Host-Pathogen Interaction (Immunology)
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Lecture: 51
Adaptive Immunity-Antigen Immunogen Properties

Hi, so, in previous session we have initiated the discussion about the antigens and we have discussed the difference between antigen and immunogen and we have discussed also about the heptane. So, heptane itself cannot induce the immune response if you tag this heptane with some carrier molecule then this can induce the immune response particularly in terms of antibody production. And this antibody is against the antibody produced is against the heptane and the carrier and the junction between haptaien and carrier.

So, in this session let us move on to the properties of antigen or immunogen more appropriately properties of immunogens.

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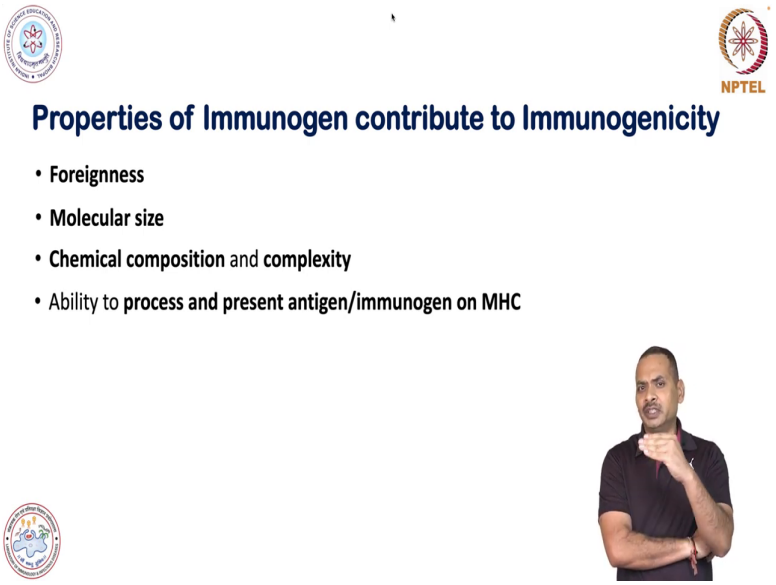

So, there are following properties of immunogen that contribute to the immunogenicity the first is a foreignness I will explain each property in more detail. So, first is foreignnessforeigners second is molecular size third is chemical composition and complexity and the fourth is ability to process and present antigen oblique immunogen on MHC. So, you are aware that the once the microbial pathogen is phagocytose.

Then this is a undergoing degradation and then after degradation some of these peptide from this antigen they are presented along with MHC Class 2 molecule. This is for this is a conventional way. Another way is that especially in case of viruses what is happening the virus in-faect the cells and you know that virus hijack the host cell machinery. So, after hijacking they start making their own protein.

So, these own protein are basically processed and then it is presented along with the MHC class 1 molecule to the CD 8 T cells or cytotoxic T cells. So, this is what I am trying to say in in last Point ability to process and present antigen oblique immunogen on MHC.

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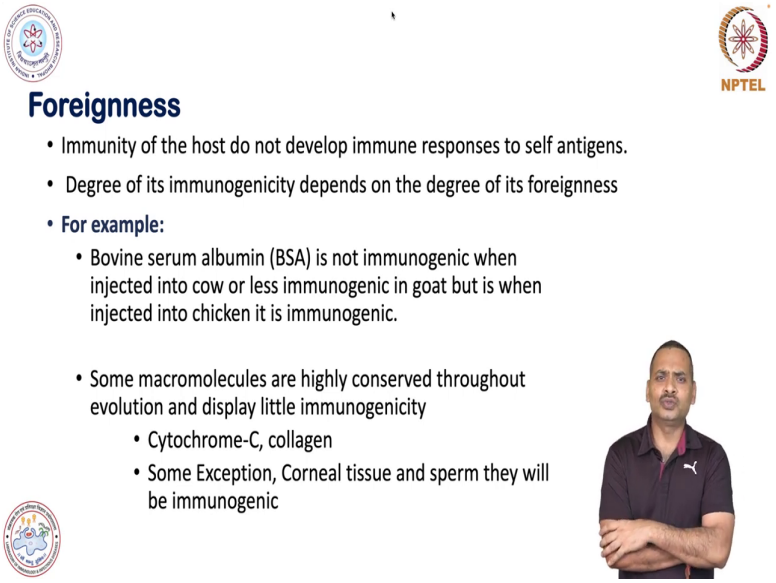

So, let us discuss about the foreignness. So, what is foreignness? So, so if you have an antigen from same host same host means for example from any animal you can use the mice or rabbit whatever for doing this simple work. You isolate some protein for example albumin protein and then you inject it the same protein in the same host. So, that will be not at all immunogenic it will not induce the antibody response.

So, in order to have a good and antibody response the protein or the molecule should be from the distinct origin. So, in a very simple word the degree of immunogenicity depends on degree of foreignness for example I will again explain you. So, if you have a bow-vwine serum albumin the wine serum albumin is originated from the bovinewine or cow. If you take this protein and then inject it in the cow then it will be not at all immunogenic.

But if you take this protein and inject it in the goat then it will be a immunogenic but if you take this BSA protein and inject it in the chicken then that will be also immunogenic. However in case of chicken it will be highly immunogenic the BSA which you inject it into the chicken is highly immunogenic compared to the BSA which is you injected in the goat. Why because there is a very less degree of difference but in case of Cow and Chicken there is a great difference.

So, that what I want to explain. So, degree of its immunogenicity depends on the degree of foreignness. So, cow and chicken is more foreign compared to the cow and goat. So, there are some examples the first example I have already explained. There are some exceptions also in this theory. The exception is there are some protein in various hosts they are highly conserved for example the cytokine cytochrome C the collagen.

So, if you take the collagen or cytochrome C from cow and inject it into the chicken then it will be almost not immunogenic because they are highly conserved. Similarly, it is true for the collagen protein. On another hand in ~~our~~ in contrast I will say because there is a very big difference if you take the corneal antigen from the host. For example you take the mice you prepare the cornea and prepare the antigen or isolate the protein from the cornea.

And you if you inject it into the same animal for example in the mice you have prepared from mice and you will inject it, in a mice then that will be highly immunogenic. Another is the sperm. So, if you take the sperm from animal and then again you will inject it into the same animal then it will be a highly immunogenic these are few exceptions. Now I will try to explain why it is like that.

So, during development of immunity at the beginning of a development of immunity at that time these antigen were not exposed and that is why these antigen is not they do not develop any tolerance against these antigen the corneal antigen and the sperm antigen or sperm proteins. So, that is why if you if you prepare this protein and inject it into the host then that will be an immunogenic.

This is one example. Another is if you take out the cell lyse~~ice~~ it. Lyse~~ice~~ at make some DNA or a stone or something and then if you will inject then that will be also immunogenic. So, try

to understand during education of immunity these molecules are not exposed that is why they are not tolerant. So, this is a about the foreignness.

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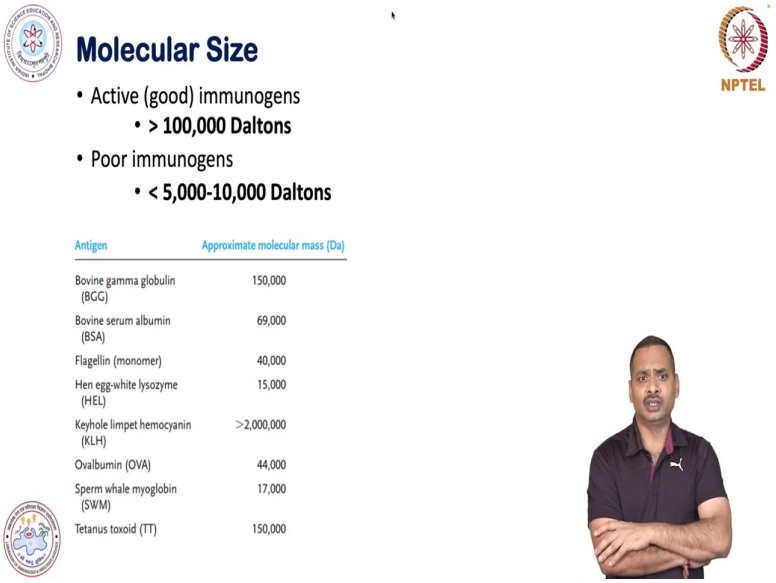

Now let us move to another part that is a molecular size. So, the if the molecular weight of this immunogen will be more than 100 Dalton this is a generalized concept if it is more than 100 Dalton 100 000 Dalton or 100 kilo Dalton then it will be a better immunogenic. Compared to the the molecule which is having only 5 kilo Dalton to 10 kilo Dalton molecular weight. So, this is also very important.

If you remember some of the lipid molecules like derivative of cholesterol why not we are able to make the antibody against those molecules there are several reason. One of the reason is the molecular size I will discuss another reason in subsequent slide. Here I am showing some common antigen which we are using in our experiments which is quite commonly used in our experiment when we when we perform adaptive immunity experiments.

One is bovine gamma globulin and its molecular weight is about 150 kilo Dalton. Another is BSA BSA is quite commonly used because it is a quite cheap it is not only used in immunization it is also used in various other experiment. So, this BSA has a 69 kilo Dalton molecular weight. Flagelylin it is not so, commonly used but people do use flagellin its molecular weight is a 40 kilo Dalton hen egg white lysozyme this is also quite commonly used.

This is a about 15 kilo Dalton another molecule which is quite commonly used is Keyhole Limpet hemocyanin this is a hemocyanin protein and its molecular weight is more than 200 kilo Dalton as you can see. Sorry it is a it is a 2000 kilo Dalton. OVA albumin this is also quite commonly used in the experiment its molecular weight is 44 kilo Dalton. A sperm whale myoglobulin this is 17 kilo Dalton and this is also used in our experiment and finally the tetanus toxoid which is about 150 kilo Daltons.

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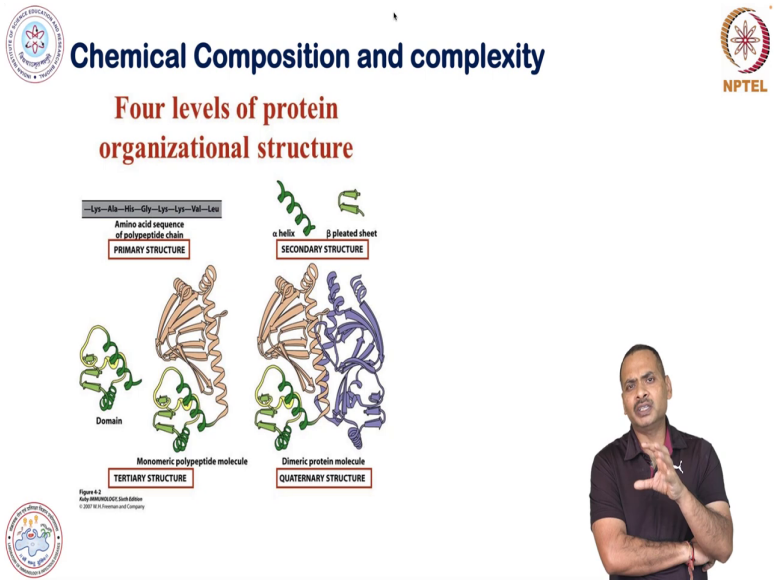

So, these are some molecule which is commonly used in our experiment. Another property is chemical composition and complexity. What what I mean to say that the chemical composition and complexity here you can see that the molecule which is very simple in a structure they do not have a a structural complexity. As I told you in previous slide that there are the lipid molecules they do not induce good immune response.

One reason is molecular weight and another reason is this they do not have any complexity they are very simple structure. So, you know that a protein molecule has a three major structure that is primary structure secondary structure as you can see in this slide and tertiary structure and quaternary structure. So, if the protein molecule or the immunogen or immuno antigen will have this complex structure then this will be a very immunogenic.

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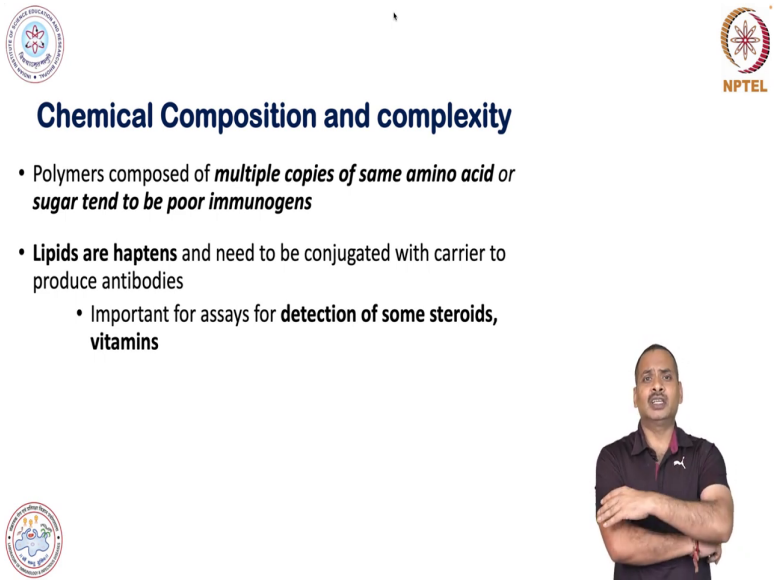

Here you can I can justify by a simple statement if you make a polymer of same amino acid then it will be very poor immunogen. So, copolymer of same unit same basic unit will be not at all immunogenic or almost no immunogenic either it is pro amino acid or it is sugar molecule. Lipids are as I told you they do not have a complexity they do not have a molecular weight that is why they are heptanes.

They need to conjugate with some complex molecule for example BSA people use PSA people use a KLH as you have seen in the previous slide. And after chemically joining these lipid molecule with this carrier molecule they use it as a immunogen. So, I have explained you the importance of this in previous session. So, we make them antibody against these very important molecule and these are mainly used in the Diagnostics.

Like a diagnosis for vitamins some steroid like molecule as you may know that vitamin D3 it is a cholecalciferol ~~cold calciferol~~ which is a derivative of steroid. So, this is basically the antibody against these molecules are made in this way.

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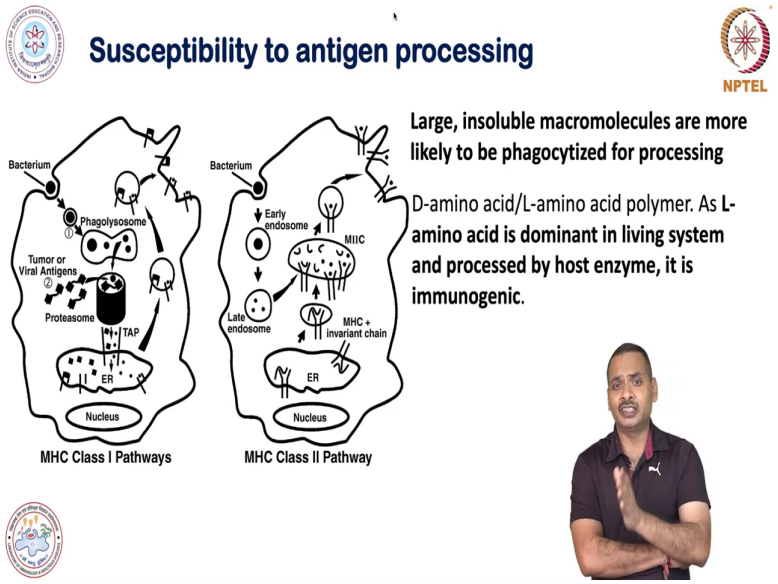

Susceptibility to the antigen processing this is also very very important. I have explained you how the antigen is processed in a brief, bacteria is phagocytos then it goes into the endosome endolysosome or phagolysosome~~fabulizosome~~. And over there this bacteria is break down into small pieces and some of these peptides are presented the protein molecule I am talking particularly about the protein molecules.

So, these protein molecules will turn to the peptides and these peptides are loaded over the MHC Class 2 molecule and then this will be transported to the membrane and over there it is presented along with MHC Class 2 molecule to the T cells. So, now you can understand this thing. So, in order to have a better immune response or antibody response this is very much needed if you remember once this antigen is presented along with MHC class 1.

Then there will sorry MHC Class 2 molecule to the Th~~H~~ cells then these Th cells May differentiate to the TH2 and TH2 immune response is important for the antibody production. So, here you can see that the TH2 cell help is needed to make the antibodies. So, if this process is hampered then this will be not very good for development of appropriate adaptive immune response or antibody production.

So, these two processes the processing of antigen and presentation is very important in order to get appropriate adaptive immune response particularly antibody response. So, here there is a few points which I would like to say that you can prove it whether this is important or not. So, as you know that human host is having the all protein is composed of L amino acids it is a polymer of L amino acid our all protein has L amino acid.

So, what you can do; you can perform a simple experiment you take a mice or rabbit and challenge this animal with the polymer of the hetrohydropolymer of D amino acid. So, when you challenge this animal with D amino acid protein and the protein which is composed of D amino acid then what will happen it will be taken up by the by the cells but it will be not processed by the host enzyme.

Because this host enzyme is consist of L amino acid and they cannot process the D amino acid protein the protein which is consists of D amino acid. So, these proteins will be not immunogenic. On another hand if you challenge the another group of animal same composition same sequence with L amino acid then you will find out that this will induce very nice immune response in terms of antibody production.

So, here I think I have explained you in great detail all these things the processing and presentation of antigen is very much essential.

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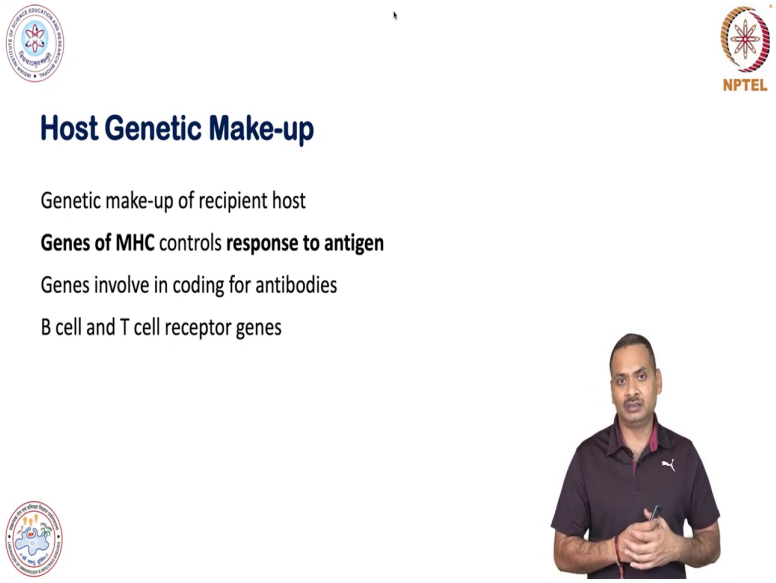

Now role of biological system in immunogenicity there are several set roles of the system one is that host genetic makeup, genetics of the host. I will explain you in more detail. Another is root and amount of challenging antigen or immunogen. By which route you are introducing this antigen and how much amount if it is extremely low then it will be not good huge use of some agent we call it as adjuvant to enhance the immunogenicity.

Now let us take up each one the host genetic makeup means genetic makeup of the host. So, you know that there are wide range of polymorphism or changes in the gene and when I say Gene then there could be a promoter there could be a UTR and so on so, forth. There is a huge things right I cannot tell you in few words. So, all these things matters a lot I am giving you one very simple example for your understanding.

For example, there is a MHC molecule and this MHC molecule is under some promoter if there is a some changes in the promoter which will Express less this ~~I might see~~MHC molecule then there will be a not. So, good production of immune production of antibody because the MHC class 1 expression is less the processed antigen is not presenting very well to the T cells. So, this is also coming in the genetics like that there are so, many genes right in B cells in antigen presenting cells and T cells and so on.

So, if anyone if some, some problem in the gene in any place which is which is essential for the expression and all those things then that will that will hamper the antibody production or that may enhance the antibody production. That what I want to say that genetic makeup is very much important. I have explained you genes of MHC that control the response to the antigen.

Another is a ~~yeah~~ another example this is another good example if there is a mutation in gene segment which is encoding antibody then that will also hamper the antibody production. Another example could be a B cell and T Cell receptor if there is a there is a some mutation or changes or polymorphism in B cell receptor or T Cell receptor then that will affect the antibody production.

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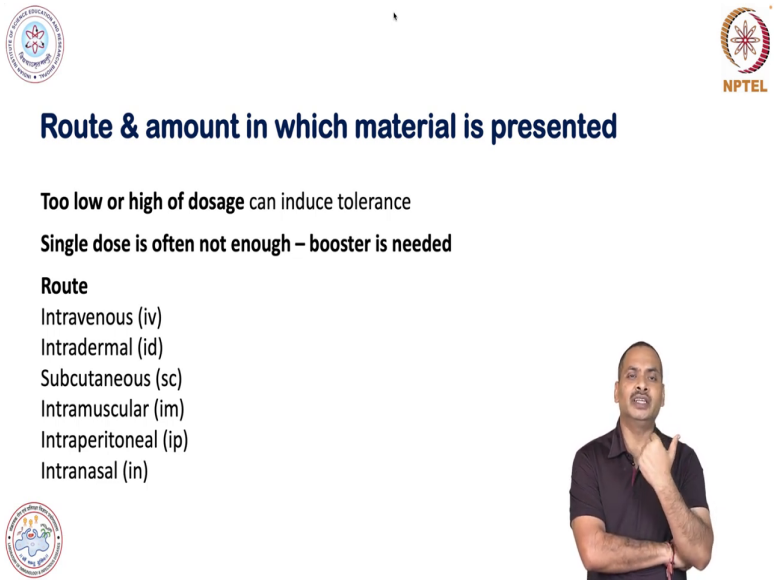

Another is root and amount in which material is presented. So, as I told you the too low amount of antigen if it is extremely low amount of antigen then that will basically induce the tolerance too low and too high. So, it is very important to find out the correct dosage of particular antigen~~integer~~ or immunogen. So, this is by experience that single dose often not enough you need a booster dose I will tell you in subsequent slide.

We give the booster dose probably now you are able to understand this concept very well because you have taken the vaccine for this Covid and then you have taken the booster dose and now there is a precautionary dose. This all these things are because you want to maintain this immune response or antibody response against that antigen if you will not take this booster dose of precautionary dose then immune response against SARS Cov2~~id~~ will be decline.

So, this is very important to give the booster dose this is this depends on antigen to antigen. For example, if you know that infection of chickenpox or similar infection smallpox and chickenpox. Once you are infected then you will develop immune protective response throughout your life you do not infect second time right. So, this depends on the antigen but in some cases you your immune response against that pathogen declines and in order to maintain those immune response you need to take the booster dose.

So, same thing is true for any antigen and immunogen. There are various ro~~o~~ute of immunization and some ro~~o~~ute is suitable for some antigen and one is intravenous generally this is not. So, common intravenous injection of antigen is a very rare if you if you give some

antigen intravenously then animal may die. When I say animal I am talking about the mice. If you give the intervenous injection of say OVA then probably that animal will die by shock.

Another is intradermal this is very common intradermal you just place the antigen just beneath the skin then the animal will develop appropriate immune response and there is a possibility that you will see a very good immune response if you challenge the animal through intradermal route or subcutaneous route also. The subcutaneous route is also very common. Intramuscular you just place the antigen in muscles in case of SARS covid 2 they gave you intramuscular vaccination particularly I am talking about the Covid Shield.

Intraperitoneal this is generally we use it in the animal not in human but in some cases probably people might be using some antigen or some vaccination may be interperitoneal but I am not aware about that in human it is common or it is an uncommon practice. Another is intranasal. So, intranasal is a. Now it is coming up quite common. So, you just introduce the antigen in nose and then you can you can have a immunity against those particular antigen.

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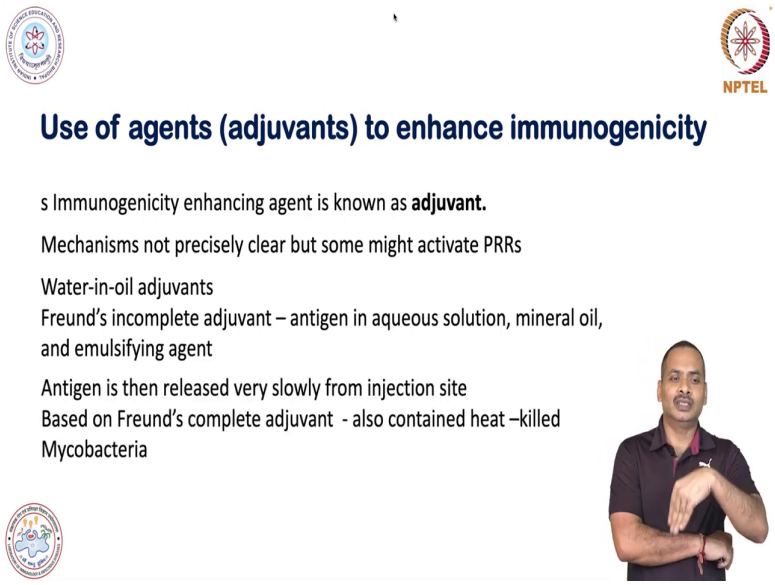

Another is a use of some agent or adjuvant to enhance the immunogenicity. So, this is also very important here you can see that immunogenicity enhances. And basically, this and adjuvant is an immunogenicity enhancing substance and there are various mechanisms. So, when you will see the composition of this adjuvant then you will understand that PRR mediated signalling or activation of PRR mediated signalling is very important against any pathogen.

And that will give the appropriate bedt for the development of adaptive immune response. So, adjuvant is basically the perhapsPAMPs it is a complex mixture of PAMP I will tell you the mechanism in a short while. So, all the mechanism is not very well understood but it is widely accepted that this adjuvant contains some antigen from or some PAMPSs from the microbial pathogen.

So, this is non-specifically activating the innate immune response this is one aim another aim is this makes the antigen phagocytosable. So, for example if you have a BSA solution if you inject it will be disappear in the host either it will cause the shock if the amount is more or if it is less it will be immediately cleaved off. So, what we do we make a Emulsion and you can make Emulsion is basically in insoluble kind of condition or a state of antigen.

For example you can here you can see the that there is a water in oil adjunct. This water in oil adjunct we use it in case of animal and particularly in mice. We call it as a Freund's fluent incomplete adjuvant it is basically the antigen is present in aqueous form and you mix it with the mineral oil. Mineral oil is like a oil kind of thing it is a high molecular weight hydrocarbon. So, when you mix it very well repeatedly or rapidly then that will make a Emulsion, a globule kind of thing.

And when you place this thing in the animal then this will remain at the site of a challenge and over there the macrophages and phagocytic cells will migrate and eat up those. So, they will eat up and then they will they will process and present the antigen to the T cells and then you will have a very nice immune response. So, this is about the Freund'sfluence incomplete adjuvanet. There is a another thing which we call it as a Freund'sprovence complete attitudeadjuvant. So, in addition to this mineral oil there is a one more substance is present which is we which is a which provides a pumps-PAMPs a wide range of PAMPs.

And the substances heat killed dried mycobacterium tuberculosis or it is a heat killed mycobacteria. So, when you mix this thing in this complete incomplete Freund'sfluency adjuvanet then we call it as a complete Freund'sfluence adjuvanet. So, here you can see that. So, this oil and aqueous thing make an emulsion and this will be easily phagocytosed by the phagocytic cells or macro charges in addition this will also have a PAMPS.

PAMPs from the mycobacteria like lipo arabinomanaan mycolic acid. So, and. So, forth it is a very complex and that will non-specifically activate the the PRR mediated signalling. So, this is a very good situation and then this will be basically induce a very good antibody response.

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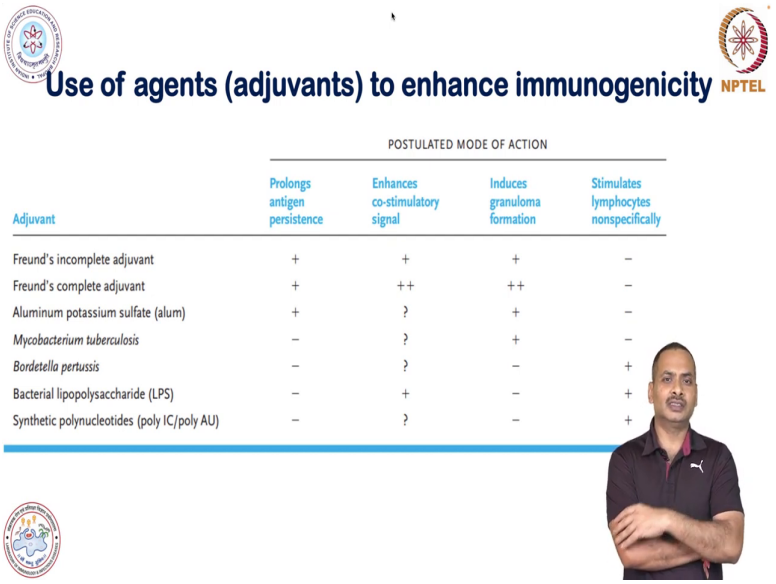

Adjuvant	POSTULATED MODE OF ACTION: Prolongs antigen persistence	Enhances co-stimulatory signal	Induces granuloma formation	Stimulates lymphocytes nonspecifically
Freund's incomplete adjuvant	+	+	+	–
Freund's complete adjuvant	+	++	++	–
Aluminum potassium sulfate (alum)	+	?	+	–
Mycobacterium tuberculosis	–	?	+	–
Bordetella pertussis	–	?	–	+
Bacterial lipopolysaccharide (LPS)	–	+	–	+
Synthetic polynucleotides (poly IC/poly AU)	–	?	–	+

So, now I will talk about some of the adjuvant which is a which is a in used in animals and I have told you there is only one adjuvant which is used in the in case of human I will I will come to that point. So, the here I have already discussed about the Freund'sfluence incomplete adjuvant. So, basically the mode of action the probable mode of action for Freund's fluency incomplete adjuvant is prolonged antigen persistence.

And this we achieve by making Emulsion and slowly there will be slow release or slow phagocytosed by the phagocytic cells. It enhances co-stimulatory signal core stimulatory signal is. So, of course I have told you the MHC along with antigen is seeing is seen by the T Cell receptor but this is not sufficient there will be some more signal is needed to the T cells from the antigen presenting cells or yeah antigen presenting cells like macrophages and dendritic cells in order to activate the T cells.

So, those other signals we call it as a co-stimulatory signals it induces granuloma formation. So, granuloma is just encasing the antigen at particular site that we call it as a granuloma. It is stimulate lymph lymphocyte non-specifically that you can understand that PRR expressing on various cells including lymphocyte they are basically stimulated by these PAMPs and then this will make the lymphocytes active.

So, Freund's~~fluents~~ complete adjuvant is doing this three things one is prolonged antigen persistence enhanced co~~re~~-stimulatory signal and induced granuloma formation. So, another adjuvant is Alum this is aluminum potassium sulfate this is used in the human. So, of course this is a prolonged the antigen persistence if you if you have a good knowledge of chemistry you can understand.

So, if you put some salt in protein solution then that will make a precipitate right and this precipitate is concentrated. And when you inject it in the in the animal then this will make a small specules. So, this is the principle of most likely the principle of this aluminum potassium sulphate. This also induces the granuloma formation at the site of challenge there will be a granuloma.

People use mycobacterium tuberculosis but it is not used in I do not know where it is used we use the mycobacterium tuberculosis in case of f~~f~~Freund's ~~fucnt~~ complete adjunt. Body tailor pertussis this is also hypothesized to used as a adjuvant. Bacterial LPS so, this is also hypothesized to use it as a as a adjuvant. However this is associated with lot of toxicity. So, in these cases the lot of toxicity is associated that is why it is not used but recently there is a one component of LPS is there that is ~~mpla~~MPLA monophosphoryl lipid a this this component is a proposed to used as a adjuvant and this does not have a

toxicity and there is a synthetic polynucleotide as I have told you poly IC which is a when we introduce in in the cell then it will mimic like a virus infection but there is no virus. So, poly IC or poly AU~~u~~ can be also used as a vaccine adjunct. So, with this I am stopping here and in next session I will discuss a subsequent part of this antigen and we will also discuss various kind of there is a one very technical term which we call it as a epitope.

Epitope is nothing it is an antigenic determinant. So, I will talk about the epitope and all those things in next session thank you.