Host-Pathogen Interaction (Immunology)
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Lecture: 52
Adaptive Immunity-B and T cells Antigenic Epitopes

Hi, so, we will continue this antigen and immunogen property and today I will talk about some more aspects of this antigen and immunogen and then we will discuss about how we can elicitate antibody response in animal or human. So, let us begin this session.

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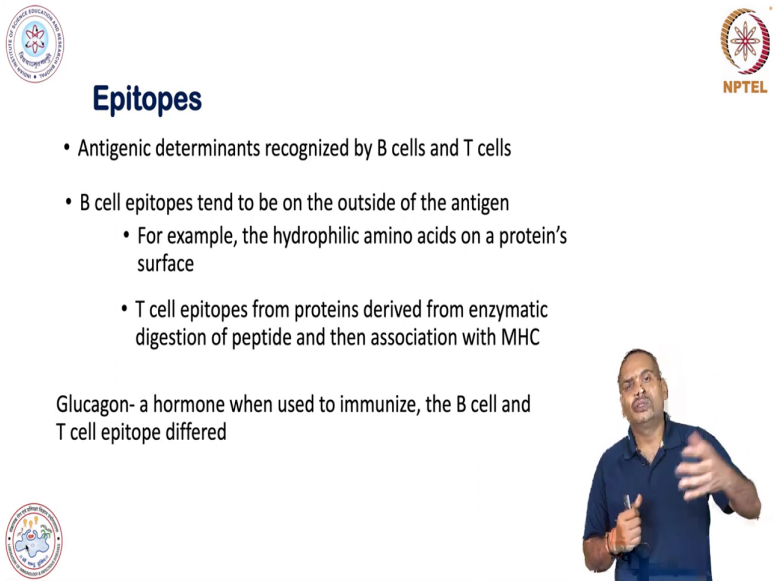

So, so there is a term known as epitopes. So, what is epitope? Epitope is nothing it is it is antigenic determinants recognized by B cells and these antigenic determinants are also present for T cells also. So, all those antigenic determinants or surfaces over the antigen which is recognized by this B cells. B cells means the antibody expressing B cells and the T cells also. The processed antigen is presented along with the MHC molecule and this MHC molecule and this antigen is recognized by the T Cell.

So, these surfaces in case of antigen in case of B cells we call it as the epitope and the surfaces which is present over the antibody the surfaces which is which is interacting with the antigen the surfaces over the antibody which recognizes the surfaces on antigen we call it as a paratope. So, just this is an important term. So, please remember that tend to be on the

outside of antigen. So, you know that when I here I am talking about the antigen I am particularly talking about the proteinaceous antigen.

So, in three dimensional structure you may very well aware about that the surface of the protein is basically consists of hydrophilic amino acid and the inner core of the protein is basically consists of hydrophobic amino acid because there is no water molecule over there but on Surface there are water molecule and generally these are hydrophilic in nature.

So, the T Cell epitope from protein is a basically derived from enzymatic digestion. So, you remember that the antigen is taken up by the macrophages or dendritic cells and then there will be action of enzyme in phagol-lysosome and then that digested protein turned to the peptide and this peptide is associated with MHC Class 2 if it is a th cell in in case of TC-c cell it is MHC class one molecule.

So, these molecules are presented along with respective MHC and then that will activate respected T cells. So, so this is in case of T cell in case of B cell this is a naive protein or native protein and this native protein is a having some surfaces and these surfaces are B cell epitope and the surfaces which is present on the antibody we call it as a paratope. So, there is a one example.

So, glucagon is a is a basically hormone when we immunize the animal with glucagon then B cell and T Cell epitope will be differ it is not the same epitopes. For example if animal is challenged with some antigen the B cell epitope and T Cell epitope will be entirely different.
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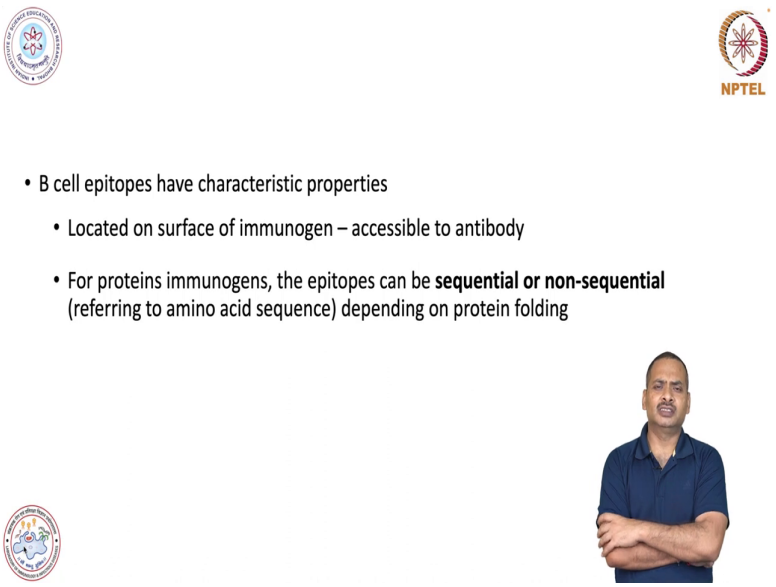

B cell epitope has this characteristic property which I have already explained basically they are located on the surface of antigen or immunogen and basically it is accessible. So, since it is present on Surface. So, it is accessible by the antibodies for proteins immunogens the epitope can be in case of B cell the epitope can be a sequential or non-sequential epitope. I will explain you subsequent slide.

What I mean to say that because you in previous session I have discussed that protein makes various level of structure. Primary structure which is a simple sequence there will be a secondary structure. Secondary structure is they make some structure like a beta pleated sheet or Alpha helix~~analysis~~. And these this secondary structure several secondary structure which is made by a particular or some sequences of the protein this will make a tertiary structure.

And this generally protein is present along with other protein in complex form and this both on both these structures means the structure of one protein and another protein that basically makes a quaternary structure. So, let me explain what is the sequential and non-sequential epitope. I have a very nice scheme with that you can easily understand.

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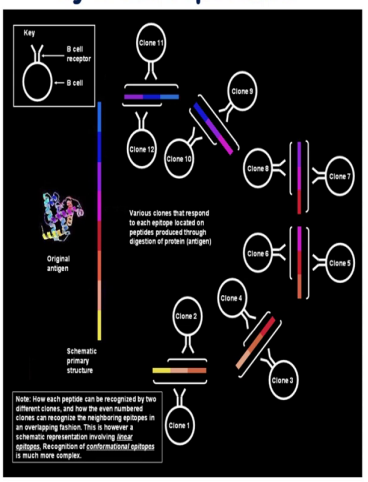

So, sequential epitope recognized by b-cell repertoire. So, here you can see that there is a there is a one polypeptide chain which is represented by very colourful you can see there is a yellow and at both hand one end has a yellow colour and another end has a blue colour. So, you can understand this is a kind of a sequence and so, the ~~C~~ sequential epitope. So, here you can see that the clone. Clone one can recognize this yellow and little orange colour and darker orange colour.

So, this is a sequential epitope. So, if you see that various clone cloned one two three four five six seven eight they are basically recognizing the sequence of this protein.

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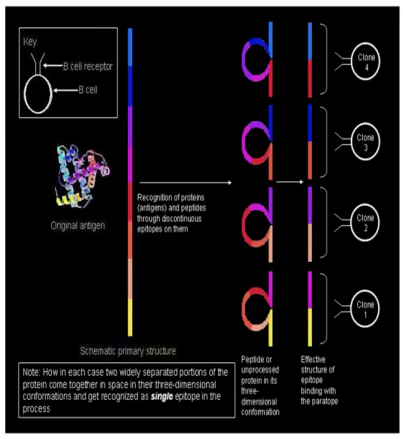

And if you see the another structure here you can see there is a non-sequential epitope. So, when this polypeptide makes some structure here you can see that this polypeptide basically

making some kind of some structure maybe there is a disulfide linkages and that makes a this loop kind of a structure. So, now the epitope is no more sequential epitope it is a non-sequential epitope.

Here you can see that the Clone one is basically recognizing the yellow and purple colour which is quite far it is not in the sequence. And why this is a recognizing yellow and purple because there is a some fold. So, this kind of epitope we call it as a non-sequential epitope. So, in general the protein which is which is inducing the immune response particularly antibody response.

The antibody is basically a mixture of or it is a repertoire of a B cell which is producing antibody and these antibody have a both kind of they recognize both kind of epitopes the sequential as well as non-sequential. So, this is a very important to understand because and there is a one technique here just I want to give you a note. So, in general we think that this antibody recognizes the the three-dimensional structure.

So, in this if this is true then when we perform the Western blotting if you if you have any idea about the Western blotting. So, in Western plotting we separate the protein in denatured condition we call it as a we separate by using a technique known as SDS page over there we denature the protein and we separate it and then we transfer it to the appropriate membrane and then we probe with antibodies. So, in that scenario the protein is completely denatured.

So, that does not have a three dimension means a native structure so, why antibody is reacting. So, most likely or in case of Western so, the the epitope which is seen by this this antibodies mainly the sequential epitope. So, this is just a note that you you should remember that the mixture of antibody which is generated against particular antigen particularly proteinaceous antigen that will this mixture of antibodies consist of both mixed both type which can recognize sequential as well as non-sequential epitope. So, please remember this thing this is very important.

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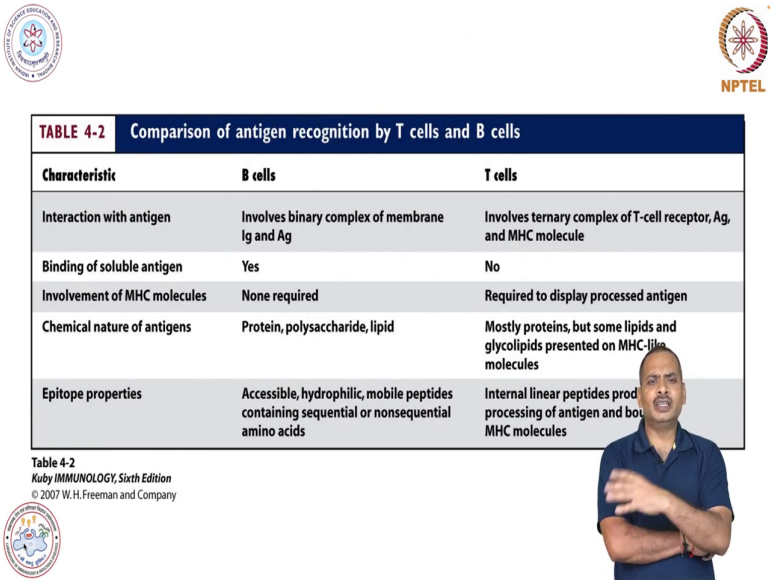

Now here I have a little comparison of antigen recognition by T cells and B cells if you see and this will this will clear your further any doubt if you have. So, interaction with antigen in case of B cell it involves bind it makes a basically binary complex of membrane immunoglobulin membrane-bound immunoglobulin and antigen. So, initially these B cells will have an antibody which is membrane bound.

And this membrane-bound antibody is basically recognizing the native antigen and this is a simple binary interaction there is no involvement of other molecule but on another hand the T cell in case of T Cell it is basically a ternary complex ternary ~~turn the~~ complex of T Cell receptor. So, T Cell receptor is a basically consists of two polypeptide chain you will see in in subsequent session that T cell in general they have a two polypeptide chain which makes the T Cell receptor.

So, T Cell receptor is basically consists of Alpha and beta chain this TCR TCR receptor is consist of Alpha and beta chain which is predominant form of T cells there is some another kind of T cells which we call it as a Gamma Delta T cells those T cells have a gamma chain and Delta chain just forget about that thing. So, generally T Cell receptor is consist of two polypeptide chain that is Alpha and beta and this Alpha Beta chain is basically interacting with MHC molecule and the peptide which is presented along with MHC molecule.

So, this makes a ternary complex. So, here the interaction is quite complicated I think you can understand binding of a soluble antigen yes in case of B cell they can bind with soluble antigen but in case of T Cell it is not at all true T Cell only see the processed antigen along

with MHC class MHC molecule over the over the cells either it will be antigen presenting cells or it will be a MHC class 1 expressing cells any nucleated cells involvement of MHC molecule.

In case of B cell there is a no involvement known direct involvement of MHC molecule for interaction or for recognition but in case of T Cell it is most important it requires the presentation of antigen along with MHC molecule without that the T Cell cannot activate or cannot recognize the antigen chemical nature of antigen. In case of B cell mainly it is a proteinaceous polysaccharides and very rarely lipids.

Lipids are you I have explained you that lipid cannot induce the good immune response but when we want some immune response against lipid molecule we tag it with the carrier molecule. So, once you tag with this carrier molecule then that can induce the antibody response in case of T Cell it is mostly the protein but sometimes lipid and glycolipid is also presented on MHC like a molecule.

So, I think I have told you in previous session if you remember there is a cell known as NMKT cells this NMKT cells basically recognizes the lipid antigen or lipid or glycolipids and this lipid and glycolipid is presented along with MHC like molecule known as cd1 family molecule and once they recognize they are they are also inducing some immune response but in general this is only the protein antigen.

Epitope properties so, in case of B cell this is a accessible by BCR or B cell receptor or BCR basically the antibody which is present over the B cell membrane this is accessible and as I told you it is a mainly hydrophilic surfaces and mobile peptides containing sequential or non-sequential amino acid. Now you can understand the sequential and non-sequential amino acid.

In case of T Cell it is a basically internal linear peptide produced after processing of antigen and it is basically bound with a MHC molecule appropriate MHC molecule it may be a cd1 it may be MHC class one or it may be MHC Class 2. So, this is a comparison of antigen recognized by T cells and B cells.

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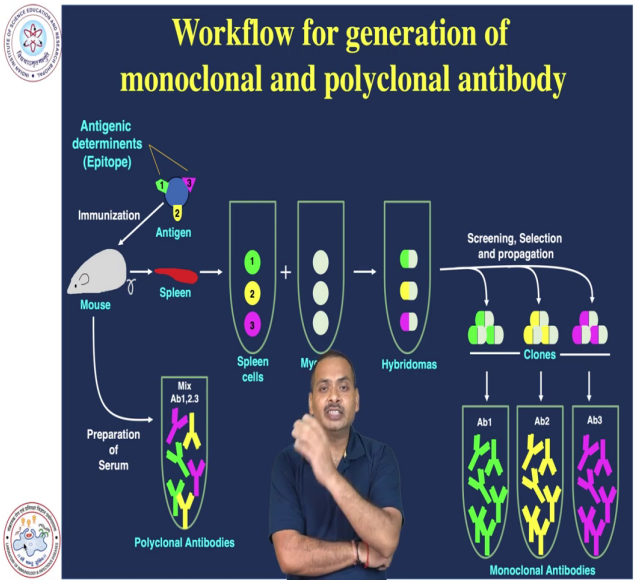

Now let us move on now I just want to so, you have understood about the antigen you have understood about the epitope. And now I want to show you what is the workflow for generation of monoclonal antibody or polyclonal antibody. So, here there is a experimental animal I have made it a mouse you can use it as a rabbit. So, Mouse is I put Mouse because I want to discuss about the monoclonal antibody which is mainly generated in the mouse.

So, here there is a mouse and this is antigen and here you can understand that these one two three they are the surfaces they are the antigenic determinant or epitope over the antigen I made it this because it will help in understanding the concept of monoclonal and polyclonal~~ethonal~~ antibody. So, when this mouse is challenged with this antigen then there will be after some time there will be a production of antibody which will recognize these surfaces.

These antigenic determinant here for your convenience I made a antibody molecules with same colour. So, one the green colour is basically recognizing the epitope one the yellow colour is recognizing basically epitope 2 and the dark purple colour is basically recognizing the epitope three. So, this is a polyclonal antibody. Now I have a challenge I want to make an antibody which is only recognizing the surface one.

So, if you take this polyclo~~n~~neal antibody and if you try to isolate this only one kind of antibody or which is recognizing epitope one it is technically very challenging to isolate that that one kind of antibody. So, in order to get one type of antibody we adopt some another

method here I am just showing the workflow and after this I will talk about how to make the monoclonal antibody.

So, the workflow will be you immunize the mice and when the mice is showing very good titler of antibody you have to sacrifice this mice and after sacrificing you take out the spleen and this spleen is basically containing the B cells which is making a particular kind of antibody here you can see a spleen has a three kind of these B cells the green the yellow and the purple. So, green will make this a green colour antibody yellow will make the yellow colour antibody and so on.

So, after making you need to fuse these cells with myeloma cells. So, this myeloma cells is basically a cancerous B cells and when you fuse with this cell then these cells these cells means the green yellow and dark purple cells. So, try to understand these cells the green yellow and purple cell they cannot multiply for longer time why because this is primary cell after they may survive for few days and after that they will die.

So, basically here what is the aim is to make the B cell line for particular cells. So, in order to achieve this thing we basically fuse this cells with cancerous cells. As here you can see that we will use the myeloma cells and after that this fused cell can survive very long very long or these cells become a Immortal and once you have this Immortal cells which can produce your antibody of your interest.

Then you can expand those cells these particular cells and then you can make a one kind of antibody try to understand from polyclonal antibody isolating one particular kind of antibodies extremely challenging you can understand the molecular weight is same only this is the this is the Affinity of the antibody with that particular epitope it is a practically impossible or I am not saying that it is practically impossible.

But it is extremely difficult in order to isolate one kind of antibody from the mixture of antibody but this is a very simple method here you basically fuse with myeloma cell and then you grow these cells individually then you can easily make this monoclonal antibody one kind of antibody and these antibody we call it as a monoclonal antibody.

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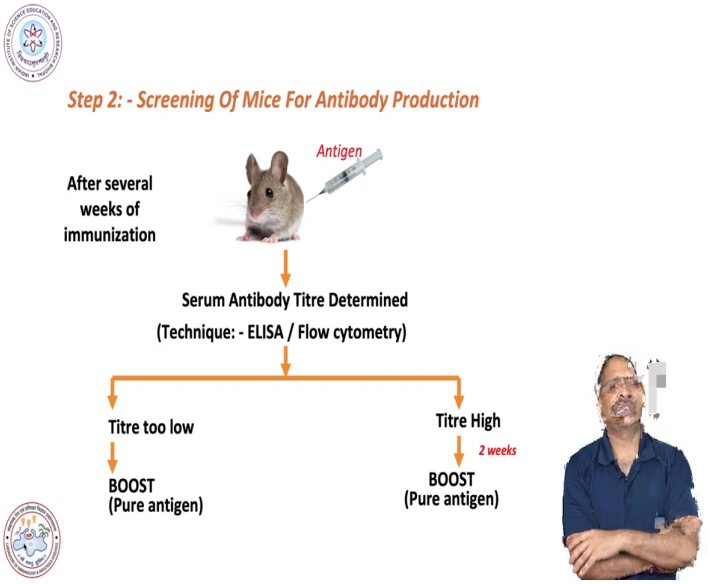

So, now I will talk how to make this monoclonal antibody or in previous slide I just showed the schematic or workflow. So, basically what we do we challenge the mice with your antigen of Interest or protein of Interest along with the adjuvant I have explained you we use the complete~~d during~~ adjuvant or incomplete and adjuvant. So, after challenge you basically check the title of antibody if the mice is showing very good titer of antibody then you basically sacrifice and remove the spleen and then you do further experiment here.

If the title is very low then you basically need to give the booster dose you need to boost again with a Fruend's~~fluent~~ incomplete adjuvant and antigen and if the titer is a good then you can you can sacrifice and take out the spleen.

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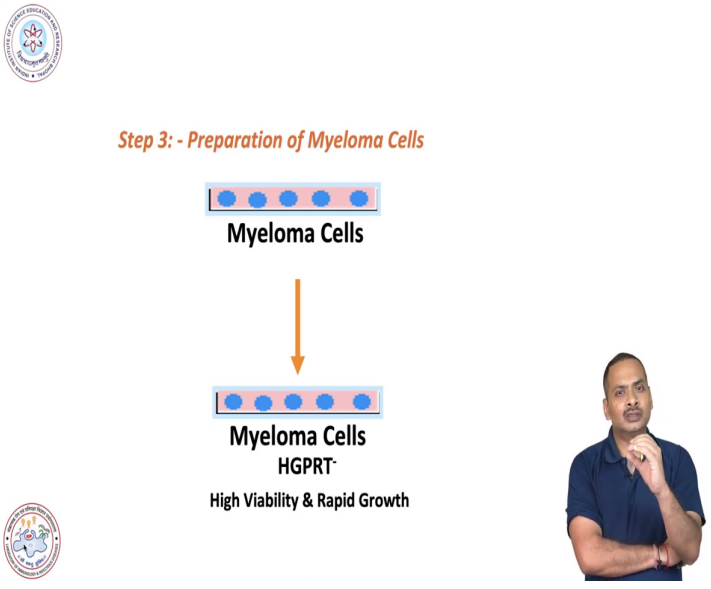

So, after that what we do we fuse with melanoma cell. now here I will explain you the molecular mechanism for screening how you will you will separate out all those cells which is fused with spleen cells the spleen cell which is fused with melanoma cell. So, this melanoma cell this is basically derived from one knockout mice which we call it as a HGPRT. HGPRT is stand for hypoxanthin guanine ~~wenin~~ phosphoribosyl transferase.

So, this mice is basically lacking this enzyme I will explain you what is the what is the use of this enzyme in subsequent slide~~ght~~. So, this melanoma cell is derived from HGPRT knockout ~~noise~~mice and it is cancerous in nature they can grow indefinitely just remember this.

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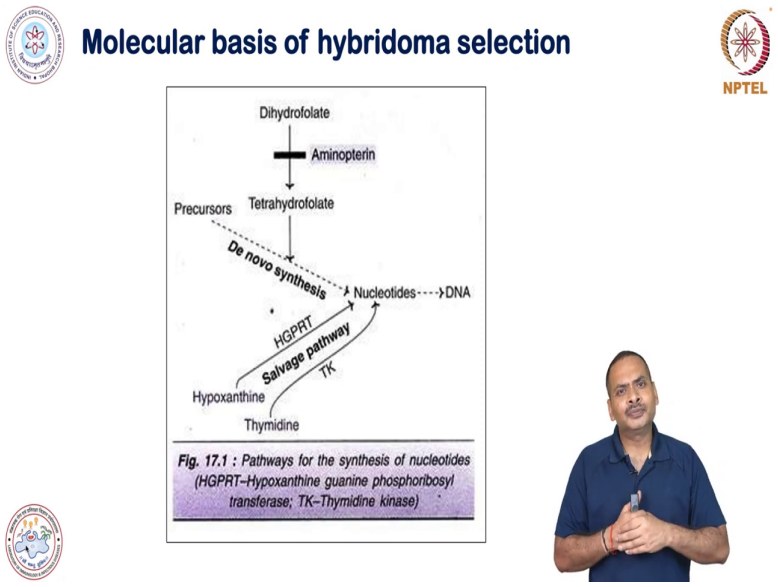

So, now I will tell you how we do the screening. So, the spleen cell the spleen cell which is which is coming from the immunized mice they have a they have a property that they can grow for shorter time but melanoma cell can grow for indefinite time right. So, what we do we is we basically we want all those cells which is fused with these spleen cells just for your information we use various agents such as polyethylene glycol.

In order to fuse these two cells sometime people use a Sendai~~unday~~ virus which basically fuses this these cells and just for your I am just trying to refresh your biochemistry knowledge. So, DNA in cells the DNA synthesis is taking place by two major pathway one pathway we call it as a de ~~T~~ novo pathway and another is Salvage pathway. So, in denova pathway all this basic units of DNA that is the base~~i~~s the ribose sugar.

And inorganic phosphate is synthesized and then this makes a nucleoside and nucleotide and then this nucleoside and nucleotide polymerize and that makes a DNA molecule this is a ~~T~~ denova pathway. In case of Salvage pathway you know that these cells are keep on dying. In Salvage pathway this broken DNA or broken bas~~i~~es or nucleoside and nucleotide are used in order to make a new DNA molecule.

You remember that there is a semi-conservative method for DNA replication right anyway don't go in that detail. So, so for this de~~T~~ nov~~o~~a pathway there is a there is a one very important enzyme is there which is needed in order to derive this de~~T~~ nova~~o~~ pathway that enzyme name is dihydrofolate reductase. So, this dihydrofolate reductase is very important in terms of controlling this de~~T~~ nova~~o~~ pathway.

If you poison this pathway then cell will die but it will not die immediately this will switch to the Salvage pathway if Salvage pathway fulfill their need for DNA replication then it will not die else it will die. So, there is a one molecule known as am~~i~~nopt~~e~~ve~~r~~in~~e~~. Aminopterin is a basically a poison which inhibits this enzyme dihydrofolate reductase. So, this dihydrofolate reductase if it is poison then this will switch to the Salvage pathway.

And for Salvage pathway you need this this these bases like hypoxanth~~a~~ine, thymidine in order to run this pathway. So, what we do we take this after Fusion we put these cells on a special media which we call it as a hat media which contains hypoxanthine, ~~hypogenthin~~ am~~i~~enopterin and thymidine~~thymedin~~. So, after putting this media this hat media what will happen then this the cells which is which is basically so, this Amino terrain will kill the ~~T~~ denova pathway.

And please remember the cells which is derived from spleen this will have a both dihydrofolate reductase enzyme as well as this will have a high percentage going in phosphoribosyl transferase which is absent in melanoma cell please remember that thing. So, what will happen. So, this when you when you fuse and put it on hat media then what will happen this aminopterin~~MN of terrain~~ will kill this de~~T~~ nova pathway. Since this the hypoxanthine ~~hypothatin guonese~~ guanine~~is going in~~ phosphoribosyl transferase is also present in this cell.

So, only those cells will survive which is fused with a spleen cell the fused cell means a spleen cell and melanoma cell which are fused they will survive. So, there will be a three or four situation. So, one is that spleen cell will fuse with a spleen cell this is first situation when you will mix this a spleen cell with myeloma cell then there will be several situation the first situation will be the spleen cell will fuse with a spleen cell.

So, those cells will die why because of this because these cells will be inhibited in this sense that the DNA synthesis pathway is inhibited by aminopterin this is first and second reason is that these are primary cells they cannot survive longer this is a property of primary cells. Another possibility is that melanoma cell will fuse with melanoma cells. So, those cells will also not survive because they will lack this HGPRT enzyme.

The third situation will be the spleen cell will fuse with melanoma cell and this spleen cell fuse with melanoma cell they will survive. Why because they have they will regain the property of immortality because they are fusing with the cancer cell number one. But they are lacking the since the melanoma cell is lacking HGPRT enzyme which is provided by spleen cell. The spleen cell then so, this will have a hgprt the fused fused cell will have HGPRT and in media you have a hypoxanthin and thymidine.

So, this will trigger or activate this Salvage pathway and then they can survive. So, in that way we screen those cells. So, here you can you can see this is a very nice schematic for molecular basis for hybridoma selection whatever I have explained you. So, in that way we can isolate the hybridoma the fused cell spleen and melanoma cell we also call it as a hybridoma.

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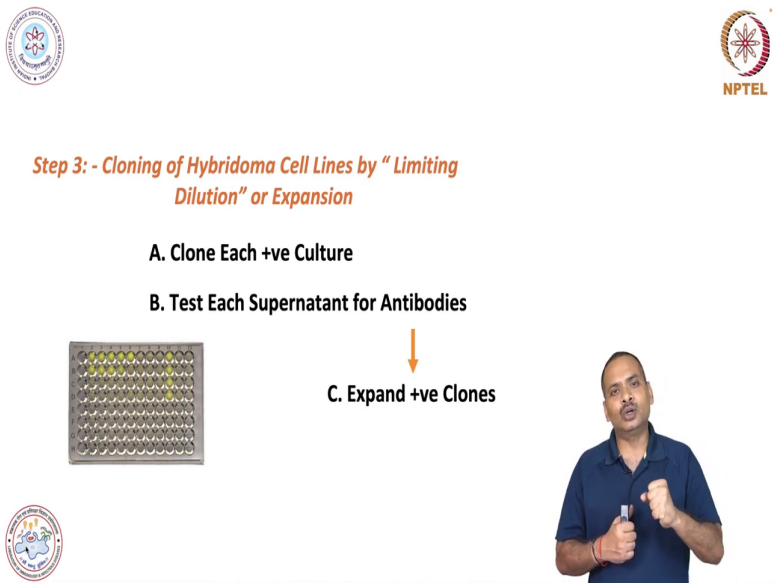

And then what we can do that we can we can also make us limited dilution basically we will what we will do we will make dilution in such a way that each microwell ~~world~~ tighter plate will contain only one fuse cell. So, here you can see that one fuse~~l~~ cells will be there and this will be basically will amplify with time and then we will test for the antibody whether this is making our antibody of interest or not.

Once you will find out this clone is making ~~our~~ the antibody of Interest then you take that colony and expand it in bigger number and then that will make a huge amount of antibody. So, there are several ways by which you can make this antibody expand these cells one is that you take these cells and inject it into the peritoneum of mice along with some some some agent. So, then what will happen this cancerous cell will grow like anything in the peritoneum and that will that will make the belly~~value~~ of this mice extremely large.

And you just aspirate that fluid and this fluid will be rich in the at monoclonal antibody this is one way but this is not very good way reason is that this will contain lot of other proteins. So, you if you need a very purified antibody then this method is not good the correct method will be you need to expand this cells in bigger flask and all those things and then you can isolate the antibody. So, that is better way.

So, this depends on your need you can make your antibody. So, now I have discussed about the polyclonal antibody and monoclonal antibody.

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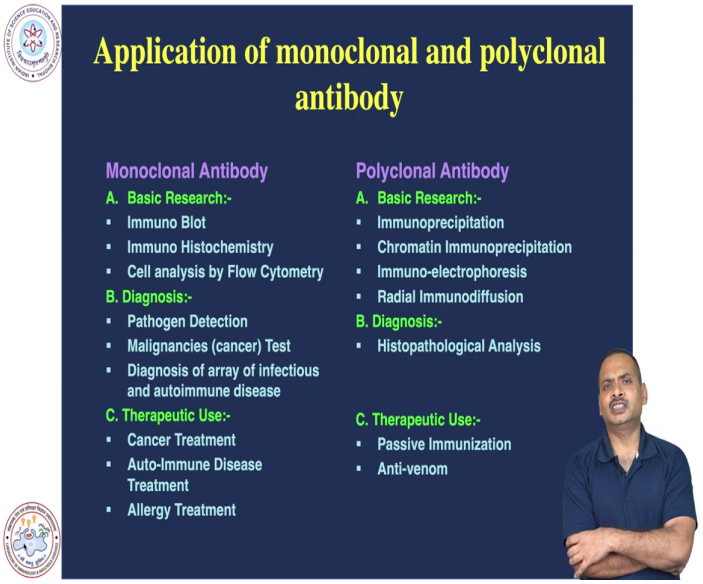

Now I would like to tell the application of these two very important things. So, here monoclonal antibodies basically used in basic research diagnosis, therapeutic and in basic research we basically use for this immunoblot, ELISA~~iza~~, immunohistochemistry, flow cytometry you do the cell analysis using this monoclonal antibody. It is widely used in a diagnosis like pathogen detection malignancy diagnosis of array of infectious and autoimmune factors.

And in therapeutic many monoclonal antibodies are used in therapeutic we call it as a Mabs or biosimilars. So, they are they are quite emerging therapeutic agent they are also known as biosimilars this can be used for autoimmune disease treatment allergy treatment and so on. So, on another hand polyclonal antibody is also used in basic research for example doing immunoprecipitation experiment, chromatin immunoprecipitation, immunoelectrophoresis radial immuno ~~defusion~~diffusion.

And so, it is also used in diagnosis histopathology and it is also used in Therapeutics. So, one of the very good therapeutic application is generating the anti-snake bite serum. So, this is quite common but in general for better results people try to use the monoclonal antibody but there is some challenges with monoclonal antibody which is derived from Mouse because this is this mouse antibody is foreign to the human and that will cause the array of problem.

So, now there is a humanized antibody and this has reduced the problem to larger extent but still there is a problem. But I know that there are some companies they made a mouse which can make a human antibody they clone the whole chunk of DNA in the in the in the mouse

they replaced this antibody encoding Gene segments. So, with this I will stop here and in next session I I will start more about the antibodies, thank you.