Essentials in Immunology

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Module No. # 21

Lecture No. # 39

Synthetic Vaccines

So, in the last few classes, you probably have been introduced to the subject of vaccines. Vaccines have been known since the last, maybe more than a hundred years.

Conventionally, vaccines were made up of live, but attenuated, pathogen. There are vaccines to viruses as well as bacteria. Now, with more and more understanding of the molecular mechanisms of an immune reaction and the molecules that were required for generating these responses– both T and B-cells– people started looking towards easy– an easy way– of obtaining vaccines.

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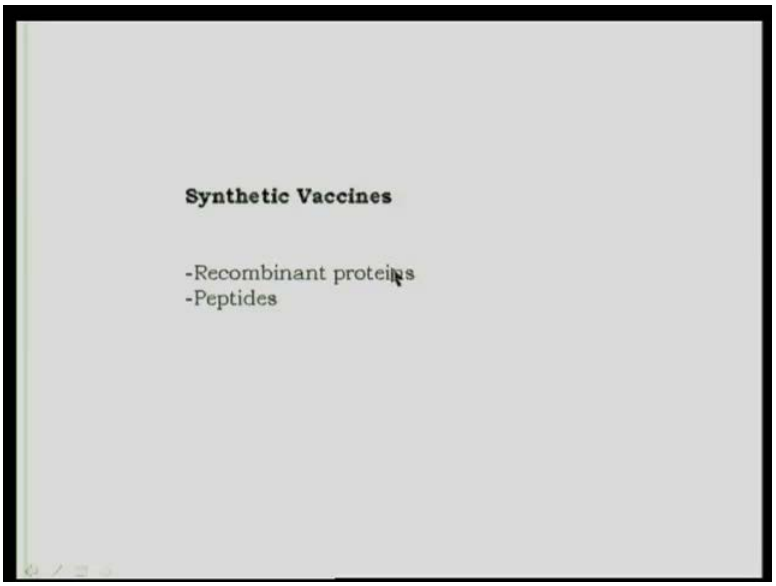

The first step towards this was use of recombinant proteins; that is, proteins that were made by or expressed by the recombinant DNA technology, and further to this, instead of

using proteins or large molecules or the entire molecule, immunologists have taken the reductionist's approach, and have been successfully using peptides, instead of the entire molecule.

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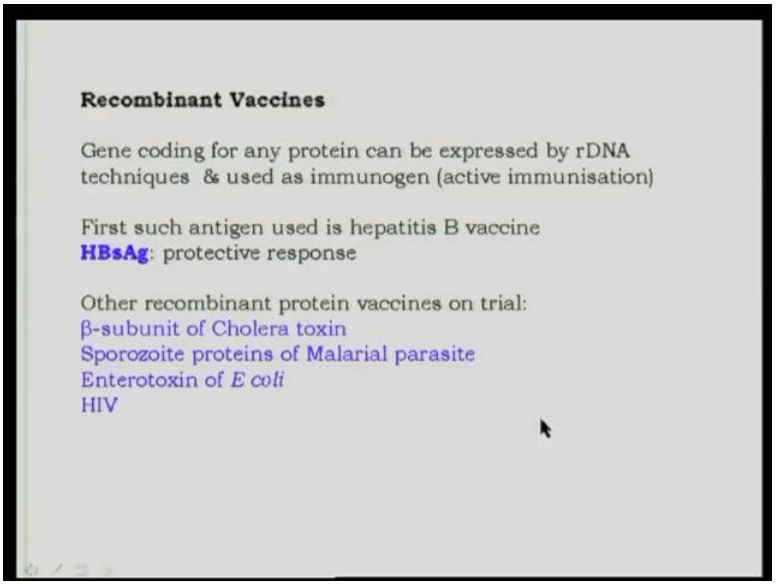

I am not going to deal with recombinant vaccines, except just to touch up on, very briefly, gene coding for the any protein can be expressed by the recombinant DNA techniques– that is very well known, and recombinant proteins have been available for a very large number of years; so also, recombinant vaccines.

Now, though most of these recombinant vaccines are still under trial, there are the first such antigen, which has been used and is in the market, is the one for hepatitis B virus. So, a vaccine which is made from the hepatitis B surface antigen– this is… this was obtained, or this is being obtained, by the recombinant DNA technology, where the gene coding for the hepatitis B surface antigen, which affords a protective response, the gene is isolated, amplified, and put into a construct, and expressed the the proteins express into the bacterial system or the eukaryotic system.

Now, this is known to give a protective response. Like I said, I am not going to deal with recombinant vaccines, except, just introduce this. There are other recombinant proteins, which are on trial: beta subunit of cholera toxin, sporozoite proteins of malarial parasite. In fact, there are extensively many research groups who are looking at different proteins–

target proteins– of the malarial parasite, enterotoxin of E coli, as well as HIV– Human Immunodeficiency Virus.

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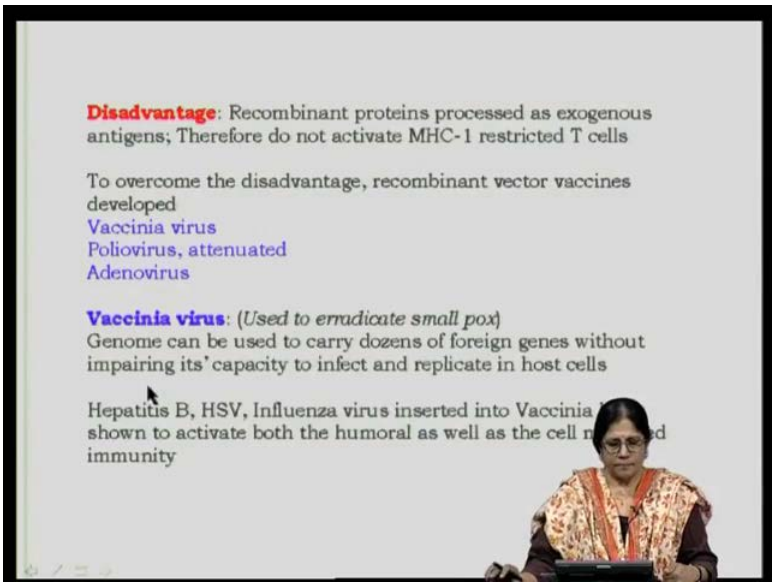

Now, are is it are the is the use of the recombinant proteins really advantageous? Does it afford protection the way the native protein or the native pathogen induces? No, it is not. So, especially if you are using a protein which is been expressed, let us say, E coli, or in eukaryotic expression system, the reason for not affording a complete immune response by recombinant proteins is because: let us say if the protein is corresponding to a neutralizing the epitope of a particular virus the virus or an bacteria.

These are well injected would be taken by the antigen presenting cells and presenting the antigen to the helper T-cells for the generation of cytotoxic T-cells, which would really be, I mean, development of which, really, would constitute a complete immune response; not just the antibodies. These are done by… The presentation of antigen is done through MHC class I, as all of you all already know, because cytotoxic T-cells are MHC I restricted T-cells. Therefore, pathogens or viral… when bacteria and viruses need to be intracellular, then the proteins corresponding to these are expressed in the context of class I molecule.

So, while recombinant proteins do have the ability to elicit a good antibody response, MHC class I restricted cytotoxic T-cell response is meagre. So, anyway, that is not where immunology stopped. To overcome this disadvantage, recombinant vector vaccines were

developed, and the ones that are quite well known are the vaccinia virus, poliovirus, attenuated adenovirus.

Now, vaccinia virus, which, of course, you might… This has been used to eradicate small pox. This is why the genome of this vaccinia virus can be used to carry a dozens of foreign genes without impairing its capacity to infect and replicate in hosT-cells, and the virus- the, the immunogens, or the vaccine that are being developed in using vaccinia virus as the vector, are hepatitis B, HSV, influenza, and it has been shown that proteins thus expressed are able to activate both the humoral as well as the cell mediated immunity, because as one can imagine that the virus– the vaccinia virus– harboring this foreign protein can infecT-cells and mimic what would happen in case of intracellular parasite.

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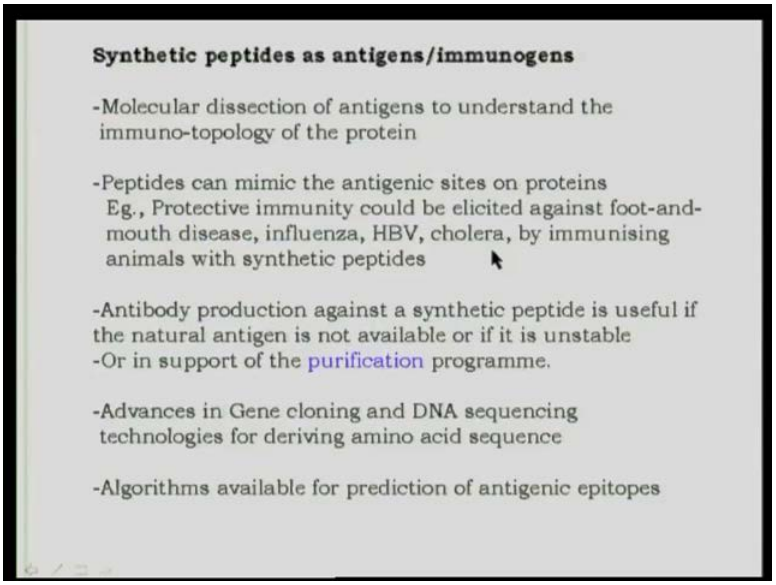

So, now, I come to what I will be dealing with the topic of the lecture today, and that is synthetic peptide. And like I told you, of course, one can use the… the parasite attenuated forms of the parasite for, I mean, as extremely good vaccines.

But it is not always easy to be able to, let us say, in case of HIV, it would be very difficult to replicate the virus in vitro. They are, of course, also, dangerous viruses and dangerous bacteria, and therefore, instead of using the entire organism, why not use parts of the organism, which on their own, cannot replicate?

Now, this has… With this understanding, immunologist had started carrying outer molecular dissection of antigen to understand. You know what is molecular dissection, as you looking at the given the entire sequence of the protein corresponding to particular virus or bacteria, which is known to afford a neutralizing– virus neutralizing, or pathogen neutralizing– response, to find out in this molecule which regions are immunogenic, both by way of T-cell response as well as B-cell response.

Now, this has been, of course, people have also looked at proteins as such from an academic interest, not necessarily only to understand or to make a vaccine, but all this information has helped people to identify smaller regions of fragments or peptides of these larger molecules, which can mimic the antigenic sites on proteins, and therefore, protective immune response can be elicited.

Now, there such… there are large number of such studies available in literature, and I am only, you know, just mentioning very few example of this peptide being…, peptides being used to mimic antigenic sites; that means, not the entire molecule, but smaller fragments, thereof. Examples are the protective immunity could be elicited through identification of, you know, by injecting peptides corresponding to food and mouth disease, influenza, HBV, cholera, just by simple immunization of these with these peptides. So, this, of course, experimental or research, now, for all these to come into the market, of course, it would take much time with appropriate validation.

Now, antibody production against the synthetic peptide, of course, would be extremely useful when the natural antigen is not available, or in adequate amounts, or if it is unstable, you know, once when it is purified or isolated from wherever, it is unstable. Also, if that pathogen is extremely dangerous, so therefore, it would be good in these situations to have a synthetic peptide.

Synthetic peptides, you know, getting antibodies to synthetic peptides would also be very useful for purifying the native protein or the native pathogen, right? So, synthetic peptides are extremely useful. Of course, one to be able to determine which peptides would yield useful antibodies is something which we will discuss as we go along. Now, advances in gene cloning and DNA sequence technologies has, actually, given enough information. Early, what people would do, I mean, much before the molecular biology methodology became commonplace in laboratories, people use to look at the, you know,

derive this amino sequence of protein, which would be pretty laborious, but after molecular biology techniques being started, you know, coming to laboratories as common technique, it is not difficult at all to obtain the DNA sequence of particular gene, and looking at complementation, it is not difficult to predict the amino acid sequence, and then, there are several algorithms that are available now the predict antigenic determinants..

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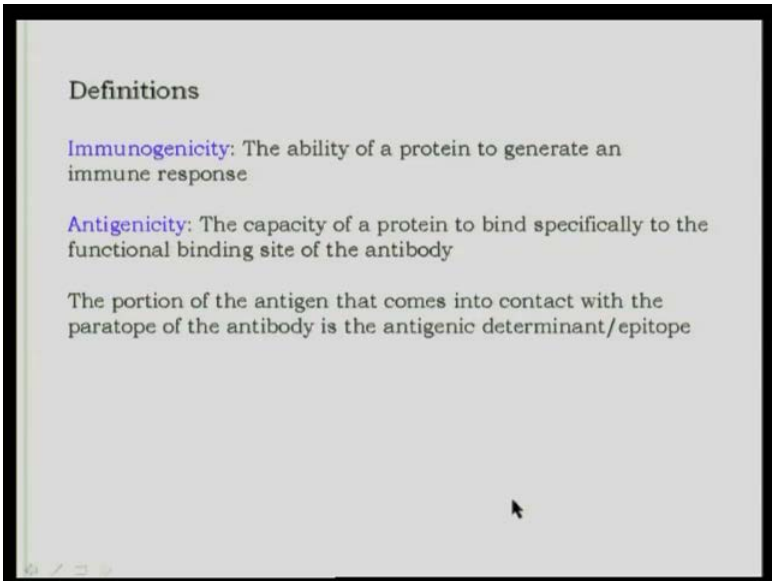

This path I have already discussed, in brief, in some of our earlier classes. Now, let us go back to the fundamentals of definitions of immunogenicity and antigenicity. Now, we already have discussed earlier that immunogenicity and antigenicity, though they are very related, these terms are different, because immunogenicity is the ability of a protein to generate an immune response, whereas antigenicity pertains to the capacity of a protein to bind, specifically, to the functional binding site of the antibody or the product of the immune response, which is antibody. Let us think, in the what is also you know what are be using constantly, and which it, of course, I was also use earlier antigen determinants or epitope– the portion of the antigen that comes into contact with the paratope of the antibody. This is known as the antigenic determinant or the epitope.

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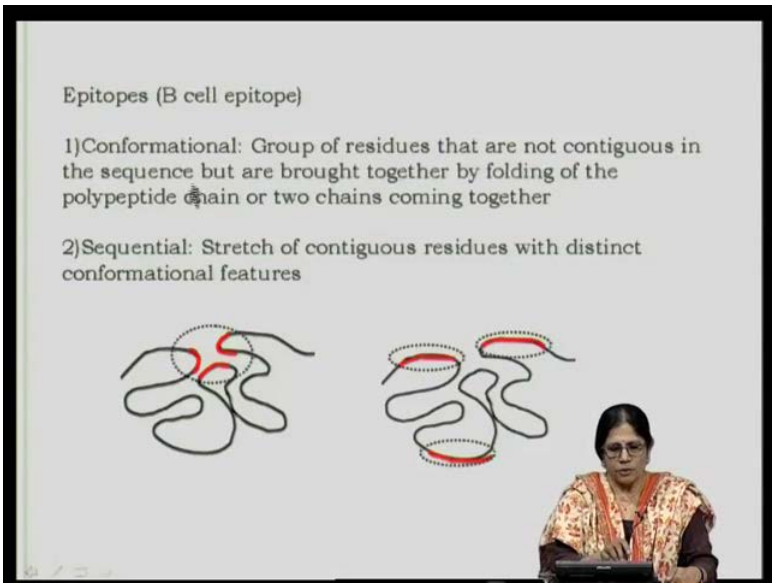

So, as you might remember from our earlier class, B-cell epitopes can be classified as conformational or sequential. So, if the B-cell epitope is conformational, it constitutes group of residues that are not contiguous; you can say, if you stretch out this particular molecule, then there would be three different regions, which are separate from each other in the primary amino acid sequence. They are brought together by folding of the polypeptide chain, or for that matter, that could also be two chains that come together.

Now, suppose a protein has two subunits, and bringing of the subunits together can constitute an epitope region; sequential, on the other hand, a sequential epitope is that which is… which comprises of a stretch of contiguous residues, which, of course, would have distinct conformational features. Now, the understanding of sequential epitope is not… One still has to remember that if the conformational of the sequence of a particular set of amino acids which constitute an epitope. So, it is in both cases, it is conformation, but while one, you know, it is… we think that one should probably say discontinuous and continuous.

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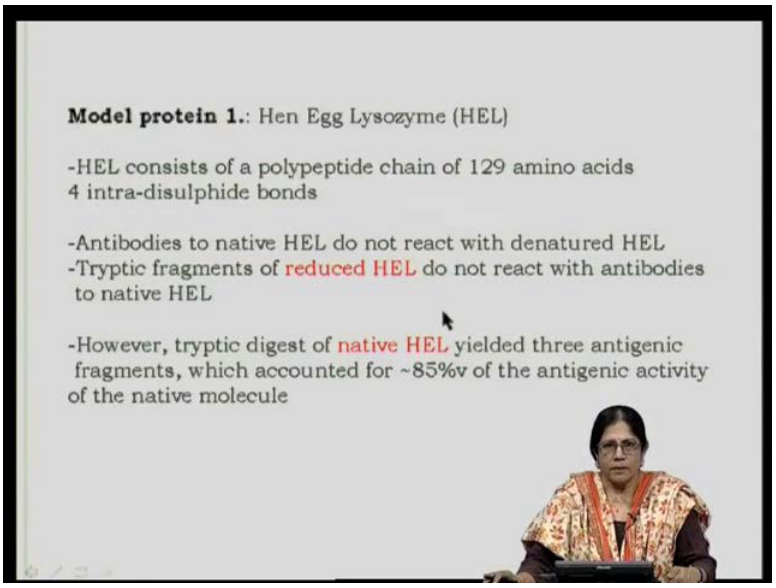

There are large number of protein that have been studied. You know, also, these proteins, which of the pathogen which afford or induce good immune responses. There are many, many, you know, reports– there are three model proteins, which I just like to introduce to you, and these model proteins have served as the basis for antigenic. You know, first they have also served as the basis for some of these algorithms that have been developed identify B-cell epitopes.

Hen egg lysozyme– I have a alluded to this molecule earlier. This molecule is made up of 129 amino acids, for there are four intra-disulphide bonds, interestingly, antibodies to native; that means, the native conformation where the four intra-disulphide bonds are, plays antibodies to the native HEL do not react with denatured HEL.

Tryptic fragments of reduced HEL– that means, when you reduce these bonds, and then, now, you cleave the protein with trypsin, which will target all lysines, now, tryptic fragments of reduced HEL do not react with antibodies to the native HEL; however, this is again, but interested that if the protein is not reduced HEL, hen egg lysozyme is in its native confirmation, and now, digestible with trypsin, which would mean only lysine which are available in this folic confirmation, would be susceptible to cleavage. So, when tryptic digest of native HEL were made, these yielded three antigenic fragments, and this constituted over 85 percent of the antigenic activity of the native molecule; that is, if there are antibodies which are raised to native HEL, and now, the native molecule is

digested such that there are three fragments, you know, the entire confirmation remains similar, and only, so, there would be, sort of, domains which are formed, which are obtained.

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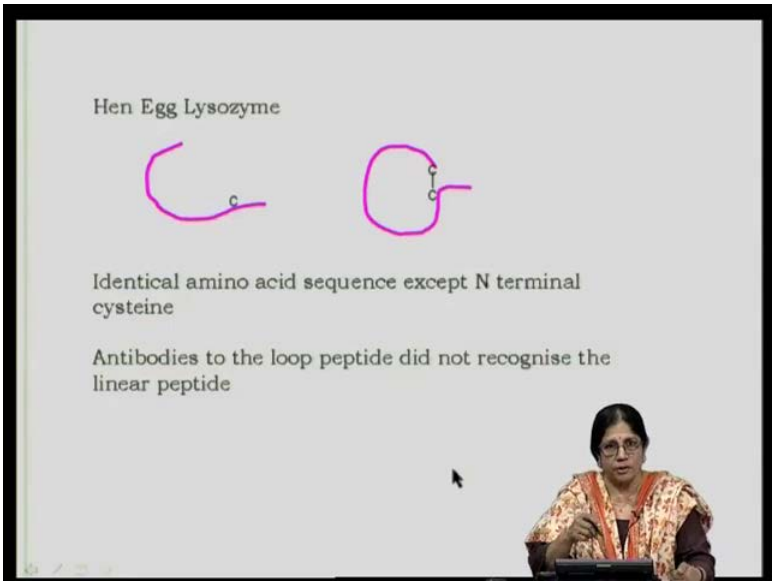

Now, 85 percent of the reactivity of the polyclinic antiserum could be accounted for in these three particular fragments. Further to the hen egg lysozyme story, this, of course, I have already talked to you about, earlier, that there were two peptides made from one of the domains of this HEL. Now, in… if the peptide was linear and was injected for making… for raising antibodies, or the peptide was now restrained by making a disulfide bond such that there would be a loop confirmation, antibodies to the looped confirmation did not recognize the linear.

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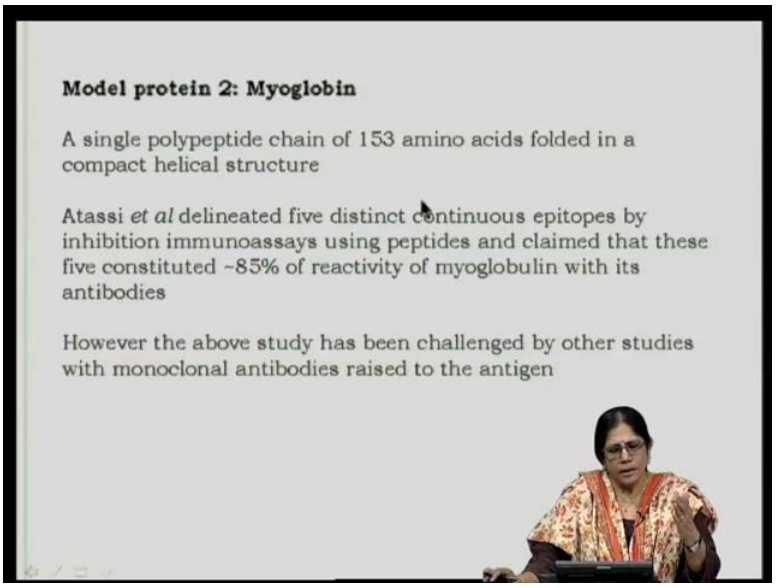

So, however, I should emphasize that antibodies to the linear peptide, to recognize that, definitely, there are distinctly conformation dependent and antibodies to continuous versus discontinuous epitopes. The third protein…; the second protein is myoglobin, which has, in a extensive study have been carried out to this protein, this is single polypeptide chain of 153 amino acids, which is folded in a compact helical structure. Many people have worked on this protein, and interestingly, one of the, you know, of course, in the study, people have found differences with respect to the immunogenic, sorry, antigenicity.

However, I will just talk about Atassi et al. who really carried out extensive analysis on the, actually, molecule dissection of myoglobulin to try to understand the epitopes present. Now, Attasi et al. delineated five distinct continuous epitopes by the following: they are made; they have injected myoglobulin, raised polyglobulin antibodies. Now, the have carried out trypsin digestion of this molecule, and taking these fragments, have looked for the arbitrary of this fragments to inhibit the binding of myoglobulin to the antibodies, to the respond that there were fine continuous epitopes which constituted, or were about able to bring about inhibition to, but 85 percent of the reactivity of myoglublin with its antibodies.

So, they said that there are more continuous epitopes, rather than discontinuous epitopes, but like I said that, you know, this above study has been challenged by several other

studies, and especially by monoclonal antibodies that was raised to the antigen. Never mind.

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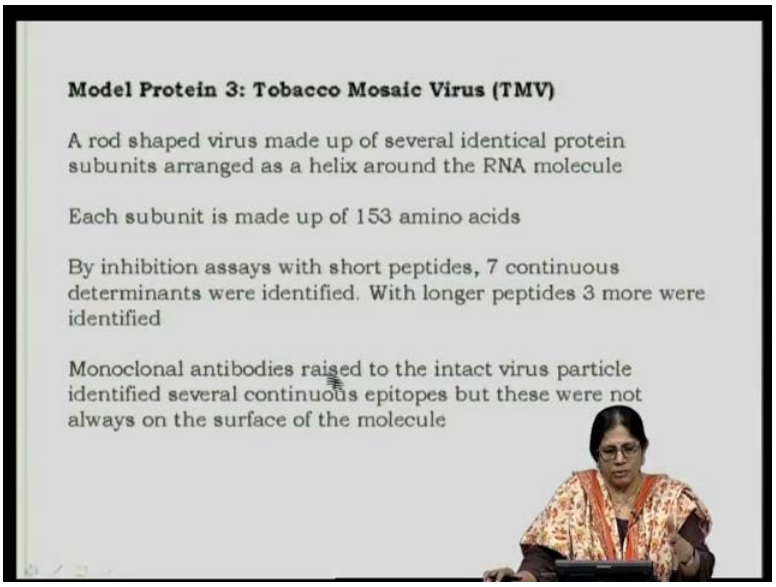

We will go to the third model protein. This is a much more complex, you know, we started off with a smaller molecule of HEL, but it has four intra-disulfide bonds, and we went on with myoglobulin, and tobacco mosaic virus is the third model protein which is, of course, much larger protein, because it is a rod shaped virus that is made up of several identical protein subunits arranged as a helix around the RNA molecule.

Now, each subunits is made up of 153 amino acids. So, what people have looked for, and these are… this will also tell you the various experiments that are carried out to dissect out the epitopes of a particular protein. Now, by inhibition assays with short peptides, seven continuous determinants were identified. So, with shorter peptides, seven continuous determinants were identified; however, when they made longer peptides, another three were identified. What does that mean that, in fact, you need to have slightly larger proteins, sorry, larger peptides, which can assume a confirmation which is similar to what is present in the native confirmation of the protein. Also, monoclonal antibodies raised to the intact virus particle identified several continuous epitopes, but these were not always on the surface of the molecule, which would mean that yes, when one immunizes an animal, you can that can antibody response, which could be to epitopes,

which are another variable when you are looking for binding of these antibodies to the antigen.

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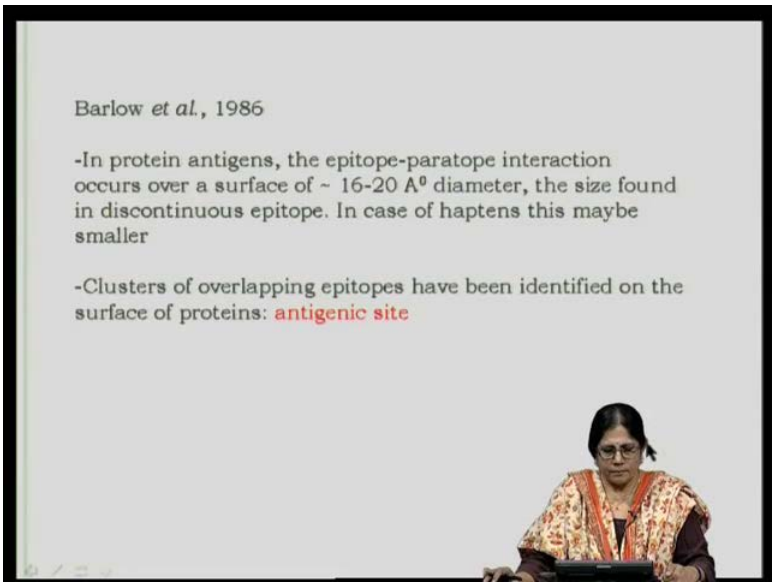

So, the contribution– I am telling you all these, because all these observations have gone on to establishing different algorithms, to be predict these epitopes. In protein antigens, the epitope-paratope interaction occurs over a surface of 16 to 20 angstrom in diameter, and this is the size found in discontinuous epitope. So, why this is an important? Because when one looks for a particular to the… tries to identify or design synthetic peptides corresponding to particular protein, then one should look at a size, which would be, at least, covering 16 to 20 angstroms. Of course, in case of smaller molecules that haptens, this maybe smaller, smaller density, to 20 above large it will be proteins it would be 16 to 20 angstrom.

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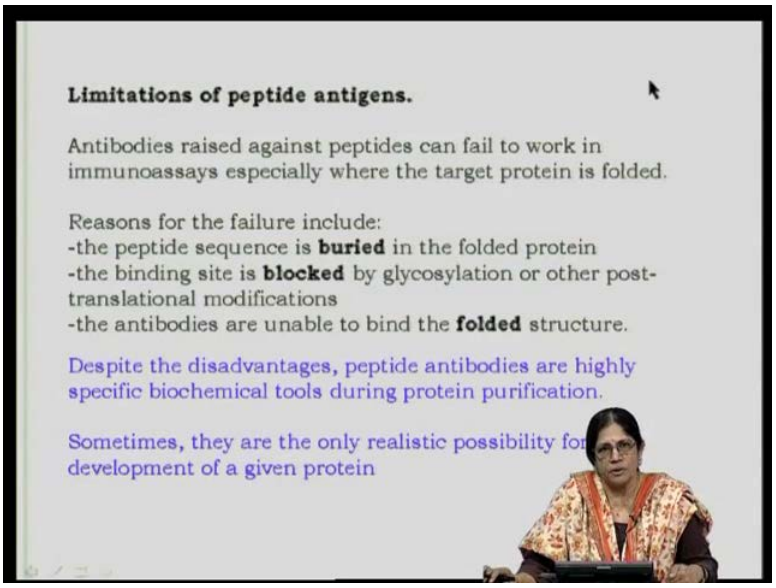

Now, will come we know that synthetic peptides are being researched upon to see, but these can induce antibodies that can recognize the native protein. A first look at what are the limitations of peptide antigens antibodies raised against that peptides can fail to work in immunoassays, especially where the target protein was, you know, folded extensively. Reasons for the failure can be that the peptide sequence is buried in the folded protein, or the binding site is blocked by glycosylation or other post translational modifications like phosporylation or acetylation. The antibodies to peptides are enable to binding folded structure, like I said in the first entrance itself, which is present in the slide, that antibody raise against peptides may not bind to the target protein if it is an extensively folded one.

However, despite all these limitations, these, which would be disadvantages peptide antibodies, are highly specific biochemical tools during protein purification. So, if you can identify a peptide, I mean, let us say from a molecule, one identifies several peptides, and I will tell you how, being looked at a recent time by algorithms, you would definitely, eventually, end of with at least one peptide which is able to induce antibody that bind to the native confirmation of the protein, and therefore, can be used the protein purification. So, extremely specific and highly, you know, extremely good biochemical tools, and sometimes, like in cases of infectious particles, peptides are the only realistic possibility for test development for a particular given protein.

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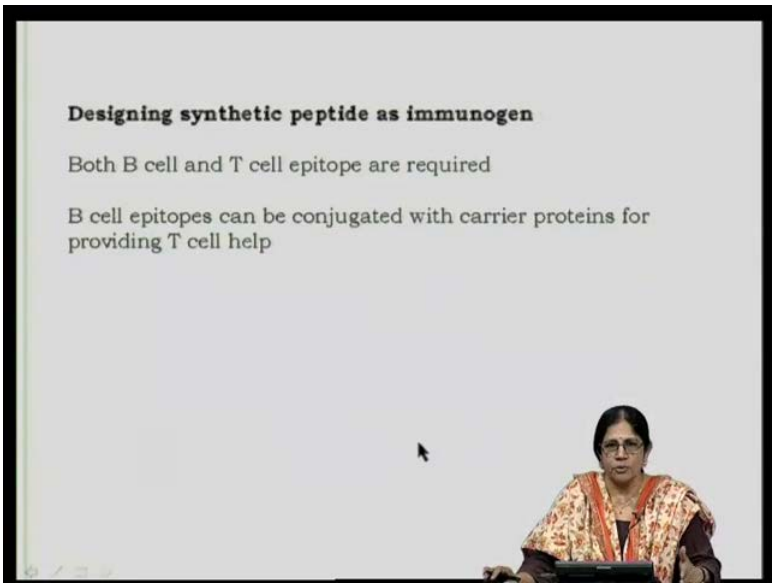

Now, we come to designing synthetic peptides as immunogen. For designing a synthetic peptide, one would need to look at both– presence of both B and T-cell epitopes. Now, why are both of them required? You would remember from my classes, that you need to have the most of the B-cells are T-cell dependent; that for a B-cell to get activated, it can do so independently, unlike T-cells which require the antigen to be presented in the context of class I or II molecules, T-cells recognize the native confirmation or the native protein. So, whereas activation process itself for most B-cells, where all B-cells, in fact, is independent of other ancillary cell, the fact remains that most B-cell require T-cell help, with respect to cytokine secretion, or you know, provided by the T-cell, as well as for providing the co-stimulatory signals.

So, automatically, then, a peptide– synthetic peptide– should have both T and B-cell epitopes, so the chances of both T and B-cells coming closer together in an activated state becomes– the chance becomes much more; however, in case the synthetic peptide has only B-cell epitopes, like would happens in case of a heptanes, the T-cell help can be provided by carrier protein. In case of vaccines, now, this would be fine in animal studies, but in case of designing vaccines for human use, of course, it would be at most importance to have both T and B-cell epitopes present in a, in the same molecules, same fragment, because one would always on the which carrier proteins to use in case of human immunization.

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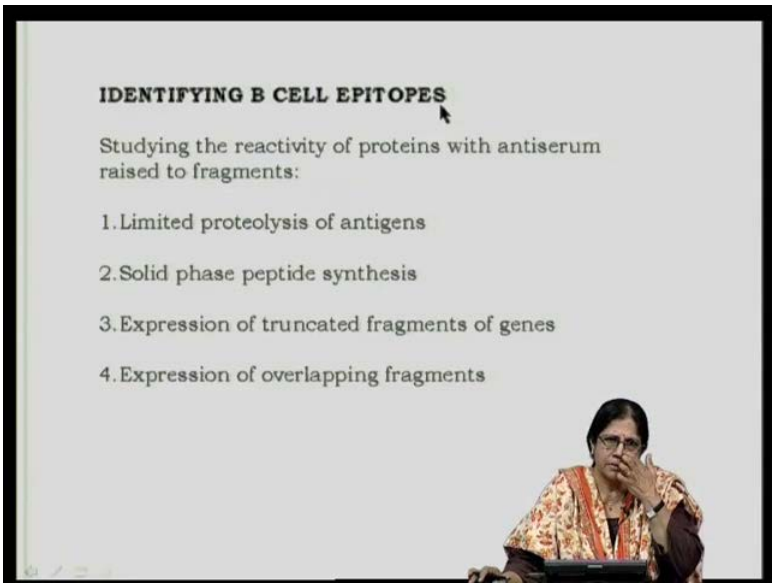

Now, how does one identify these synthetic peptides, which you know, I mean, what tells you the T-cell would be relevant? These are the sequences that could be… this is the region of the protein which would induce, or then, the antigen to the relevant protein that is also able to neutralize, that becomes important to do. That is the part of this seminar– this lecture– I have already dealt with in my lectures on antigens.

How these one identified B-cell epitopes? Since we talking about synthetic peptide, I will go over this a realistic. Briefly, let us say you have protein. Now, from pathogen, you have the amino acids sequence, that is like to find out which of these regions are B-cells epitopes. Studying the reactivity of proteins is… can be done by studying the reactivity of the proteins with antiserum raised to the fragments. Which fragments? Those made by, you know, obtained by limited proteolysis of the antigen which are made by synthesis– a solid phase peptides, or if one uses recombinant DNA technology and expression of truncated fragments of genes. So, have the gene of the particular protein corresponding to the protein, then it is not difficult to now truncate gene from either the 5 or 3 prime, and then, you know, get smaller and smaller fragments, and find out which of these now can, let us say, yield antibodies of any consequence, of any significance, and alteration of obtaining truncated fragments have expression of overlapping fragments.

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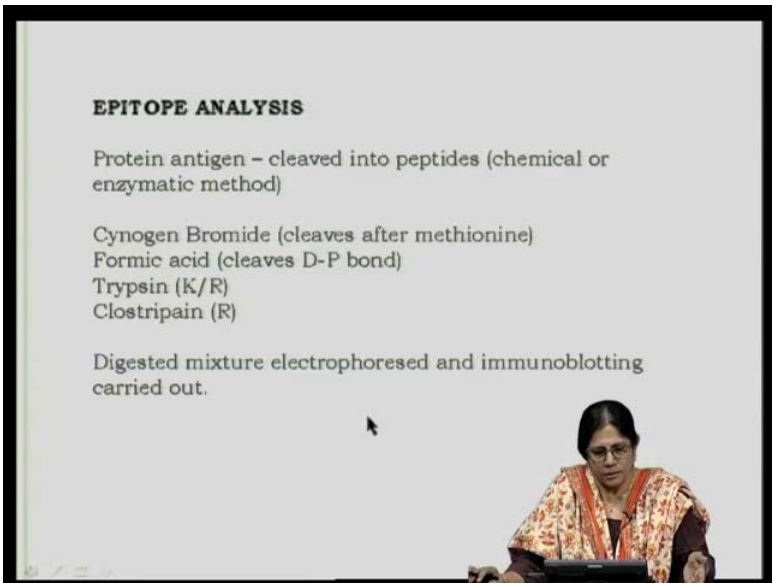

I have, again, this also repetition of what I dealt with, now. This emphasize could be different one, can also carry out epitope analysis by cleaving protein antigens by chemical or enzymatic method; ones that are method commonly used, you know, to be able to identify, let us say, you have monoclonal antibodies to a particular protein, and, you know that one of the monoclonal antibodies is able to inhibit replication of that particular virus or bacteria. This is only an example that I am giving you, or let us say, it is a particular and an you have enzyme as the antigen, and you have monoclonal antibody to this particular enzyme, and you find one of the monoclonal antibodies is able to inhibit the enzyme activity. That means, it is not allowing substrate binding, let us say, or you have an anti… you have a toxin molecule, and you find that amongst, let us say, group of five monoclonal antibodies, one of them is able to neutralize the toxin activity, or is able to prevent toxin from binding to its receptor, or preventing the toxin from gaining entry into the cell, and therefore, toxicity.

Now, I like to find out, instead of using the toxin you cannot use the toxin as a whole for injecting, you know, for active immunization, because the toxin would be toxic. So, instead, suppose you would like to use a fragment of this particular toxin, which would afford, and you would like to see whether this can, you know, protect the cells or an individual from toxic activity, or may be inhibiting the transport of that toxin. So, to do that, one can fragment. Thus, the premise here is that you already have monoclonal antibodies, and that is what we started off with, that one of the monoclonal antibodies is

able to affect this. So, using the monoclonal antibody, you can identify. How would do that, is cleave the protein by different methods into smallest fragments– cynogen bromide which cleaves after methionine, formic acid which cleaves the aspartic acid-proline bond, and this, of course, not to allow molecules have, but if you molecule has it, there you can use that; trypsin which cleaves after lysine or arginine, clostripain which cleaves own your arginine, and let us say you use all these four different procedures, by which you can cleave the fragments of the protein into fragment, and this digested mixture can be electrophoresed.

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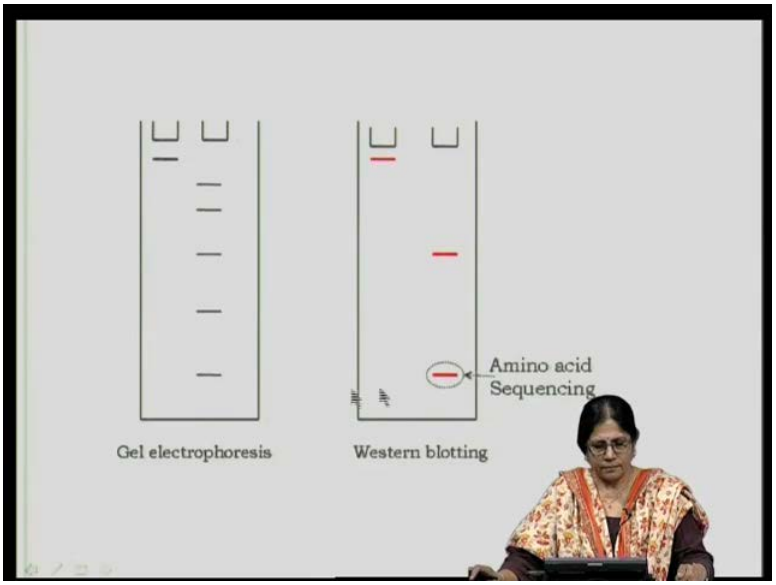

This is one such very simple diagram, but you have the undigested molecule, and that one that is fragmented by it could be any of the four methods that I have mentioned, and you have an identical gel, which is transferred to a blotting membrane. Then, using the monoclonal antibody, which I said would be of… which has the capacity to neutralize toxin activity, this, of course, is not monoclonal antibody. Let us say one monoclonal antibody identifies this, another monoclonal antibody identifies this. In any case, when you have different fragments, you may… you can get more than one fragment that is identified even by a monoclonal antibody. This smaller stretch would definitely be a part of a larger stretch. So, if you do N-terminal amino acid sequencing, you know where does this fragment come from, and if you have identified this around 1500, so that, say a 100, and you know what small fragments of amino acid corresponding into that.

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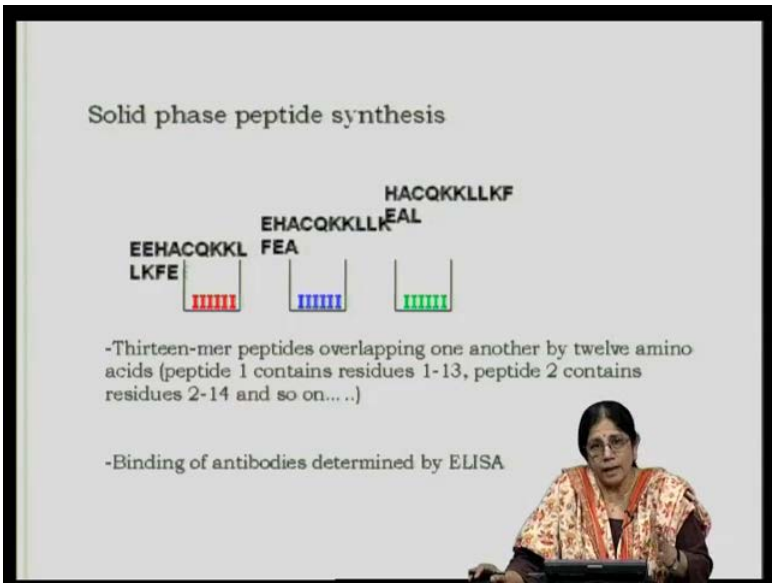

Then, we can go on to identifying the peptides by solid phase synthesis, while you can literally find out the core epitope region. This is a little bit more expensive procedure, but it gives you precisely or peptide corresponding to approximately one to eight amino acids, which forms the core of the epitope. The solid phase peptide synthesis is done in the following way: there are… this is typically done on pins, which are well depicted here, not as pins, but the pins have reactive groups on which one can start synthesizing peptides, up to about 13 to 15 residues starting from the C-terminus, and then you can make a series of peptides with just a shift of one amino acid.

These can then be checked for binding to the monoclonal antibody. Remember, the one that I said a little while ago has been identified to have neutralizing activity. Now, we already have identified, from the previous experiment, a stretch of large peptides, like I say, 25 or, maybe, 35, which are capable binding the antibody, and now, you go to fragments of peptides generated in sequence from the stretch. Let us say from the you can you may 13-mer peptide overlapping one another by 12 amino acids; that means, peptide one contain residues 1 to 13; peptide two contain residues 2 to 14; and then peptide three would contain the residue three to 15 and so on, and then you check for binding, and definitely, you will be able to identify the precise sequence to which the monoclonal antibody… Then, it is a question of just synthesizing the peptide.

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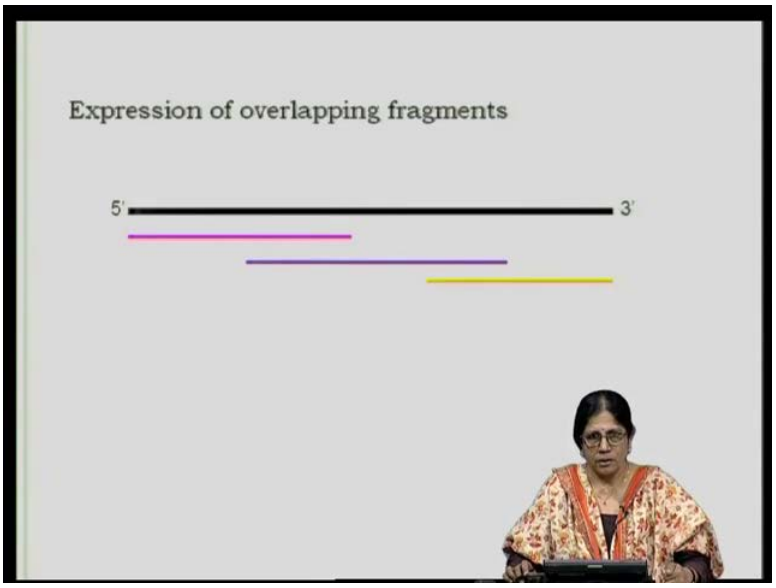

Alternatively, one can express different overlapping fragments corresponding to a particular protein by taking the gene, and you amplify fragments of the gene, cloning each one and expressing the protein. So, now, you would have fragments number 1, which, let us say, is from 1 to 100, the next one is from 50 to 150. So, you have fragments of 100 amino acids, and now these can be checked for binding by ELISA, by western blot, and then you can narrow down the region to which that monoclonal antibody, which has a relevance, has an epitope. Then again, one can, either by molecular biology approaches itself, then make, let us say, you have the region which is here, because peptide 1 and peptide 2 of fragment 1 and fragment 2 bind.

So, you know it is in the overlap region, and then, by subsequent deletion and addition, one can narrow down even further, so that one can make a synthetic peptide. Another approach would be gene constructs made with truncations on the 5 prime side, the 3 prime side. The protein fragment is expressed and checked for binding with antibodies. So, you have another gene sequence, 5 prime 3 prime side, and you can start, now, truncating the protein, and this one can, if you have to crystal structure of the protein, actually, it becomes even easier, because you then know that the truncation can be made in such a fashion that you do not abrogate any of the secondary structure. You know the protein can be folded correctly when expressed, and as recombinant protein. So, you can, again, you are able to identify.

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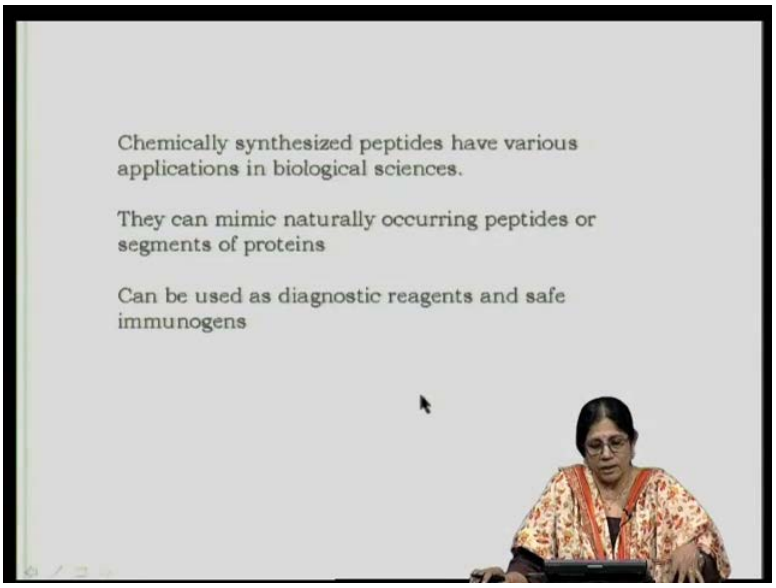

So, chemically synthesized peptides have various applications in biological sciences. We know that they can mimic naturally occurring peptides, or segments of proteins, and can be used as diagnostic reagents and safe immunogens. Now, chemically synthesized peptides have various applications in biological sciences. This surmises, of course, only based… now, this can be only be if these synthesized peptides are able to mimic the corresponding region in the context of the entire molecule. This is something that I cannot emphasize more. Only then can they mimic the segments of the protein, alright? But we know now, that of course, peptides are being used. So, obviously, it is possible; you just have to hit upon the right synthetic peptide.

Now, where synthetic peptides, so far, have not come into the… you know there is no successful story which respect to synthetic peptides as immunogens. Synthetic peptides are extremely useful as diagnostic reagents. One very good example I can tell you is synthetic peptides to the human immunodeficiency virus– AIDS virus– is being used– are being used– not is, are being used. There are certain peptide which are being used in diagnostics. HIV, you know, to be able to work with the virus, one needs a very restricted and very safe laboratory zone. Therefore, people would, of course, not be able to use… work with the viruses, but one can always work with synthetic peptides corresponding to the virus.

Now, taking… making observation from antibodies generated in human, this has also lent a lot of information, but one can take– people have taken– antibodies, looked at the antibody response from various individuals– infected individuals– infected with HIV, of course. Those that have been infected and have come down with the symptoms, those who have been infected, but are long time protected; those that have been infected with the virus, but have still have shown mild symptoms. So, looking at the neutralizing, you know, this gave information on which antibodies were relevant with respect to neutralization of the virus.

There are individuals who have been infected and have, you know, just not had any symptoms whatsoever. So, that means, they are able to combat the infection, combat the disease, combat the virus itself. So, taking a cue from all this, a large number of peptides corresponding to the envelope protein of the virus, which have been identified, and these peptides are been used to coat ELISA plates, to be able to find out whether the person is infected or not. Peptides can be used as diagnostic reagents and extremely safe immunogens.

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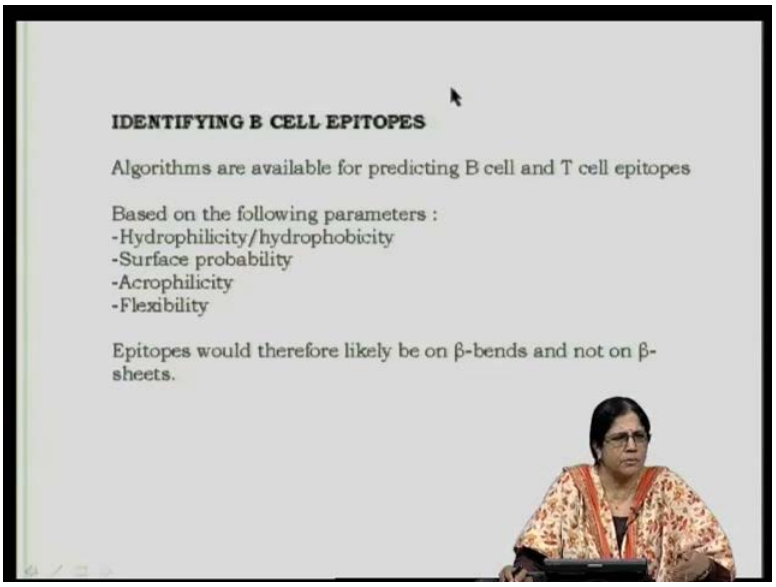

Now, how does one… I talked about HIV– Human Immunodeficiency Virus– and there, it was possible for people to look at the immune responses and find out which of these might give a clue with respect to the infectivity status of a person, whether a person has diagnosed it, but in other cases where such an information is not available, or such sera

are not available, how can one identify B-cell epitopes? Like I said a little while ago, algorithms are available for predicting B and T-cell epitopes. T-cell epitope prediction is little bit more difficult, because remember, predicting T-cell epitope means one would need to predict not only the region that could bind of the peptide that binds to the T-cell receptor, but also that part of the peptide, now, which would dock on to class I and II molecules. We do know that for docking on to class I and II molecules, there are certain sequences which are absolutely essential.

So, not all peptides that are generated by cleaving of the proteins intracellularly, actually, can be immunogenic with respect to T-cell epitopes. So, for predicting T-cell epitopes, one also needs take into consideration the sequence of the molecules of the peptide, which can dock on to the highly polymorphic class I and II molecules of humans. B-cell epitope prediction, however, is much simpler compared to T-cell epitopes. So, how does one identify B-cell epitopes? The algorithms are based on the following parameters: each of these, of course, also has relevance.

B-cell epitopes can be predicted by the four parameters: hydrophilicity; hydrophobicity of that particular stretch of amino acid; the surface probability of this stretch of amino acids on the molecule, you know, they are when we mean the surface probability, it is with respect to the folded confirmation of the protein and availability of this on the surface of the molecule, because remember, I always kept saying that B-cell epitopes, when B-cells recognize through the antigenic receptor, native conformations– the protein, as such.

So, therefore, all molecules or all antigenic determinants, therefore, would be on the surface, or at least, these are the ones that would be relevant– acrophilicity, as well as flexibility. So, acrophilicity is accessibility even if the molecules is not… even in the molecule is on the surface, but if it is not accessible because of presence of glycans or phosphates or any post translation modification, then that is not available for B-cell interaction.

Now, flexibility is that, you might remember, that when we dealt with antigen-antibody interaction, though antigen-antibody interaction is dependent totally on… is dependent on complimentarity between the two paratope and the epitope, we know that after the first interaction, there can be induced fit process taking place which allows these

interactions to become tighter with time. So, therefore, the molecule on the surface, or the epitopes, become should be flexible. People have shown that though the initial binding may have a particular, the first contact may have give a binding of an affinity X. This might increase– would increase– because of flexibility of the antigenic epitopes, which can now flip the paratope and thereby, there is an increase in the affinity or stronger binding, so flexibility. Also epitopes, because of all these, epitopes would be more on bends than beta sheets, because beta sheets always usually in the interior of the molecule, and the beta bends would be exterior of the molecules. So, epitopes would, therefore, likely to be on the beta bend.

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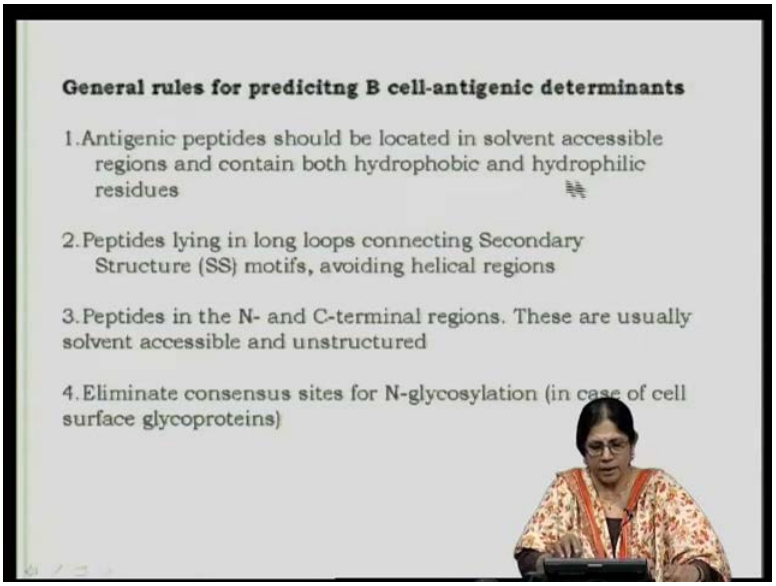

So, there are also general rules for predicting B-cell antigenic determinants. The rules are, again, based on the four parameters I mentioned– antigenic peptides should be located in solvent accessible regions and contain both hydrophobic and hydrophilic residues. One, that which would mean that, again, these would be more likely to be surface exposed, because in an aqueous solution, in the protein would always have a folded confirmation such that there would be more hydrophilic regions outside of the molecule, so, solvent accessible. Predicting B-cell antigenic determinants on peptides, which lie in long loops connecting secondary structures, and avoiding helical regions. Now, of course, there are reports where antigenic determinants have been found in helical regions, but much more on beta bends. So, in the secondary structure prediction, if a region is corresponding to a beta bend, and also, this region happens to be, definitely,

on the surface, then it is more like most likely to be an epitope or an antigen determinant of the protein.

Now, third rule– peptides in the N and C-terminal regions are, usually, the immunogenic ones or harbor the antigenic determinant, because these are usually solvent accessible. The N and C–terminal, again, I am saying, usually, and these are unstructured. So, they are likely to be epitopes. Again, I will reiterate what I said earlier with respect to acrophilicity– consensus sites for N-glycosylation, specially in case of cell surface glycoproteins, can be deleted, you know, as being an epitope, because post translation modification, even you know it happens to be an region which is hydrophilic and surface probable, may not be an epitope because the glycan would inhibit the B-cell receptor from binding the epitope.

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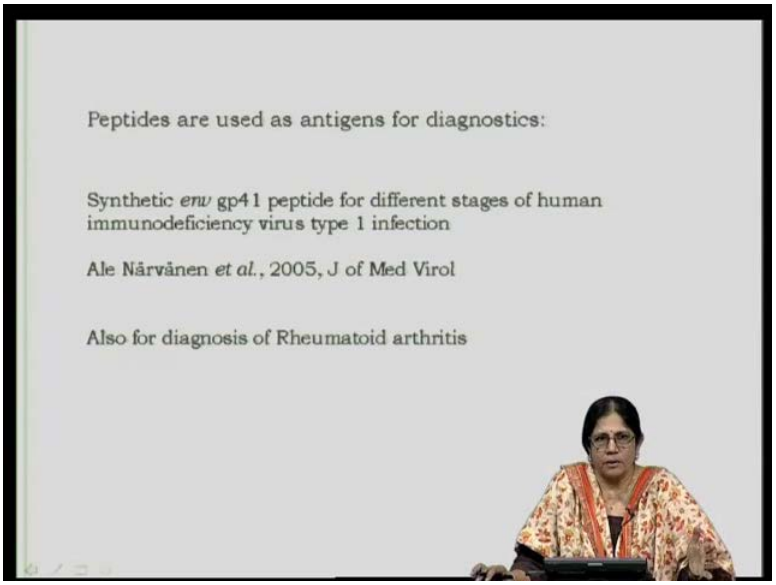

Are there peptides which are been used as antigens for diagnostics? There are several– but the ones that are known are synthetic envelope GP 41 peptide, which is used for different, you know, to diagnose to the infection– at different stages of infection– in Human Immunodeficiency Virus type I infection. So, synthetic… This is very much in the market, and there are, like I told you earlier, that glycoprotein 41– there are different epitopes that are being…, now simply peptides corresponding to different epitopes. This is our success story. Also, what is in the market are peptides corresponding to rheumatoid arthritis.

Now, how does if you might remember one of the reasons for autoimmune disease rheumatoid arthritis is antibodies– accessibility of a particular region on the FC region of immunoglobulin G, which becomes accessible, because of, let us say, lack of appropriate glycosylation. Now, to be able to find out if the arthritis is because of an autoimmune disease, it is a rheumatoid arthritis, than inflammatory arthritis or osteoarthritis, then antibodies can be measured in the blood of these individuals to region– a synthetic peptide– corresponding to the region in the FC portion, which is available in these individuals because of lack of appropriate glycosylation.

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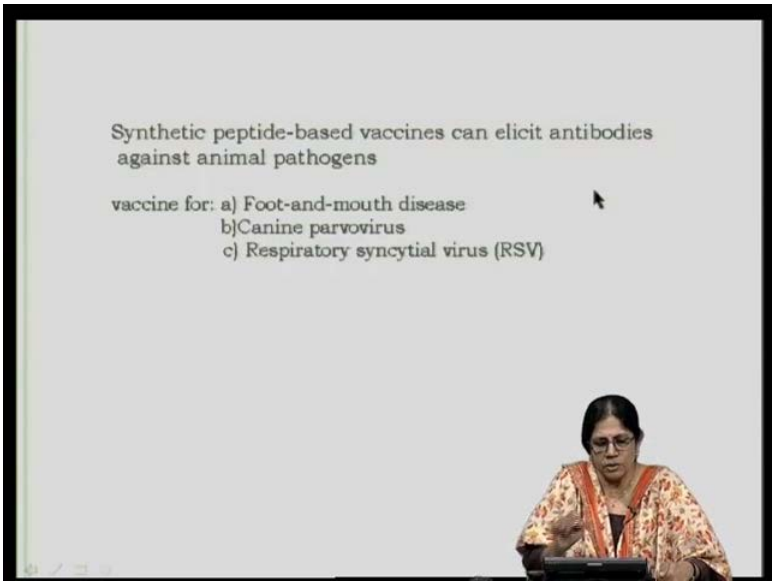

So, this is also other success story, not in human, so much, but synthetic peptide based vaccines have been shown to elicit antibodies against animal pathogens, and the again a success story is foot and mouth disease and canine parvovirus, alright?

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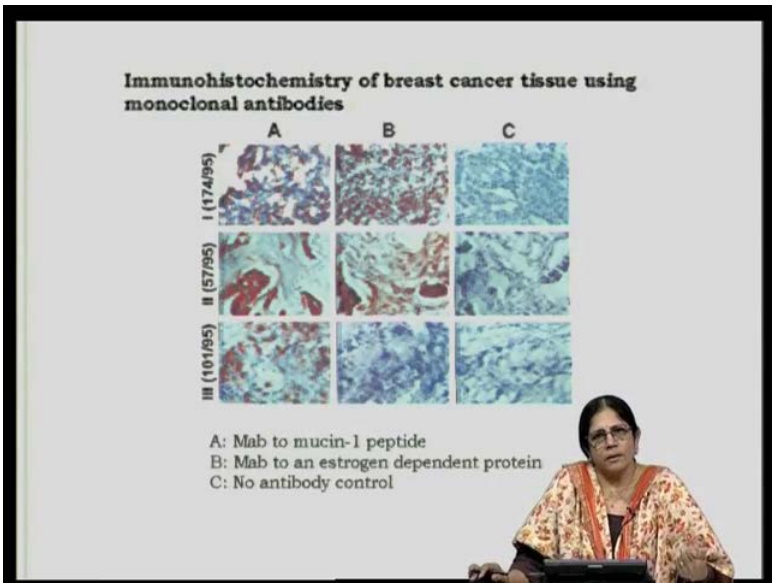

Now, let me go into success stories with respect to synthetic peptides which have been used to raise antibodies against antigens which are very difficult to obtain from from cells, let us say. Now, let us say, one is looking at large molecules– large antigens– which are found intracellularly. Now, the concentration of proteins is extremely low. Even if few one is able to… one decides to isolate the protein, how does one do it, especially if do not have antibody? Like I said in little while ago that synthetic types can be used, really, in those areas, you know, in those contexts where is difficult to purify the protein– the native protein– to even be able to purify the native protein, ultimately, to be able to characterize it.

Suppose you obtain the synthetic peptide corresponding to this protein. Let us say you know the gene for the protein; you know the gene sequence from which one can determine the amino acid sequence, and now, using different algorithms, one can, you know, one can use, let us say 3 4 algorithms, which are available for predicting B-cell epitopes. You make the synthetic peptides corresponding to the most probable B-cell epitope, and now make antibodies to this. Antibodies is to this can now… first, one can check whether the antibodies can actually recognize the protein which is present in the cells. After one gets the go-ahead signal, then you can make immunoaffinity column from the anti… you know, the antibodies to the synthetic peptide, and now, using this, one can hope to purify the protein from a multitude of proteins present in the cell lysate.

One such experiment I would just like to tell you– there are several, I mean, millions of such reports which are available in literature. Let me just point out: this is a immuno histo-chemistry micrograph of breast cancer. There are three different breast cancer tissues, and this panel shows– the A panel shows– the sections of the tissues– breast cancer tissues– has been stained using monoclonal antibodies, which are raised to a peptide corresponding to a protein, which is expressed in very high concentration in upon carcinogenesis– upon cancer establishment– and one can see that the antibody, which is raised to a peptide, is able to bind to that particular mucin I protein present.

Now, this is a monoclonal antibody to an estrogen dependent protein. This is a larger protein, and of course, this is the control. So, one can use this. Now, you know that this mucin I peptide over expression occurs in… upon the transformation of normal breast tissue to cancerous one. So, the monoclonal antibody is not to the whole molecule mucin peptide, but it is to a fragment thereof. In fact, if one is able to purify the mucin I, the entire protein one immunized for getting antibody, it is very difficult to get the antibodies, because especially in case of breast cancer, the protein becomes highly glycosylated– the mucin one protein.

Therefore, if one makes the peptide, then you can get, definitely, antibodies to the protein and can be used, too, in diagnosis. So, you can see, very clearly, this is just an example. There are very large number of such cases where antibodies to synthetic peptides can be used in diagnosis, not only what I mentioned earlier with respect to diagnosis of diseases such as HIV, but also to find out whether a particular tumor is benign or malignant.

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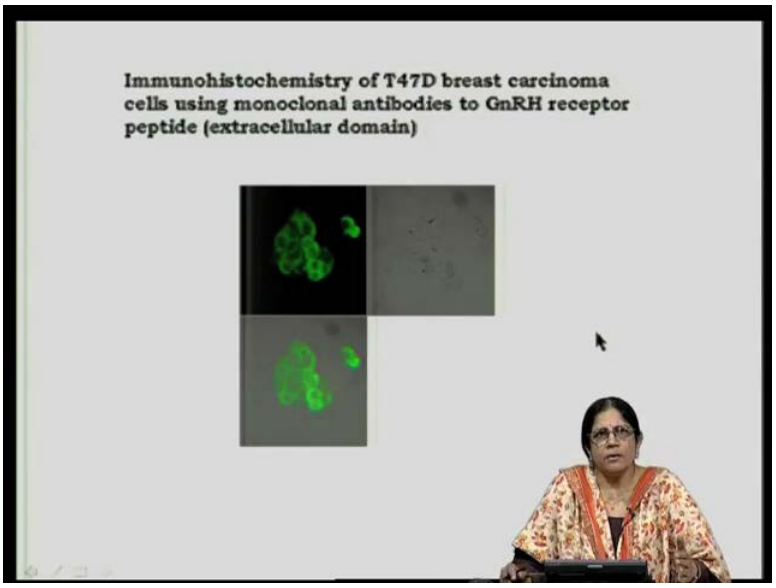

This is yet one more example of antibodies made to a receptor. Now, especially, you know, receptor are difficult to purify, especially receptors like gonadotropin releasing hormone receptor, which is made up of… which is… which belongs to the family of seven trans membrane receptor. So, this polypeptide spans the membrane of the cell 7 times . So, therefore, extremely hydrophilic– difficult to extract the protein from the cell membrane. It is difficult to obtain the receptor, to purify the receptor.

Therefore, now, once the sequence of the receptor became known, then using algorithms to the extracellular domain and the loops, a synthetic peptide was synthesized and this was injected into mice, monoclonal antibodies were made, a few of the monoclonal antibodies recognized the native receptor, and one of these monoclonal antibodies, shown here, to bind to T 4 7 D– a breast carcinoma cell line– which overexpresses this receptor. Normally, GNRH receptor is present in the pituitary gonadotrophs, but upon carcinogenesis, some cancer cells overexpress this receptor, and therefore, these antibodies can also be used as diagnostic tools.

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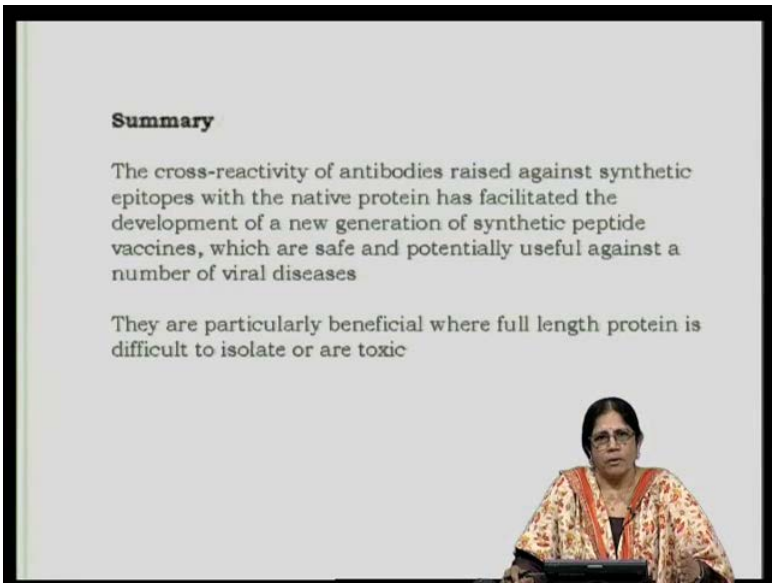

So, I have just given you a glimpse of where and in which context synthetic peptides can be used. Synthetic peptides can be used both in diagnosis as well as vaccines. So, in summary, the cross reactivity of antibodies raised against synthetic epitopes with the native protein has facilitated the development of a new generation of synthetic peptide vaccines, which are safe and potentially useful against a number of viral diseases.

Why are these safe? You might remember that in case of certain viruses, which have been used, even though in an attenuated form, have been use as active immunogens. In some cases, they have induced the disease itself because of reversal of this attenuation. Synthetic peptides– this does not arise; this danger does not arise. So, synthetic peptides are particularly beneficial when full length protein is difficult to isolate or are toxic. So, these are the benefits of synthetic peptides and use of the synthetic peptides in vaccine development as well as in diagnosis. Thank you.