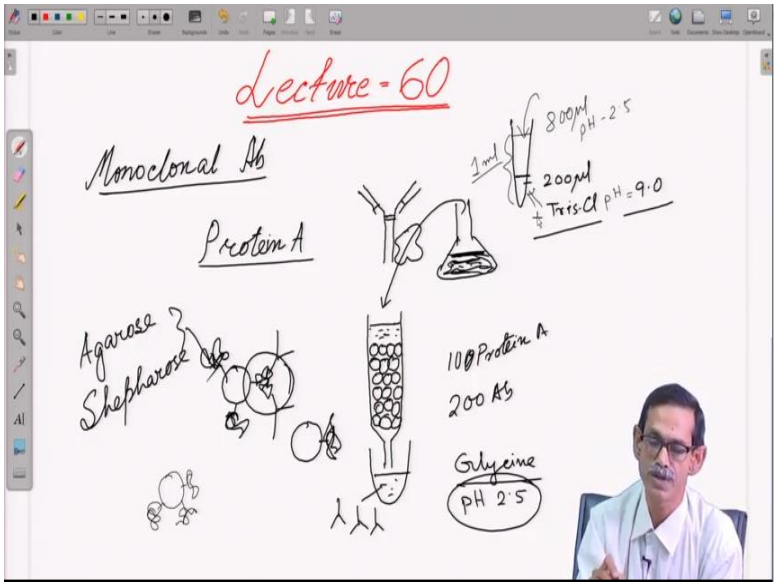
Immunology
Prof. Sudip Kumar Ghosh
Department of Biotechnology
Indian Institute of Technology, Kharagpur

Lecture No -60
Monoclonal Antibody (Contd.,)

So welcome you all, the last lecture of NPTEL course on immunology. So I hope all of you enjoyed the course, I mean those who are continuing from very beginning to end and we tried our best I mean in this situation actually what happened we are habituated to take class in board and chalk system. So this is kind of not we are very familiar as even after that I hope you understood what I or we would like to tell you and definitely if there is a scope we having a live interaction or so forum you had different questions.

And if there is any more questions I mean definitely we will be here and our teaching assistant will definitely help you and we will be here too. So and in the last part of the lecture or the last lecture what I am going to talk about, I am continuing the thing what I did in the last lecture.

(Refer Slide Time: 01:27)



In last lecture we are talking about monoclonal antibody right. So now you have liters of culture a monoclonal antibody and this is there I mean there are many other things because they are growing in medium so when they are secreting the antibody in medium it is the medium contains

so many other things. So we have to purify that many way we can purify that but the most common technique that we are going to use.

And one previous class I do not remember which lecture it was but we told that staphylococcus protein A, the surface protein staphylococcus Aureus has a surface protein which binds to IgG. Normally monoclonal antibody you go for IgG, IgM monoclonal is also there I that staphylococcus Aureus that protein A is the surface protein of that bacteria which binds the constant region of antibody that mostly the Fc portion of the antibody.

So if this is the antibody, suppose this is the antibody it will bind the Fc region. So what scientists did and that is the bacterial defense mechanism bacteria produce it, but scientist used it in different way what they did in a sepharose or agarose bead they immobilize this protein A, that you can do cyanogen bromide activated bead c and b are activated bead you can buy so in that bead so those bids are no actually those beads are activated. So they have a free bond any protein you give it will bind there.

So that way that Agarose or Sepharose bead either one attached with protein A, so we will just do the affinity chromatography very simple. We have a column affinity chromatography which is packed with that bead, I am not drawing this protein anymore we have packed with this bead the same you know the gel exclusion chromatography or gel filtration so this is packed with the bead this bead is a special bead what if you see I mean in amplifier or magnified version you will see that each bead has protein A attached to it.

It may not be one it may be multiple protein A, because beads are big bigger or much big enough to hold multiple protein A. So what will happen now if you have so and if you have some solution where you have monoclonal antibody in it or the antibody in it definitely, the source is one clone so it is a monoclonal you can do it for polyclonal also. Even if you have a serum containing lot of antibody you can use the same technique.

Because serum has lot of albumin protein almost 60% of the serum protein is albumin so if you want to separate the antibody from that serum albumin same technique we use, what will happen

we add this here so we add this liquid here, so what will happen? It will gravitational force wise it will go down and if you collect it in a tube, what will happen? So medium will cross and while going through this medium what will happen, I mean all possible antibody are going to attach to this bead because here the antibody are going to bind one protein A molecule combine two antibodies.

So if there are suppose in this bead there are 100 protein A molecule in this whole column, so that means 200 antibody will be bind here and rest will go to flow through. So we do not throw this thing we save it because we do not know how much antibody is there because we can keep it in 4 degree at least for years. We can keep this antibody solution for years so if you pass this is what will happen 100 protein A molecules are here.

So 200 antibody will be attached here and rest will go then we wash, wash, wash all non specific binding or attachment will go away then we what we do is so we will take out this and we will put another tube which is fresh tube. Now what we are going to do is we are going to add some buffer the any type of buffer is possible either very high PH or very low PH, if you just put glycine buffer PH 2.5, what will happen?

If the PH is too low suddenly this because protein A is one protein it binds the antigen and this is a protein, protein interaction one protein is like this another protein is binding here and as soon as, you change the PH either too high like PH 12 or too low like PH 2.5 what will happen that conformation will change and they will not bind anymore. So as soon as you add glycine buffer PH 2.5 all antibody because this is covalently attached protein is covalently attached with the Agarose bead but protein and antibody interaction is non covalent interaction you change the PH they will not bind anymore.

So what will happen when the buffer will come from top to bottom it will bring antibody also, so this solution will have all the antibody clear, but in PH 2.5 antibody cannot stay for longer time, so what normally we do is I mean so suppose this is the tube in this tube I am telling you very laboratory scale not the industry scale. Industry scale is completely different in laboratory scale

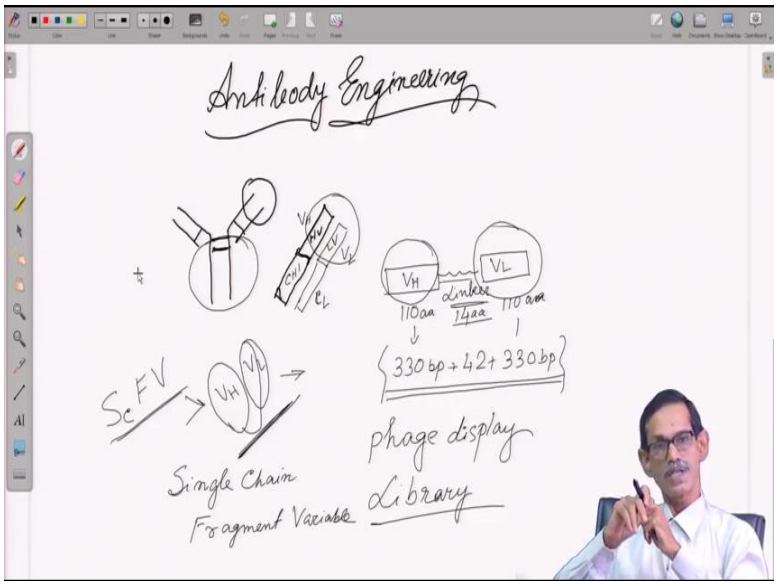
what we do is in this tube we normally take 200 microliter Tris Cl buffer PH 9.0, Tris Cl buffer PH is equal to 9.

So if this is here and now something is coming say 800 microliter some solution of PH 2.5 when we collect so total is 1 ml approximately, so when we collect 800 microliter PH 2.5 and 200 micro liter PH 2.5, if you mix it will come very much to the normal or the neutral PH 77.2, so antibody immediately denature but we have to denature also that is it and now you have multiple tube you collect them check them and you can store it.

So that is how we lab scale we purify this is the basic principle like you have to have affinity purification here where is the affinity? Affinity is between protein A and monoclonal antibody so what I told you, I told you in that bead activated bit any protein will bind. So if you have your antigen enough amount you instead of your protein A you can also add your antigen also that will be much better because protein will purify all antibody particularly when you are purifying the polycrylal antibody if you can attach your own antigen, antigen of interest.

So you will get in the I mean in the collection tube you will get only antibody which is specific to your protein no other antibody will be there, so that is how we purify the antigen. So this is one part, so now we can scale up and produce as much as antibody you do not have to go to the animal anymore because you have the cell line you can store it whenever you need you culture them grow them purify the antibody. So you do not need any other animal handling or antigen purification no; you do not need it.

(Refer Slide Time: 11:14)



Now we are going to talk about another thing it is called and very briefly I will tell, I will not go detail this is antibody engineering. So what is that we can produce antibody inside the lab just by molecular biology or recombinant DNA technology. What if you remember I am drawing again this is the heavy chain right this is light chain attached to it disulfide bond, so which part of the antibodies attaching or interacting with antibody? Only this region.

So what is there we have one so it is heavy chain variable region, heavy chain constant region 1 or CH1. This is CH1 this is from heavy chain and we have another light chain variable region what we call we call it VL and this is VH and light chain constant region right, then it is CL which part is actually interacting with the antibody antigen this region. So now if I make a construct like which will have herbicide variable region like VH then, there is a linker then Vl.

So if I make only this what will be there this is 110 amino acid you remember your that bdj recombination class 110 amino acid and this is 110 amino acid approximately it may be 105 or 115, 110 amino acid that means how much that means 330 base pair, here it is 330 base pair because if you go to DNA level from amino acid you have to multiply by three. 1 a minus 83 codon and the linker is normally 14 amino acid this is synthetic.

So what I am going to make is I am just mimicking the antibody structure so I will make one heavy chain domain there is a linker and another light chain domain. So they will, I will make I

will give this 14 amino acid such way that this heavy chain binding domain and light chain binding domain are linked with the thread kind of thing. So they will go and fold so we are instead of making the whole antibody I am making a DNA constant which will have variable region of hexane variable region of light chain only.

Which is connected by a 14 to 15 amino acid linker which is already known, which kind of combination will hold them better that is already known. So we do not have to do research for that so ultimately what happened this is VH this is VL. So we are not making the whole antibody it is a small part only one part of the fab fragment antigen binding we are making a small part constant domain also we deleted.

So this is how big will be the total constant, so this will be 14 means 42 nucleotide right, so total will be maximum 700 or very close to 700 base pair constant. So now this constant is just for a constant which will express a protein that you can express in bacteria that in express in phage bacteriophage. Normally, we express it in bacteriophage it is also when we express this we also call phage display library.

So phage display library can be used for many other things but it is also used to display the antibody molecule and this small I mean version of this antibody which has just the VH and VL and synthesized in the laboratory also can do the same purpose it will also neutralize cannot do the other effector function. It cannot do optimization because heavy chain is not there it cannot do complement activation but neutralization it can do, it is very small and you do not have to go to animal you just have to know which VH and which VL.

That means which variable domain and which variable domain of heavy chain and which variable domains of light chain actually are going to specific against your antigen. That definitely needs some research and how I am I mean time is not allowing me to explain it because if I would like to explain this phage display library how to construct from very beginning to end it will take at least an one and half to two hours minimum.

This is not the only thing because you have to do PCR lot of strategy are there then packaging how what kind of phage what protein what to do, so it will take lot of time. But for this basic class you just have to know that antibody gene can be engineered and we can make it in the lab because it is after all its a protein the way we are expressing recombinant protein for all other cases we can also express the antibody protein in this in the laboratory by recombinant.

And this is called this as a special name this is called single chain fragment and in short ScFV, so the ScFV is this molecule. So passive when we need it? We need it because more we have the requirement of the passivability more supply of antibodies there when required, population is increasing so we cannot depend on animal purification so new techniques or new things are coming up.

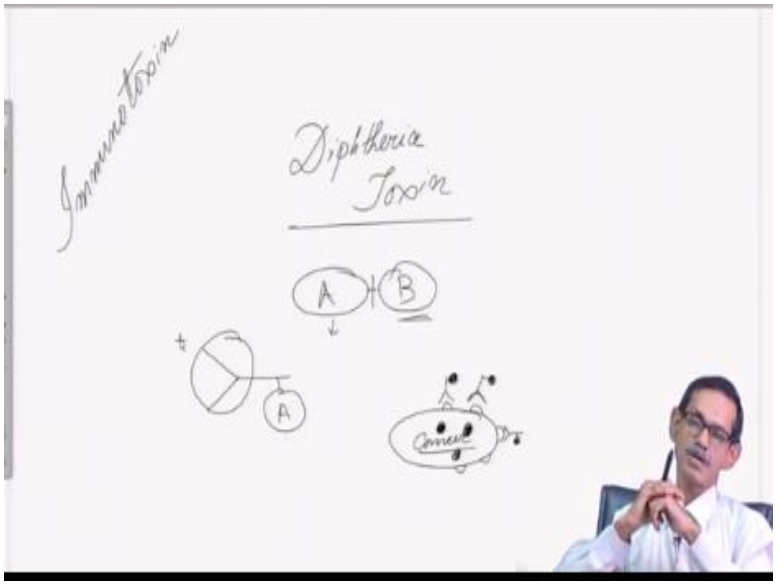
Now here is one beauty is there that when you do the treatment with like antibody raised in other organisms a mouse antibody, if you used to treat human it will be treated as foreign right. So that need to be changed we have to make a humanize antibody so human body should not make so if we can replace this Fc portion making humanizing humanized antibodies will be much much easier.

And that is why the ScFV is very popular, ScFV is very popular so this technique is very common now and people are using and one more thing is I am just going to very quickly, I will tell you is called immunotoxin.

(Refer Slide Time: 19:36)

I should say this is very important, so this is one kind of very wonderful strategy after discovering what is there. So at the end of antibody molecule they put a toxin, toxin means suppose diphtheria toxin.

(Refer Slide Time: 20:14)



So diphtheria toxin is dangerous it will it can kill the cell, so and most of this exotoxin has two part, one is called A AB toxin which is covalently linked A B toxin emit toxin B binds to the cell and A do the toxin part or the killing part what people are doing and what scientists do it is actually first proposed and demonstrated by professor Ira Pastan in NIH National Institute of Health in USA.

So what he did his idea was very smart, so he this is the antibody molecule so instead of because B is not doing the talks, I mean doing any harm B, A B part or the way I used to remember B is stands for binding and A is the active part. So A is actually the toxin, so I used to remember B is stands for binding and A is the active. So what he did instead of B, so he cut it so he attached A part with this antibody.

So he made this is because this is toxin and this is immunology, I mean immunoglobulin. So he named this whole complex and which is very popular even today immunotoxin what is this purpose what he did and he demonstrated, now I mean people are trying also I mean for treatment suppose this is a cancer cell. If somebody has cancer in their body what is going to happen some part is different cancer cell is going to express, some protein which is not expressed by our own protein our own cell.

So cancer cell converted to I mean normal cell converted to cancer cell and they started producing some antigen which is not present in our all other cell or the normal cells of the body. So cancer cell will have some extra antigen which is not present and these antigen is going to be presented by MHC one definitely, so what he thought that if I can make antibody against this which is very specific to cancer cell.

Now if I inject this antibody into blood what will happen? That antibody will go and get bind to cancer cell only and now second thought comes so if this I can make antibody which will bind only cancer cell, why do not we attach a toxin here, so what will happen so the antibody will go and bind to cancer cell which is attached with toxin and as soon as antibody binds to cancer cell antigen it will be endocytosed that mean it will be internalized that is a normal receptor mediated endocytosis that we have talked so many times.

So ultimately what will happen this toxin will go inside and as a result cancer cell will die so inside the body specifically cancer cell will die and that was so brilliant I mean idea and it is and now it is working many patients are now cured by using this technique and this is very specific, only problem you are giving a very bad toxin just in case if it is released something happen. So that modality you have to fix, I mean there is a lot of research went on.

But in that case you cannot use this antibody from animal source you have to use antibody from if it is possible antibody from that individual person already. So that will not be treated as foreign and this is immunotoxin, which was long back in early 90s like just around my I mean 1890 to 1900 that time, Paul Ehrlich proposed the magic bullet. Magic bullet means he said that we have to find something which will go and kill the target cell or targeted way, so that it will not harm to any other part of the body.

So targeted and all of us, I mean those who are interested in drug delivery now are thinking for targeted drug delivery. So here I will end my lecture here today and I hope again I am telling you enjoyed and definitely it is not it was not possible in this lecture the whole immunology there in huge amount of interesting thing we could not discuss.

Because infection immunity is there immunotherapeutics is there so disease wise, so cancer immunity or immunology, HIV immunology all are interesting immuno manipulation. So many things but I hope you are now in a position that you can those who are interest get I mean got interest after this course or from before can understand and read these books and the chapters, thank you all.

So now this is the time to introduce you all other member who helped us me and professor Agneyo Ganguly to make this successful the all this teaching recording and all other part there, because what you are seeing it is not only our effort it is the effort of many other people and I will request them to come here to just introduce all of you to know that who did the background work.

Thank you very much, so I already told that I would like to introduce the team who work background and for whom this course has been successfully recorded and you are seeing. So here myself you know me and professor Ganguly and next to me Shiv Sankar Das, I mean without whom this course would not have been successful because he is running after me for many years to take a course and finally it happened.

Ramu who helped me in recording and guiding what to do what not to do how to see it and how to take care of that. Priyanka help me like help us for like making question and all other mail and all this thing. Saurabh also helped in recording and all other arrangement. So thank you very much, thank you all and many others who is recording right now you cannot see him, so for all of us all of them actually this course has been successful, thank you very much and I hope you enjoyed the course thank you.