Essentials In Immunology

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Module No.# 20

Lecture No.# 37

Vaccines

Hello, welcome to this lecture on Vaccines. Before going on to this lecture, let us recapitulate some of the basic facts about the immune response.

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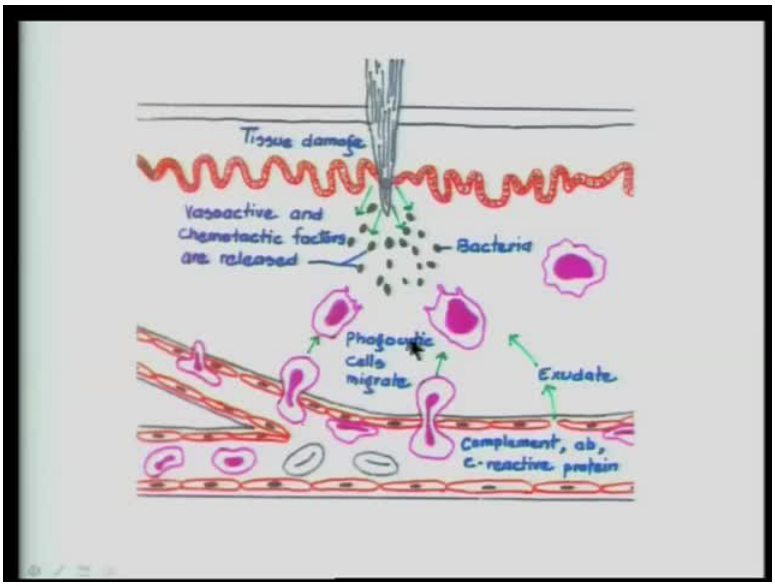

So, if one looks at immune responses to pathogens, we went through the earlier lectures that the bacteria that replicate within the own site. For example, in this slide, it is tissue damage due to the breaking of a throne; the bacteria that multiply within that site actually secrete certain material which is chemotactic for the cells of the innate immune system. Basically, macrophages and glycoside that migrate to the site of bacterial replication with the ultimate goal of phagocytizing this bacteria.

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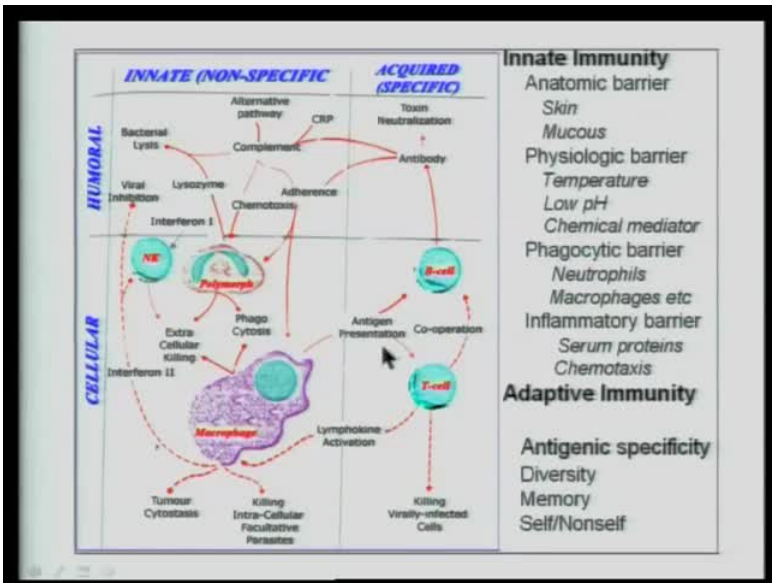

So, if you were to look at the immune system say, you have two arms of the immune system called as the innate and acquired; the innate immunity participating in certain reactions such as Lysozyme secretion, Chemotaxis and importantly the activation of complement due to the binding of the immunoglobulin molecules with their specific antigens via their portion of the immunoglobulin molecule. The activation of the complement itself causes, further chemotactic due to the release of complement fragments and allow these macrophages to come to the site of bacterial replication or immune complex mediated complement activation.

Now, the other components are n k cells, the secretion of various kinds of lymphokines which by themselves are nonspecific. So far, as the immune response is concern, but they are specific to their receptors such as interferon, IL2, so on and so forth. In addition to this, this is supported by the acquired immune arm of the immune system, that is initiated as a result of several innate immune reactions including the response or a binding of various ligand's to the toll like receptors, which are an important part of the innate immunity. It acquired immunity system has the participation of B cells for the secretion of specific antibodies, that recognize specific antigen, if it is a toxin, it is antitoxin or if it is antibacterial, there are different polyclonal antibody that are secreted in the serum that will bind to the bacterial surface antigens.

In addition to this, the activation of T cells by the presentation of antigens via dendritic cells or antigen presenting cells leads to the cooperation of with the B cells for the secretion of specific antibodies, as well as the activation of several kinds of T cell mediated immune responses, such as cytotoxic T cell, as well as T helper cells.

So, all this is to ensure that, there is diversity of the immune response, so that immune response can recognize multitude of antigens, having recognize the multitude of antigens or pathogenic infections, the immune system is endowed with a memory to remember that it had come down or face the similar infection earlier the life of individual. And therefore, able to respond faster by the secretion of IgG responses of the right type of so far as affinity maturation is concerned.

The self, non self-recognition via the MHC molecule, therefore, if efforts were to be tried and put into action that would result in protection of the individual, which is what vaccination, is all about. Vaccines stands for vacca meaning cow because, that was in the history when Edward Jenner used these cow pox material to immunize various people; therefore, in the Latin the word, cow stands for vacca and therefore, it was named as vaccination at that time.

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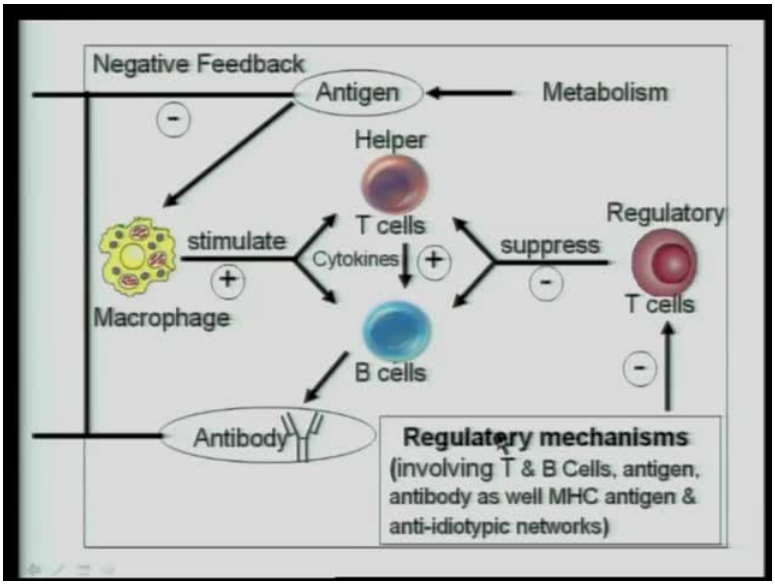

So, if you look at vaccination, then you must then use approaches that will help to strengthen the immune response. So that, it is prepared for an incoming infection and the various places, where the immune system can be strengthened is the macrophage in

terms of innate immunity itself. Therefore, the activation of the immune innate immunity either by causing better phagocytosis or by activation of macrophages by optionization or immune stimulating complexes or activating the process of antigen presentation itself, by providing the right kind of cytokines and the right kinds of antigens that would then stimulate both the T cells and B cell arms of the immune system.

Also it so happens that, these T helper cells and the reactions that they orchestrate is different from individual to individual, because the MHC haplo type itself is different and therefore, as we went in to in the earlier classes, there seems to be diversity in individuals responding to different kinds of peptides that are bond to the MHC complex.

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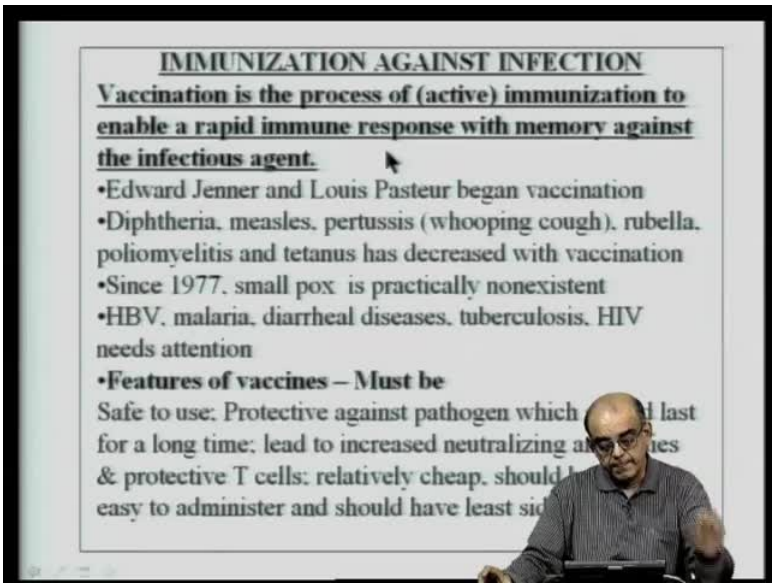

In other words, all of us are not equal in the way we respond to various kinds of antigens or even various kinds of protein antigens from the same virus and therefore, one ends up being either susceptible or resistance to a particular kind of infection. Therefore, vaccination approaches have to deal with this sort of hydrogenate also and of late ones speaks about personalized medicine, where ones take into account the genetic sequence of an individually, in order to find out how that person is going to respond to a particular infection or a particular pathogen.

B cells is a lot of algorithms to find out, what sort of epitopes are suitable for a protective immune response and these approaches are being put into action, in order to make vaccines that are better able to make protective antibodies. But, some total of protection

needs the participation of helper T cells and therefore, this sort of hydrogenating, how T cell respond various kinds of antigens need to be looked into.

So, going on further, looking at the aspect of vaccination itself or immunization auto strengthen the immune response against infections, vaccination could be defined as the process of immunization meaning, active immunization via the injection of a particular material into the person, so that it enables the rapid immune response preferable with memory. So, it can be long-lasting against the particular infectious agent; as I allowed to earlier, Edward Jenner and Louis Pasteur began this whole process of vaccination in history.

Now, this vaccination has now being tried out and has been found to be successful in the case of diphtheria, measles, pertussis which causes whooping cough, rubella poliomyelitis as well as tetanus, all these diseases have decreased fallowing the onset of vaccination affords. Now, since 1977, small pox is practically nonexistent other diseases that are pathogens that require attention because they have not been able to eradicate them with Hepatitis B Virus, malaria, diarrheal diseases, tuberculosis, HIV, all these kinds of diseases need attention and the more complicated the pathogen, the more complicated becomes the effort to vaccinates against it.

So, what are the features of a vaccine? The vaccine must be safe to use, in terms of not causing the disease by itself, it must be protective against the pathogen and this protection should last for a fairly long time. And it should lead to increased neutralizing antibodies; neutralizing antibodies are the ones that confer protection against the particular disease. Neutralizing stands for those types of antibodies that bind to a virus or a pathogen and stops it from infecting a particular cell, it neutralizes the virus and therefore, it is going to be protective.

As a humeral immune response that orchestrated against any pathogenic infection can lead to the elicitation of antibodies; however, these antibodies need not always be protective, there can be antibody that are against surface antigens of a particular pathogen, but it will not stop infection. In fact, there are types of antibodies called as antibody dependent enhancement phenomena, where these binding of these antibodies actually increase the infection in a particular individual, by binding to f C receptor and so on.

So, therefore, protective T cells also have to be activated as an essential feature of any vaccine that is thought about. And this is something that needs to be addressed, it has to be relatively cheap, it should be stable and easy to administer and should have least side effects. It should be relatively cheap, since many of the poor developing countries need to afford them, they should be stable because they have to be stored for long periods of time, one cannot afford to make these vaccines in a using all the complicated procedures that I am going to describe in a next few slides, it cannot done so easily all the time.

So, they have to be stored for some times at some place in order to be used for long period of time and so, there should be they should be stable and they should be easy to administer because children are the one that, that receives these many children are in a majority of cases are one, that receives these vaccinations attempts or against various pathogens. And they should have least side effects, because if they have side effects then, everybody is afraid to take the vaccine and then vaccine becomes useless.

And also you will see, many of these vaccination affects some small side effects that are reported which may not even be due to the to the vaccination attempts per say, but due to other causes can lead to a lot of public reaction that will cause the vaccination to be withdrawn for a small period of time, before it is ascertained that the vaccination of safe in the first place. But, this withdrawing of vaccination actually causes these diseases took pert back again and it involves a term called as herd immunity, which I will try to cover as we go on.

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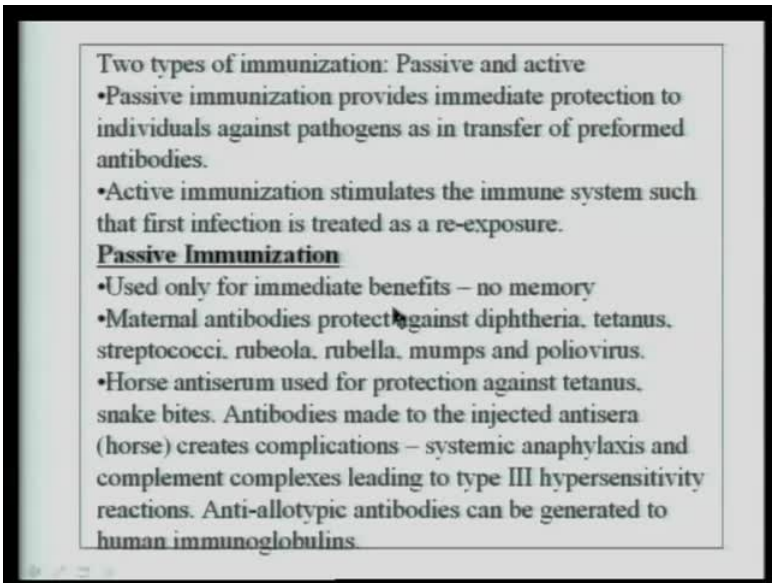

What are the types of immunization that one refers to? These immunizations actually fall into two categories called as passive and active immunization. Passive immunization provides immediate protection to individuals against pathogens as in transfer of preformed antibodies. This is something that is resorted to when immediate protection is needed for example, during snake bites, you need to have antibodies against the snake venom and they have to be administered as soon as the snake bite occurs; there is no point having a vaccination for snake bites before itself, because all the population is not likely to be bitten by snakes.

So, therefore, those who are in danger, who have been bitten by snakes at that point in time, they have to have access to this sort of antisera, which can be administered passively. As oppose to passive immunization, active immunization stimulates the immune system such that, the first infection is treated as a re exposure; in other words, this becomes a secondary episode rather than a primary exposure to the antigen itself or the infection.

So, what are the features or what are that properties of passive immunization? It is use for immediate benefits because, it is just transferred from let us say, pure antibody that has been prepared and stored in a while, because it has been transferred into an individual, it has no way of inducing memory because it just antibodies. So, T cells are not activated and therefore, memory T cells or memory B cells are not going to be

activated because the person with B cells of the immune system is not activated against the toxin.

Another example of passive immunization is maternal antibodies. Maternal antibodies that are protected in the home such as going across the placenta like IgG, maternal IgG that can cross the placenta and protect the fetus from various kinds of infections that is coming from the maternal blood stream. It also has to do with the transfer of antibodies through the maternal milk, during breast feeding.

So, many of these antibodies against new borne infants are actually transferred through maternal milk during breast feeding and these are protective antibodies that protect infants, against a variety of infections. So, such maternal are protective against diphtheria, tetanus, streptococci, rubeola, rubella, mumps and polio virus.

In addition to this, one has to consider that in various situations or various parts of the world, everybody is not 100 percent healthy; in other words, there could be regions where there is malnourishment in such situations, mother who are who are malnourished will not be able to confirm 100 percent protection to infants, because of deficient transfer or deficient secretion of this kind of antibodies, that are needed for the infant.

Usually horse antiserum used for protection against various kinds of toxins, specially tetanus toxoid, tetanus all of you probably remember that, if a boy falls in the football ground, he is immediately taken to the doctor for a tetanus injection because, you can have toxin production, if they wound get infected. And tetanus toxin is actually very dangerous and as I mentioned snake bites. The way one makes antiserum to snake venom is to take much diluted or very small portion of these venom and injected into horse. So that, the horse is do not die of the dose that is given and slowly in a dose over a period of time, because the horse ends up making antibody.

So, the second time you inject the same venom, it makes more antibodies so on and so forth up till a time, when the horse serum has got sufficiently high (( )) of snake venom antibodies. Now problem with passive immunization is that, transfer of these antibodies into humans always elicits a reaction against the transferred antibodies, because all of us make antibodies to for a species.

In fact, that is how for various kinds of diagnostic, one make antibodies like in a Liza, you make antibodies to a various kind of a let us say sheep antibodies made in rabbage, in rabbit antibodies are made in the goat by injecting purified rabbit antibodies and so on. Therefore, when horse antibodies, antisera are given to the humor, the humans end up making antibodies to that horse antibodies, that creates complications not only does it inhabits action of the horse antibodies over a period of time, it will also end up making the person allergic, you can have systemic anaphylaxis and there could be lot of these compliment mediated complexes and which lead to type three mediated hypersensitivity reaction.

In addition to that, these antibodies can elicit what are called as anti allotypic antibodies which is which are directed against the allotype of the Fc portion of the immunoglobulin of the horse. And therefore, these passive immunizations are resorted to only when there is a requirement for immediate effect and is not preferred to be used for given protection over a long period of time.

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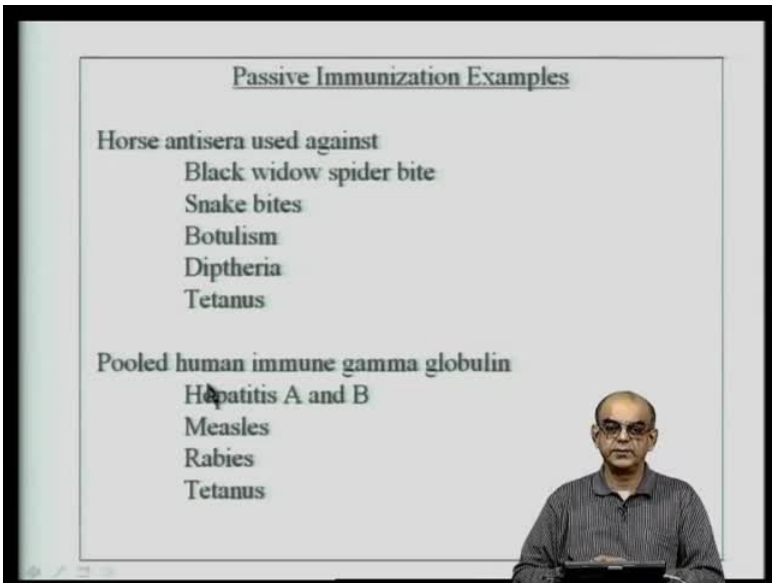

So, what are the examples that are involving passive immunization? These horse antisera as actually been used against black widow spider bites, snake bites botulism, diphtheria as well as tetanus. The other hand, organisms like Hepatitis A and B, Measles, Rabies and Tetanus has been combined with pooled human immune gamma globulin. So, in other words, people who are immune to such viruses they have antisera against these

viruses. So that, immune antisera is pool from various kinds of individuals and given to individuals or infants who are suffering from these in acute face of the disease. In other words, when the clinician things that the disease has gone too far and cannot be protected simple by intervention, by injection of various kinds of either drugs or other kinds of vaccines, one resorts to this kind of passive immunization.

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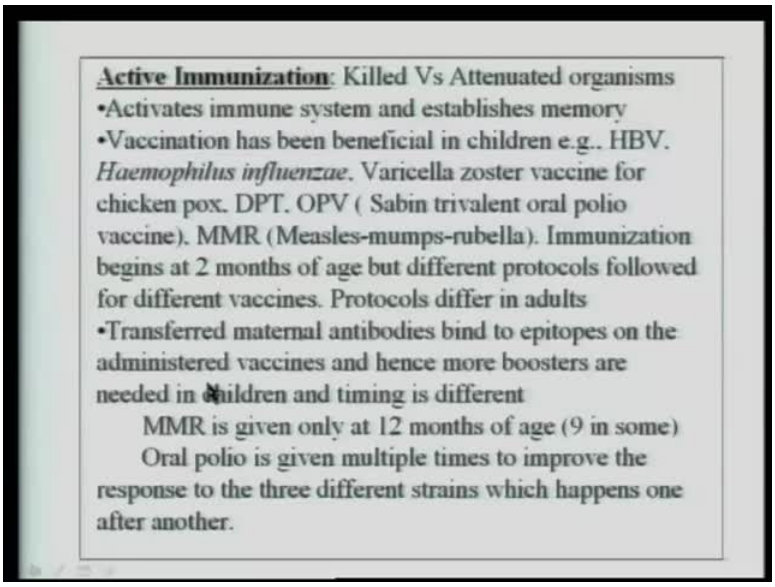

Now, coming to active immunization, active immunization is the process of immunizing an individual with the pathogen or pathogenic material itself. Now, you cannot inject pathogenic materials by themselves, because they themselves will cause disease. Therefore, that pathogen or bacteria or virus has to be killed or it has to be attenuated. Attenuation is the process of weakening the capacity of the organism to cause disease, it is done by passaging, these or making these viruses grow in different kinds of tissues or different kinds of cells, other than what is supposed to be its natural host.

So, this active immunization ends up activating the immune system and in many cases, establishes memory. This memory of course in many cases, need not be established also. So, one ends up trying to look for approaches or candidates vaccines that actually establishes memory because one cannot go on re immunizing individuals although that has to be done in many situations. Vaccination has been beneficial in children, example hepatitis B virus, Haemophilus influenza, varicella zoster vaccine has been use for chicken pox, DPT is very famous that the diphtheria (( )) vaccine, OPV which is Sabin

trivalent oral polio vaccine which is delivered, orally OPV stands for Oral Polio Vaccine, MMR stands for Measles Mumps Rubella. This immunization of infants actually can begin as early as 2 months of age, but in different geographical regions of the world and depending up on the immunity of the population, different protocols needs followed for different vaccines and protocols could differ very much in adults compare to infants.

Now, the factor that complicates this issue of the time of immunization is actually, the maternal antibodies that are transferred. These antibodies actually because they are binding to these different kinds of pathogens transferred from the mother, they can actually bind to the epitopes of the administered vaccine, if it is a killed bacteria or attenuated bacteria.

Therefore, the ability of the killed bacteria to Eliciton immune response in the new borne becomes weaker therefore, clinician actually look at what time period this vaccination has to be put into effect depending upon the prevalence or the type of antibodies, maternal antibodies that are there in the new borne, which is passively protecting the new borne against the same kind of organisms.

Therefore in such situations, when the injected vaccine is not as efficient more boosters may be required in children and timing could differ in different regions of the world depending upon whether the mother is malnourish or whether the infant is immune suppressed and so on. Usually, MMR is given only a twelve months of age, oral polio is given multiple times to improve the response to 3 different strains, which are there in this Sabin oral polio vaccine and because of this, more number of boosters are required because the immune system responds by making antibodies to one, the one strain towards one strain of this vaccine then, next the other strain elicits the formation of antibodies and finally, third strain elicits the formation of the antibodies.

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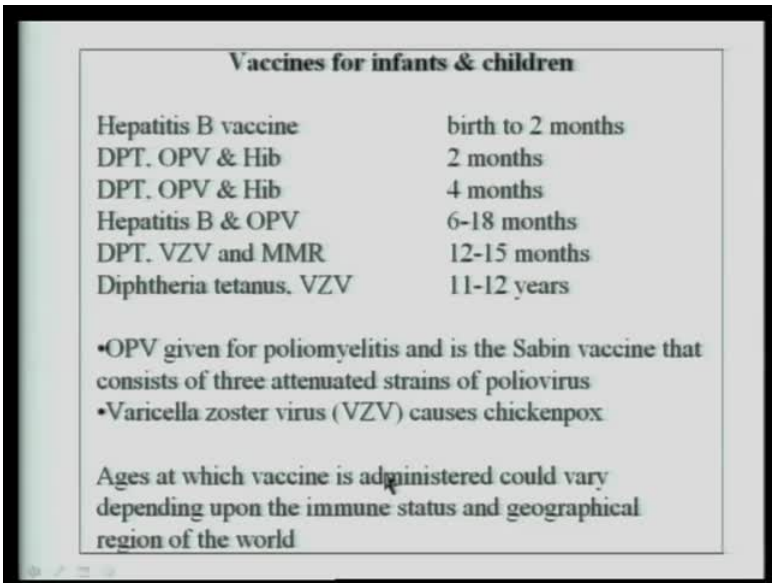

So, vaccines for infants and children, some of these examples are given here. For Hepatitis B, vaccines it is birth to 2 months, DPT, OPV and the B antigen of Haemophilus, this is HiB to OPV and HiB its 2 months and DPT, OPV and Haemophilus influence are B antigen four months of age, hepatitis B, OPV it is about 6 to 18 months and so on and so forth. Some of these characters, I have just listed out here and as I was telling you in the previous slide, ages at which this vaccine is administered could vary depending upon the immune status and geographical region of the place.

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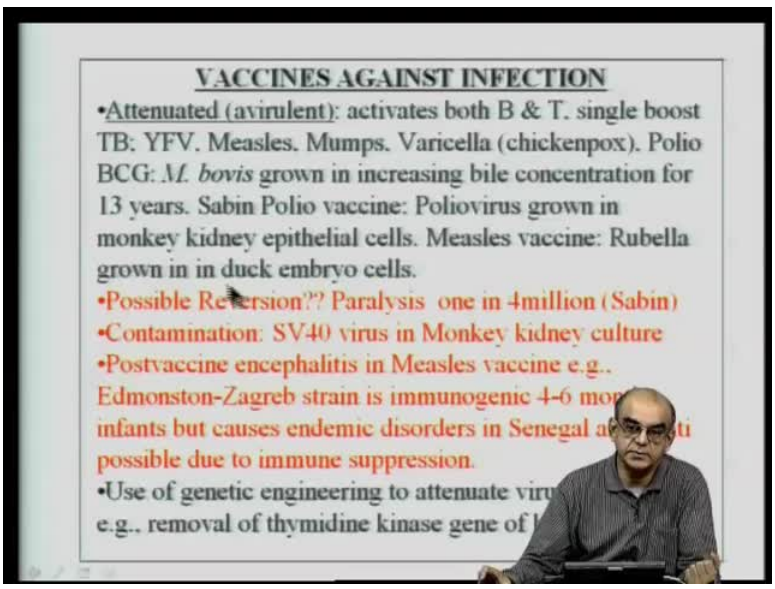

Attenuated meaning as I told you, it is a virulent, it is not able to cause the disease. Now the ability of attenuated organism activates both B and T cells, examples are TB they have used it in the case of TB, yellow fever, virus, measles, mumps varicella, polio. Now in the case of BCG, which is a well-known vaccine, which has been successful in some parts of the world, but it has been unsuccessful in many parts of the world including India.

This vaccine is nothing but, mycobacterium bovis, which has been grown in increasing bile concentration for about 13 years. It has resulted in several mutations that has attenuated mycobacterium, the Sabin polio vaccine has been prepared from passaging or growing polio virus in monkey kidney epithelial cells, the measles vaccine is rubella grown in duck embryo cells.

So, some of these attenuated organisms do have a chance of reverting back, but the chances of a possible reversion are 1 in 4 million, but this has come up from time to time in the history of vaccination and caused lot of problems in terms of withdrawal of vaccines. Such preparation of attenuated viruses or pathogens can also be contaminated for example, in monkey when one grows monkey kidney cells, SV40 virus is contaminant that can come up with some of these preparations.

So, one has to examine these particular vaccine batches in order to remove those which are contaminated with contaminants like SV40 virus. The other complication is the post vaccine encephalitis in some of these measles vaccine. For example, in Edmonston Zagreb strain is immunogenic in 4 to 6 month infants, but it causes endemic disorders in Senegal and Haiti because of possible immune suppression of these infants in those regions.

So, therefore, the immune system of the one of individuals over being vaccinated is also important, when one uses attenuated strains, because if an individual is immune suppress then an attenuated organism can retain or develop a capacity to cause damage or the pathogen so called, becomes infective in an immune suppressed individual. Now a day, genetic engineering is being used to attenuate viruses completely.

So, rather than relying on the natural process of passaging or growing these viruses in different kinds of tissues in order to cause attenuation, which is ultimately due to different kinds of mutations, why not cause the mutations by design. So, you mutate

pathogenic antigens or viruses that are involved in making a virus virulent and make them non virulent. We can also choose some of these genes that you would like to mutate so that, they will not be able to revert back again.

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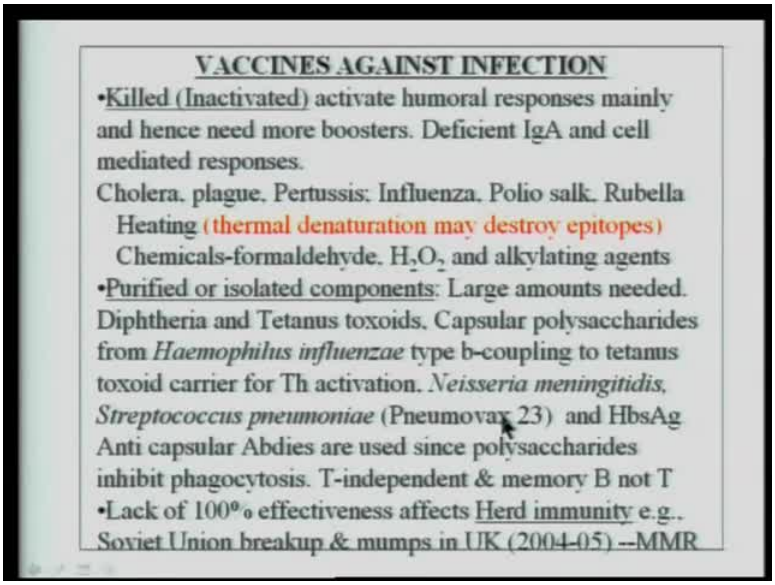

So, some of these approaches involved the removal of thymidine kinase gene, in the case of harpies for vaccination in pigs. As oppose to these kinds of attenuated organisms killed vaccines, activate humeral responses only, they do not response or they do not activate memory responses. And hence they need more boosters, they are deficient IgA and cell mediated responses when one use killed preparations. Some of the examples are cholera, plague, pertussis, influenza, salk polio vaccine, rubella.

This killing can be achieved by heating by thermal denaturation, but many cases thermal denaturation may destroy many of the epitopes may require, in such cases, chemical such as formaldehyde, hydrogen peroxide and alkylating agents are used. Purified or isolated components, you can purify material from bacteria, large amounts can be purified and for example, in the case of toxins, diphtheria and tetanus toxoids, capsular polysaccharides from Haemophilus influenzae type b has been has been purified.

And they have been coupled to Tetanus toxoid carrier because Tetanus toxoid also acts as an adjuvant, we come to that little later on being very highly immunogenic by itself, this carrier coupling enables the helper activation for the establishment of memory

because capsular or polysaccharides are thymic or the T-independent response activating type of antigens.

So, therefore, one needs to activate the helper cells which can be done by coupling these antigens to Tetanus toxoid, which by itself is very immunogenic and causes the helper activation. Other cases are Neisseria meningitides, Streptococcus pneumonia, which has 23 different kinds of polysaccharides that can be used and the hepatitis b surface antigen itself. One of the important aspects of using polysaccharides that, these polysaccharides are used by these bacteria in order to evade macrophage mediated phagocytosis.

In fact, that is one of the reasons why some of the antibodies that are made to incoming bacteria, they are made against polysaccharides, so they can go and bind to the pathogens and optionize them. Optionization is a process by which antibody coated material or antibody coated pathogens can and gulf macrophages and activate them further.

So, anti capsular antibodies are therefore used, since polysaccharides inhibit phagocytosis. And polysaccharides as I told you that, their T-independent and only memory B cells may be activated, but not T memory cells. Many of these approaches they have lack of 100 percent effectiveness and therefore, it effects what is called as herd immunity. This has actually seen examples during the breakup of the Soviet Union, many of these vaccination of infants where discontinued because of obvious problems in vaccination because, the whole country was breaking up.

Now, because of that, these diseases actually increased in the population and made many of the infant susceptible. So, this property of immunity, the whole population is termed as herd immunity and this can vary because, if you do not vaccinate when the child is born, they become more and more susceptible, more individuals in the population become more susceptible as they become older. Another example is mumps in the united kingdom went up quite a bit in 2004 and 2005, because the type of MMR vaccines that was used was altered just a couple year earlier and that made mumps go up in UK, then finally, the MMR vaccine was brought back to control such sort of a resurgent of a certain of mumps.

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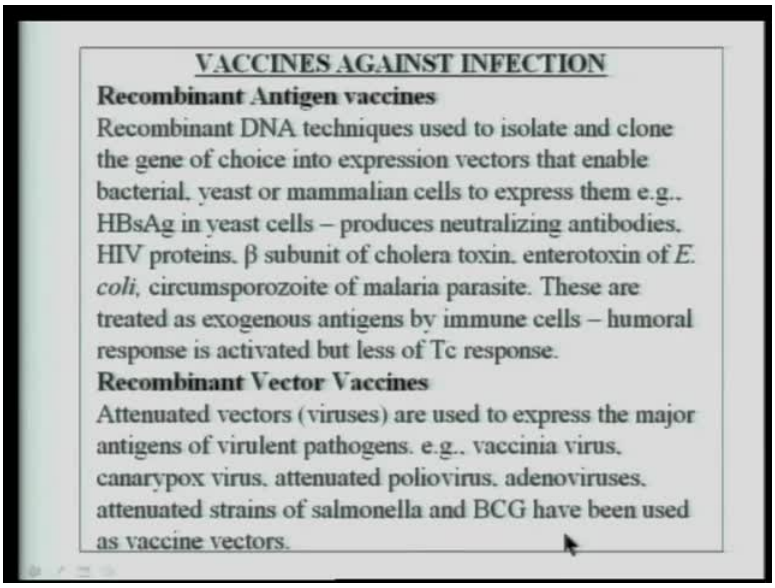

Now, different kinds of vaccines can be made recombinant antigen vaccines, use the recombinant DNA techniques to isolate and clone the gene of choice, which is from the pathogenic organism, which can elicit the formation either antibodies or T cell. They are put into expression vectors, those which can express this antigen and like for example, bacteria or yeast or even mammalian cells, once they are express, they are purified and they are then used to immunize an individual. For example, HBs surface antigen can be expressed in each cell, this has been known to produce neutralizing antibody.

One interesting aspect about yeast cells is that, these antigens can be expressed on the surface of yeast cells and yeast is something that is there in the normal food and one can use it for overall immunization also. HIV proteins, beta subunits of cholera toxin, entero toxin of equalize, circumsporozoite antigen of malaria parasitne, these are all examples where they have used recombinant DNA techniques to express these antigens and purify large amounts of them and then give it an immunize individuals.

These are treated as exogenous antigens by the immune system in visa vi the participation of MHC class 2 mediated responses, because the humeral response is activated in responds to purified antigens expression, there is less of class 1 mediated response which involves the activation of cytotoxic T immediate killer cells.

Therefore, they have come up with recombinant vector vaccines, you make a vector that express a particular antigen of choice, you take this virus or vector and attenuated by

mutating the virus, mutating the antigen that actually causes the infection or which is responsible for it is virulence. And therefore, these virus attenuated viruses, they can be engineered in such a way that the antigen of choice can be put into the gene nom of this engineered attenuated vector.

And the virus can be allowed to infect the individual and why because of the attenuation, the virus itself will not be able to cause disease, but it will multiple, because it has all the rest of the components, viral component that allow to infect the whole cell. But, in the whole process, the other antigen that have been cloned into this particular gene nom will be expressed in the mammalian cell or in that individual, immunizing that individual against both a class 1 as well as class 2 mediator response.

So, some of the gents that have actually being used widely is vaccinia virus. Vaccinia virus has been use in a variety of cases for example, rabies virus or rabies antigen some of these gene have been put into vaccinia's virus and they have been used to in fact, they have been used to immunize dogs as well as well as foxes in the forest to prevent rabies from spreading. Canary pox virus, attenuated poliovirus, adeno viruses these are all have been used as vectors even bacteria such as salmonella has been use as attenuated vectors to express different kinds of antigens as well as BCG.

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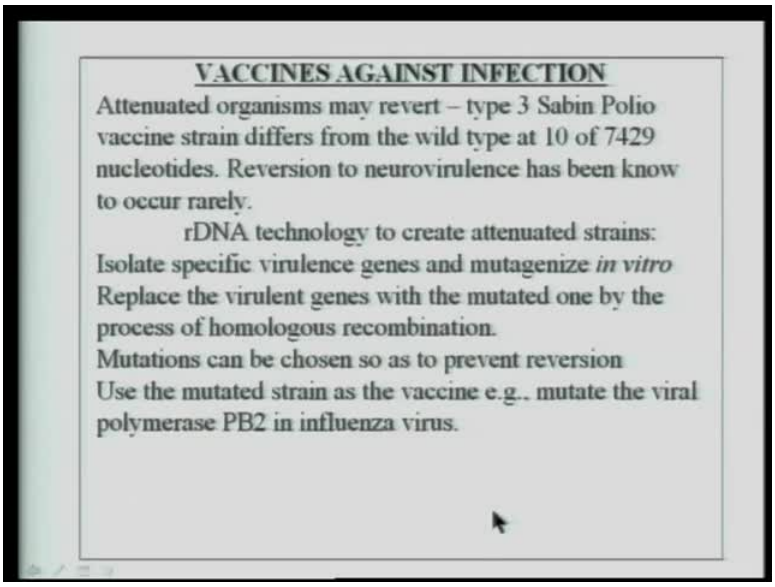

Now, vaccines against infection, going into this little further, attenuated organisms are prepared normally by passaging in different kinds of cells can actually revert. In fact, this

is one of the reasons why attenuated vectors by using recombinant DNA techniques have been resorted to. So, for example, in type 3 Sabin polio vaccine, this strain differs from the wild type at 10 positions or in 10 nucleotides out of a total of 7429 nucleotides, means point mutations.

Now, reversion causing Neurovirulence has been noticed, but it has happened only very rarely, but even this rarity can cause hawk in a vaccinated individual because the individual becomes susceptible to death because of the Neurovirulence and the bad effects of this particular reversion. Therefore, in many situations during the history of vaccination, many of these attempts or vaccinations has actually been withdrawn for a couple of years and then brought back after verifying that it was not the bad effects, where not because of the vaccination per say, but because of some other problems in the individual. Recombinant DNA technology has been used to create the attenuated strains, as I allowed to in the earlier slide.

So, the steps involved isolation of the specific virulence genes of that virus and mutagenize that particular gene in vitro. So, you take out the gene and you cause mutations in that particular gene. So, that it is not able to cause the virulence again. So, then you take this particular gene and you replace the virulent gene of the vile type virus, with this mutated gene by the process of recombination.

So, you can actually get the mutated gene or a mutated DNA into the vile type virus in a infected cell and because both the DNA are there together in the same cell during the process of virus recombination of the DNA, the mutated gene goes and recombined the region, where the virulent gene. Therefore, the gene nom that has got this mutated gene becomes a virulent.

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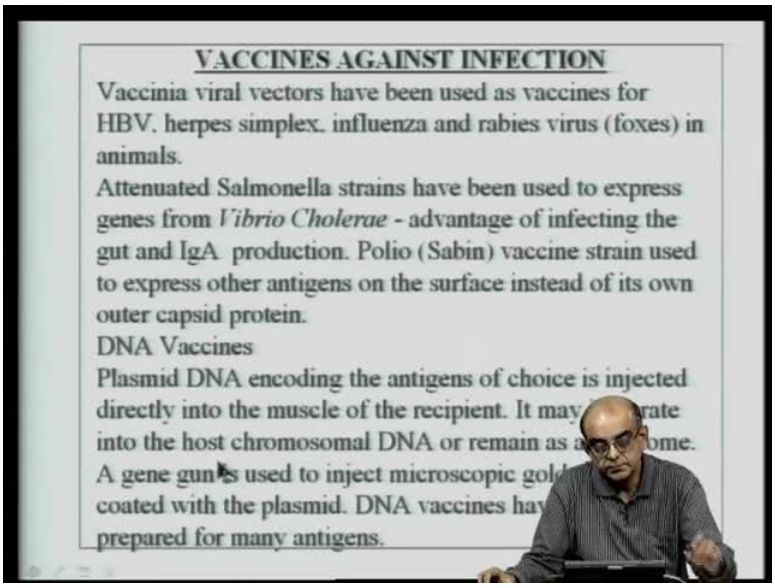

So, such sort of process involving homologous recombination has in fact been used in a variety of cases. The advantages being that, these mutations can be so chosen, so as to prevent reversion. And examples being as the vaccine they for example, influenza virus has been used in such cases, where they mutate the viral polymerase gene called as a PB2. Now, as I told you earlier, vaccinia viral vectors have been used as vaccines for Hepatitis B virus, herpes simplex influenza and rabies viruses, and salmonella strains have been used to express genes from all these are all different examples of what was just described in the earlier slides.

For example, vibrio cholera, genes form vibrio cholera has been express in salmonella strains. So, the advantage being that salmonella infecting the gut of the mucosal immune system and many of these organism that are spread through the oroferprofe fecal route benefit by using those kind of vector that actually cause in faction in the oroferprofe route or the gut.

So, therefore, if one uses antigens that get expressed in the oroferprofe route, it is an advantage to having activating immune response against those organisms that call infect oraferprofe route, mainly by IgA production and so on. So, DNA vaccine is another very popular effort that is being tried out in the recent history, this is nothing but, plasmid DNA that encodes for the antigen of choice. This plasmid DNA is actually injected directly into the muscle of the recipient and this has been found to protect individuals

against various kinds of organisms and the exact manner of protection has been debatable, because some of these protect through a cell mediated immune response and some of them are disclaim that, there have been activation of the production of antibodies via a DNA plasmid immunization.

Now, this particular plasmid may integrate into the host chromosomal DNA or remain as an episome; that means, outside of the gene nom, but because of this fear that this DNA integrate into the host chromosomal DNA, a lot of skepticism still remains as to the use of plasmid DNA although several efforts have shown that, this sort of integration is practically not there, or it does not happen all the time. Now, this particular DNA immunization has been used in what is called as a gene gun, which enables the piercing of the particular plasmid along with the microscopic gold particles, which enhances the potential of this DNA to immunize an individual.

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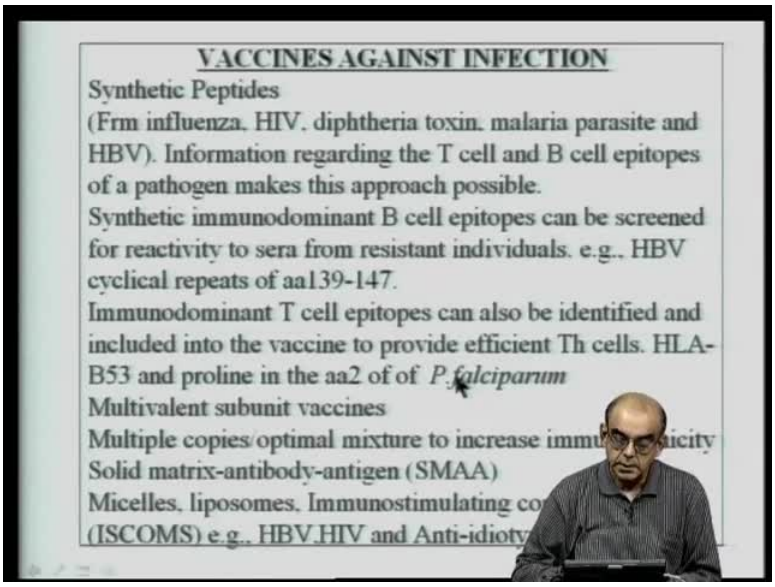

So, these gold particles are used to coat the plasmid DNA and DNA vaccine effort as involved a variety of antigens, one can look it up in any text book, need not go into a detail in this vaccination class. The other aspects, the more popular one that is interesting aspects that need to be covered today are, one of synthetic peptides. Now, these approaches have been tried for in the case of influenza, HIV, diphtheria toxin, malaria parasite, as well as hepatitis B virus. The basics behind the use of synthetic peptide is that, as one knows more and more about T cell and B cell immunology, one can look at

what sort of as earlier that antigen presentation involves the presentation of small peptides and as they are bound to the group of the MHC molecule whether they are class 1 or class 2.

So, when one looks at the sequences of these peptides that are normally there on in the groove of the MHC molecule, one can take purified MHC molecule and elute these peptides from the MHC groove by a process of acid precipitation and then analyze them by mass spectra of Spectric mass spectrometry to get at the sequence of these peptides. So, if one looked at these peptides, one gets an idea as to what sort of peptides may actually be found in protected individuals or protected alleles of the HLA molecules as oppose to those who are actually susceptible. So, you have now susceptible and resistance individuals or susceptible or resistance HLA alleles.

So, if one looks at the sequence of the peptides that are bound in those grooves, one can try and say the these peptide sequence are more protective in a particular population, but because of the variation or the number of HLA alleles, one is not able to come up with a particular sequence of peptide that is protective across the population. So, in such cases one looks for epitopes that bind to many HLA alleles, it is called as a promiscuous epitope.

So, these sorts of approaches have been used in order to construct synthetic peptides. So, if a particular epitope is immune dominant in a particular HLA allele, if one knows that the population has more of these HLA alleles, one can synthesize this particular peptide couple them carriers and use them as immunizing agents against those individual in those individuals.

In addition to this, you have a lot of algorithms or ways to predict B cell epitopes because many antigens have been identified over a period of years in the history of immunology to look at what sort of epitopes are bound by different kinds of immunoglobulins. So, one can look at a particular sequence, primary amino acid sequence of a protein and then come up with a particular hypothesis that, this particular reason can be immunogenic or dominant B cell epitopes. So, if that is given into the individuals or used for vaccination, it would end up, eliciting the formation of beneficial antibodies.

So, before which one can screen those synthetic peptides for reactivity to sera from resistance individuals. So, if those individuals who are resistant to a particular disease react to all of those synthetic peptides which have been prepared, then one sure that such sort of a peptides can be used in a vaccine preparation. For example, in a Hepatitis B virus, there are cyclical repeats of amino acids 139 to 147 have been used in a vaccine preparation. Now, immune dominant T cell epitopes can also be identified and included in a vaccine to provide an efficient T cell help.

For example, class 2 molecule, HLA class 2molecule can be looked at see what sort of peptides are there. This is in fact called as a reverse immune genetics approach, some of these efforts have been useful in the case of HLAB53 allele, which shows that B53 allele had always a proline in the second position, and this was useful against plasmodium falciparum because the peptide came from the plasmodium falciparum organism.

So, they use this particular peptide from the plasmodium which had proline in the second position to vaccinate against the against the stage of the parasite that infects the liver, the hepatic stage. In addition to this, the multi subunit vaccines which include multiple copies in order to try and optimize the mixture, that would increase the immunogenicity of a particular antigen. For example, solid matrix antibody antigen complexes or what are called as SMAA complexes, where you take a solid matrix like Agarose or some such solid of matrix and you couple an antibody against the particular antigen, then you give that antigen that you want to immunize against. So, the antigen goes and couples to that antigen and this entire complex is used as an immunogen and this because it is a complex, it activates the immune system and phagocytes much better.

In addition to that, using detergents you can prepare micelles along with the protein antigens, mix them up and give them to the individual. Liposomes are preparations that utilize phosphate molecules along with the protein antigen of interest. So, you have a lipid bilayer that is prepared and the protein antigen is incurred in this lipid bilayer of phospul lipid.

So, the hydrophobic portion is directed inside and the hydrophilic portion is directed outside, which when injected can stimulate or being bound by antibody baring B cells or immunoglobulin receptor barring B cell. In other word, in another attempt, you have what is called as immune stimulating complexes, which is just like liposome, which have

already different kinds of lipid material for so lipid is also has other material called as [qua lay] along with the protein antigen of interest, to stimulate again and deliver these complexes to the cell into the cytosol of the cell.

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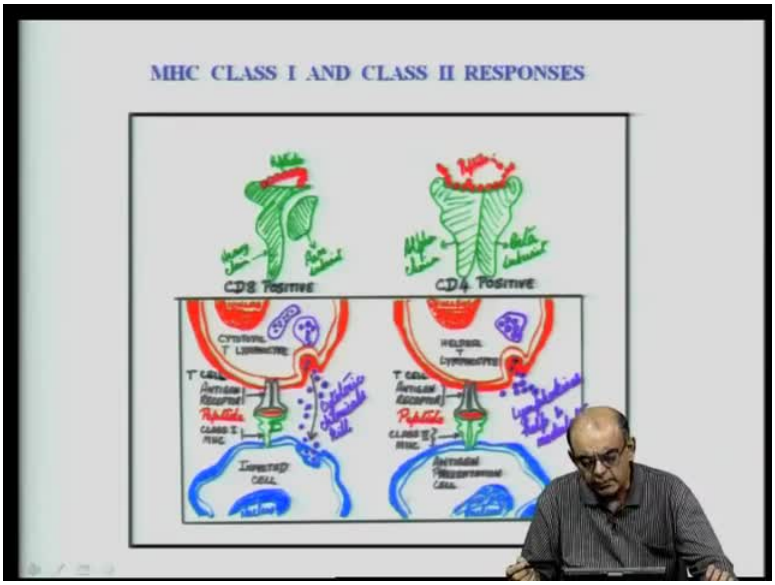

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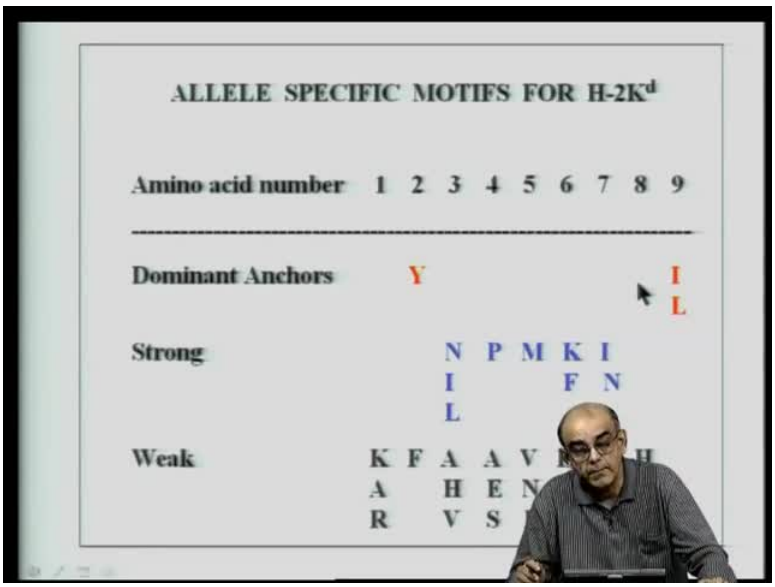

So, these Immunostimulating or ISCOMS go and bind with the antigen presenting cell let us say dendritic cell and release the antigen into the cytosol, they benefit being that this can be taken up for a class 1 mediated, antigen mediated response by the tap protein transporter associated with antigen presentation. Some of these things HiB, NBV and

HIV have been use in such examples. Then you have coming into the anti idiotypic vaccines, which I will cover to just after these two slides. This is the peptide it is bound to the class 1 molecule and this can be eluted as I told you, and the sequence is worked out.

So, can that is what I meant in the previous slide, that you can look at the look at the sequence for example, HLAB53 was experimented in this way, in this reverse immune genetics approach. This is just to show you in the mouse the particular strain of mouse called as balb cif, you elude those peptides from the class 1 molecule, you will see that these are called as dominant anchors residues, tyrosine is always there in the second position, Isolosin or Losin is there in the nineth position.

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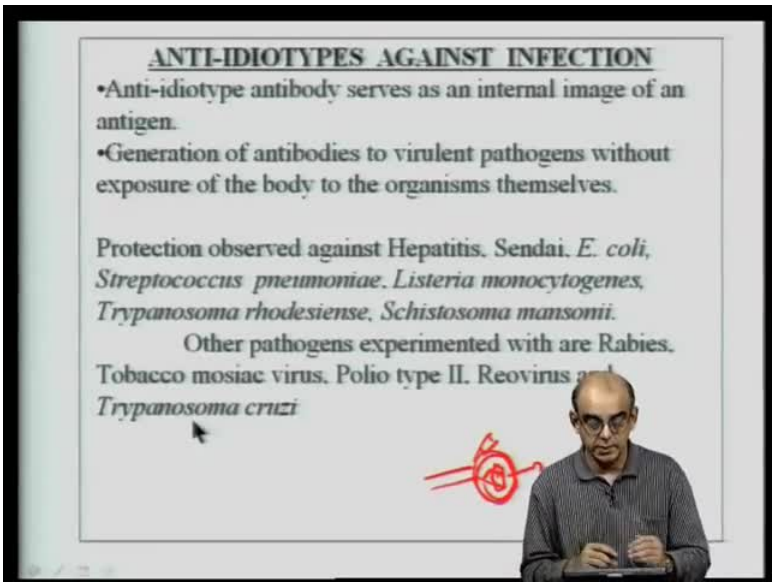

So, if you look at the antigen and you have tyrosine the second position, Isolusin and Losin in the nineth position, you can at least hypothesized although it may not be 100 percent true that, these particular nano peptides are always going to be bound to the class 1 molecule. But, many of these peptides are in fact, bound to the class 1 molecule.

So anti-idiotype antibodies, you have already learnt that, it serves as an internal image of an antigen. What is this anti-idiotytpe? If you look at the antibody, you have the Fc portion and you have the way the light chains attached here by disulphide bonds. Now, because of their variable nature, they themselves can be a foreign antigen in a particular individual. In fact, immune system supposed to be controlled in a feedback regulatory

loop by having an antibody, in an antibody being formed because an antibody is being made against a particular pathogen. So, they find this anti-antibody all these variable regions can make aconglomeration of epitopes, which elicit the formation of anti-antibodies.

So, this collection of these variable region epitopes is called as the idiotype. So, if you make anti if you make antibodies to these variable regions, they have form that many of these anti-idiotypes actually are surrogate antigens. In fact, many of these anti-idiotype has in fact being used to try and see how we can suppress allergy, but in the case of vaccination, you can make use, this as internal image of the antigen and generate antibodies to virulent pathogens, without using the pathogen itself, that is a advantages of using anti-idiotypes. So, there has been protection observed against hepatitis, Sendai, E coli, streptococcus, pnemomiae, listeria, monocytogenes, Trypanosome rhodesiense, Schistosoma mansonii, these are all the pathogens where anti-idiotypes have in fact, being used to protect against these diseases.

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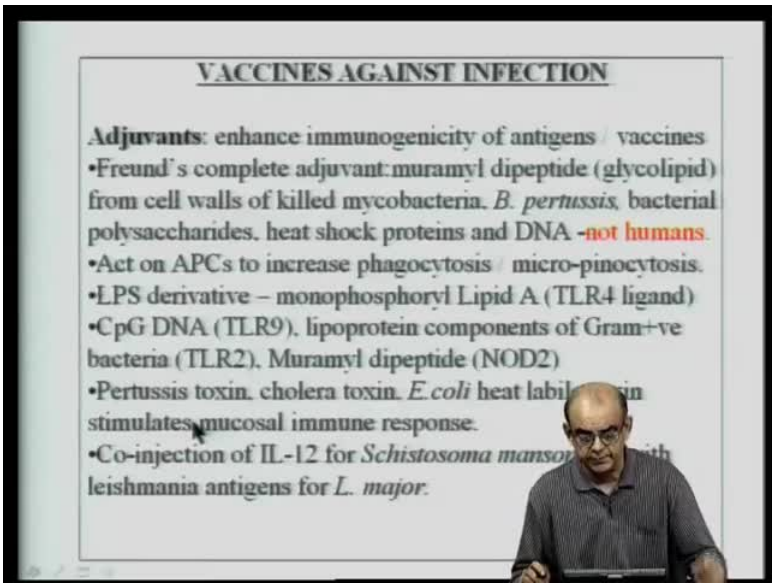

So, protection has been observed by using these anti-idiotypic approaches in animals. Other pathogens that have been experimented with are rabies, tobacco mosaic virus, polio type 2, reovirus and trypanosome cruzi. However, although you had the formation of antibodies in such situations, protective immunity did not result in these examples. So, in order to summarize this class, we need to look at several aspects of the immune

response that has been activated against the variety of organisms by the reactive process of immunizing or passive process of immunization, but before that, simple little bit about adjuvants which are used to increase responses to immune response to an antigen; they enhance immunogenicity of antigens or vaccines.

So, they are along with of vaccines one of the popular one that are used in mice is Freund's complete adjuvant which is nothing but, the muramyl dipeptide that is present in the bacterial cell wall. In fact, you have heat killed mycobacteria in this point's complete adjuvant. The adjuvant activity is by a process of a dipole effect or a delayed release of this antigen because mineral oil also is incorporated into this adjuvant and in addition to that, the activation of macrophages because these muramyl dipeptides activate macrophages.

Other one is the Bordetella pertussis antigen, which also acts as an adjuvant material to activate macrophages. Bacterial polysaccharides heat shock proteins as well as DNA these are all have been used as adjuvants. They act on basically antigen presenting cells in order to increase their activity to increase phagocytosis as well as micro pinocytosis. The LPS derivative because LPS by itself is very toxic and very small doses it cannot be used as anadjuvant, but some of the components of LPS has been purified called as monophosphoryl lipid A, it is a TLR4 ligand, similarly CpG motives of DNA binds TLR9, lipoprotein components of gram positive bacteria bind to TLR2, and the muramyl dipeptide bind to the NOD2 receptor.

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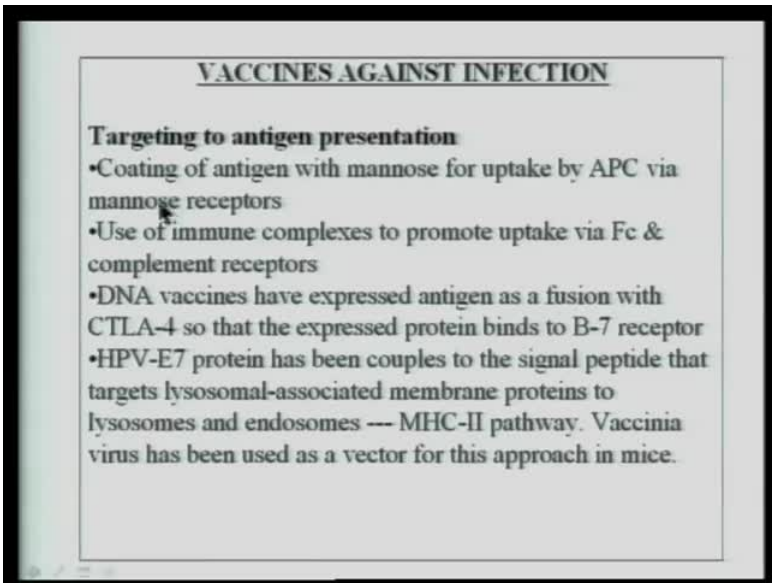

So, all these various kinds of adjuvant have been used; in addition to this, the co injection of IL12, some of the cytokines have been use for a variety of disease not only by exogenous administration, but also as a co fusion with the particular gene that you want to immunize against. So, IL12 gen is just supposes to a particular gene of interest, then IL12 is also made at that time I am activating a pro inflammatory kind of a situation in vivo.

So, they can also be these kinds of targeting approaches have been used in this particular slide, they can coat antigen with particular kinds of residues like for example, mannose to coupler to (( )) receptors, immune complexes why are the Fc receptors, DNA vaccination as I told you have been made as a fusion product with CTLA4, so that they can bind to B7 cause co stimulator activation and so on and so, forth.

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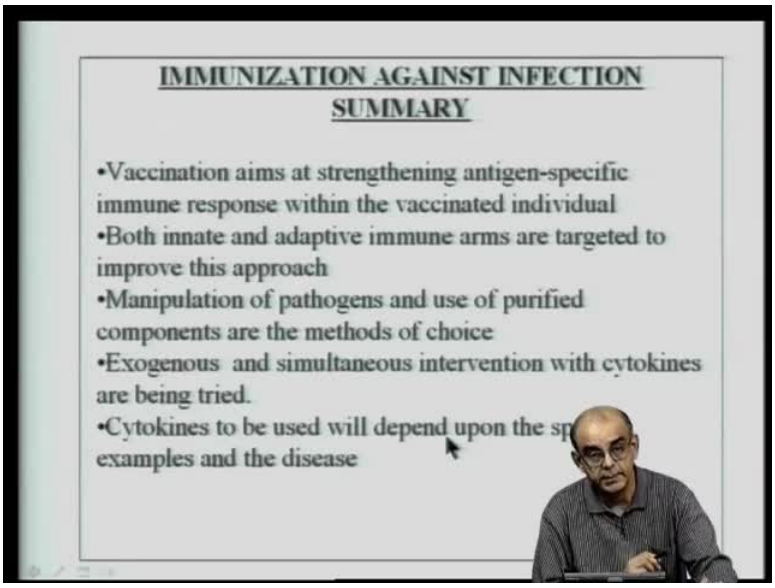

In addition to that, fusion with the signal peptides they can be targeted to the MHC2 pathway. So summarizing, you have all these different kinds of attempts which are basically aimed at strengthening the immune response, both innate and adoptive immune response are targeted to improve this approach. And you manipulate the pathogen to make it less virulent, purified components are used exogenous and simultaneous intervention with cytokines are being tried as I just mention and cytokines to be use also then depend upon the specific example and the disease because each one of us respond differently to different kinds of organisms.

So, while certain kinds of cytokines can actually trigger a cascade which is involving inflammatory and pro inflammation cause deliria side effects, others may not have the same kind of response. Therefore, you see that varieties of efforts are in fact moving towards kind of a personalization of medicine, although that is a far way of.

Thank you very much.