Essentials in Immunology

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Module No. # 21

Lecture No. # 38

Antigens and Immunogens

In the last several weeks, we have been discussing B cells, the receptors present on B cells, and the kind of structures present on the cells we know as immunoglobulins, which serve as antigen receptors. We have seen how B cells produce antibodies, which are nothing but, again, the same receptors in soluble form. We have also seen the structure of these immunoglobulins, the classes, the sub classes; we have also seen the functions of immunoglobulins.

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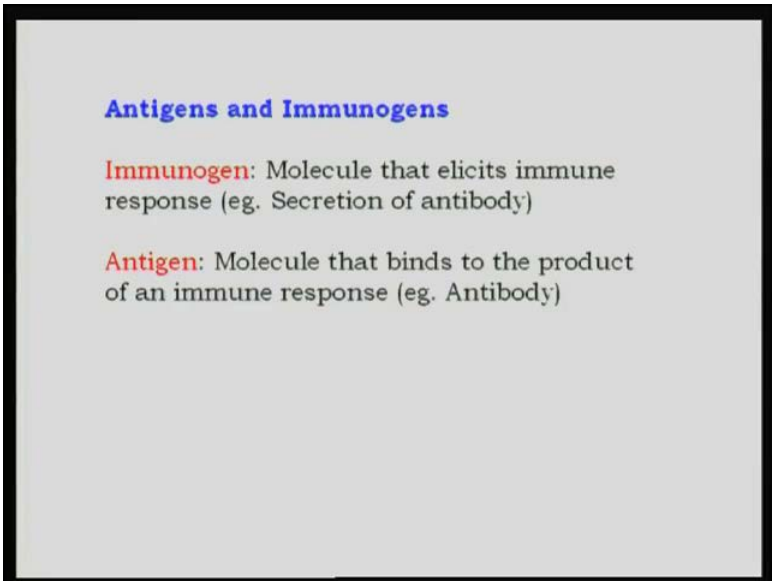

Now, let us look at the other aspects. Let us look at what these cell surface receptors as well as antibodies recognize. Of course, we know them as antigens. Now, let us look at

two words– antigens and immunogens. Antigens and immunogens are, sometimes, used to describe, not at a very specific manner, what these molecules are– are there any differences between antigens and immunogens? Both are, of course, extremely related, but there are subtle differences, and the definition of which I am going to start my lecture with.

Immunogen is that molecule that elicits an immune response. It triggers an immune response, for example, in case of the humoral response or the B cells, an immunogen would trigger the cells to proliferate, differentiate, to become a plasma cell that secretes antibody. In case of T cells, it would be that– an immunogen would be that molecule that can elicit an immune response in T cells, again, for proliferation and, subsequently, secretion of interleukins or cytokines, which help other cells for generating an immune response or for differentiation into cytotoxic T cells, now, that would be an immunogen– that which elicits an immune response.

On the other hand, an antigen would be a molecule that binds to the product of an immune response, and in case of B cells, you have guessed it right– the product of the immune response would be antibodies. So, an antigen is that molecule, which would bind to the product of the immune response, in case of B cells– antibody, and in case of T cells, it would be that which is able to bind to. For example, now, product of the immune response in this case would be, in case of cytotoxic T cell, would be the target cells.

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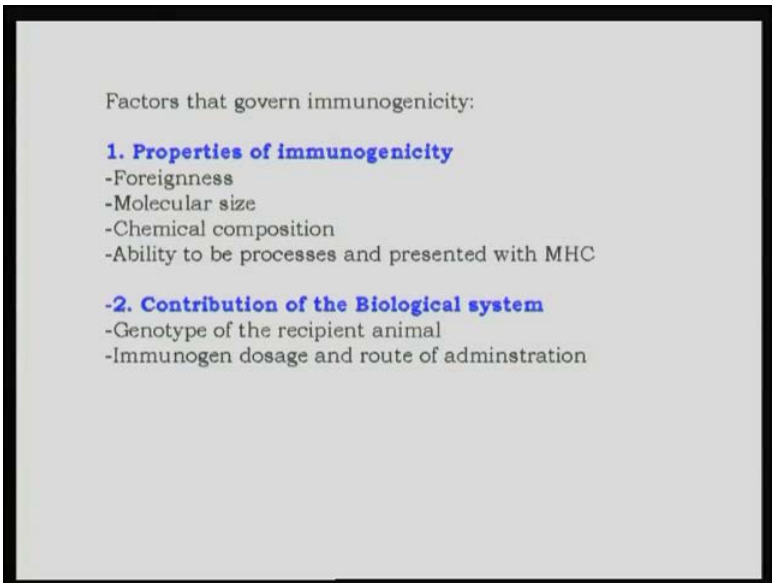

Of course, there are large number of factors that would be governing immunogenicity. Let us first talk about immunogenicity– what were these factors? Those that would be the properties of the molecule itself, and the second would be contribution of the biological system, that means, the host. Properties of the immunogenicity with respect to the molecule would mean what kind of a structure does it have; how foreign is it.

So, based on that, there are at least four different properties that confer immunogenicity, and which regulate the level of immunogenicity. Now, is there something called level of immunogenicity? Yes, of course. If a molecule is very immunogenic, or we would say, a molecule is very immunogenic if it elicits an immune response or antibody secretion, which has a high titer and which is also over a long periods of time.

So, therefore, that this would mean it is a better immunogen than that which elicits an immune response, but the antibody secretion, the amount of antibody secreted or the affinity and the duration is much lesser. Let us just go over the words– properties of immunogenicity would be determined by foreignness, molecular size, chemical composition, and the ability to be processed and presented with the class of molecules– MHC– which probably, you are going to listen to from others. Contribution of the biological system– there are two that come under this genotype of the recipient animal and immunogen dosage, and route of administration. Now, let us look at this in a little detail.

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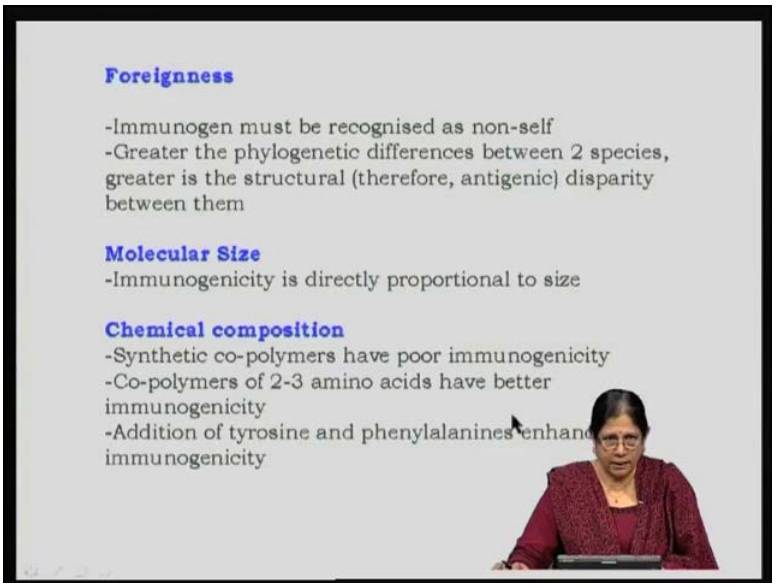

Let us start off with the first, that is, foreignness of the immunogen. We know, already, by now that the immune system is based on the central dogma, that it has the capacity to recognize self and non-self. So, we know, therefore, that the immune response can be activated by molecules that are foreign– that are not presented in our bodies, because if you might remember, because during the development of B cells, those cells which have the capacity to recognize self molecules are deleted from the system, or at least most of them are deleted. A few of them, of course, continue, but most of them are deleted, which would mean that the first thing, immunogen must be recognized as non-self.

Now, let us take an example of serum albumins. A serum albumin, as the name suggests, these are molecules which are almost the same molecular size and present in serum of all mammals. Now there are of course, differences between albumins from one species to another; however, there are also several communalities. An individual from one species would be able to recognize albumin from another, but because of similarities, which are…, which would be seen in between species that are closely related, one can say that a host would recognize albumin from the species to which it is not closely related. Therefore, greater the phylogenetic differences between two species, greater is the structural disparity– structural– therefore, antigenic disparity between them, and therefore, species which are phylogenetically far from each other would definitely be able to mount immune response to one and another's molecules. I have already given

you the example of albumin. Let us say, now, if I can go with examples, a monkey would not respond to human albumin.

However, a monkey would be able to respond by way, of course, secreting antibodies, would respond to albumin from chicken or from rat. Albumin from rat, when injected into mice, may not evoke a immune response, because these are closely related rodents; however, a rabbit, which is close enough, but yet far away with respect to the immune response, would be able to recognize and amount an immune response to mouse immunoglobulin, sorry, mouse albumin.

So, therefore, greater the phylogenetic differences between two species, greater would be the immunogenicity, alright? Now, here, because I am talking about structure, I have antigenic, because we are talking about the molecule. What we are dealing with, right now, of course, is the immunogenicity.

So, foreignness– molecules have to be different from each, from I mean, structurally, for them to be recognized, of course, because self, I mean, B cells recognizing self, or therefore, closely related species– molecules form closely related species– would be recognized, also, more or less self. Let us go the next molecular size.

Immunogenicity is directly proportional to size– this, one can understand very easily, because as I have already described to you that if it is a large molecule– a large immunogen– the number of antigenic determinants which are present on that molecule will also be more than a smaller molecule. Let us say, a molecule of size 1,50,000 versus a molecule which is only 50,000, there would be at least three times the number of antigenic determinants on the larger molecule.

So, therefore, the number of B cells that would be triggered to generate an immune response and make antibodies, would also be more when we are talking about a larger molecule. So, therefore, immediately, immunogenicity is directly proportional to size. One can also look at it from another angle; larger molecules are retained for a longer period of time in our system, therefore, allowing B cells to recognize and generate an immune response.

What is the size of the molecule which can be immunogenic? Now, in earlier time, I mean, which is about 20 years ago, it was thought that any molecule which has a size of

more than 5000 or 5 kilo dalton is has the capacity to generate an immune response, but later on, with most sensitive assays and the ability to even detect small quantities of antibodies generated in an experimental animal, now, we know that even small peptides can generate an immune response, provided both, I mean, they the peptide has both T as well as B cell epitopes. Now, we will, of course, come to that a little later.

So, size is important; the larger the molecule, the better would be the immune response generated. Chemical composition is the third. Now, of course, when I say large size, automatically, it would mean that, I mean, would it mean that the molecule is diverse or complex immune structure? It need not always be.

However, when we come to, I mean, we do not think of, we come to natural conditions and not experimental, it holds to that, mean you have an immunogen which is large in size would definitely have more complexities, more structural variations than a smaller molecule. Experimentally, let us look at whether this would have any relevance. What people were, I mean, when people were trying to understand immunogenicity, they were looking at synthetic polymers.

So, when they generated synthetic polymers of, let us say, simple amino acids, let us say, simple one, like glycine, they found that if you had, you know, a glycine chain– a chain of only glycine, the amino acid glycine– and there is absolutely no immune response generated to this; however, synthetic copolymers which have more have more than 2 to 3 amino acids, and in random sequence, have some immunogenicity– better immunogenicity– than just synthetic polymer of one amino acid, they were able to see as the complexity grooves, which, I mean, which are not written here. They are, actually, determined the immunogenicity for each of the molecules, but when they had polymers which had more than 2 to 3 amino acids in the sequence, of course, the complexities grew, and therefore, the immunogenicity slowly started to increase.

Now, interesting here is that when they added to these 2 to 3 amino acids, which would be, let say, hydrophilic, they added tyrosines and phenylalanines at regular intervals. This enhanced the immunogenicity several fold. Now, there is this kind of data available, and one can refer to that which amino acids appear to be, which appear to be harbored in epitopes, are there such repetitions of amino acids? It is something that one can look at

the database and derive, but we know that if there is complexity with respect to structure, there is better chance of the molecule being an immunogen.

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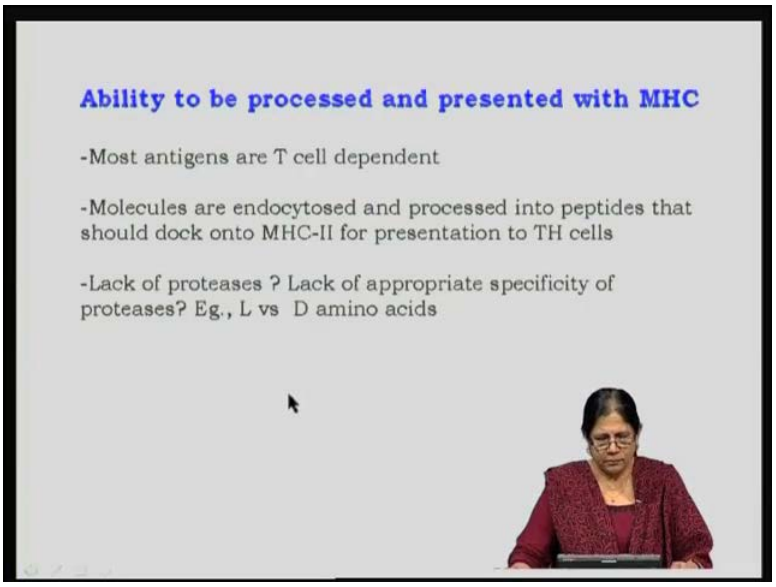

What is this ability to be processed and presented with MHC? What does this mean in the context of B cells? You know that B cells recognize through their antigenic receptors or their B cell receptors, which are immunoglobulins. They recognize native conformations, by now, you also already know that T cells, on the other hand, need to be presented with peptides, either in the context of class I molecules as in case of cytotoxic T cells, or in the context of class two molecules as is the case with the helper T cells, but most B cells, though they recognize native structures, they require T cell help for that proliferation and differentiation.

So, most antigens are T cell dependent– that we know, which would mean we also have to think in terms of the immunogen being… having the capacity to be endocytosed and processed into peptides. Now, why is this relevant, because after all, one can imagine that antigen presenting cells would definitely have the capacity to also present peptides, but now, they have to generate the peptides. So, presentation is one– presenting to T cells is one, but can they generate these peptides?

In a regular system of course, all are cells– all are nucleated cells– have proteasomes, and you must have studied this already, which can break peptides into, I mean, proteins into small peptides, and which can be docked on to class I molecule in the endoplasmic

reticulum. Similarly, with respect to class II molecules, now, which are… this is the extrinsic pathway, antigen presenting cells such as macrophages and dendritic cells and B cells have the capacity to endocytose these foreign molecules, and now, degrade these molecules, and peptides which are generated can be docked on to class II molecules are presentation to TH cells, but is it is there a possibility that some proteins cannot be processed? Yes.

Now, in mammals, you have amino acids which are made up of the isomer D– D-amino acids. So, therefore, the corresponding proteases would also be able to cleave only D amino acids, sorry. I am very sorry; I will go back; L amino acids. Now, if we come across peptides, let say from parasites of plants which have D amino acids, then we will not be able to cleave. We will, we do not have enzymes– the proteases– that can cleave. Therefore, we will lack of cleavage would mean there would be no peptides generated or very few peptides generated, and thereby, they will not… they will not be… cannot be docked on to class I or II molecules, and therefore, the immune response is curtailed. Please remember that what I… when I talked about L versus D, we have L rather than D, when we counter any molecules which have D amino acids, and whether if that would be the cleavage site for a particular protease it cannot cleave.

Therefore, lack of appropriate specificity of proteases would also, now, make an immunogen less immunogenic, just because T cells cannot be presented with peptides cannot get activated cannot, therefore, differentiate to helper cells or cytotoxic T cells, and if they do not differentiate to helper cells, then B cells will not be able to get those cytokines which they require. Now, again, I will repeat what I had talked about in my second or third lecture. B cells require interleukin 4 and 5 for the immediate proliferation, after their activation by any antigen or immunogen, now.

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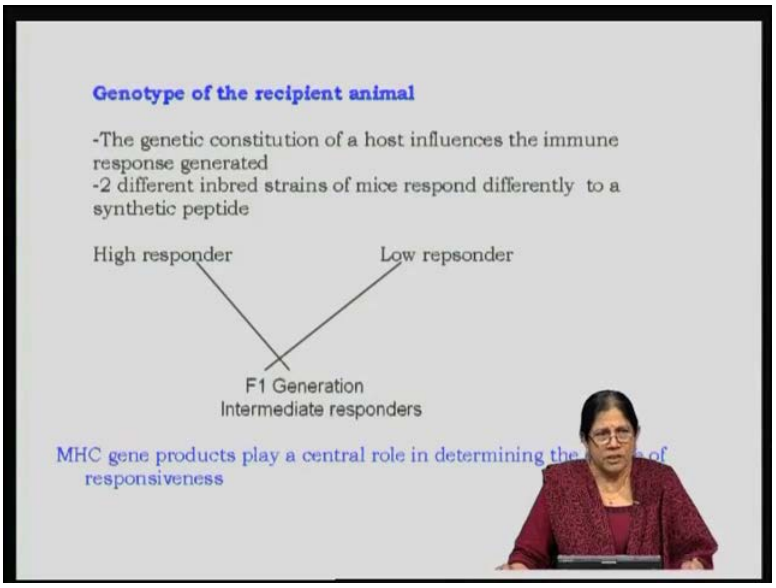

Now, we come to this second half– we have talked about the molecule itself, how it can contribute by its properties to be a good immunogen or not. Now, let us talk of genotype of recipient animal. Does the genotype… would the genotype have anything to do with the way the animal responds to an immunogen? Yes, of course. That is very true, because if you just look around, and you can see that even in a condition where there are… there is, let us say, there is an… there is infection. You know you must have heard about these SARS virus, or you must have heard about, now, the other viruses, which the influenza virus, you know H1N1, and you know that for any people in a locality to get infected, would mean that the other people in that locality would have an equal chance to get infected. Now, why did not they get infected?

Now, when we say they got infected or not, we always consider the symptoms with which they come, or the symptoms that are present– they present– a person might get infected; that means, the virus would have entered the person's body, but because of a good and well-developed immune system, in fact, the symptoms you know the virus is killed, and therefore, there are no symptoms of the disease, which would mean that the immune response is, in fact, is governing the way the infection is settled in or takes route in a particular person.

So, let us say ninety percent or more can mount a good immune response, so that those ninety percent people or more do not get come down with the disease symptoms. They

get infected, but they get, get immediately, you know, have have a good response, and response which is already been there or develop a response which can eradicate or completely obliterate that virus. So, therefore, obviously, then people with the good immune system or robust immune system would be able to respond better to any infection.

So, the genetic constitution of a host influences the immune response generated, that is obvious from simple observation, but has this been proved experimentally? Yes; there are large number of experiments that have been carried out, and people have seen that, you know, people– immunologists– have tried different strains of in mice.

Now, in there are several inbred strains which are used in experiments and these are very homogeneous they are. In fact, they are identical one strain is identical; all the progeny in one particular strain is identical to each other, and you have another strain which has a completed different haplotype different MHC molecule.

So, now I am just going to discuss with you a very simple experiment that people have carried out. They have taken high responder and low responder; what I mean by high responder, they have already, now, taken strain of mouse a strain B, and now they have injected with the same peptide, or let us say, an immunogen, and then looked at the immune response which is generated by way of antibodies, and they found that some mice, even at the same dose to a particular peptide, respond by way of large or high titer antibodies, and let us not talk about affinity high titer antibodies, whereas, low responders do respond, but have very little circulating antibodies to that.

Now, when this high responder is crossed with low responder, it was seen, immediately, that the F 1 generation always is intermediate with respect to its response. So, obviously, by Mendellian genetics, you know, already, that genotype of the recipient animal, thus dictate to a great extent how the host influences the immune response generated.

So, MHC gene products, now, with respect to B cell immune response, you can already, immediately, say is the helper cells, which are required for generate… for getting helper T cells required, which will recognize molecules or peptides which are presented to them in the context of class II molecule– MHC class II molecule– and therefore, the type, or the type of MHC gene present would now determine whether the peptides can be docked on or not, and therefore, since now it is the helper cell which are play a key role in

modulating a B cell response, MHC gene products play a central role in determining the degree of responsiveness.

Now, let us say the low responder one would have the capacity to, let us say, only present two peptides, and where the high responder has the capacity to, or has the polymorphism at the MHC class II molecules, so that this high responder can now can present eight peptides. Definitely, the eight peptides presented to that many T cells, versus just two, would after all determine that this particular strain has a capacity to generate a robust immune response.

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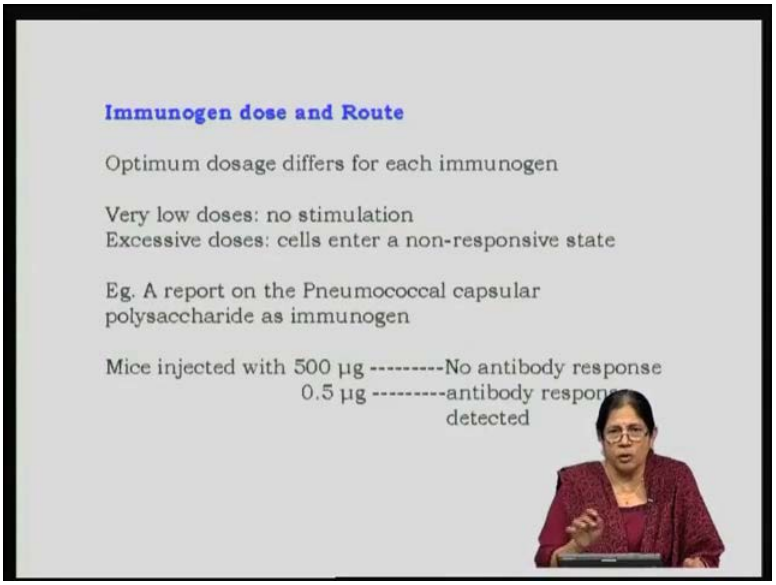

Finally, with respect to what is contributed by the host, the immunogen dose, and the route, these two are extremely essential. Is there an optimum dose for any immunogen that determines immunogenicity? Now, again, immediately if you are thinking in terms of an outbred population of even animals, let us say, an outbred strain of rats, of course, it does not, and therefore, when we come to humans of course, there is no optimum dose with respect to one immunogen and another.

However if you come down to experimental analysis, then, even then, optimum dosage differs from each immunogen, because you already know that the complexity of the molecule, the size of the molecule, all these also would play a role on the dosage. If it is the small molecule, less complex, you definitely would require more. If it is the small molecule, it be it will be thrown out of the immune system– thrown out of the system

much faster, will be retained for very short duration, not adequate for the B cells to recognize, and thereby, again, the dosage need to be… needs to be increased.

So, optimum dose varies. Very low doses may, well, by the whatever discussion, I mean, whatever I said now, there would be no stimulation at all of B cells, because even before the B cells recognize the antigen as immunogen, such low concentration that it is already thrown out of the system.

However, it does not mean that an excessive dose is going to alter the responsiveness. If there is excessive immunogen, then B cells enter a nonresponsive state. That is too much, that is too much of activation. Let us think in terms of phosphorylation of this molecule– they are just too much now; too much of anything is bad– it is bad also for cells. Cells would undergo B cells which are too activated also, there is… would go into a nonresponsive state, will not be able to proliferate.

So, very low doses and excessive doses– both of which are difficult to define for any immunogen and has to be done experimentally. I would like to substantiate this with an example, and I find this example very, very interesting. Now, this is will not be true of most; however, because there is such fantastic variation, I thought I will share this with you.

A report, which was sometime in, you know, in the 60s, immunologists were looking at the immune response to a Pneumococcal capsular polysaccharide, which they use as the immunogen in mice. When they injected mice with 500 micrograms at one time, they found that there was absolutely no antibody response to this Pneumococcal polysaccharide.

So, they started to decrease the concentration from 50, and when they did it tenfold, they arrived at a concentration of 0.5 micrograms per mouse, and they found there was a good antibody response. Now, in between, I do not remember, of course, now, but I might guess that 5 micrograms not 0.5, but 5 micrograms– that is tenfold low higher than the lowest dose it generate an immune response, but 0.5 dictated, I mean, in fact, generated a better immune response.

So, for this Pneumococcal capsular polysaccharide, 500 micrograms is too much and 0.5 is best. So, you can imagine for every immunogen, in fact, if you want to study the

optimum response, one needs to do an experiment. It would be impossible to, to find this out in any other way, alright?

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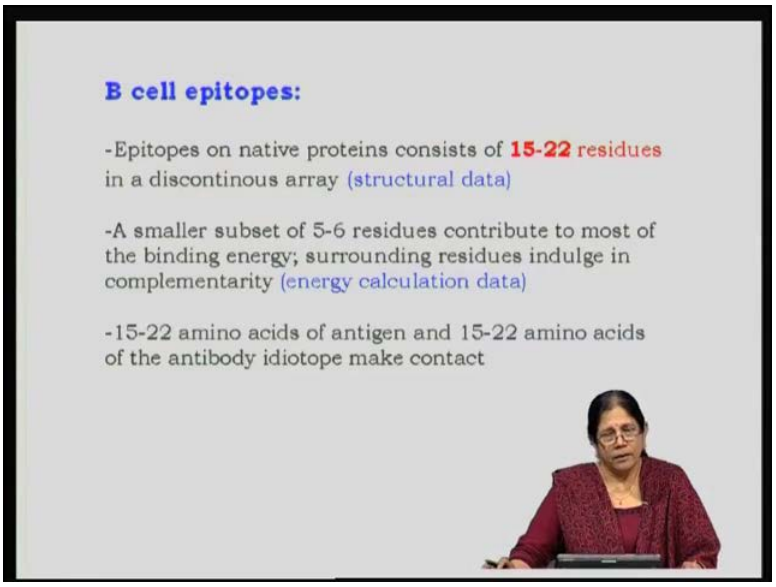

So, now we know what governs, or what regulates the amount of antibody generated or the type of antibody generated to an immunogen. You know that it is both host cell-derived factors as well as the properties of the molecule itself. Now, we come to the next step. I have been talking about antigenic determinants, and I have been telling you that B cell antigen receptors recognize B cell epitopes. They have to be, therefore, then defined region on an protein molecule. Now, what are these defined regions? The defined regions would mean that yes, in a given sequence of amino acids, there should be a set of amino acids, which would define the antigenic determinant. These are B cell epitopes.

So, epitopes corresponding to this B cell receptor– how large are they? Epitopes are native proteins consists of 15 to 22 residues. Now, this can be 15 to 22 residues if it is in a discontinuous array. Now, I will be talking about this discontinuous-continuous versus discontinuous. In case of a sequence in a not discontinuous but continuous array, the number comes down, and it can be, instead of 15 to 22, it can be about 8 to 12.

Now, epitope on native proteins consists of this; it would mean that you require that many, anyway for a good antigen-antibody interaction or receptor-immunogen interaction, now, a smaller subset from the 15 to 22, a smaller subset of 5 to 6 residues contribute to most of the binding energy, but the surrounding. So, 5 to 6 adequate for

that, actually, the core epitope, you know, because of which there should… there will be absolutely no interaction, but the surrounding residues indulge in the complementarity also contribute to the forces that govern an antigen-antibody interaction, or what is true of antigen-antibody would also be true for the B cell receptor-immunogen interaction.

Now, people have actually determined this to find out the core epitope, and we will come to that later. So, about 15 to 22 amino acids of the antigen and 15 to 22 amino acids of the antibody at idiotope– idiotope is that region or at the variable region of the antibody molecule which is…, which comes into contact, binds to the the antigen determinant that would be the idiotope.

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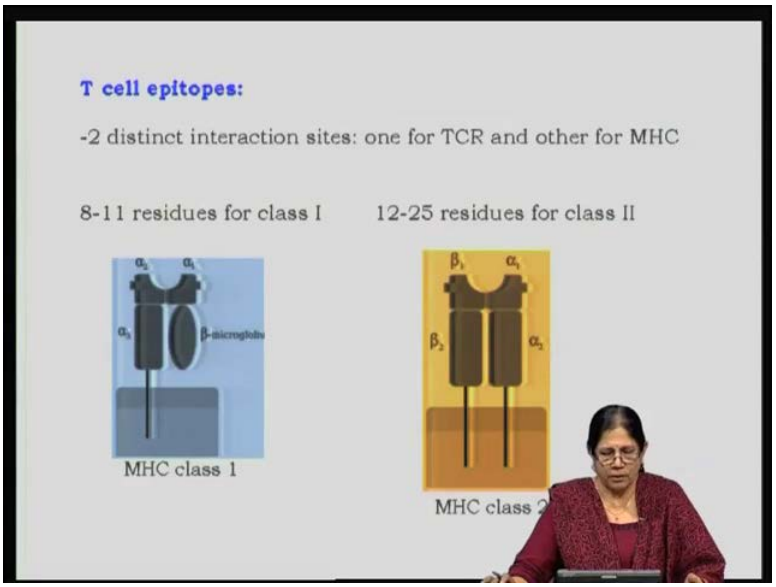

So, the same number of amino acids, approximately, on the immunogen or antigen now comes into contact with the same number of amino acids on the idiotope of the antibody. I am not going to talk too much about T cell epitopes, because that is something that will be talked to you or discussed with you when you talk about when the T cell and the T cell receptor is, is presented. I will just like you to tell you what would be the difference between T and B cell epitopes, and therefore, I have to introduce at least a part of the T cell epitope.

There are two distinct interaction sites. This is very different from what you saw in B cell epitopes; there are two distinct interaction sites for T cell epitopes– one for the TCR and other for MHC, because we do know T cell receptors recognize antigens– antigenic

peptides– to be precised only when they are docked onto either class I or class II molecules.

Now, are there any differences with respect to the number of amino acids that make a T cell epitope? There is a difference because of the way these molecules need to be docked on to the MHC molecules. In case of class I molecules, the peptide should be about 8 to 11 residues, whereas, for class II molecules, the peptides should be at least 12 to 25 residues.

Now, the details of the openness or the groove that is formed by the MHC molecules to… how is the peptide is different from class II and I molecule? Just to suffice, you have to say that class I molecule has a docking site which is closed, and therefore, cannot accommodate peptides which are longer than 11 residues, whereas, the class II molecule is a bit, and therefore, can accommodate larger peptides.

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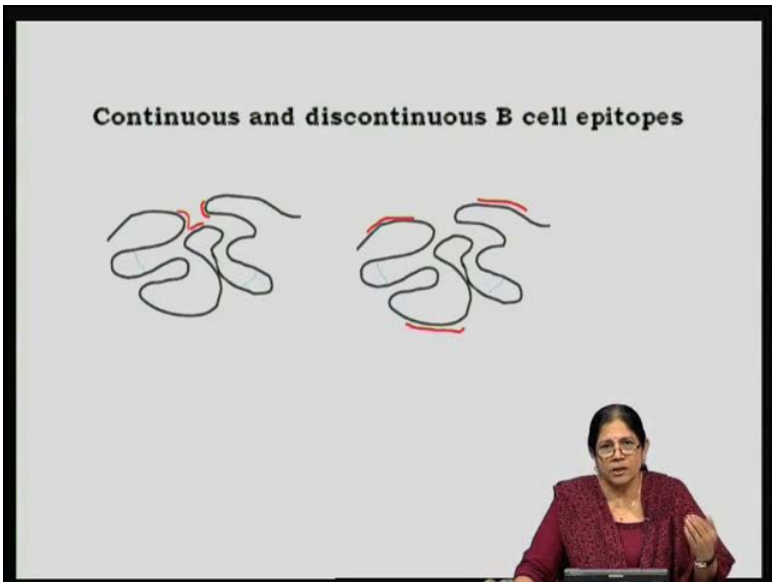

Let us come back to now B cell epitopes. I did say there is something, something like discontinuous versus continuous. Now, I have drawn a very simple diagram; this does not really tell you which molecule it is, but we know polypeptide chains are have tertiary structures– secondary and tertiary structures. Now, there are epitopes on molecules that are formed by the coming together of regions of that polypeptide chain, because of disulfide bond or by the site chain interaction, of the from the site chain of the amino acid.

Now, in this particular molecule, there are two disulfide bonds. In fact, there could be more depicted too, because of which there are three regions of same polypeptide chain which come together, and what you see, here, in red, is the epitope. Now, this epitope, therefore, would be called discontinuous B cell epitope, because on the primary amino acid sequence of the molecule, I mean, if you open this out into a chain– a linear chain– then these three would be very far away from each other, but they have come together to form the epitope because of the folding of the molecule. Now, these are discontinuous B cell epitopes. On the other hand, the same molecule, you know, which is folded the same way, can also have antigenic determinants in a sequence of amino acids in a continuous array.

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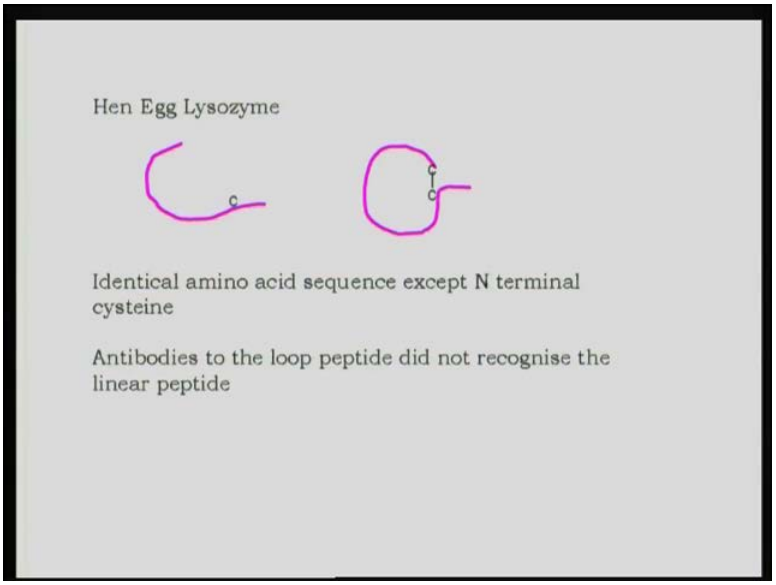

Let's look at what these two mean, you know. So, let me come with an example. Lots of studies have been done by immunologists, immunochemists, on the molecule hen egg lysozyme. This molecule has four disulfide bonds and of course, I am not depicting all that over here. What was an interesting experiment for me to discuss with you is that hen egg lysozyme, now, one– they were able to identify one region of the molecule, which forms in the native structure, a loop, and the loop is stabilized by disulfide bond, cysteine disulfide bond.

So, what immunologists did was, now, they made the linear peptide which does not have that one of the cysteines. Therefore, now this cannot form a loop structure. So, they had a peptide which is linear, and the same amino acid sequence. Now, they just introduced a cysteine in this particular peptide; it would be at the N-terminus.

So, if you look at the structures, peptide A would be linear and peptide B would be a loop. When antibodies were generated to peptide A, as and then, in other animal to peptide B, then they tried, they started looking at the interaction, whether the antibodies generated would be able to recognize, you know, cross interact. Now, it was interesting that antibody that were raised to the peptide A, in fact, gave a response. The Y antibodies that recognized this, but the response first slightly lower. This said that the structure, you know the complexity in the structure always induces a better immune response.

Interestingly, antibody generated to the peptide 1 recognize peptide 2, but antibodies generated to peptide 2, which is a loop structure, did not recognize peptide 1 at all, because this was linear. What does this mean that, now, if you think in the context of the native molecule, this would represent the native structure and this D nature.

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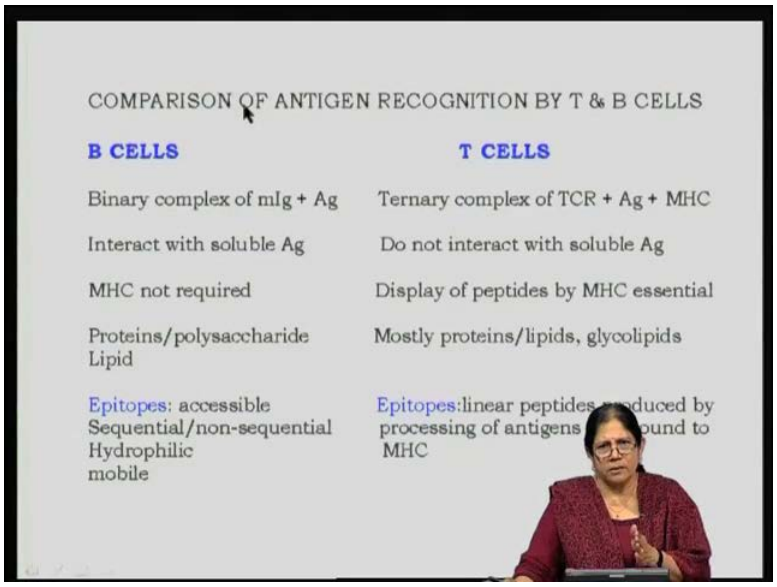

Now, if you go back again with lysozyme, I have told you that the hen egg lysosome has four disulfide bonds. So, if you now break these disulfide bonds, the tertiary structure is gone. The molecule, therefore, would be denatured, and now, if you look at the specificity with respect to the immune response antibody generate to the native structure,

does not recognize– in this particular context– does not recognize at all, but if you think in terms of the entire molecule would have recognize the denatured form, but to a much lesser degree.

Just let us look at, now, we say that B cells recognize antigens. So also T cells. You already know one aspect, that is, of difference, that B cells recognize native structures– mostly native– whether it is discontinuous or continuous native antigenic determinants, T cells require the antigen to be processed and presented in the context of class I or II molecules. Therefore, denature are there any other differences.

Now, B cells the receptor is a binary complex of membrane immunogen and the membrane immunoglobulin and the immunogen T cells, on the other hand, it is a ternary complex, because there is requirement for the TCR to recognize antigen which is… which is docked on to class… the MHC molecules. B cells interact with soluble antigen; that means, when the antigen, immunogen enters our body, B cells can immediately recognize, whereas T cell need that step where the soluble antigen is converted to peptides, which is docked on to the MHC molecules. Again, obviously, therefore, B cells do not require MHC, whereas T cells require display of peptides by MHC, and this is absolutely essential.

B cells recognize proteins polysaccharides and lipids. So, they recognize proteins, essentially. So, proteins would, therefore, evoke a very strong response; then comes polysaccharides, and lipids, last; T cells– mostly, proteins. Now, there are, of course, reports that lipids and glycolipids, also, can bind to the MHC molecule– not in the way that the peptides bind– but can bind, can occupy that, and can activate certain T cells.

B cells– the epitopes are accessible– why? Again, because we know that B cell receptors recognize native structures, which would mean that in that aqueous phase, epitopes need to be outside on folded molecule. It should be accessible, because it is only because of the hydrophilic environment, the hydrophilic regions would be or hydrophilic patches on the molecules are the ones that are likely to be outside. So, therefore, accessible B cell epitopes can be sequential or nonsequential as we have discussed, but they need to be outside and, of course, like I have already said, the region should be hydrophilic should also be flexible. We will come to that in a little while. T cells, on the other hand, the epitopes are linear peptides produced by processing of the antigen and bound to MHC.

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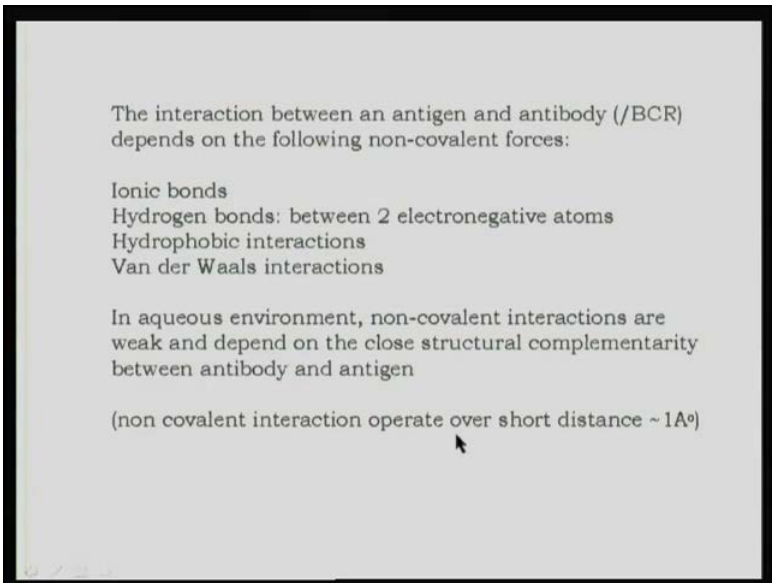

The interaction between an antigen and antibody depends on non-covalent forces. First, of course, is complementarity, and these between the antigen and antibody and there are, of course, large number of structures of complexes of antigen with its antibody, which show that complementarity is what actually drives the antigen-antibody interaction, stabilized by ionic bonds, hydrogen bonds, hydrophobic interactions, as well as Van der Waals interaction. This is just a pictorial representation– this is the antigen in red, and this is the control of the antibody, and this is just to highlight that there is complementarity; look at the surfaces of the antibody or the idiotope of the antibody, and the epitope of the antigen.

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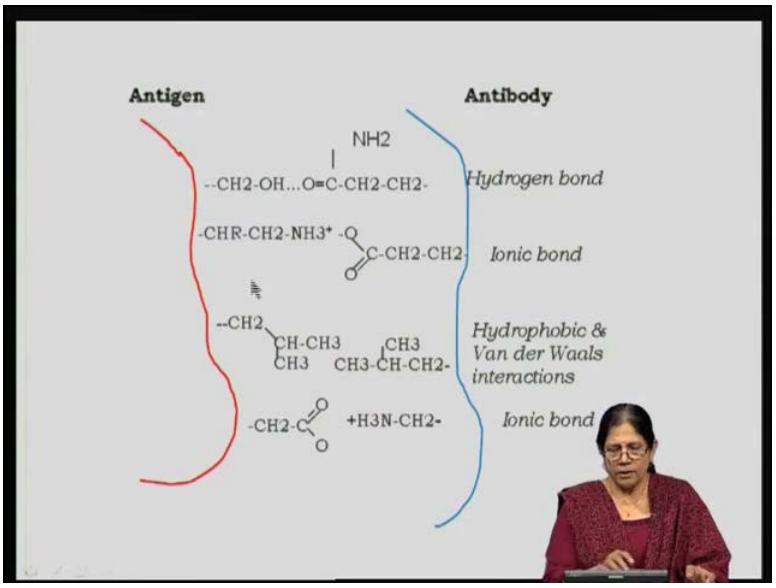

Now, this when, you know, when they are they come close together, they both would, actually, have something like a fitting of two– not exactly like lock and key, but similar to that. That is complementarity, and then, there are these hydrogen ionic bonds as well as hydrophobic van der waals interactions.

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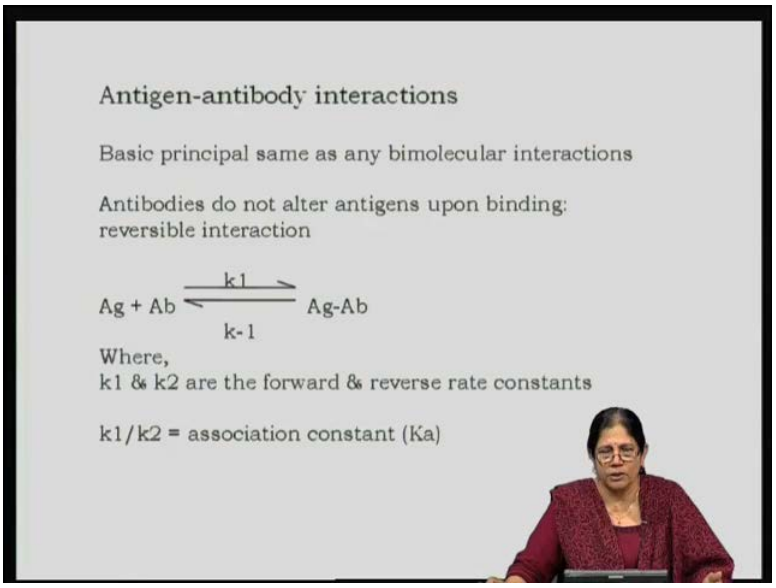

Antigen and antibody interaction are based on the same principle as any bimolecular interaction, any protein-protein interaction. For that matter, antibodies do not alter antigen upon binding, so it is a reversible reaction. Antigen and antibody forms the complex of antigen-antibody, and the forward reaction for forming the complex is k 1, and there, of course, this is the dynamic process, and this is, you know, reversible. Therefore, k minus 1 for the complex dissociating antigen antibody, separately.

So, what are the small k 1 and k 2? They are the forward and reverse rate constants, and k 1, sorry, there is, yeah, k 1, ratio of k 1 k k minus one, there there is a small mistake here this k minus one is what I have written as k 2 it is the same as k 2 and k minus 1 are the same. So, I should have either had k minus 1 here, or I should have put k 2 here. So, k 1, ratio of k 1 by k 2, or ratio of k 1 by k minus 1, determines the association constant.

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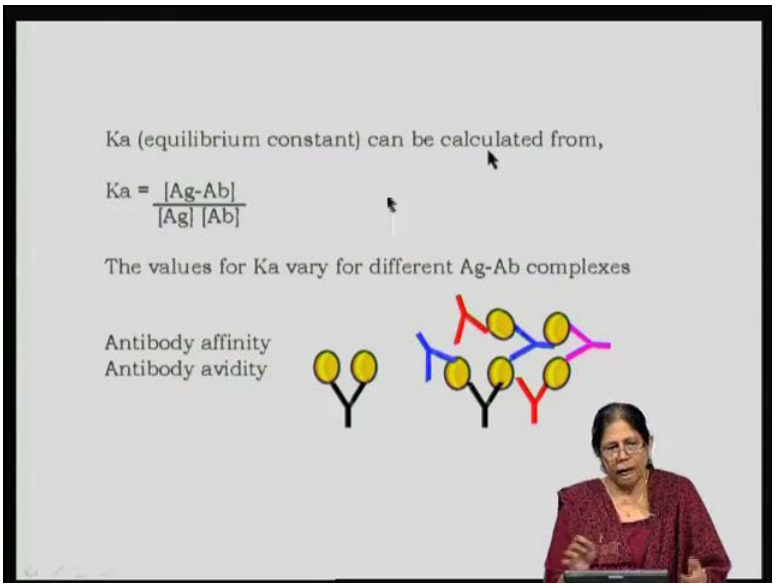

Now, in how do you…, how do you discuss this, that if you have an antibody which has very high affinity, then k 1 would be stronger than k minus 1– association would be more than k minus 1– the dissociation. So, naturally, the k 1 by k 2 is what is going to tell you that the interaction is very tight. Can one calculate this affinity, which is the k a? Yes. One can…, one can calculate by measuring the complex the molar concentrations of the complex by free antigen and antibody. So, it is governed by the k a or the equilibrium constant. The affinity is equal to the concentration of antigen and antibody complex, divided by the concentrations of the antigen antibody which are free from each other. Of course, needless to say, here, the values for k a vary for different antigen antibody complexes.

Now, this becomes even easier to describe in terms of monoclonal antibodies, because now, one can generate polyclonal antibodies and monoclonal antibodies to the same antigen, and if one measures the affinity of the monoclonal antibodies, then you can have affinities in the range of 10 to the power 10 moles per liter, versus 10 to the power 6 moles per liter, so a difference of 10000, or even more. So, affinity is very easy to define. It is the strength of the antigen-antibody interaction. So, high the affinity, more will be titer will be the binding.

What is avidity? One may have come across the word avidity– is it different from affinity? Yes. Now, when one describes avidity, it will mostly pertain to polyclonal

antibodies, because in case of monoclonal antibody, as is shown here, a monoclonal antibodies, specially to globular protein, will be able to recognize and bind to two separate identical antigenic determinants, because both arms of the immunoglobulin would, I mean, the heavy and light chain are identical. So, both the arms will bind the same epitope.

Now, in the polyclonal, in the context of the polyclonal response, let say that the antigen which is shown here, in yellow, each molecule can recognize three, at least of different antibodies, which would mean recognizing three different antigenic determinants. Now, it is because antibodies are bivalent, and if the antigen is more than bivalent, then you can have a complex formation like this. Anyway, that is not I am here to say; what I would like describe is the avidity.

So, what has been seen is binding of one antibody to one particular epitope on the antigen can bring about a conformational change in the antigen molecule, which may allow the binding of the second one or it may also abrogate, but it can allow, and in fact, it has also been shown that binding of one antibody can bring about a conformation, in such a way that the second antibody to the second epitope binds with the better affinity. You know better, because the conformation alteration is allowed. Now, the antigen-antibody interaction the second one to be tighter now that. So, a larger number of molecules binding to one or antibody molecules binding to one antigen would determine the avidity of the molecule, and therefore, avidity of a polyclonal antiserum can actually enhance, or will will enhance the affinity binding to a much greater extent than ever achieved by a single monoclonal, no matter how the high the affinities.

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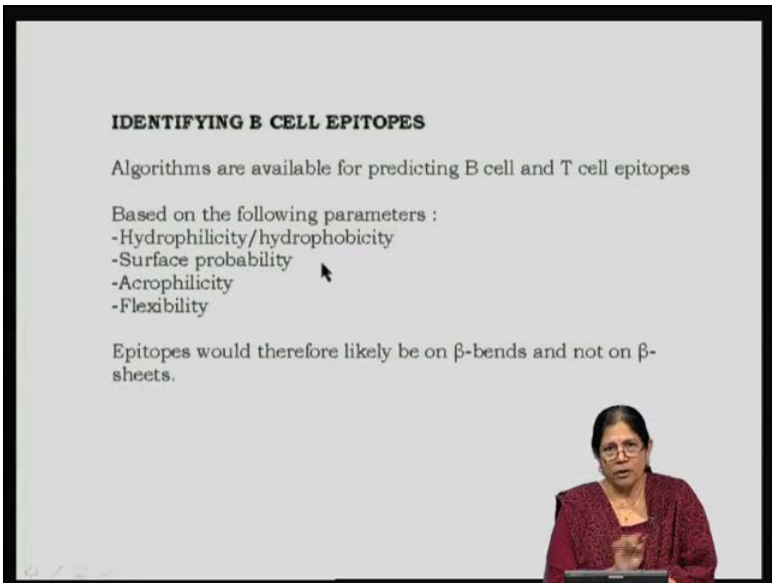

Now, we talked about B cell epitopes. Can one identify B cell epitopes? Yes. There are, of course, large number of algorithms that are available, now, that can predict B and T cell epitopes, but how were this algorithms generated? They have to be generated on certain parameters. So, by way of description of these B cell epitopes, these algorithms were generated, which dependent on whether a region is hydrophilic or hydrophobic. Like I told you, when we talk about antigen-antibody interaction, we are always talking about aqueous phases, which would mean that the most hydrophilic region, or let me put it the other way around that an antigenic determinant has to be hydrophilic in nature. Only then, can it be available for B cell receptor interaction.

For them to be hydrophilic, you know, the accessibility of hydrophilic, they should also be surface probability like I, when I started off, saying that, you know, hydrophilic, there could be other regions of the molecules that are hydrophilic, but yet, not, you know, part of the antigenic determinant, because they are not surface exposed, when a protein is getting folded, then, of course, there is a possibility that you can have hydrophilic regions, which happen to be in the inside of the molecule just because of the folding and nothing else.

So, therefore, you also need surface probability, which is accessibility, acrophilicity, and flexibility. So, surface probability– one can predict. Acrophilicity is again being accessible– flexibility. Now, why is flexibility important? We have always been talking

about molecules undergoing conformational change; it has been proven by experimental condition. In experimental conditions, that antigenic determinant on a protein molecule can undergo changes– slight conformational change– which allows the B cell receptor or the antibody to bind better.

So, when kinetics of this binding was looked at, it was found that though the affinity with which the first contact was made after stabilization or allowing the reaction to become stable, it was seen that same interaction, actually, resulted in a higher affinity, which would mean that this can only happen when there is flexibility. So, therefore, now, all these four parameters go to make a B cell epitope.

Now, since we talked about all these hydrophilicity, surface probability, etcetera, at epitopes would, therefore, B cell epitopes. Again, epitopes would, therefore, likely be on beta bends, you know, loops, and not on beta sheets, because usually, in all molecules on protein molecules, the beta sheets are stuck inside the molecule, are internal, and it is the loops that join these beta sheets and the determinants would be on the loops.

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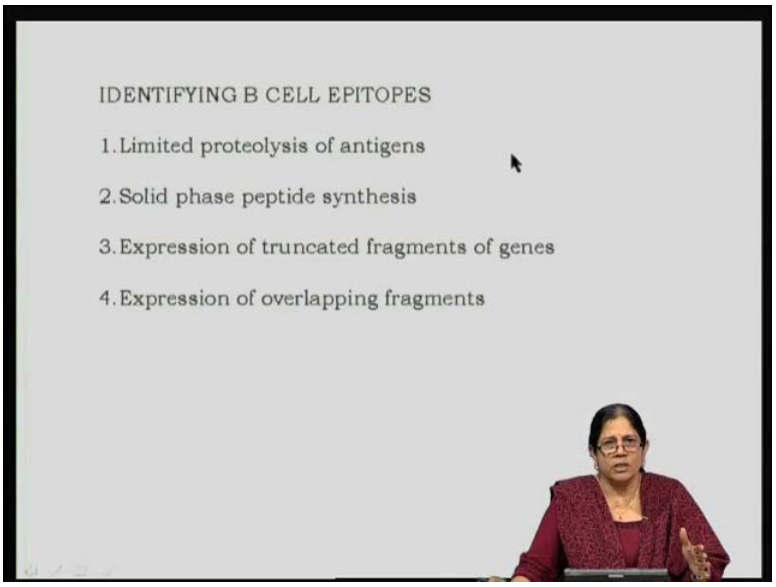

Can one identify B cell epitopes? By experimentally, yes. One can do several experiments, but I would like to mention here that identifying B cell epitopes is easily done experimentally, but they would identify mostly sequential epitopes, because for all these methods, it is difficult to have the secondary and the tertiary folding or sequences, structures, because all these, for example, now limited proteolysis of antigens, solid phase peptide synthesis, all of them would only talk about linear epitopes, but let us go to them anyway. So, that is the identifying B cell epitope it is not. So, easy to identify a discontinues one

The only the one can identify the epitope is when one is able to determine the structure of an antigen with this cognate antibody by crystal structure. So, any other experiments it is not difficult- it is not easy.

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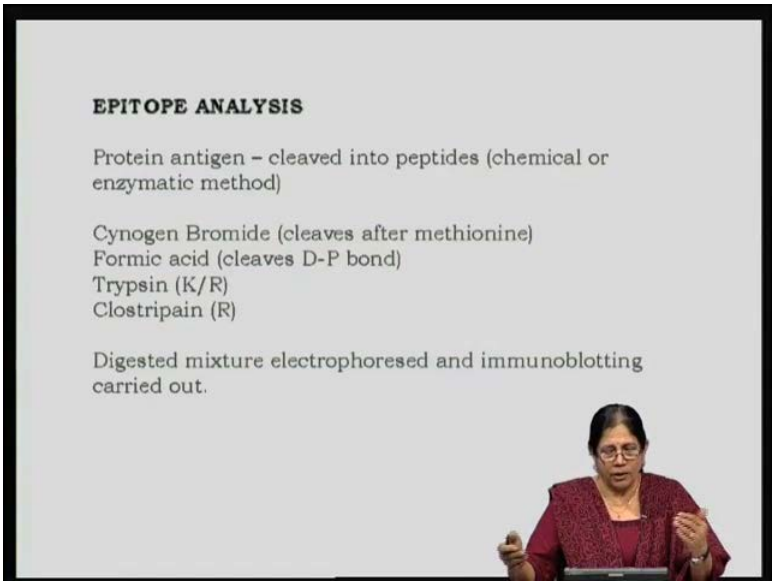

However, one can, at least, identify, you know, map the epitopes present on by these methods; epitope analysis can be carried out by cleaving. One of the method is cleaving. The proteins antigen into peptides by enzymatic or chemical methods, and now, you can, you know, let me just tell you the specificity, you know, you can use chemicals such as formic acid, which cleaves, very specifically, a D-P bond, that is, aspartic acid-proline bond. Not all proteins have this, of course, but if your protein has, you can do that. So, you know, already, where the proteins has been cleaved. So, you generate those

fragments– trypsin cleaves at lysines and also arginines; clostripain cleaves only at arginines; cynogen bromide cleaves after methionine.

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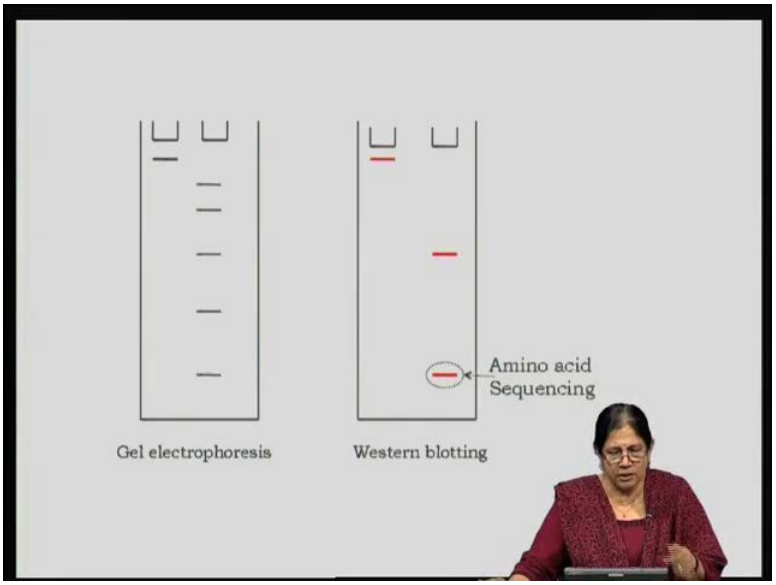

So, you can look at the amino acid sequence of your protein, and you can cleave. Now, mix molar fragments and the digested material is then electrophoresed. You can electrophorese this– this is a gel with the digested antigen and this is undigested one, and then, one can do a western blot, you know, immuno blot, and then see which are the fragments that get picked up. Now, if it is an monoclonal antibody, you will have very few fragments that get picked up. As compared to polyclonal, you will get almost all the fragments picked up, because there would be that many antigenic determinants.

However, now, one can then sequence the smallest fragment, and you know the primary amino acid sequence of your protein; you know this is the this particular fragment which would be smaller; this could be along one kilo dalton and so, at least, you narrow down to the region which has the epitope corresponding. Easily, here, I can say with respect to monoclonal antibody. Polyclonal– which still be more difficult– but it can be done.

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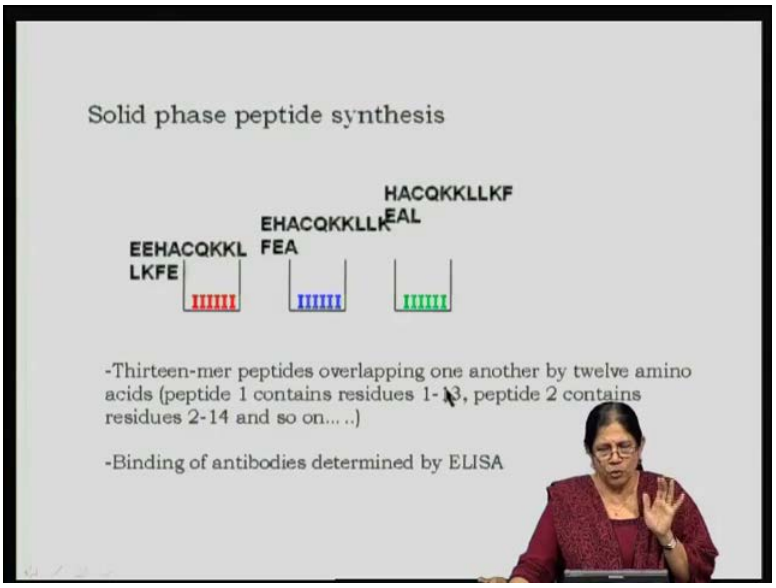

Now, solid phase peptide synthesis can narrow down that region further by taking, you know, making small peptides corresponding to that region which was identified. Let us say by, you know, the earlier experiment of fragment generation, and then you have the amino acid sequence you can make. In this particular case, I have written 13-mer peptides where generated are made on solid phase on pins, and which are…, which are in each of these wells, and there is a frame shift of every amino acid.

So, therefore, now you have peptide 1, which is dissimilar, which is identical to peptide 2 except for the first amino acid, and this one has at the C-terminus another amino acid, which is from the sequence itself. Now, one can look at the binding of antibodies and then decide.

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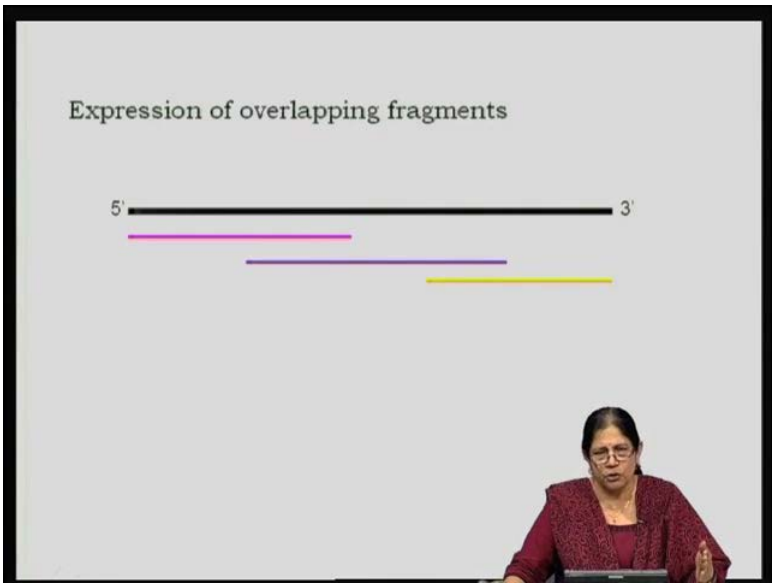

One can do the same thing with molecular biology approaches; for that, of course, you have to have the gene corresponding to your protein, and then you can make overlapping fragments. This is the gene 5 prime to 3 prime, and you can make overlapping fragments sequentially, like this, or you can truncate the gene either from the N-terminus or the C-terminus, and now, express each one of them and check for binding to antibodies, and then, you know, where likely and by several such experiments, one can now say that this is likely to be the epitope, and later on to peptide generation, if you really want to know the core sequences.

Now, why would you want to identify B cell epitopes? What is the reason? There can be molecules, such as HIV, you know, which are very…, one would not to work with viruses in the lab, and only if you have B 3 facilities, and, in fact, much more robust containment facility where you can work with viruses; however, one can– if one is able to identify epitopic regions, then you could be dealing with only proteins and peptides, and therefore, you can go ahead and now design peptide vaccine, which is going to be another lecture. And there are pros and cons of identifying peptides as vaccines, but again, that is a matter for a discussion which is related, but not identical, to this.

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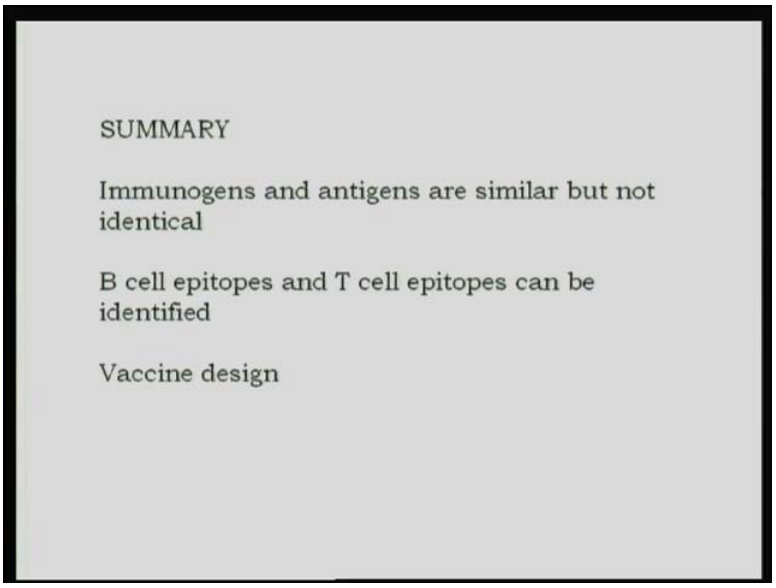

So, now, let me summarize: immunogens and antigens are similar, relevant, of course. All immunogens are antigens; however, not all antigens are immunogens; B cell epitopes and T cell epitopes can be identified based on whatever I have discussed, so far, and one can use these for vaccine design. Let me just tell you why all immunogens are antigens, but the reverse is not true, and I will give you an example– you have now happens which are have not even began discussing, and I will do that when we deal with vaccine design.

Now, peptide vaccine design– haptens are small molecules; they can be antigens, or they cannot be immunogen unless they are conjugated to a carrier protein, and then injected. The antibodies that are generated will be to the hapten, as well as to the carrier protein. So, therefore, now it will be, in fact, the antibodies will also recognize the hapten alone, not only as conjugated by carrier protein. Therefore, we know that haptens can be antigens, but not immunogens. They can, of course, be made immunogenic by conjugation. So, we will be dealing with, you know, vaccine design and what are the pros and cons in a subsequent lecture. Thank you.