Immunology
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Lecture No -59
Monoclonal Antibody

Welcome to the last but one class of this course. In my last lecture we were discussing about the passive immunity and their future trend what can be done with the passive immunity. So today what I am going to do is I want to talk about antibody that we can generate in laboratory, because whatever the passive immunity is the antibody raised in other organism or other individuals which we can use for that treatment or curing or the neutralization of the toxin this for this kind of reason.

But the thing is the supply of antibody from animal there are certain problem that we have discussed in the last class, but so we should have a system from where we can generate a huge amount of antibody. So that we can treat the patient for either say snakebite or any disease already like tetanus or rabies which is already developed because the immunity or active immunity development we do not have that much time.

So we need a readymade antibody in our hand and that should be a good amount and in animal definitely there are a lot of antivenoms we are still producing in horse, but if we have certain facility or the system where we can generate antibody without going to animal will be better. So before going to do the monoclonal antibody, let us discuss one more time what do you mean by or what we understand about the polyclonal and monoclonal antibody? So let us start like today we are going to talk about the number of lecture is lecture number 59.

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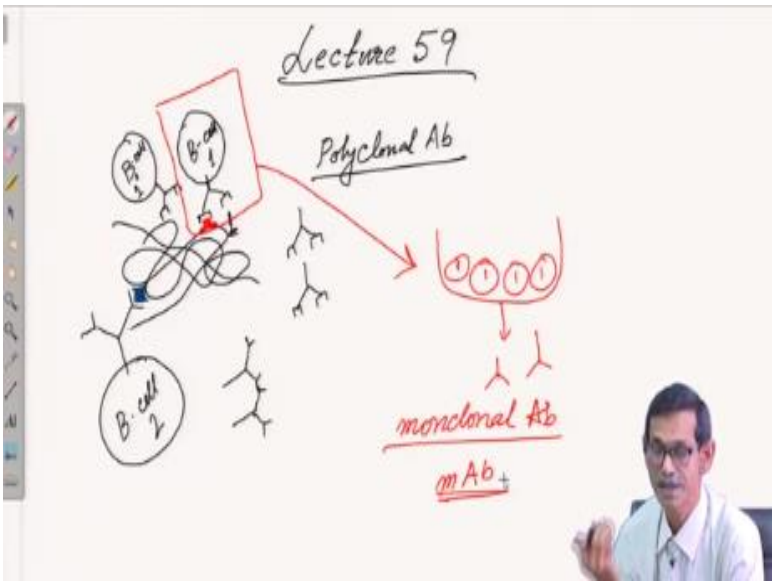

So first let me discuss or start polyclonal antibody. So you know what is clone? Clone is the same thing multiple copies of the same or identical thing this is called clone. So polyclonal means there are multiple clones are there how multiple clone can developed in a regular immune system or active immunity? Suppose this is the protein antigen that we are injecting and they are say for example two epitope so this is one epitope and another epitope say this region is the another epitope.

So why I am showing this is the linear epitope, the red one is a linear epitope and the blue one is the conformational epitopes. So now if this antigen we administer into animal or any pathogen attack what will happen this antigen will enter into our body or in any organism and then immune system will react. So immune system what will happen B-cell receptor will recognize this epitope, this is that I am talking about B-cell epitopes so red and blue are the blue cell epitopes, B-cell epitopes.

So that B-cell epitopes, what will happen this suppose this B-cell epitope is look like this and the blue epitope is look like this, so this is the three-dimensional structure. Now what will happen so if this is there is antibody, antibodies receptor will be like this. So this is the B-cell epitopes now if I needed this polyclonal part I am talking or discussing or the polyclonal antibody, so now so this is B cell and another antibody will be like this which is again attached to it.

So this is again a b-cell receptor first recover I mean the epitope will be recognized by the B- cell so this is another B-cell, suppose this is B-cell 2 this is B cell 1. So in this I am just considering only one B-cell reacting with red epitope another B cell 2 is reacting with blue epitope, what will happen both the B cell ultimately will be converted to plasma cell and produce antibody so what we will see after 7 days or take around 10 days if we see so there will be antibody like this.

And from this B-cell what kind of antibody will be produced these B cell antibody will be like this, so this will be antibody. So in serum both type of antibody will be there and both are going to recognize this antigen because in antigen I do not know how many epitopes are there it may be a single epitope it may be multiple epitope, multiple means 2, 3, 4 depending on the size of the antigen if the protein is very big we will have multiple epitope.

So if there are 4 epitopes at least there will be 4 different B-cell will be activated, 4 different kind of antibody. So in serum we will get the pool of antibody which comes from multiple B-cell so this mixture when we get this is called polyclonal antibodies. This is going to make polyclonal antibodies, but now assume there is only one epitope suppose this blue is not there only red is there.

Even after that if I inject that antigen into anymore what will happen so one B-cell one will come here another B-cell may come which may recognize the same epitope but it is slightly different in sequence, suppose in the epitope binding site the paratope of the antibody or the B-cell receptor suppose in one B cell it is aspartic acid in another B-cell it is glutamic acid, rest of the sequence are same.

So what will happen both will bind right so what will happen then so another B-cell will come. So I assume that this epitope is not there only one epitope is there, so another B-cell will come say B-cell 1 prime which has similar epitope. So even suppose in that case both B 1 B cell 1 prime or 1, 2 B-cell are going to produce a therapy antibody which is almost identical but they are originated from two different B-cell.

So 2 different B-cell means 2 different clones their sequence are very similar but 1 or 2 amino acids in the interacting region or the paratope region are different, then also you will call it polyclonal. So even antigen has only epitope still the animal or whole organism will produce antibody from multiple trope. What is happening actually after presenting by the antigen presenting cells a dendritic cells T-cell will be activated and all the B cell which is recognizing this epitope will be activated by that T-cell.

So all of them are going to convert it into plasma cells and produce antibody, so this is then polyclonal antibodies. Even there is only one epitope there is a very tricky question sometimes so if one antigen has only one epitope whether the antibody produced in any animal whether it is a polyclonal or monoclonal, it is polyclonal because multiple B-cells 1, 2, 3, 4 multiple number of B-cell may be activated and give the pool of total antibody which is coming from or which are coming from multiple results.

Now then also if only one epitope is present only one is present then also it is a polyclonal if it is raised in animal. We have no control, very unlikely that one epitope has only one B-cell in body so even if it is that we cannot make sure. It will be a lot of you have to isolate purify sequence and then confirm. So normally we say it polyclonal. Now if any one of them suppose there are 4 such or 3 such B-cells which are producing antibody against a epitopmial of number 1.

So now if by any technique if we can identify this or separate this and grow them in a culture or somewhere where all the B-cells are of same type, say all are one. Then what will happen it will produce only one type of antibody then if this is we can purify and cultured in a system so that we can get antibody from that all antibodies are going to be identical then it is called monoclonal.

That means the antibody is getting monoclonal antibody, so antibody we are getting from a single clone it also called m A b, many time you see m A b so this is monoclonal. Now we are going to discuss how we can get monoclonal antibody. So it was first discovered or the technique was first discovered long back it is 1975, the George Kohler and Cesar Milstein. George Kohler

and Cesar Milstein who developed this technique and they received Nobel for just developing this technique in 1984.

So there is the Nobel Prize for this technique so it is a very I mean beautiful technique I mean now many labs are producing that but initially the idea was fantastic what they did. So now we will see what they actually did and how we can plead the monoclonal antibody.

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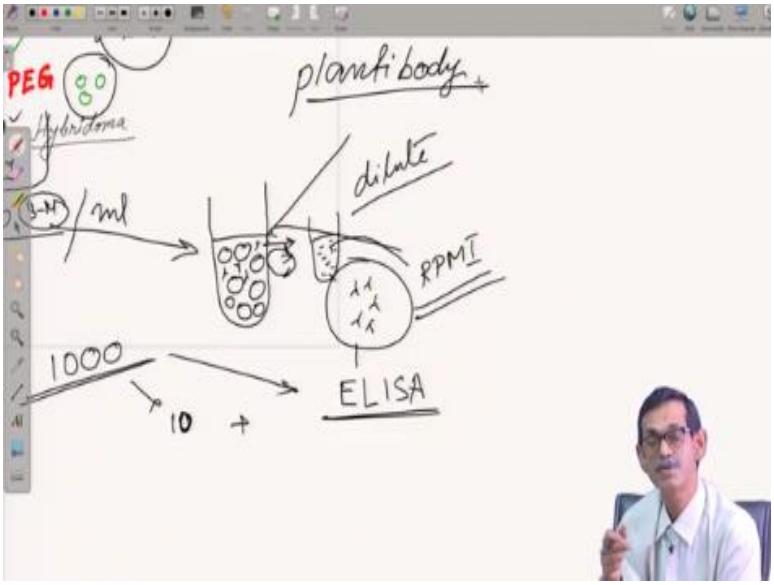

So let us see that I have 1 B-cell now how will get 1 B-cells of this is 1 B-cell, how I will get 1 B-cell? It is not possible normally, what we do? Suppose I have one antigen say antigen X, I would like to make monoclonal antibody against this antigen X so what I have to do is I have to take a mouse. So these mouse I will inject this antigen X into it so what will happen this mouse will be primed with this antigen X.

So if I would like to raise antibody in any animal what I do I just discussed in last class that we are going to use adjuvant in case of animal we are using Friend's complete adjuvant that means oil in water emulsion plus it kill bacteria with it so first injection then first booster, second booster with incomplete adjuvant and to raise the antibody level high so what will happen if I inject antigen say for 1 time, 2 time, 3 times depending on how good your antigen is.

So; after say second booster if I isolate spleen because from spleen I will get many B-cells which are already primed with that. So what will happen if you take a mouse and directly isolate the screen you will get a lot of B-cells but activated B-cell against this antigen X will not be that many. So as I am interested to develop monoclonal against antigen X so better is you prime that animal so that number of B-cell activated against antigen X will be more.

So after you isolate the spleen you can get the B-cell from it. So it is normally spleen cell in total called splenocytes and spleen is a secondary lymphoid organ so we have lot of B-cells lot of T-cells. So here what will happen you will have you can purify or isolate the B-cell in different way. So you know the flow cytometry of the facts we can use at this moment so we will have so I am making small B cells, now all are red. So lot of B-cells will get.

So these B-cells I do not know which one is I mean there are millions, so out of this millions of the I do not know exactly which one is actually activated and which one is against this antigen X it is not known. So suppose at the beginning we do not have to know, but I can say that the number of B-cells against antigen X is better or more than if you take a mice directly. If you take one Mouse directly then number of B-cell is not that many. You can pull multiple more number of Mouse also, so suppose one now so isolate the spleen then from there you take B-cells, so these are already activated.

So if you grow them in cell culture medium, medium you know medium is just a food source like you I am definitely all of you grown bacteria in LB or other nutrient block. So that gives the food and the basic materials of food, so similarly there are medium specialized for animal cell culture. There are many say RPMI is one R P M I Rosewell Park Memorial Institute, this is a name of one Institute in Buffalo New York.

They developed a series of medium, so RPMI there are different number depending on how they develop one medium is there is another called Dulbecco Modified Eagles Medium DMEM so there are various kinds of animals and culture medium that the way we have in material like Luria Broth, Luria Bertani and then Nutrient Broth, Potato Dextrose Medium. So these are the

animal cell culture medium, but if we grow in this medium what is going to happen these cells has an mean I mean a limited lifespan they will not grow forever.

After certain generation all of them will die, so that is not a permanent solution. So people have tried that. So then in this Milstein and Kohler, they figure out how to make them immortal? So what they did they took another cell which is cancerous B-cell this is cancerous B-cell myeloma. This myeloma cells they are B-cells cancerous, that means they are immortal they do not die, so they become transformed or after transformation they became immortal.

But what they figure out that should be some property what first this should not produce antibody, so myeloma cell they purify which has the immortality they are also B-cell but they cannot produce antibody. So then they thought these cell are able to or capable of producing antibody but they are not immortal. These cells are immortal but they cannot make antibody or they selected that also they are not making antibody.

So they thought if we put them together and just fuse them and select the cell which are both the property that means half property that immortality will come from this myeloma and antibody producing property will come from this B-cell. So what will happen we will get a cell so which will have half the property of myeloma that means they will be immortal, they will be mortal and half the property of this B-cell that means they will produce antibody.

So, how to do that? So they make many such I mean they figure out particle you can do two different way. One if you put them in electric field they will fuse basically you have to fuse these two cells, so B-cell and myeloma cell you have to fuse and make them one having both the property. So you can do electro fusion or can use PEG Poly Ethylene Glycol. Poly Ethylene Glycol PEG has the property they fuse the cell.

So now this kind of hybrid cell is generated and that is why this cell is also called I mean the technology also called Hybridoma technology that was actually discovered, but now what will happen if you give PEG both red cell red cell will also fuse right too many B-cell will also fuse

and similarly the green cell to Myeloma cellulose also fuse, because PEG will not selectively fuse only green and one green one red.

Say they will make both fusion to I mean many B-cell sorry many B-cell will be fused like and make like B-cell B cell fusion some this is BB fusion and this is my myeloma myeloma fusion and this is B cell and myeloma fusion and we want this. So in the mixture both myeloma myeloma fusion B-cell B- cell fusion and B cell myeloma fusion. We have to select only this one because we do not need these two.

For selection of this is it not it is I mean to get rid of this it is not a big deal because they were they are not going to survive after few cycles anyway, right they are going to die. But the problem is to get rid of this myeloma myeloma fusion, because there will not of any I mean after this experiment you have huge number of cells millions of cells so from they are finding out the right one which will produce antibody against antigen X is one of the very tough job.

It is cumbersome lot of work you have to do and in that if you have a mixture of this myeloma myeloma, it will make the technique even or the system more tougher or tougher than the only B-cell myeloma cell fusion. So what I have to do we have to figure out something so that they will not also survive. So now we will put in a medium only this cell will survive for what they did these myeloma cells was chosen in that way they are mutant in HGPRT.

HGPRT is an enzyme hypoxanthine guanine phosphoribosyltransferase, what this enzyme is doing? This enzyme actually involved in nucleic acid biosynthesis. So, all the nucleotide synthesis there are two pathway in every cells, in every cells in our body we have two different pathways. One is de novo pathway I hope you already know this from your metabolism course in the biochemistry one is de novo pathway and number two is salvage pathway.

So de novo and salvage this HGPRT is a very important engine of this salvage pathway, so what is happening this myeloma cell as this major one of the key enzyme that HGPRT is not there so they cannot use salvage pathway. Even I mean but they do not care normally because their de

novo pathway is active, so they can make their nucleotide and survive but in this medium where we grow mixture of all this because in after PEG you have this, you have this.

These we do not care much because they will die after fuse cycle of their like cycle, but this cell and this cell both will survive so we have to get rid of that they do not have a HGPRT fine but they have de novo pathway so what we do is we in that medium we add hypoxanthine, we add aminopterin and we add thymidine. So hypoxanthine, thymidine both nucleotide they are going to use for then you nucleic acid biosynthesis or DNA synthesis.

But they cannot use the myeloma cannot use this hypoxanthine, because they do not have hypoxanthine one in phosphoribosyltransferase. So for them for myeloma cell or the myeloma myeloma fuse cell, so some this myeloma cell will also be there so in this mixture there will be five type of cell, what are these five types of cells? One is BB fused some and some unfused cells of B type and some unfused cells of this myeloma cell type.

So 5 type of cells so this one this fuse B cell or unfused B cell will die but both this myeloma and myeloma myeloma fuse and hybridoma all three will survive but these two does not have a GPRT so they cannot use this hypoxanthine or salvage pathway, but we have to stop this de novo so that they cannot synthesize their nucleotide at all. So aminopterin this is the inhibitor of one of the enzyme of de novo pathway, what is that enzyme?

This is tetrahydrofolate I mean the folic acid so to stop their de novo pathway to stop the de novo pathway we add one specific inhibitor called aminopterin. Aminopterin actually innovates, the de novo pathway by inhibiting one very important enzyeme of that pathway. It is dihydrofolate reductase. So what is as a result what is going to happening they cannot use hypoxanthine or the salvage pathway they cannot use the de novo pathway because aminopterin is there as a inhibitor.

So both nucleic acid synthesis pathway de novo and salvage arer blocked. So what is happening with type both these fuse cells as well as single myeloma cells all are going to die and these cells

also are going to die by natural death because they cannot survive multiples more than few cycles. So as a result ultimately we will have only this hybridoma where B-cell and myeloma cell get fused, clear.

So then what is happening? So finally after the death of both the fused myeloma as and single myeloma cells and B-cells will have only hybridoma cells, so these hybridoma cells are suppose I mean then we have to figure out which hybridoma cell is actually I need or we need so how we will select because that hybridoma cell are going to produce antibody against this antigen X. Suppose there are thousands of cells, there are many suppose there are thousand such hybridoma cells are there.

So from there we have to select how many of those are producing antibody of what I need or I want, so I have to dilute in such a way so normally we use 96 well microtiter plate. So we will dilute in such a way each well should have one cell, so thousands cell if we have so what should be the dilution you have to dilute it thousand times of thousands cell per ml for example. So we have to dilute in such a way so there are one cell part well, so we have to dilute thousand times so thousand time dilution and that dilution if we draw in individual cell so I have I mean not as a 96-well plate means I may have to take 12 96-well plate.

So each well I will give this cell in such a way so one microliter each so they all will get one cell. It is possible that some well does not get any cell another will get two cells, then I will grow for say 14 days so what will happen from one they will increase they will grow up to certain level and during that growth in I mean if I draw this in bigger way I mean little bigger format. So what is going to happen suppose in that this well is big so in that there are now there are multiple cells.

So while they are growing the hybridoma cells, all are hybridoma I am talking. So while growing they will also secrete antibody, so with growth they will release the antibody also in the medium. So if we take the medium out send I mean take some sample and take the medium out and so what will happen in that medium I will have only antibody. So if I centrifuge the cell and collect the supernatant I will have the so centrifuge and then collect the supernatant.

In supernatant I will have only antibody so whether that antibody is against my protein of antigen of interest or not what I can do you know that now this is simple ELISA, so if I do ELISA I can if the ELISA is positive then I can say that is the real well from where my cell. So say thousand ELISA I have to do because I distributed in thousand wells and I have to do individually the product from each well.

So now you can imagine what is the volume of total work? So if the number is more if it is 10 to the 6 we have to make so many plates. So now this thousand well I have to do thousand independent ELISA from there I am a gets a 10 out of 1000 are giving ELISA positive. So this is the first round of tests then what I will do, I will take that 10 cells and grow them again in different well freshly I will grow.

Again I will do ELISA then I will see maybe next ELISA first time sometimes false positive may possible so next time if I get all 10 or again positive that I am very lucky. Many times it happened only two were positive or only 3 or 4 are positive. So from there I have to select the single clone, so after first round of screening I will what I will do I will dilute them again, so that by any chance what I was saying in some well in some well there are 2 cells it is possible, in the well there are 3 cells also.

So I do not know whether the positive cells are from product of 3 or product of 2. So what I will do from there a second round I will dilute them again, so suppose there are two cells so what will happen and total again 1000 cells so provide your statistics wise there will be 500 and 500. So if everything is equal so 500 cell is one type and 500 cell is of another type, again if I dilute so every two wells I can have a single clone.

So that way I have to make sure that these antibody what we are getting is actually from one B-cell or one hybridoma cell, the advantage is now once it is ready then it will leave life long and as many cycle you want, whenever you want what you have to do again you grow this cell, and when we are growing them in RPMI that medium. So whenever I need, I will culture them increase the volume.

There are varieties of bioreactors are available normally this case the hybridoma cell we grow hollow fiber bioreactor. We grow the Saline hollow fiber bio reactor, so in that case we can increase the volume we can produce more and we can separate or purify the antibody and that can be used for different purpose and that antibody later on variety of techniques developed we can make human eyes so that anti-dutrite reaction will not happen.

Because once you have the clone then or the cell isolated then you can clone the gene that we will discuss in the next class, you can clone the gene and that clone you can express in yeast bacteria plant. If we express the antibody in plant there is a very nice name is there it is called plantibody. What will happen you grow the plant purify the antibody from total plant protein; you do not have to go cell culture.

You clone the gene in plant make a transgenic plant with this particular antibody and then it is not that easy but possible. So now once you have a monoclonal you can do many things you can maintain the monoclonal purified antibody do your I mean serve your purpose or you can clone the gene and express in heterologous system like yeast, bacteria other system like plant and purify whenever you need, so there are few things like how we can make the antibody in lab by molecular biology technique, we will discuss in the next class.

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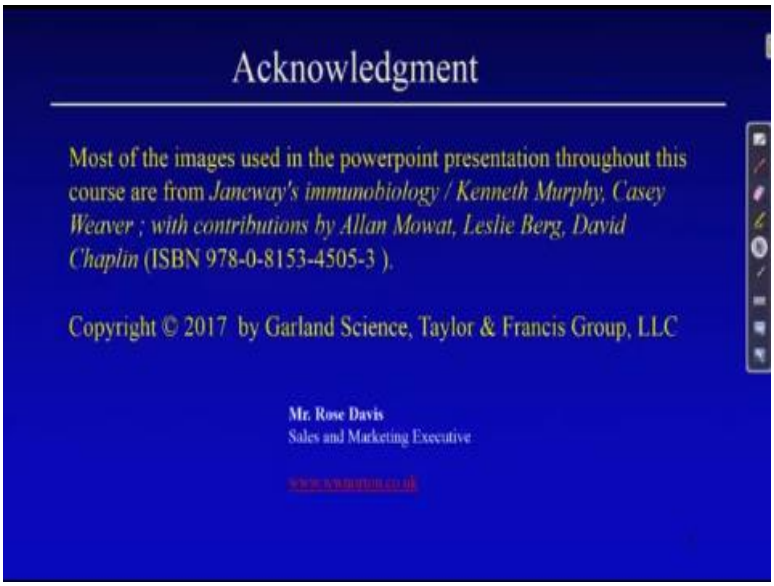

For till then I mean I just what I would like to show one slide what I tell because this course is almost going to be finished, so in my lecture all the slides or most of the slides rather I use from this book the Janeway Immunobiology and it is a copyrighted, but I am also thankful to Mr. Rose Davis sales and marketing executive of WW Norton company who is now the authorized agent of this book and seller publishers though and they are kind enough to give me permission to use this all these materials for this course. I am very much thankful to them, thank you very much.