. So, plasma is nothing.

So, you take out the blood and put the anticoagulant. So, you collect the blood in with anticoagulant then blood will not clot. So, if you spin that blood you will have a straw colour fluid which we call it as a plasma. So, the difference is very simple between serum and plasma. So, in case of plasma all blood clotting factors will be remain there. In case of serum

there will be no blood clotting factors like fibrinogen and all those things you probably studied in earlier of your education.

So, antibodies are also found in other secretion this was also kind of established by these worker and this work was a basically a very simple work and this was basically given by these two people that is Arne Tiselius and Elvin Kabat ~~Arne Tisalis and Elvin Kabot~~ or Tiselius and Kabat experiment this is quite famous and they performed and they reported in 1939. This is very simple experiment which clearly demonstrates there is a some protein molecule which will be involved in defense.

So, now I will explain this experiment this is very simple you should understand. So, what they have done they have they have taken the defined antigen or for example BSA I do not know precisely what protein they have taken but I am giving the concept. So, they have taken the protein antigen and challenged the animal most likely they use the rabbit. And after challenge they collected the blood after 14 days or in at particular duration and then they have isolated the serum.

So, after challenge they collected after some time they collected the blood and they prepared the serum and when they have prepared the serum after that they divided this serum into two portions two equal portions it is simple right. And one portion what they have done they have put the antigen in insoluble form and they allowed it to react. So, what will happen you can understand very simply when you prepare the antigen in insoluble form it is very simple.

You just tag this antigen with some insoluble Matrix like beads some beads like agarose bead or something like that and when you will put this antigen and beadt in the serum in one portion of serum. So, what will happen there will be antibody against this antigen right. So, this antigen will react with antibody which is present in the serum and once you will do this process after that if you spin the serum which contain these beads antigen beads.

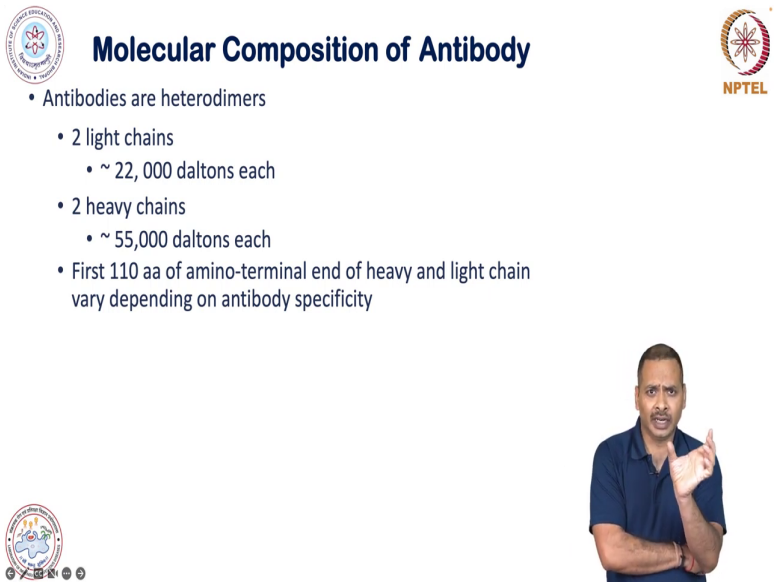
So, what will happen all antibody will be removed. So, there after that they have performed a simple electrophoresis experiment here you can see that there is a gamma portion. So, we also call it as a immunoglobulin antibody as a immunoglobulin we also call it as a gamma globulin. So, this gamma globulin is basically here you can see that. So, then they have

performed the electrophoresis of both one is treated the serum which is treated with antigen and bead.

And another is just a whole serum and then they can find out that this is a reacting portion the gamma portion is basically reacting here you can see the gamma portion is reacted and removed from the serum. So, in that way this antibody was discovered it means it the molecule people can see that ok this molecular weight molecule is responsible for the defence. After that there was a several challenges.

And the one of the most important challenges what is the molecular composition how big what is all those things.

(Refer Slide Time: 11:07)



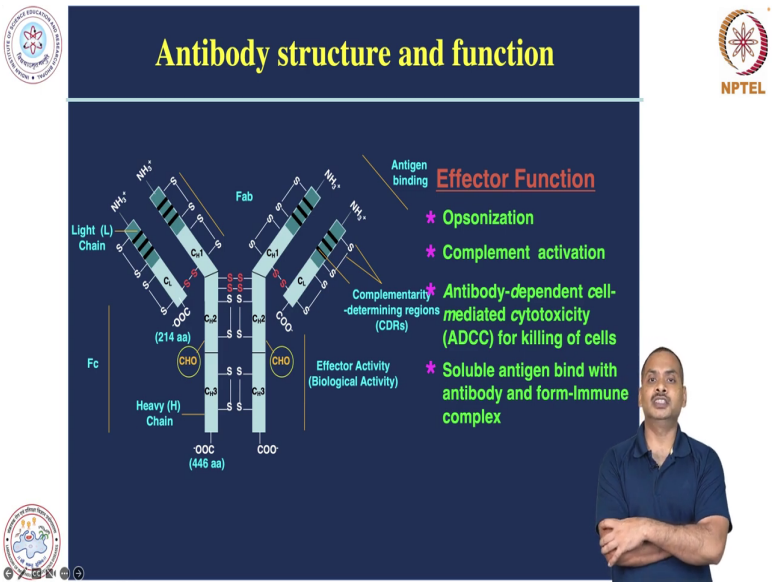
So, this molecular composition of antibody was again deciphered by a lot of people by very simple experiment at least by this way they can tell that the anti antibody molecule is consists of that many polypeptide chain. So, what is the molecular composition so antibody is basically now we know that antibody is a basically it is a heterodimers. There are two heterodimers which combines and that makes antibody molecule.

And this antibody molecule is basically consists of two light chain I will show you in a in a short while and each light chain is basically consists of 22 kilo Dalton polypeptide chain and it has a two heavy chain which is consists of 55 kilo Dalton each. So, how this. So, this is basically giving an idea that the antibody molecule is having that molecular weight but this is not giving any idea about how they are arranged.

So, in subsequent experiment they have they have people have deciphered how this molecule looks like but before that I just want to show you the structure. So, that you will be easily understand how this work result to the to the determination of structure of antibody and then the functional aspect of antibody. So, the first 110 amino acids in both light chain and heavy chain towards ~~end~~ N terminal they are basically variable.

And this depends on antibody to antibody and the antibody specificity depends on this 110 and terminal 110 amino acids.

(Refer Slide Time: 13:21)



So, here I am just giving you the structure I have discussed this structure earlier but I quickly showed to you when I was discussing the classical complement. Here you can see there is a here you can see there are two light chains and here you can see that there is a N terminal and there is a C terminal. And in light chain you can see one the towards N~~n~~ terminal there is a darker region and towards C terminal there is a lighter region if you see in colour code.

So, this lighter region is the constant portion of it will remain same it depends on the kind of light chain. And here you can see that there are black strips over this towards ~~end~~ N terminal in darker region these regions are basically hyper variable region and this light chain is consists of about 240 amino acid. There is a heavy chain here you can see there is a heavy chain and this has also the darker region which is variable region and there are CH 1 CH 2 CH 3 region. So, these regions are basically constant region.

And now here you can see in the structure in both light chain and heavy chain there are lot of disulfide linkages. And this disulfide linkage is basically makes a very unique structure which we call it as a immunoglobulin super family structure. So, immunoglobulin this makes this disulfide linkages makes the immunoglobulins super family structure. And this heavy chain is basically consists of approximately 446 amino acid.

And this light chain and heavy chain this is a heterodimer and this heterodimer again make a bind with another heterodimer and then that makes a functional antibody. And this this two heterodimers are basically joined by the several disulfide linkages here you can see. And this the black strip which I told you that this we call it as a hyper variable region or CDR we also call it as a CDR complementarity determining region.

This is closely associated with the antigen. The portion which is binding with the antigen so, this hyper variable region or the darker colour region in both light and heavy chain basically they interact with antigen and we call it as a antigen binding region in short form we call it as a Fab ~~A B~~ the fragment antigen binding. There is a the constant region we call it as a F~~C~~c region and this is this F~~C~~c region is playing very important role in various biological activity.

I will tell you what is these biological activity or more precisely the function of antibody. This constant region is also heavily glycosylated. So, gamma globulin or immunoglobulin is highly glycosylated molecule. Here you can see that the CHO is basically representing the glycosylation the throughout the region. What are the effector functions of this antibody you know some.

One is opsonization, so, once the antibody is generated against any microbe then this antibody will coat th~~i~~se microbe and then it will be readily phagocytose it is not like a complement thing in case of complement the microbe is basically coated with C3B or C4B. So, that the term is same opsonization. So, opsonization~~oxidization~~ means the coating of this this microbial pathogen with particular molecule here it is antibody in case of complement it is C3B or C4B.

So, antibody has one important function that is opsonization another is complement activation I have discussed in a great detail. So, I am not going to talk any about complement activation basically it activates classical pathway. Antibodies are also playing very important

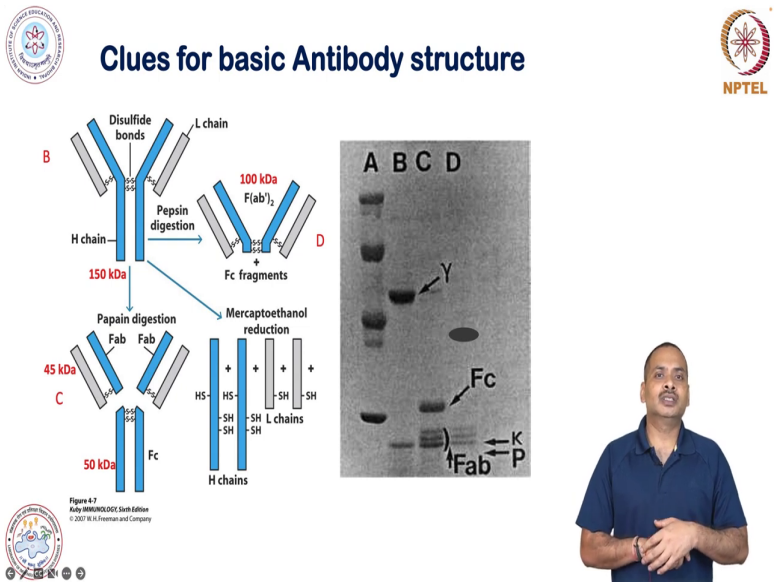
role in antibody dependent cell mediated cytotoxicity for killing of the target cells virally infected Target cell.

And ADCCS are basically triggered by NK cells if you remember. The soluble antigen bind with antibody and this basically makes a immune complex and this immune complex is basically insoluble and it can be phagocytose it can be removed in the kidneys and all those places and then this will keep the system clean. So, these are the one some key function and besides this they the antibody has a different constant region.

And each constant region has some specific function. If you remember I have discussed in during this course there is a some FC gamma receptor. FC Epsilon receptor so, FC gamma is basically there is a one class of antibody IgGG. So, IgGG bind with FC gamma receptor. FC Epsilon receptor if you remember the I discussed a little bit about the hypersensitivity over there is a FC Epsilon receptor there are high affinity low Affinity.

So, FC Epsilon is basically IgGE is interacting with FC Epsilon receptor. So, these are these are some simple and they have a some particular function. So, the structure of antibody this is not a precisely true for all antibodies this is one standard antibody molecule which is close to the IgGG immunoglobulin Gg.

(Refer Slide Time: 20:40)



Now I will talk how the how the structure was a kind of determined by simple biochemical method. So, this structure was determined by very simple experiments using electrophoresis and by generating antibody molecule against particular fragment. So, this structure was a

determined by simple method here you can see that if you digest this antibody purified gamma globulin with pepsin.

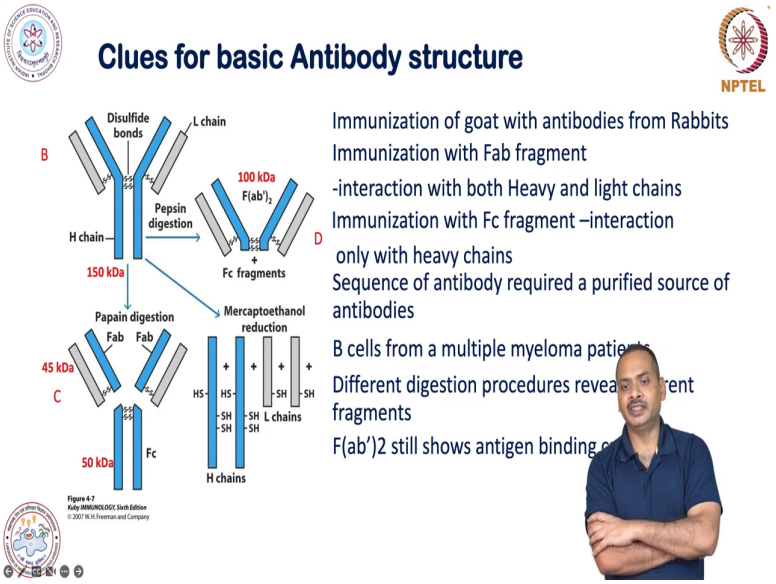
So, then it will generate. So, here you can see that the intact antibody molecule has a molecular weight of 150 kilo Dalton if you digest with pepsin then it will generate the FABab2 fragment. Here you can see that the disulfide bonds are there and both light chain and heavy chain heterodimer are still in joint form that will generate the 100 kilo Dalton fragment. And if you perform this papaine digestion then you will see there will be FAB fragment and there will be a FC portion.

So, these fragments can be can be determined by using specific antibody I will I will tell you in subsequent slide. And if you treat this antibody molecule with some reducing agent like a beta mercapto-ethanol then you will get a heavy chain and light chain. So, this is a if you if you if you see this simple experiment and this is a kind of puzzle if you do this thing then you will get this kind of thing if you do this thing then you will get this kind of result.

And when you put all these result together and think then you can you can find out the structure of the tentative structure of antibody. At least how these polypeptides are organized and how they are present in the molecule. And after that this can be easily detected by simple electrophoresis here you can see that there is electrophoresis electrogram. And in these electrogram they have detected these particular fragments like 100 kilo Dalton or this 45 kilo Dalton and 50 kilo Dalton by simple by making the antibody against these molecules.

For example if you have a rabbit antibody if you take this antibody from rabbit and inject it into the goat then you can generate the antibody against that antibody means you can generate anti-rabbit immunoglobulin in Goat and you can use that antibody in order to detect. So, this is the method by which this complicated puzzle was solved how this polypeptide chain is basically arranged.

(Refer Slide Time: 24:05)



Here I am giving you the key points how this experiment was performed. They basically immunize immunization of goat with antibody from rabbit you as I have told you earlier the immunization with FAB fragment. So, you can you need to isolate these fragments. So, at that time electrophoresis and chromatography was quite well established. They do not have a had a very sophisticated techniques and methods.

Interaction with both heavy and light chain. So, if you make an antibody against say for example FAB as you can see the 45 kilo Dalton the antibody which is generated against that FAB this can also react with FAB Square as well as this can react with a heavy chain and light chain after treatment with mercapto-ethanol. So, this is a kind of puzzle you need to think then you can understand very well.

Immunization with a fc fragment that also generate some antibody and that you can see this interaction on that is interacting with only heavy chain sequence of antibody required a purified source of antibody here there is a one challenge generally antibody when you challenge the animal with antigen you will get the mixture of antibody and if you use the starting material as a mixture of antibody that is very difficult to proceed.

So, from where they get the purified antibody the simple answer is they got it from cancerous cell they induced some cancer in the in the animal and that animal that that animal will produce only one kind of light chain heavy chain or one kind of antibody. So, they use that antibody. So, B cell from multiple melanoma patients in case of human they also use this B cells from multiple melanoma patient.

And by doing this different digestion and by chemical treatment they could determine the structure the structure as it is shown over there. This FAB2 is still show the antigen binding capacity after all this digestion this has a capacity to bind with the antigen. So, in that way the structural information was or a structural Insight was gained and of course later on the crystal structure and all those things were there.

So, with this I will stop here and in next session I will discuss about various kind of antibody. And how this antibody diversity was generated it is a it is a very complicated molecularly it is a very complicated but I will try to give you at least a snapshot about how this antibody diversity is generated, thank you.