Essentials In Immunology
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Module No. # 10
Lecture No. # 21
The major histocompatibility complex – Part 3

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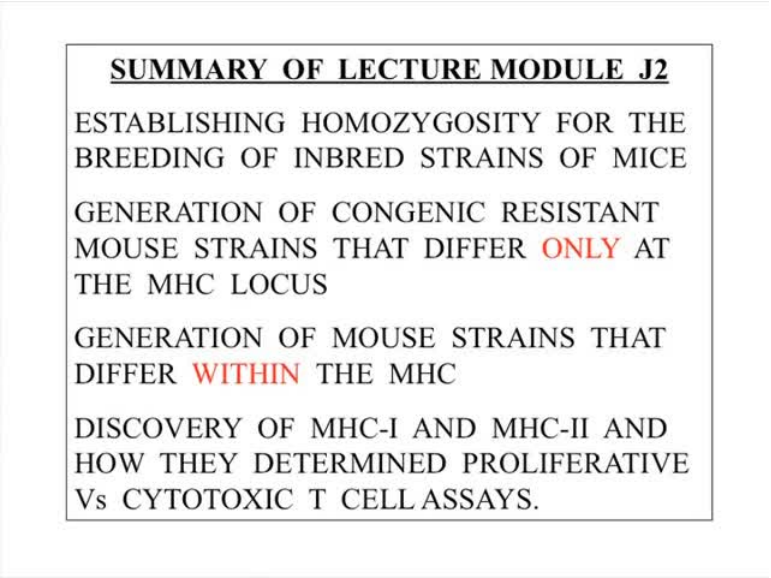

Hello and welcome to this series of lectures in essentials in immunology. To summarize the previous lecture, we had looked at how to establish homozygosity for the breeding of inbred strains of mice, the generation of congenic resistant mouse strains that differ only at the MHC locus and also, the generation of recombinant mouse strains that differ within the MHC locus, which actually led to the discovery of the two types of MHC, MHC I and MHC II, and how they determined the proliferative versus cytotoxic T cell assays.

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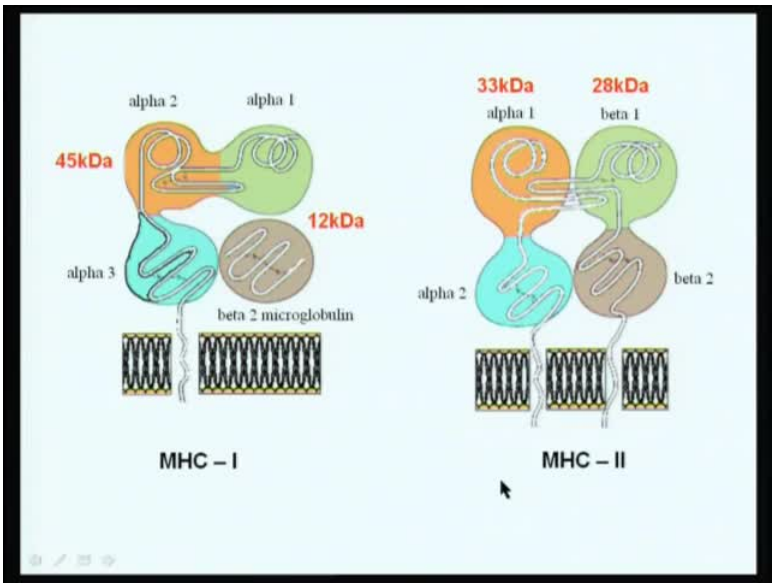

Now, going into the structure of the MHC antigens, we also learned how the MHC I antigen or MHC I molecule has got the heavy chain of 45 kilodaltons, which is associated with a lighter subunit of 12 kilodaltons called as the beta 2 microglobulin and how only the heavy chain was anchored into the membrane and the different domains of this heavy chain alpha 1, alpha 2 and alpha 3. In contrast, the MHC II molecule had two subunits, both of which were anchored into the membrane. These two subunits being the alpha 1, which was 33 kilodaltons in size and the beta subunits which was 28 kilodaltons in size; the alpha subunits having two domains, alpha 1 and alpha 2 and the beta subunits having two domains, beta 1 and beta 2, and we also took note of the fact that the structure was represented this way, having 2 alpha helices and the floor, in both the cases, and the floor having a cleft, which we referred to as the peptide binding cleft, which would bind the peptide antigen.

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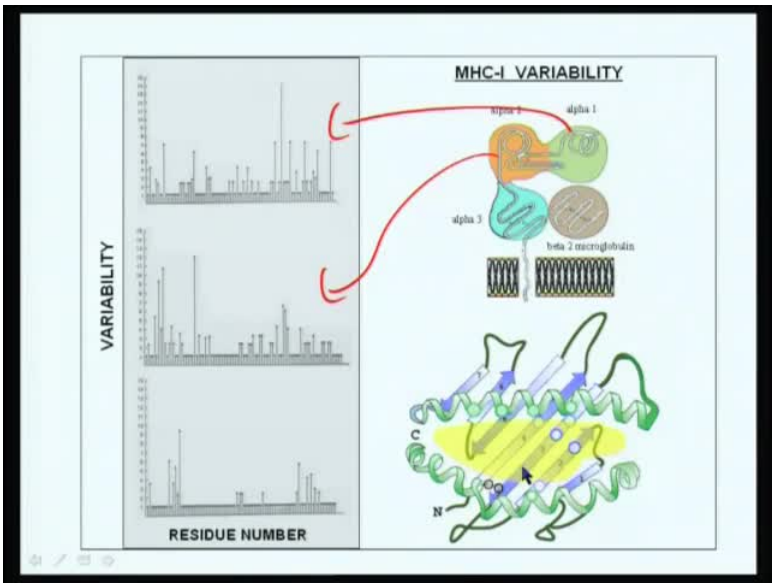

So, looking at the variability plots of these subunits, both for the MHC I as well as the MHC II and in this case, especially the MHC I variability, we saw how to were the alpha 1 and alpha 2 domains were more variable in nature compared to the alpha 3 domain, which was just above the membrane. If one looks at the MHC molecules from the top, that is, if you look at this structure from the top, this is the view that you will get. This is the model that has been arrived at, looking at the x ray diffractions patterns of all of the HLA molecules, especially in the human, HLA molecules was purified and then the detail structure was worked out.

And this is the reason, why the structure is written with two alpha helices because there are two alpha helices over here and then the beta pleated floor made up by 7 beta pleats, as you see in the numbers, in the blue arrows here 1, 2, 3, 4, 5, 6 and 7 and these are anti-parallel in nature and if you looked at the variability of the amino acid residues, they all confirm to these circles that have been are found here on the alpha helix and you also find the variable residues in the beta pleated floor, the blue circles that has been represented over here. These are the 2 disulfide bonds that you see over here.

This is the N-terminal portion and this is the C-terminal portion. Of course, the N-terminal portion the C terminal portion continues into the third alpha 3 domain, then gets into the membrane. Since this is the top view, you are not looking, you will not be able to look at the alpha 3 domain. So, this shows us how the sides of this molecule are variable

as well as the floor and this yellow background that has been represented over here is the place, where the antigenic peptide sits within the groove of the MHC; this groove is called as the antigen binding groove or the peptide binding cleft.

Now, the side chains of the amino acids are in contact with this beta pleated floor as well as the alpha helix. So, one can imagine that the variable nature of the amino acids in these two alpha helices and the beta pleated floor will also vary with the different alleles of the MHC. The different the haplop type of the immune strain of mouse or for that matter, the allele of the HLA complex, you will find these amino acids are different and therefore, would accommodate a different the peptide that is accommodated within this groove will have a different property to it and this then leads to the diversity in the nature of the peptides that are binding to the MHC antigen binding groove. This variability is what enhances the diversity of the recognition by the immune system.

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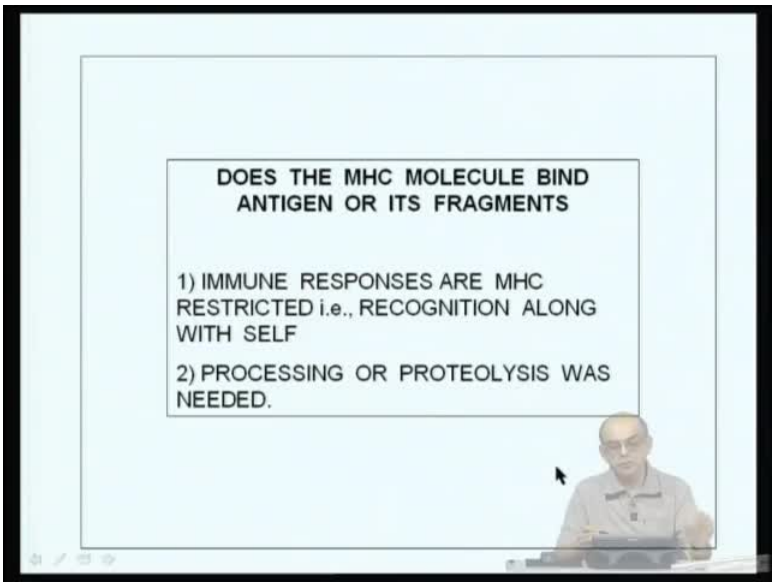

So, going on further, to look at this molecule in a different way as to ask the question does the MHC molecule bind antigen or its fragments because we have allowed it to the groove as the antigen binding groove or by the peptide binding cleft, especially the observation that immune responses are MHC restricted and that is the recognition along with the self MHC allele takes place and that the processing or proteolysis of the antigen was needed before the activation of T cell hybridoma or T cells that would then lead to the proliferation. This was referred to in lecture number 3, as to what these macrophages

do to the antigens they encounter. Because of these two observations, they hypothesised that the antigen present in cell was taking up the antigen and fragmenting this antigen by proteolysis within the lysosomes of the endosomes and then this proteolysed fragment was then brought out on the cell surface somehow in association with the MHC complex.

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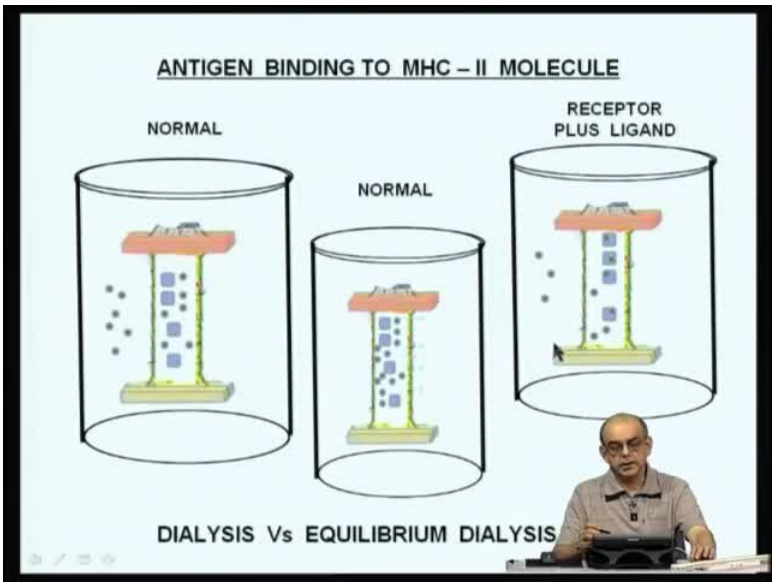

To look at this, various experiments were done to demonstrate the binding of the MHC molecule with the antigen of the proteolytic fragment derived from the antigen that the immune system was exposed to or the macrophage was exposed to and this one of these experiments was equilibrium dialysis experiment and you all know that a simple dialysis experiment involves the diffusion of the low molecular weight substances from inside the dialysis bag to the outside; in fact, this is a method in protein in enzymology and when during the purification of proteins, where you want to exchange the buffers that were used with a different buffer, one dialysis solution containing high salt buffer with the low salt buffer outside the dialysis bag and these low molecule weight buffer salts move out from the bag into the outside and therefore, you establish a equilibrium and the higher protein molecule do not cross the membrane because this membrane is semi-permeable in nature and you have different dialysis bags of different cutoffs like 8000, 12000 and so on and so forth; 8000 meaning it would retain protein molecules that are below 8000 that are above 8000 and allow the molecule that are relatively below 8000 to move back and forth.

So, in this example, we have in this example starting from here, the central figure, you have about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, just as an example to demonstrate to you, there are 12 small molecules and if you allowed them to diffuse through the membrane, you have 6 of them coming out 1, 2, 3, 4, 5, 6 and at equilibrium, there would be 6 molecules outside and 6 inside the bag, in addition to the protein molecules of higher molecular size, which will not move out to the bag. Instead of being a simple dialysis, this equilibrium dialysis experiment that was used to demonstrate the binding of antigenic peptide to the MHC, utilize this principle, where if you consider that these protein molecules that are inside the dialysis bag given here as squares, if these were to be assumed as receptors and you were dialyzing out the specific ligand that was binding to this receptor, one would find that in these 12 molecules putatively being the ligands for this receptor, if there are 4 protein molecules or 4 receptors, it would bind these 4 ligands and therefore, of the 12, you would have only the remaining 1 of them being available for dialysis.

So, in this particular case, you have 10 ligand molecules and out of the 10, 4 are bound by this receptor and therefore, there were only 6 that would be available for diffusion or exchange and they would equilibrate 3 inside the bag and 3 outside and with further changes of the outside solution, even these 3 would come out, but essentially to look at this, you find that if you had a radioactive ligand outside, you put it you put it outside into the into the beaker outside the dialysis bag and if these were to be radioactively labeled, you would find that these molecules would progressively move inside and accumulate in the bag because they would be bound by the receptor and therefore, become unavailable for dialysis.

So, basically then, the principle would be to look at having purified MHC molecules inside the dialysis bag and put the radioactively or fluorophore coupled ligand and antigenic fragment outside the dialysis bag and look at the movement of the radioactivity of the fluorophore into the dialysis bag and then plot versus time because this is called as equilibrium dialysis, you allow it go to equilibrium and look at the number of counts or the fluorescence that has accumulated within the bag that has the purified MHC molecules.

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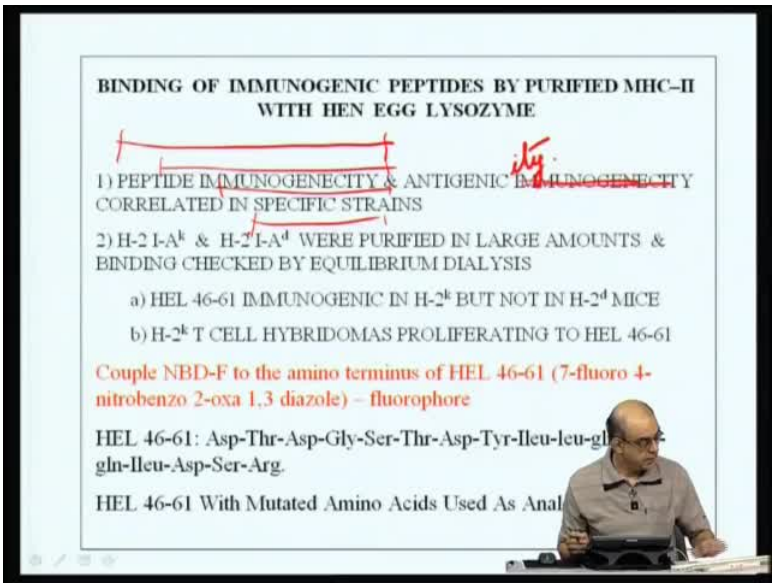

This was essentially the experiment that was done to demonstrate the binding of ligand by the MHC molecule. In addition to these, we need to take into consideration, certain other observations that allowed this experiment to be done and that was as I referred to in the end of the previous lecture that peptide immunogenicity and antigenicity correlated in certain specific inbred strains of mice. Now, immunogenicity is the term that is used to characterize those substances that were good immunogens; that would mean that injection of this substance into animals would lead to a good immune response in terms of both T cells as well as antibodies.

Now, the term antigenic, antigenicity, peptide immunogenicity and antigenic immunogenicity, supposing you were to use an antigen that were that was smaller fragments of a particular peptide and this antigenicity would mean that it would also bind to the antibodies that were made and this there is small mistake here in this slide; this is not antigenic immunogenicity, this should read as peptide immunogenicity and antigenicity. So, these peptide immunogenicity and antigenicity correlated in specific strains.

The experiments that were done with certain model antigens like hen egg lysozyme as I told you earlier, one would immunize hen egg lysozyme the whole molecule into a particular immune strain of mouse and then go on preparing smaller and smaller fragments of this hen egg lysozyme to see, whether these smaller fragments were also

immunogenic and they found that hen egg lysozyme fragments 46 to 61 turned out to the both immunogenic and antigenic in H 2 k strains of mice, but not in H2d strains of mice; in other words, H 2 d would not respond very well to this particular model, but the H 2 k strain would respond very well to this fragment by making antibodies and also activating T cell. So, this 46 to 61 had the ability to bind to bind to T cells and activate them after being presented.

Activation of T cells, as we as we learned in lecture number 3, would lead to the proliferation of T cell. So, the preparation of T cell hybridomas involved the preparation of immortal T cell clones had been immunized with 46 to 61 from the H 2 k strain of mouse. Now, these T cell hybridomas retain their ability to recognize HEL 46 to 61 and respond by the secretion of IL2 of the consequences of recognition by the structure that recognizes the presented HEL 46 to 61 on self-antigen presenting cells or HEL 46 to 61 that was associated with a H 2 k molecule on the surface of antigen presenting cells.

So, once they had these regions, the coupled amino terminals of HEL 46 to 61 with fluorophore called as NBD F. We will not going into the nature of this fluorophore, except remember that it is fluorescent. So, this is the HEL 46 to 61, the amino acids that are present in hen egg lysozyme 46 to 61. They also prepared HEL 46 to 61, which had certain replacements in one or two positions that led to the abrogation of it being a good antigen in H 2 k mice. So, what was the result that was obtained when they used these reagents?

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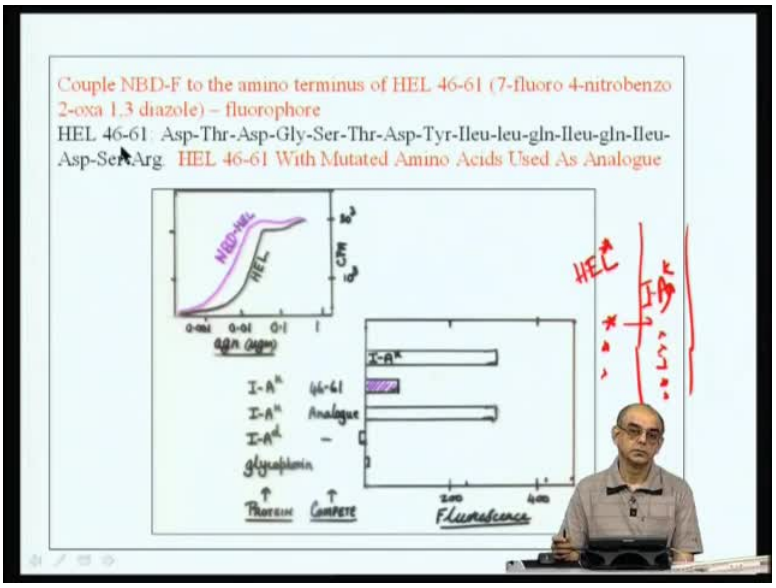

So, again on the top, you will find HEL 46 to 61 and they used 46 to 61 with the mutated amino acids as an analogue. Now, looking at the T cell proliferation or the release of IL2, which was again measured by using an IL2 specific cell line, which would proliferate in response to any presence of IL2; this is called as bioassay. So, looking at the thymidine incorporation of the IL2 specific cell line would give one an idea as to how much IL2 will be liberated. So, as you had more and more of the antigen on the x axis and the cpm indicates the proliferation of the IL2 specific cell. So, when these T cells were exposed to in H 2 k mice, H 2 k T cell hybridomas were exposed to HEL 46 to 61, you will see that this is the nature of the IL2 secretion, which is measured by cpm incorporation by a cell line that is responsive to the liberated IL2.

When the HEL or the hen egg lysozyme is modified by the fluorophore, you would find the same result in that it was very efficient in stimulating IL2 release. Again to reiterate, IL2 release and cpm incorporation are similar or they go hand in hand, in this particular assay. So, once having made sure that the modified HEL 46 to 61, referring to the modification that was done by coupling the fluorophore, was as efficient as the native HEL in stimulating the IL2 released from a T cell hybridoma that was specifically recognizing HEL 46 to 61 in association with H 2 k molecules, so, then, they went on to do the equilibrium dialysis experiment using purified large amount of purified class II molecule especially the I A of k molecules; that means I A molecules was expressed using recombinant DNA methods.

This recombinant I A molecule that was having the k haplotype or having the amino acid sequence that is present in the k haplotype mice was used to look at how it bound to HEL 46 to 61 by using the fluorophore couple HEL 46 to 61. So, basically, you are looking at the amount of fluorescence that is going in from outside into the inside of the bag. So, let us say you have I A molecule that is purified inside and you have the fluorophore coupled HEL 46 to 61, which I will put as a star.

So, you are looking at this star going into the bag; this star then gets coupled to the HE I A of k molecule by a HEL 46 to 61. So, therefore, you will have more fluorescence inside the dialysis bag as time goes on. So, at equilibrium, you would have to have more fluorescence insides the bag than outside, but at the beginning of the experiments you would have more stars outside the experiment because you had introduced the fluorescent HEL molecule 46 to 61 outside of the dialysis bag.

So, looking at this, it was observed that when you had I A of k inside the bag and the fluorescence went into the bag, so, you had more of fluorescence over here as indicated by these numbers over here, and when you used along with the fluorescent fluorescent 46 to 61, you mix them with non-fluorescent or native 46 to 61, which would not be the fluorophore and therefore, not having the fluorescence molecule, it competed for a labeled HEL 46 to 61. In other words, if you had the star marks without any star mixed up without any star, there would be competition between the two and therefore, you had a competition of the fluorescents from the high fluorescence to the low fluorescence.

On the other hand, if they used an analogue, which had been mutated at the residues, which would not allow it to bind to the class II molecule, then also, you had the star or the fluorescent HEL 46 to 61 going inside and binding to the I A of k molecule. On the other hand, if you use the one that it was not the strain of mouse or purified I A of k molecule that was derived from the strain of mouse that would not respond to HEL 46 to 61, you found that there was no accumulation within the bag at all. In other words, this experiment actually demonstrated that the I A of k molecule would bind to HEL 46 to 61. So, these were some of the experiments that were that were done in order to look at or demonstrate the binding by purified class II molecules that they actually would bind to the ligand that they were specific to.

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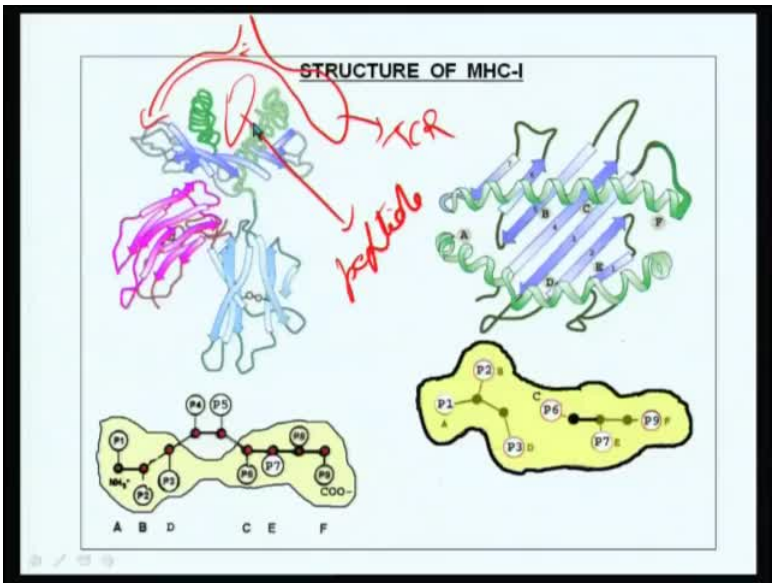

Now, looking at the structure of MHC I, in contrast to the MHC II molecule that was used in the equilibrium dialysis experiments, the MHC I could not be demonstrated as being successful equilibrium dialysis experiment. This had to be arrived at by looking at the x-ray diffraction pattern of the crystallized MHC I molecule, especially the HLA molecule, where they found certain patterns that were characteristic of a peptide sitting in the groove. Now, why is it that the MHC I did not succeed in equilibrium dialysis experiments? The reason being the difference between the MHC I and MHC II molecule is in the nature of how these alpha helices are arranged. In the MHC II molecule, these 2 alpha helices are set to be open; in other words, the distance between these two ends are little further apart and as a result, you would be able to look at a peptide that would sit across this groove and extend across the alpha helices.

Whereas in the case of MHC I, the peptide since the groove was closed at these ends, the peptide had to be located within this groove and could not extend outside and therefore, you will see that if you look at MHC class I molecule, you see the presence of certain grooves which is there even in class II molecule; these groove these pockets are called as A, B, C, D, E and F. So, there are 6 pockets over here and these are the beta pleated floor. As I mentioned earlier, these are the beta sheets anti-parallel in nature. So, if you look at these pockets, these pockets are actually binding the peptides that are sitting within this groove in the class I molecule. For example, the A pocket binds to the first

peptide residue, the B pocket binds to the second residue and the C pocket actually binds to the sixth residue.

Note the difference the C pocket is binding to the sixth residue, whereas the D1 binds to the P3 residue and so on and so forth. So, these are actually called as anchor residues in different alleles of the MHC molecule. In other words, the peptide for the class I molecule has to first anchor into these pockets. When they anchor into these pockets, depending upon the nature of the site change, you will have a bulk. So, I have demonstrated this groove here as in the previous slide, as the yellow background over here. So, you are looking at this peptide sitting from the top and when you look at it from the side, peptide actually show the 4 and 5 coming outside or rising above this particular cleft.

So, in other words, these 3 residues are in contact with the floor and these 4 and 5 are not in contact with the floor. So, if you look at the MHC class I again, as I told you earlier, these are from the side, in order to visualize the alpha 3 domain, which you cannot visualize, when you look at it from the top, you see the alpha 3 domain alpha 3 domain in the blue over here. This is the beta 2 microglobulin that is helping the class I structure stabilize on the surface and this is the place, where the peptide is sitting and to make the future classes a little easier in concept, the T cell receptor which is supposed to recognize this peptide that is associated with the class I or the class II molecule comes and recognizes in this fashion. This is the T cell receptor. and therefore, the T cell receptor This is the T cell receptor and this is the antigenic peptide.

Therefore, the T cell receptor has to recognize the class I molecule as well as the class I molecule as well as the antigenic peptide fragment that is derived by proteolysis by the antigen presenting cell. This is the reason why MHC restriction occurs; in other words, this antigen that has to be recognized only in association with this particular allele of the MHC. So, coming back to the variability of amino acids, if these particular alpha helices are variable, that means there would have to bind to different peptides and different peptides are recognized by different variable regions of the T cell receptor molecule. The T cell receptor molecule is somewhat similar immunoglobulin molecule in that they have variable regions and constant regions.

So, this then ensures further diversity in the immune systems in the sense, the diversity is associated not only with the variable nature of the T cell receptor molecule, but there is further diversity in terms of different antigenic peptide being bound by the MHC allele. So, if you had a peptide of a low affinity to this MHC allele, the T cell receptor would not recognize it with the same high affinity as it would recognize a peptide molecule that is bound with high affinity by the MHC class specific MHC class I or the class II allele. So, this is the nature of how the antigenic fragment is recognized by the self MHC molecules and this complex leads to the recognition by the T cell receptor.

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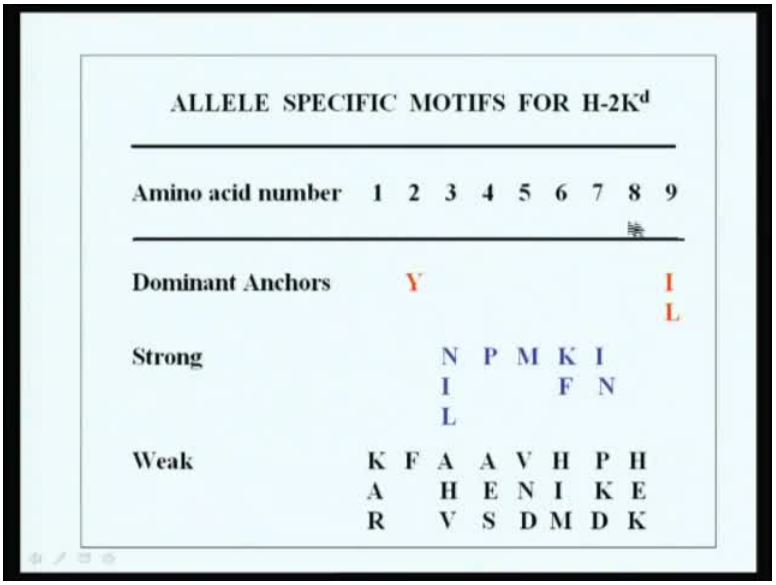

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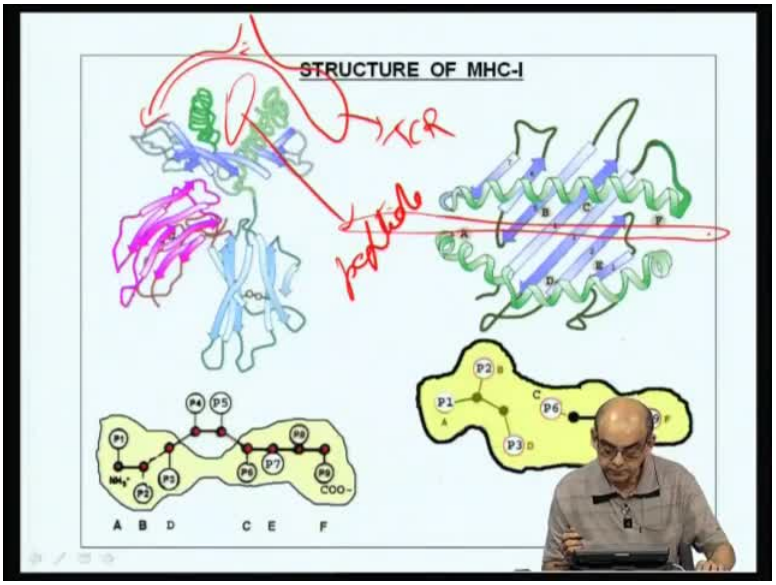

So, when they looked at the nature of antigenic fragments that was bound by MHC molecules, they found that in the class I molecule because of the closed nature of the groove, one had to elute these antigenic peptide sitting within this groove of MHC class I molecule by using acid; this acid, eluted peptide fragments were then sequenced by mass spectrometry purified by HPLC and then sequenced by mass spectrometric method and they found certain very unique properties to the peptide that are associated by different MHC alleles or different MHC proteins, especially the class I molecule. They found that only monomers were associated within this groove.

So, in the class I molecule, there is only space for 9 amino acid peptides as shown here; peptide residues 1, 2, 3, 4, 5, 6, 7, 8 and 9, beyond which, it could not be accommodated in this space. There were only 9 amino acid peptides sitting in the class I groove as opposed to the class II molecule, which had open ends at these two places and therefore, as I told you, I drew the line of the peptide longer than the groove and they concur confirming this, they actually found that the peptide that was sitting within this groove extended beyond 9 amino acids up to 13 to even larger; more than 13 amino acid could be accommodated in the class II groove; basically, because the class II groove was open.

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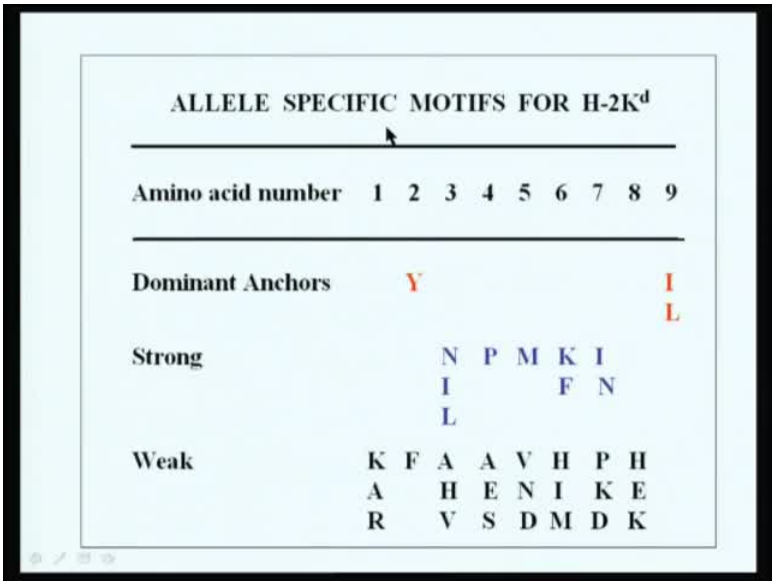

So, looking at the property that was associated with the acid eluted peptide from class I molecule, they found that, especially, this is just an example for one class I molecule called as H 2K of d or K is also class I antigen and d is the haplotype; this just designates

the primary amino acid sequence of this H 2K of d molecule. They found that in the groove, the peptide had tyrosine always in the second position, always tyrosine and in the ninth position, there was always isoleucine or leucine. So, in other words, all peptides that were eluted from the K of d molecule had tyrosine in the second position and isoleucine or leucine in the ninth position.

So, one could actually predict by looking at the protein amino acid sequence or for that matter the nucleotide sequence of the H 2K of d gene as well as the nucleotide sequence of an antigen of putative antigen or a putative protein, if it had tyrosine in the second position spaced later at the ninth position by isoleucine and leucine, one could predict that it was a putative motif for the H 2K of d MHC molecule. When they looked at the amino acid residue that were present between tyrosine and, isoleucine and leucine, they found these residues given in blue being present in more molecules that were isolated; that is, majority of the acid eluted peptide had these residues and therefore, a strong presence as opposed to weak presence of some of these residues in many of the molecules that were eluted from H 2K of d molecule. So, this is called as an allele specific motif because this motif having Y tyrosine or isoleucine or leucine as the dominant anchor was present only in the H 2K d molecule.

If one was to look at H 2D d or if one was to look at H 2K k or if one was to look at a different haplotype, the presence of allele specific motifs was indeed demonstrated in all of them. However, it was demonstrated that the residues would be different and therefore, the term allele specific, that means, they would be specific to a particular allele of the class I molecule.

The same sort of principles having the pockets and the binding to certain areas in the floor of the MHC molecule was also present, when looking at the class II molecule. So, there are certain broad rules that are obeyed, when this particular peptide is bound to the MHC groove, whether it is class I or class II.

Now, despite the prediction of the presence of these allele specific motifs in particular antigen, it does not however mean that all those predicted motifs would find the way into the peptide binding cleft. Basically, because the antigen presenting cell would vary in their capacities in order to produce those motifs or clip them at the exact position that was required for them to have tyrosine in that position and isoleucine or leucine, in the

case of H 2K d molecule and therefore, the presence of these particular amino acid residue and the presence of the particular motif does not actually mean that it is finally finding its way into the peptide binding groove. Another implication of all of the facts that are learned in the lesson today is that these peptides would vary in their affinities of binding to the class I or class II molecule.

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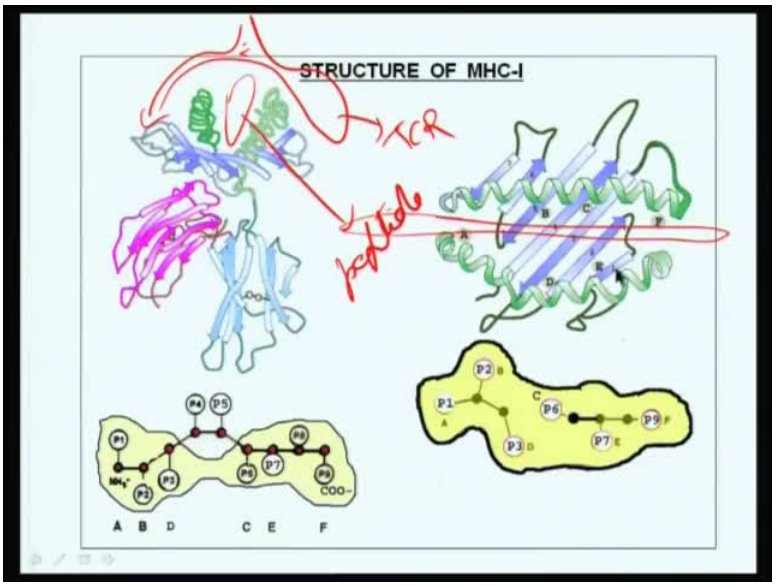

The stronger the affinity, the stronger is the activation by the T cell receptor; the weaker the affinity, the less is the activation by the T cell receptor. So, if one had a peptide that would bind with a very strong affinity, it could actually trigger a self-immune response because the MHC is blind to the self-derived antigens. In other words, the peptide that are binding to the class I and class II groove can be derived from non-self-antigens or foreign antigens or pathogens that are infecting as well as those that are self-protein that are there within that particular cell. In fact, both self-derived peptides and non-self-derived peptides have actually been eluted or have been isolated from the MHC molecule, both class I as well as class II.

So, in other words, this MHC molecule is actually blind to the fact that it is self or non-self. It is just that the self-molecule is binding to a peptide that is generated within the antigen presenting cell. The specificity of self and non-self is actually conferred by the T cell receptor because T cells learn to distinguish self from non-self, when they are differentiating within the thymus. Although this learning process is not perfect, in that T

cells bearing T cell receptor that recognize self and self-peptide also escape into the periphery. However, they are less frequent in number.

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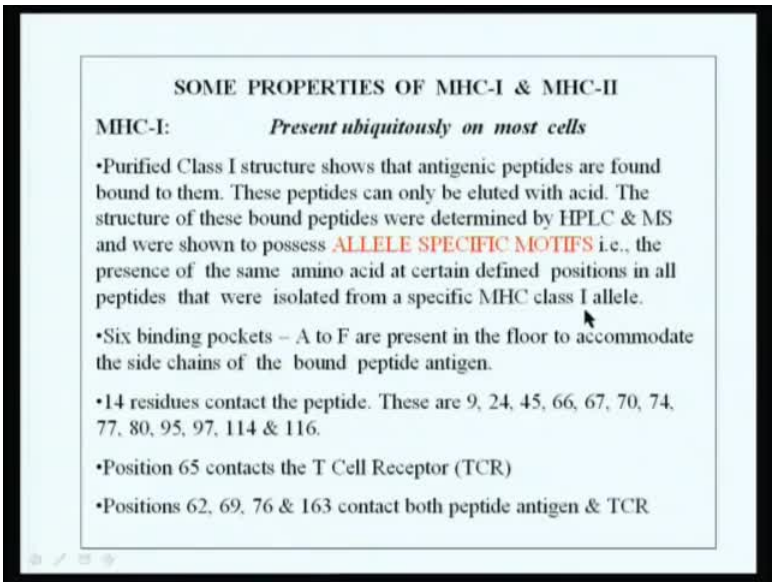

Therefore, going on further, just to look at some properties of MHC I and MHC II, it is which is described in this slide. One of the important thing being that the MHC I is ubiquitously found on most cells, except those derived from the central nervous system or the neuronal cells which express very little MHC on the surface because these neuronal molecules need to be protected from the immune response. One should note that the presence of MHC on the CNA cells, although less, could be brought about by a variety of regulatory factors. The presence of the 6 binding pockets, the presence of the allele specific motifs and the number of residues actually binding or contacting the peptide and that which contacts T cell receptor and those that contact both peptide antigen as well as the T cell receptor is given in the slide.

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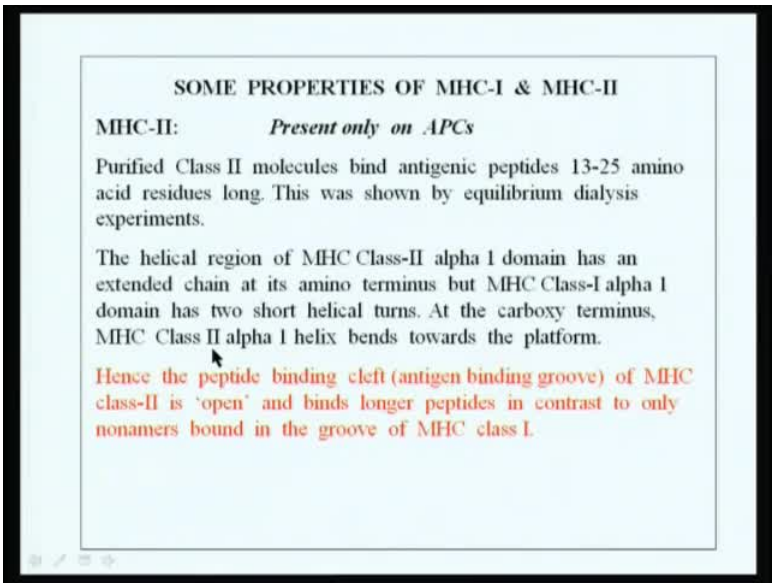

Going on further to the next slide, you find that MHC II is present only on the antigen presenting cells or those cells that present the antigen like professional antigen presenting cell, generating cells so on and so forth. These have the ability to bind antigenic peptides that are longer than 9 amino acids and that the helical region of class II alpha 1 domain has an extended chain at its amino terminus and allowing it to become be open and therefore, allowing it to bind peptide of a longer nature. So, so much for the properties of MHC I and MHC II molecules; which can other more details can be found in established text books like Kuby and so on and so forth.

Going on further, to look at the applications of MHC molecules and how it can be applied to skin graft or the graft rejection of the grafting process. Now, the grafting process is not simple. The donor and the recipients have to be HLA typed in order to match for the HLA. If the HLA is different between the donor and the recipient, it would yield to immune response and therefore, a rejection reaction. So, in order to look at what type of HLA molecule is associated during the grafting process, one has to type for the HLA; this is called as HLA typing.

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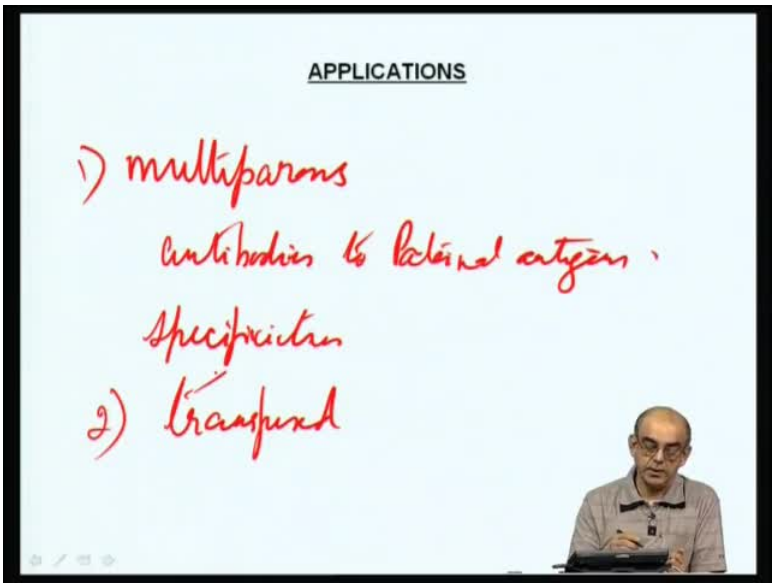

How was this facilitated in those days, when the modern recombinant DNA methods like RFLP was not available? This is called as the microcytotoxicity test. What was observed is that women who are multiparous, multiparous meaning multiple pregnancies, they had the presence of antibodies to the father of the foetus, to paternal antigens. The foetus derives half its components from the father and half from the mother. So, you have both the paternal MHC molecules and the maternal MHC molecule being inherited and co-dominantly expressed on the foetus. This leads to the recognition of the paternal MHC molecules expressed on the surface of foetal cells by the mother, which makes antibodies. The mother makes antibodies to these paternal antigens and these were discovered and characterized as to the specificities that they bound and these were catalogued in the serum bank.

And this was actually what could be used in order to type a donor and the recipient for the HLA type. In addition to that, they observed that patients who had been transfused multiple times with blood, also had antibodies to the lymphocytes or the minor fraction of the lymphocytes that were taken in during the transfusion, which got transferred in the transfused blood. So, these were also used to type HLA molecules or for HLA typing. So, how did they use these antibodies for the HLA test?

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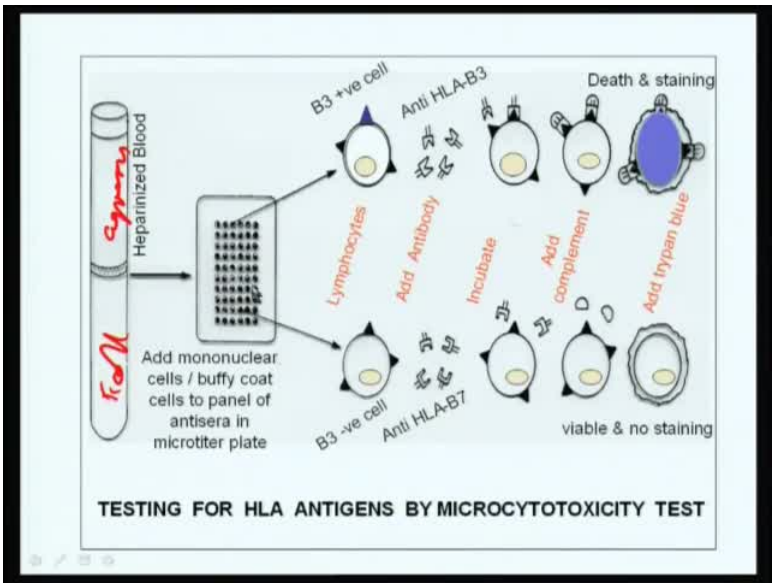

So, if you look at this slide, one isolates the lymphocytes or the mononucleocytes from the buffy coats of the blood that was drawn from the recipients or from a donor and centrifuging it on what is called as Ficall. This Ficall material actually enables the isolation of lymphocytes, mononuclear cells as a buffy coat, which comes at the junction of the Ficall and the aqueous layer of the blood; this is basically, a density dependent sedimentation. This buffy coat that is isolated is then put into plates, called as Terasaki plates and reacted with the typing sera that would be available in serum banks, which had already been characterized.

For example, in this case, the lymphocytes that were reacted with the B3 positive cell, then anti HLA-B3, which had already been characterized as opposed to anti HLA-B7 anti-sera that had been characterized to bind to B7 molecules. This antibody bound to the surface antigen HLA molecule that are expressed on the surface and after this incubation, one adds compliment and because compliment binds to this antigen-antibody complex on the surface, it would punch a hole into this cell as we saw in this previous lectures and then you put a dye like trypan blue, it would stain the cell blue and therefore, if you had a positive microcytotoxicity test, these cells would be positive for HLA B3.

So, when you use a panel of antisera, one could actually type the person in terms of what type of HLA molecule is present on their lymphocytes and then match for the donor and

the recipient. So, this is the microcytotoxicity test that was used for typing individuals in those days, before the availability of modern methods.

The modern methods of course, look at the DNA sequence and because the HLA complex of the MHC complex is allelic in nature, you have variations in the HLA nucleotide gene nucleotide sequence. So, one could synthesize, probe and allele them to those regions, which would vary between individual to individual and once the sequence, if you know the sequence of the probe, you know the sequence of the DNA that it binds to and therefore, you can type the individual bind by using these different recombinant DNA probes.

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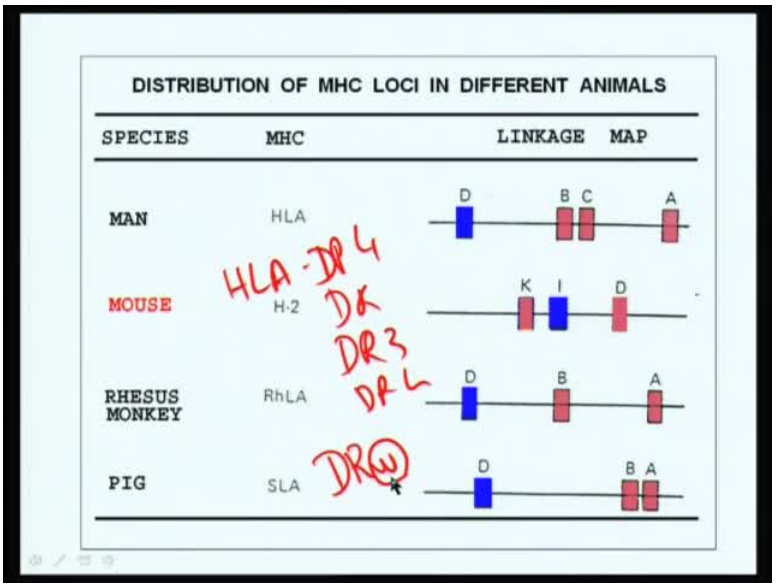

Now, looking at the DNA of the MHC loci in different animals, we found this the red stands for the class I molecule and the blue stands for the class II molecules, you find certain differences in the way they are organized in different animals. For example, the mouse as we saw earlier had the class II between the class I loci, the K and D forming the class I locus and the I region standing for or coding for the class II antigen as opposed to the mouse. If you look at the HLA antigen, you find the HLA antigen being characterized by the A locus, B locus and C locus; this A, B and C are class I molecules and the D locus stands for the class II MHC class II molecules. So, these are actually written like this HLA-A and the allele that follows for the A molecule, for instance like HLA A3, A7, A4 or for that matter, HLA B7, B3 and so on and so forth. As opposed to

the class I molecule, the HLA the HLA is written as D; there are sub loci within the D. They are DP, DQ and DR. So, you have HLA DR3, DR4 or DP4 or DP3, with the number and so on. In certain cases, you will find that the HLA DP, DQ, DR is usually, in rare cases represented with the small w - HLA DR w something.

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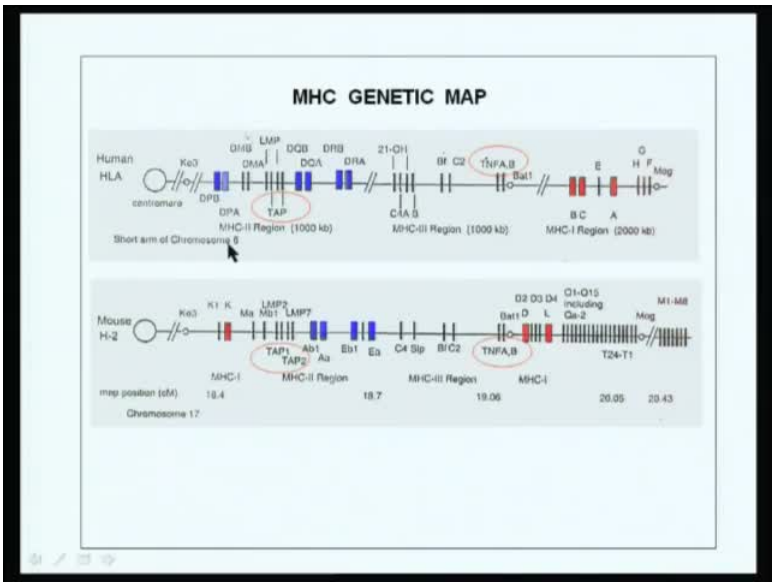

This w stands actually for workshop. This workshop meaning that this particular allele is not yet established and when it is established by different immunologists all over the world, they drop the w and it becomes a definite specificity like DR3, DR4 and so on. So, looking at the DNA or the genetic map of the human and mouse MHC complex, you have the human HLA being form in the short arm of chromosome 6 and mouse H 2 found on chromosome 17. So, this is the details of the MHC region that is found in different text books. So, the same thing if it is not legible in this slide, one could refer to Kuby and you would find the same information.

I would like you to note the following point which is circled over here - TNF A and TNF B; now, TNF stands for tumour necrosis factor, which is a cytokine that is found in the same region, on the same chromosome as that of the MHC and of course, you have the MHC III region, as I as we learnt earlier having the hydroxylase and the compliment proteins and other proteins that are found on the MHC genetic map.

The finding of the genes coding for certain cytokines like TNF actually, points out the fact that these cytokines could be coordinately regulated along with the MHC molecule.

In other words, those regulators that pushed up the MHC molecule or induced the MHC gene would also have the ability to push up the TNF under similar circumstances. So, these molecules would be coordinately regulated. Now, TAP is also found in the similar same location; transporter associated with peptide antigen, which is involved in antigen presentation and processing. LMP is another molecule that is involved in the processing.

In the addition to the HLA A, B and C which is shown in red, you have the class II antigen shown here like the DQ, DR and DP over here. This is shown for the mouse here and you find these are the K region, D region and L region and these are some of the minor histocompatibility antigens that are associated with the innate immune response. The TNF also, being found similarly, just in the same location, just like the human molecule.

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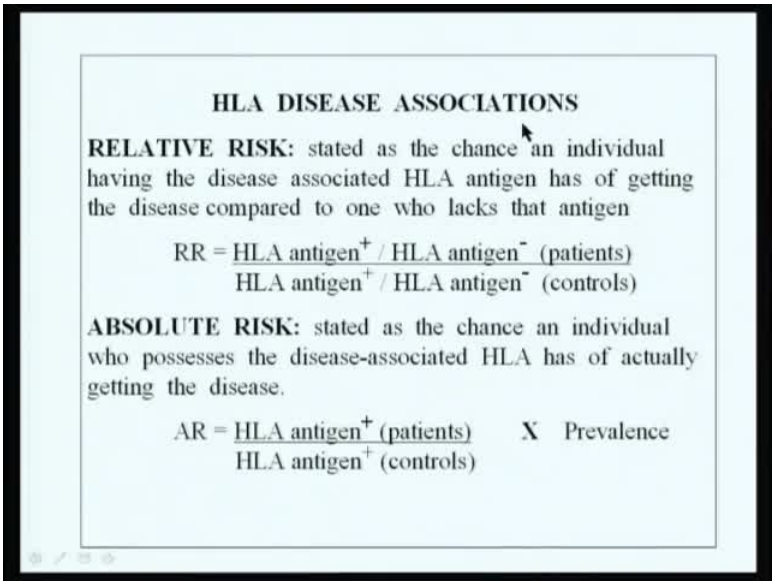

The off shoot of all these observations with these HLA typing, they found that certain HLA allele was always associated with certain diseases. So, these are called as HLA disease associations. In other words, certain HLA alleles bearing human, would always be associated with certain HLA diseases. When they had done all this typing for several human beings like several patients who come to the hospital, they found that these HLA disease association could be defined by two terms called as the relative risk and the absolute risk. The relative risk is the chance an individual having the disease associated HLA antigen has of getting the disease compared to one who lacks that antigen.

So, this is actually, calculated by the patients, who are possessing that particular HLA allele, divide that by the patients who are not having the HLA allele; you divide this entire thing by the controls, who have that particular HLA allele which is again divided by controls who lack that particular antigen. So, this is called as the relative risk. So, relative risk is an important parameter in order to advice patients, whether they were at a risk for getting a certain kind of disease. In contrast, the term absolute risk actually gives the chance an individual has of actually getting the disease, who has the particular HLA allele.

So, AR is defined as the patients, who have that antigen divided by the controls, who have that antigen multiplied by the prevalence of the disease in the population. For example, if you have a vector that is contributing to the spread of the disease like mosquitoes, if you have mosquitoes, more of those mosquitoes in a particular location, you are absolute risk would be higher. Therefore, looking at these HLA disease associations you find that certain HLA alleles are always associated with a particular disease.

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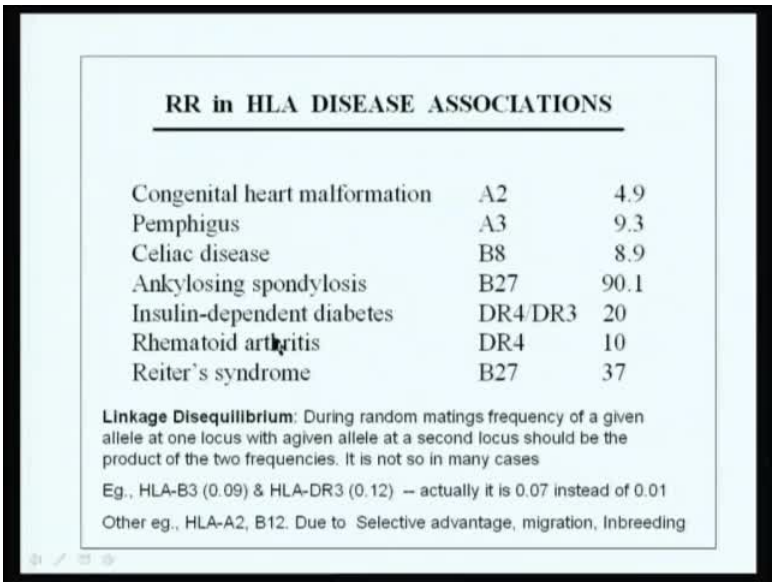

For example, congenital heart malformation is consistently associated with the higher relative risk with HLA A2 and coming down to Ankylosing spondylosis, you see the relative risk being 90.1 for HLA B27. In other words, those patients or those individuals

having HLA B27 as their allele, have a higher risk for suffering from Ankylosing spondylosis.

Similarly, insulin dependent diabetes mellitus - you find that people, who are having DR4, DR3 allele or the primary amino acid sequence corresponding to the class II molecule DR4 and DR3, would be more risky of getting this particular insulin dependent diabetes. Now, as a derivate of these observations, you find that there is a linkage disequilibrium. What does it mean? This means that during random matings frequency of a given allele at one locus of HLA with the second locus of the HLA should be the product of the two frequencies according to statistical principles.

However, we find that it is not so in many situations. For example, HLA B3, which has a frequency of 0.09 in the population and HLA DR3, which is listed as having a frequency of 0.12 should have an overall frequency of 0.01. That means HLA B3 and HLA DR3 coming in the same individual, but it is much higher; instead of 0.01, it is much higher being 0.07, which means that these two alleles for some reasons being derived in an associated manner.

What is the reason for this association? It is due to selective advantage. Perhaps these two are conferring a selective advantage for immune responses again certain kinds of certain kinds of pathogens or certain kinds of diseases. It also indicates certain migration during the history of man and admixture of populations. In addition, certain populations are noted for their inbreeding or marrying within the family like close cousin marriages that happen in India. These are the reasons why certain HLA alleles are associated; other examples being HLA A2 and B12.

So, what we have seen now, in these lectures is the nature of the MHC molecules and how it is involved in the recognition of the peptide antigen and how the peptide binds with varying affinities to different alleles of the MHC molecules and the concept that needs to be learnt over here is that due to this variation in affinities of peptide binding to the different alleles of the MHC and taking into taking into or remembering that we all have different alleles, it is possible that some of these alleles that we inherit, actually bind to certain antigenic fragments better than the different kinds of other kinds of pathogens leading to a certain kinds of inequality in how we are susceptible or resistant to particular kinds of pathogens or viruses.

For example, if I came down with a particular virus or children sitting in a class room can come down with cough, but a different child sitting in the same on the same bench in the class room perhaps may not come down with the same cold and cough as his neighbour. This perhaps is one of the one of the reasons resulting to this kind of distribution or non-recognition because HLA allele [red] a person who is coming down to the cough may not be binding to the peptide as efficiently of that particular pathogen that is causing that cough.

As opposed to a person where there is good affinity of binding to the pop to the fragment that were derived from that infection and therefore, there would be a better immune response in a resistant individual than a susceptible individual, but this is not across the board. Some of us are resistant to certain pathogens, but are more susceptible to certain other pathogens and therefore, the diversity in pathogen recognition, due to the diversity HLA in the HLA alleles. This then leads on to how these HLA alleles are going to be recognized by the T cell receptor, which will come in the next series of lectures. Thank you very much.