Essentials In Immunology

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Module No. # 10

Lecture No. # 20

The major histocompatibility complex – Part 2

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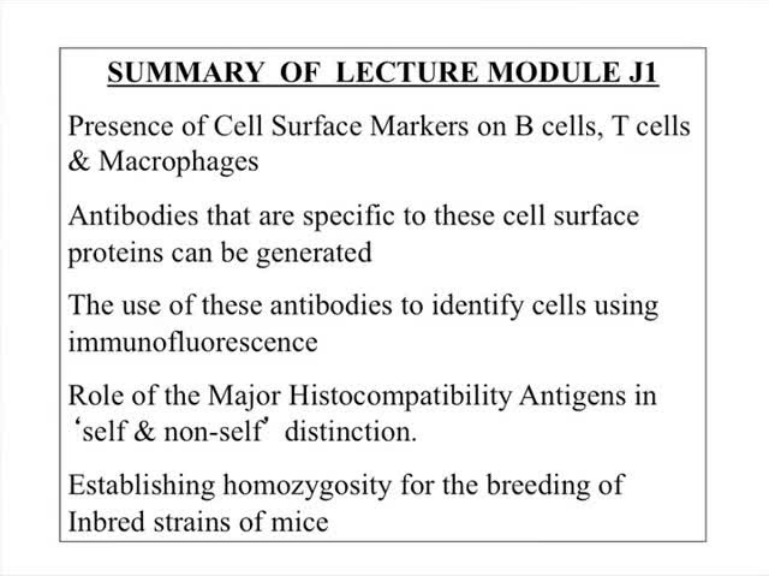

Hello and welcome. To summarize points that were covered in the previous lecture, we covered the presence of cell surface markers on B cells, T cells as well as macrophages and how antibodies that are specific to the cell surface proteins could be generated and the use of these antibodies to identify cells expressing these molecules using immunofluorescence and we also went into the role of the major histocompatibility antigens in self and non-self distinction or recognition and this involved the establishment of homozygosity for the breeding of the inbred strains of mice, which were so essential to look at self and non-self distinction.

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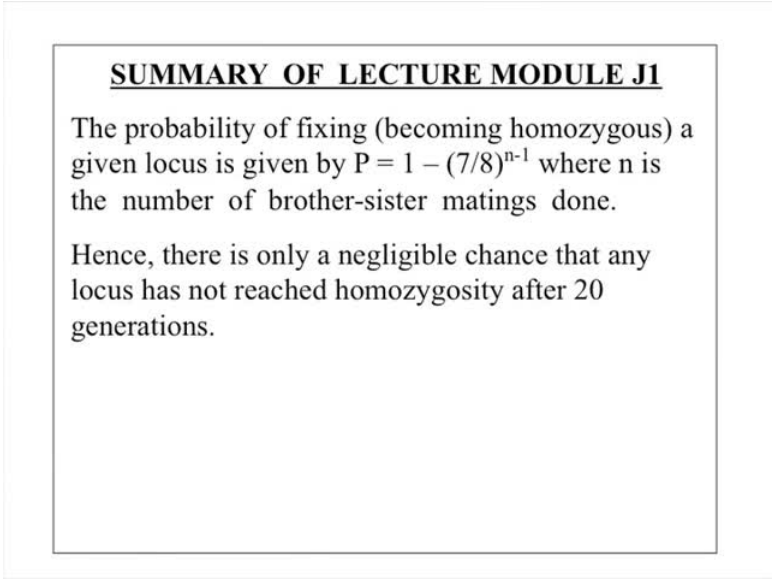

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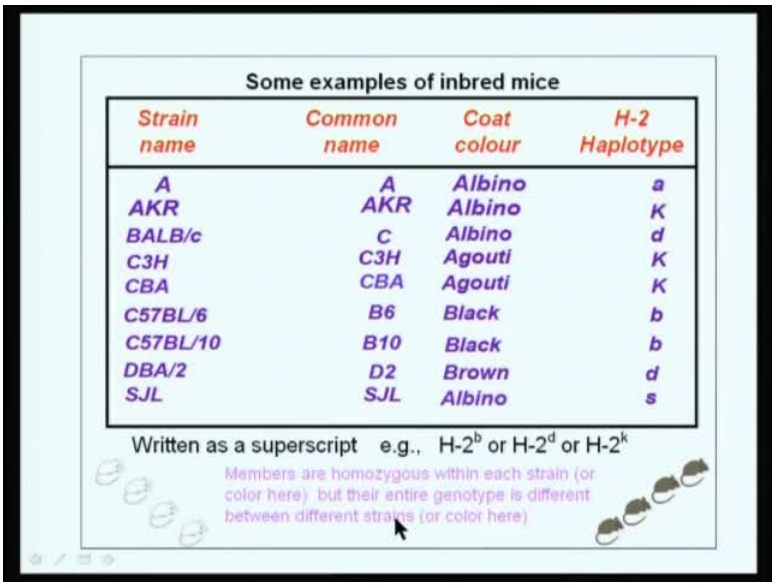

This breeding of inbred strains of mice had the principle of the probability of fixing or becoming homozygous given by this formula that is indicated over here and which also indicated that, if further system mating was done for a pair of mice for over 20 generations, then their genome would essentially become homozygous. Going to some examples of inbred strains of mice and how they are represented, which is represented in this slide and this indicates the different strain names given over here and the common names as it is usually known by. Incidentally, the common white mouse that you see in most animal houses, the balb c mouse, which are used mainly to make monoclonal

antibodies and other experiments, and the coat colour, which is also a distinguishing feature of the breeding experiments.

Now, the H 2 haplotype is given over here and the way the genotype, so far as the histocompatibility or the immune responses are concerned, is written as H dash 2 and the superscript is represented by a small alphabet, like in this case b and this haplotype is indicated over here for all these strains of mice. For example, a and AKR as K; incidentally, this has to be in small; it is not caps, it is small k. Likewise, in these two cases also.

So, you have it written as H 2, the superscript being the alphabet. So, for different mice, it is a different alphabet. All it indicates is that that these different mice have a different sequence of genes in their H 2 complex. Now, the members of each inbred strain are homozygous within each strain or if we take over here these white mice represented here and the black mice represented over here, between these mice, all there haplotype is the same; that means they are all homozygous and they are set to an inbred strain, whereas when compared to the black mouse and the white mouse you will see that the sequence of genes are very different at the MHC locus.

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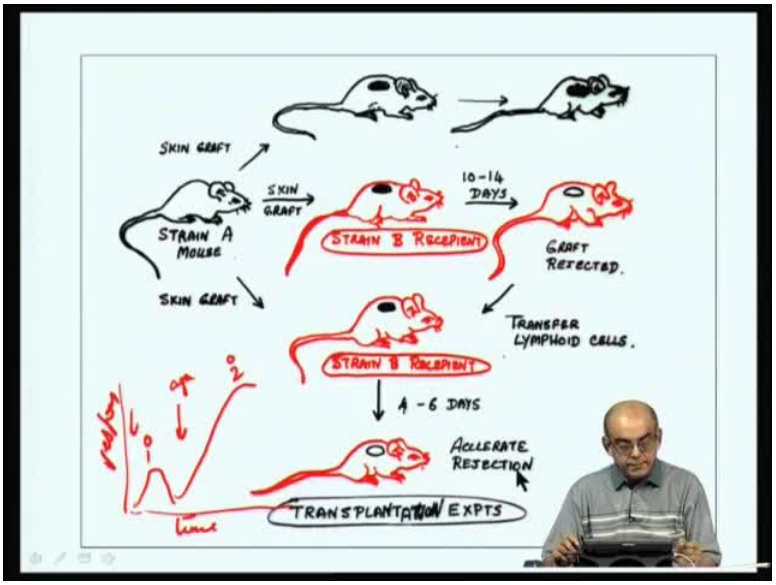

So, you have the entire genotype being different between different strains of mice as indicated by this coloured mice over here. Now, once they recognized that these were inbred strains of mice, they could reproducibly do experiments involving skin graft or

transplantation experiments, which is shown in this slide over here. So, skin grafts that were exchanged in the same mice or the same inbred strains were accepted; skin grafts that were grafted on to a different strain of mice, a donor being the one that is giving this skin graft or donating the skin graft and the recipient being the one that is receiving the skin graft, as in this case, this graft survives for about 10 to 14 days, after which that piece of skin becomes dry, the circulation is cutoff and the piece of skin drops out.

So, we refer to it as being the graft being rejected. This they have noticed, during this graft rejection, when they look at the morphology of lymphocytes as was covered in the in the class involving different types of cells of the immune system. They underwent morphological changes indicating the participation of immune cell in this process of graft rejection. Incidentally, if the mouse was grafted again with the same piece of skin from the same inbred strain of mouse, you would find that there would be an accelerated rejection, reminding you that it could be a immunological phenomena, where a memory response was involved.

Also, if lymphoid cells were transferred from this recipient to a naive recipient of the same strain and grafted on to the back again, you will see the same accelerated rejection of the skin reminding you that these are experiments or results that would be seen in immunological experiments, where one would see a primary an immune primary and a secondary immune response and when an antigen is injected for the first time, you will see a primary response. This is the immune response and on the x axis, you would have the time. When the antigen is exposed again to this immune system, you would see a heightened response, which is called as a memory response or the secondary response which is what is happening during the accelerated rejection of the skin graft indicating that this rejection of skin graft was in fact, immune mediated.

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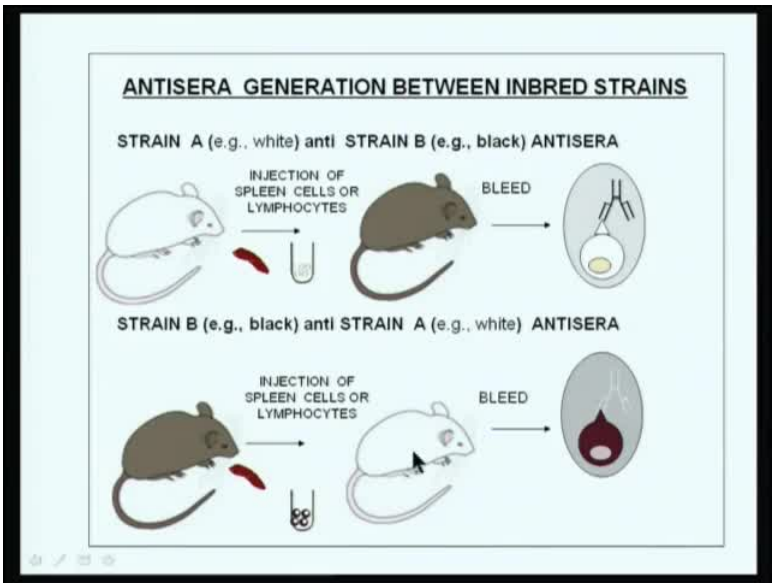

Now, once several inbred strains of mice became available and the suggestion of the immune responses being involved in this skin graft rejection or the histocompatibility reaction, experiments were dedicated to antisera generation between inbred strain of mice. For example, you could immunize the spleen cells or the lymphocytes that were taken from one strain and in the case, as an example, a white strain which is denoted here as strain a and injected into the into the strain B mouse, which is indicated by a black mouse here and after certain period time have to booster injection, one would see the presence of antibodies again strain a in the blood of these mice and these antibodies would actually recognize a strain a specific antigen.

In other words, it was possible to generate strain specific antisera by cross immunizing these mice. Similarly, the injection of the black mouse of the strain B mouse with the splenocytes or lymphocytes taken from the other strain or the strain A would generate anti antisera or antibodies that would specifically react to the cells derived from the black mouse. So, in other words, this strain specific antisera generation made it possible for immunologists to see the presence of the expression of this so called self specific antigen.

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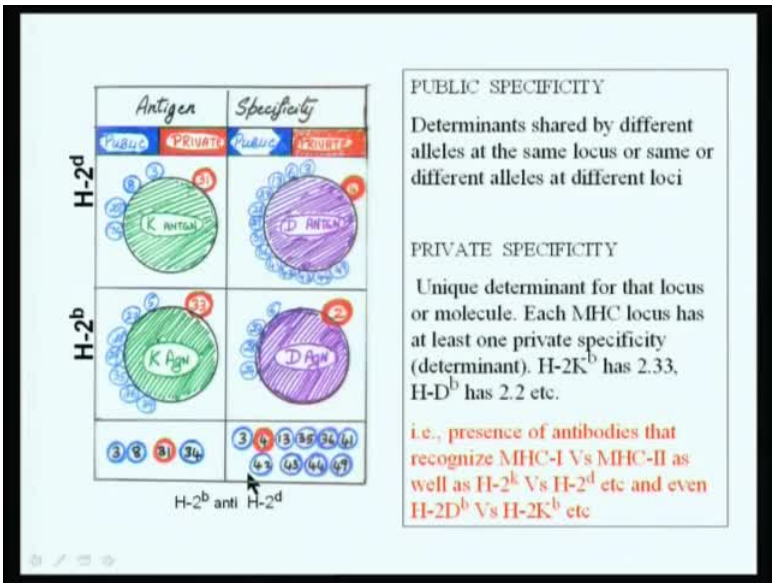

In fact, serology is such a complex field involving the cross immunization of these different strains of mice. In fact, when all these different strains of mice were immunized with the splenocytes taken from each other, you would get antibodies that would be specific, not only for different kinds of strains, that means a common determinant that was spread across different strains, which is called as the public specificity.

So, these antisera recognized determinants that were shared by different alleles at the same locus or same or different alleles at different loci. Now, this will become clear as we look at the MHC complex in later lectures. A private specificity, on the other hand, had the ability to recognize a unique determinant for that particular strain or for the particular type of MHC or locus in that particular inbred strain of mouse.

So, in fact, the number of sera that could be generated would actually come, could be calculated by this formula, where you have 2 to the power n minus 2, where n is the number of inbred strains involved in these cross immunization studies. Suffice it to say that these serology experiments involving the cross immunization of inbred strain of mice with their splenocytes or lymphocytes made it possible to generate antibodies or antisera that could specifically recognize these inbred strains of mice, in terms of an antigen or a determinant that would be expressed on protein antigens on the cell surface. In addition, these antisera had antibodies that could recognize common antigens that would be distributed across the different strains.

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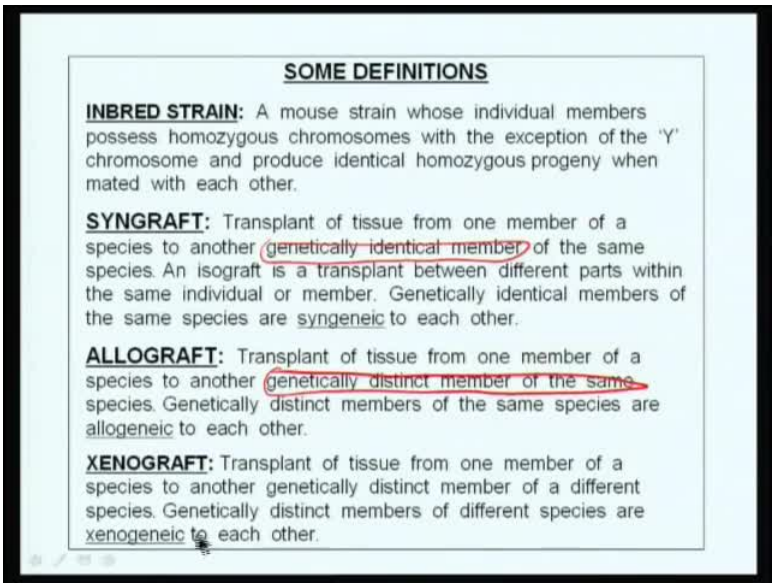

Coming now to some definitions that are arising from these transplantation experiments and immunological experiments, one is the inbred strain, as we have we are now referring to the generation of inbred strain of mouse. It could be defined as a mouse strain, whose individual members possess homozygous chromosomes with the exception of the Y chromosome because it is a male; the difference is the it represents the difference between the male and females and these strains actually produce identical homozygous progeny when mated with each other.

Now, during the grafting experiments, these are the definitions involved, being syngraft, which is a transplant of tissue from one member of a species to another genetically identical member of the same species. So, in other words, this grafting has to take place between members of the same inbred strain. As opposed to a syngraft, an isograft is a transplant between different parts within the same individual or member of the or member of that inbred strain of mouse. Genetically identical members of the same species are termed as being syngeneic to each other.

The term allograft, on the other hand, represents the transplant of tissue from one member of a species to another genetically distinct member of the same species. Genetically distinct members of the same species are said to be allogeneic to each other. Therefore, the terms syngraft and allograft have to do with the genetically identical members of the same species or genetically distinct member of the same species. The

xenograft, on the other hand, is the transplant of tissue form one member of a species to another genetically distinct member of a different species. So, genetically distinct members of different species are referred to as being xenogeneic to each other.

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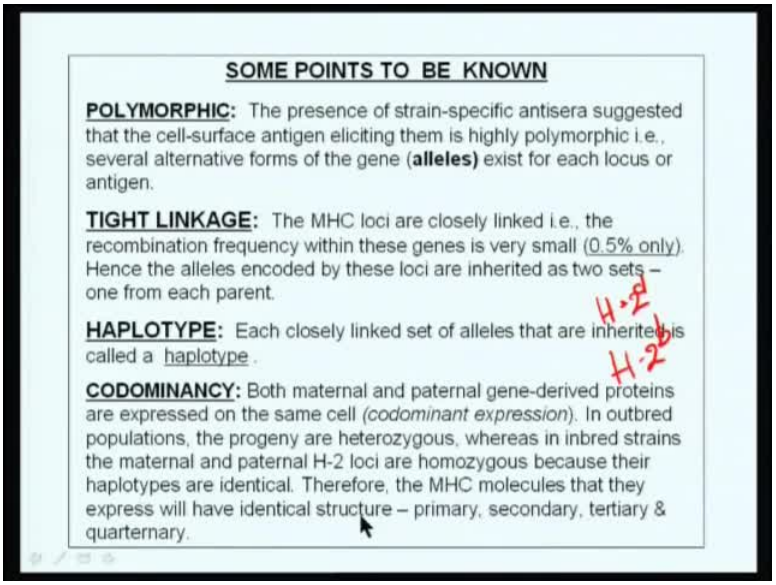

Now, continuing with some points that needs to be known is the term polymorphic. Now, the generation of these inbred strains of mice and the concept that these inbred strains of mice actually expressed different alleles of the same locus on the cell surface brought out the point that these strain specific antisera actually indicated the presence of cells of antigens that elicited them as being highly polymorphic; that is, several alternative forms of the gene or the proteins referred to as alleles for the genes exist for each locus or antigen and that all these loci, which where termed as a major histocompatibility complex, since these were discovered by histocompatibility experiments or graft transplantation experiments.

These loci are closely linked; that is, the recombination frequency within these genes is very small of the order of 0.5 percent. This resulted in the observation that all these alleles encoded by these loci are inherited as 2 sets: one from each parent, one from the father and one from the mother and these alleles, which are closely linked, which are which are inherited as a set is actually called as a haplotype. So, the haplotype is defined as each closely linked set of alleles that are inherited is called a haplotype. This haplotype, essentially and crudely speaking represents the genetic sequence of the or the

nucleotide sequence in the genes that are involved and therefore, being different between different inbred strains of mice and when a super script is written for the mice as H 2b, this alphabet actually represents a certain nucleotide sequence for these genes, which will which will be different in a different inbred strain of mouse, which may be having a d haplotype.

Going on further, the term codominancy refers to the fact that these antigens are genes or genes are expressed in a co-dominant fashion, both the maternal and paternal gene derived proteins are expressed on the same cell. Now, we are referring to the MHC locus. In outbred populations, the progeny are heterozygous, whereas in inbred strains, the maternal and paternal H2 loci are homozygous because their haplotype or genetic sequence at the H 2 locus is identical. Therefore, the MHC molecules that they express within inbred strains or members of the same inbred strain will have an identical structure, both primary, secondary, tertiary as well as quaternary.

Now, when it is an outbred strain of mouse or in heterozygous populations like in human populations, you have both the maternal and paternal MHC gene derived antigens or protein molecules on the cell surface. Incidentally, it has to be pointed out that since these serology experiments involved the cross immunization of spleen cells and the antibodies generated were set to recognize a particular determinant on an antigen that is expressed on the cell surface derived from these inbred strains, the term antigen was also used for the MHC molecule and therefore, when there are descriptions involving the major histocompatibility complex, you will see in text books that it is referred to as the MHC antigen. This does not mean that this is always used as an antigen, but the term became associated with the MHC molecule. The term antigen can also be used for other protein molecules, which are being immunized into different kinds of animals.

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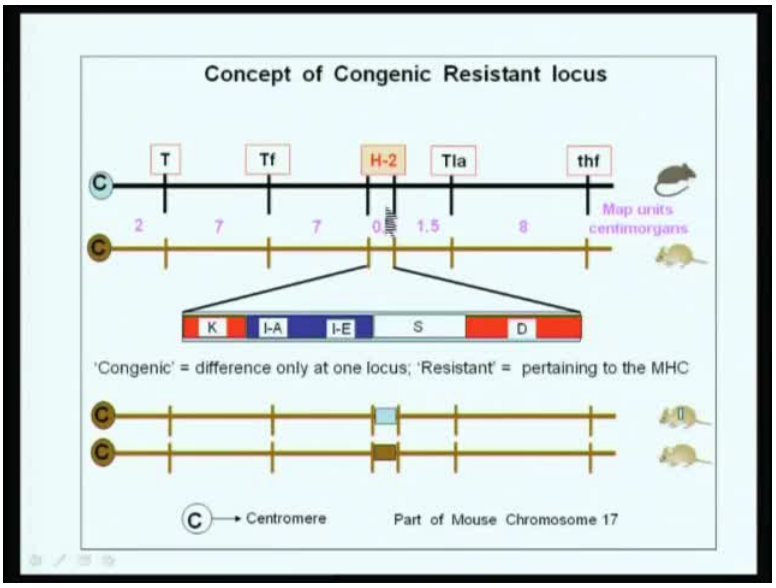

So, going on further, you find that the generation of inbred strains of mice led to mice that are having different genotypes or different haplotypes, but this haplotype actually represented the sequence or the nucleotide sequence across all the chromosomes. There was no specific locus or no specific region that was identified within all these different chromosomes as being responsible for the skin graft rejection and therefore, experiments were done in order to find out or try to locate a locus that would be responsible for the skin graft rejection reactions that where that that they were observing and this could be done by the same inbreeding strain inbreeding experiments that were shown to you earlier.

Conceptually, just to indicate to you what congenic resistance locus means, the word congenic means difference only at one locus and when you are following the major histocompatibility locus, this had to be the locus that determine skin graft rejection and the resistance or the word resistant meaning pertaining to the MHC because one was referring to the resistance or acceptance of skin graft.

So, therefore, when you look at these two representations over here, one being the brown and one being put in the black, this being C standing for the centromere. These are all the different loci that are used as markers which are around this particular region that determines the skin graft rejection, which as I referred to you in the previous slide as H 2. Now Tla stands for thymus leukemic antigen and Tf stands for transferrin and so on

and so forth. What is shown in purple over here is the map units or the recombination units in centimorgans.

So, when you look at the black and the brown lines, you will see that they are different across entire chromosome. So, you look at mouse chromosome 17, which is where the H 2 is located. One had to have mice that were different only at the H 2 locus in order to say that this difference is what is causing the skin graft rejection. The H 2 locus further is subdivided into sub loci. There are two types of MHC molecules or H 2 molecules called as the class 1 and class 2; class 1 loci being represented by the K gene and the D gene and the class 2 antigens being expressed form the I gene, I A as well as I E. So, there are two sub loci in this class 2 locus. Then you have the S region, which is the class 3 MHC molecule, which is not pertaining to this lecture.

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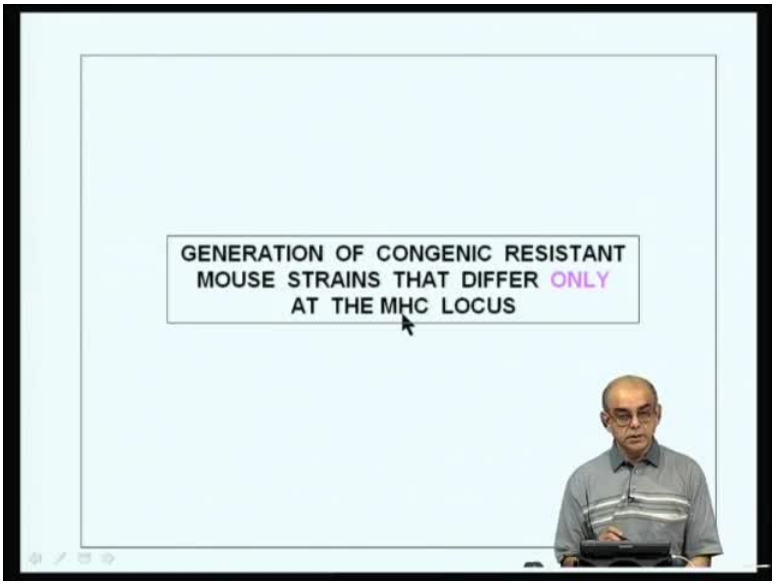

So, how did one derive mice or generate mice that were different only at this particular locus. So, in order to do that, we need to find out what sort of breeding experiments which were done in order to generate this type of congenic resistant mouse strains.

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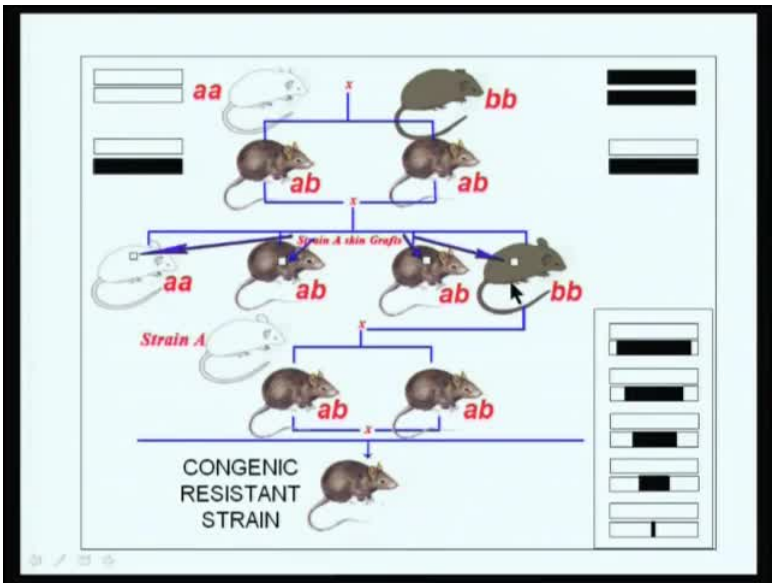

This involved the inter breeding and cross breeding of these mice including inter breeding as well as back crossing to the parental strain. So, as you see in this slide, 2 inbred strains were taken and they were mated to get the heterozygous F1. These heterozygous mice were brother sister mated, in order to get this ratio of 1 is to 2 is to 1. So, aa being homozygous and bb being homozygous, and ab being heterozygous.

Now, these pups that were there in the cage were mated back to the parental strain A mouse. The assay that was used during all these inbred strains of mice was the ability of these mice or the F1 or F2 generations to reject a piece of skin derived from the parent that they were back crossing it to. In other words that these mice had to reject the strain A skin graft. As you will see here, only the black mouse, which is homozygous would reject the A strain skin graft and these heterozygous progeny as well as the homozygous A strain would actually accept this A strain skin graft.

Now, using this assay to follow the generation of these mice during the breeding experiments, they continuously back crossed these heterozygous mice to the strain A skin graft. So, after this one strain A back cross, they went about doing the intercrossing again. They got the next cycle, where they back crossed to the A strain parent, all the time assaying them for the ability to reject the strain A skin graft and therefore, as you saw in the previous slides, as the principle of the formula suggests, doing this cycle more than about 10 to 15 cycles would generate a mouse that would become homozygous to

the parent, which is a, but on the other hand, you are always picking mice that were that were that were having the ability to reject the strain A skin graft and therefore, that would remain the haplotype that had the ability to be different from the strain A parent. So, these congenic resistant mice are having the same sequence everywhere except for the H 2 locus.

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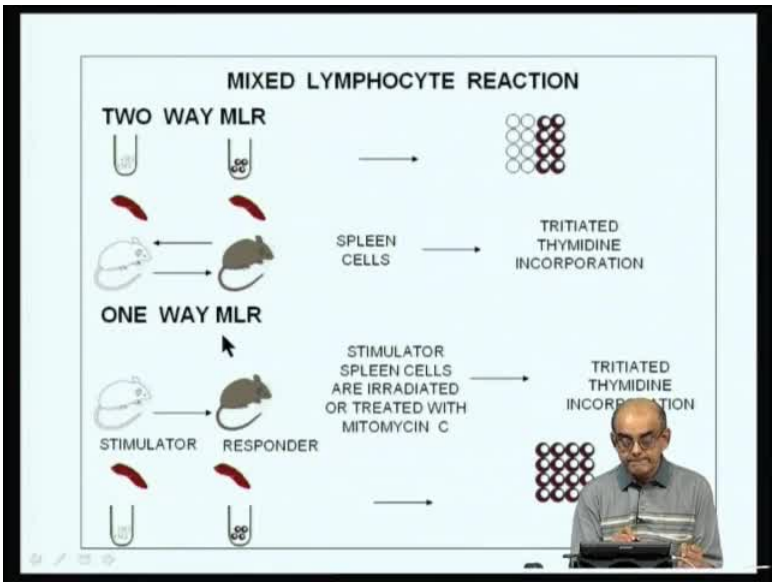

Now, after having generated these congenic resistant strains of mice, they found that since these were known to be immunologically related phenomena, they mixed these splenocytes together to see what would be the result. So, taking congenic resistant strains, in other words, these congenic resistant strains were different only at the H 2 complex or MHC locus, which was the locus that determines skin graft rejection.

So, you had two types of assays. What was called as the mixed lymphocyte reaction; as the name suggests, they mix these two kinds of splenocytes together from these 2 congenic resistant inbred strains of mice and they found that these 2 splenocytes would react against each other. This reaction could be followed because there was the consequence of this mixing was proliferation. This proliferation could be followed by tritiated thymidine incorporation into the DNA of these cells.

So therefore, these two strains of splenocytes would recognize each other and proliferate. On the other hand, they made this assay little more simpler to follow it, where they took one of these strains of mice; it is called as the stimulator because they were used to

stimulate the responding cells. they treated them with a They irradiated these cells. So, they destroyed their ability to proliferate or treated them with the drug called mitomycin C, which would again block proliferation by incorporating into the DNA and this would then result in stimulating the responding cells. So, when you measure thymidine incorporation, this would give an indication of how the responding spleen cells were proliferating.

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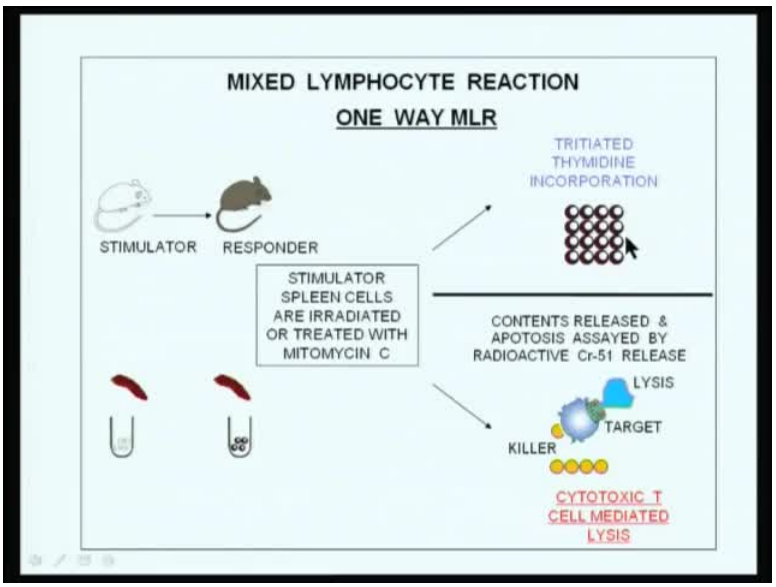

So, these were the different kinds of assays; they would they would do the assays in reverse and so, they could have a one way MLR setup for different kinds of inbred strains of mice. During this one way mixed lymphocytes reaction, which is usually done for a period of 3 to 5 days, two different kinds of results are noticed. One is the thymidine incorporation as we just now gone through.

Now, during this culture for 5 days, there also results a differentiation of killer cells in this mixture. So, these killer cells, when they are exposed to or brought in contact with the cells that were stimulating them, would actually kill them. So, these killer cells that had differentiated during this mixed lymphocytes reaction would actually engage the target cell. These target cells being derived from the same haplotype is that of the stimulator cell. These targets could either be the spleen cell that had been treated with the mitogens so that they had become bigger in size or they could be tumor cell that had

been derived from such mice or having or bearing the same haplotype or expressing the same MHC antigen on their cell surface.

So, the differentiated killer cells would recognize that particular MHC antigen and kill them resulting in lysis of these target cells and the contents being released to the outside, in addition to which apoptosis or apoptosis had are was also induced in these cells, which means that the DNA was being fragmented. Now, this assay could easily be followed by a radioactive chromium 51 release assay. The targets were labeled with chromium radioactive chromium 51, which would be taken up actively and kept inside, essentially becoming a balloon of chromium 51 and when the killer punched a hole into these cells, this chromium 51 would be released and one would one could calculate the percent killing. Why is this important? These 2 assays were very important in establishing the presence of the subloci that was that was seen in the H 2 complex.

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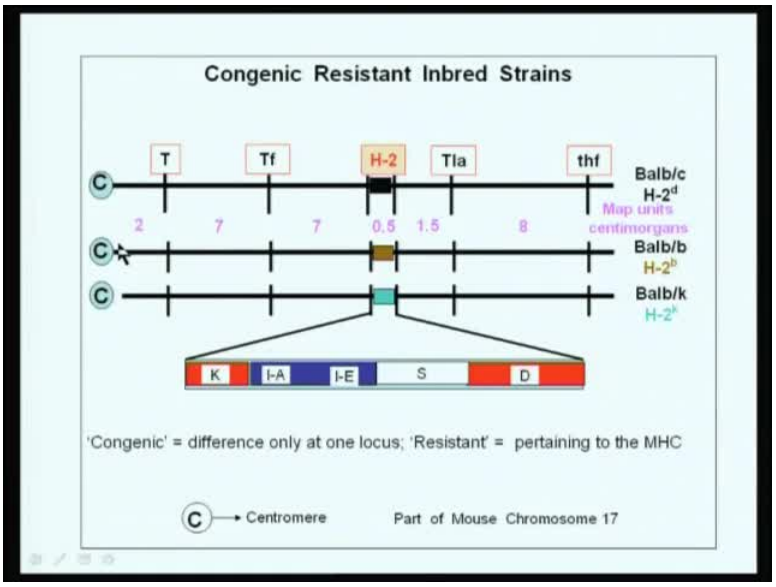

As you see here, this is the slide that I showed you earlier, in which 2 inbred congenic resistant inbred strains of mice were differing only at the H 2 complex and the other regions including the other chromosomes had the same sequence.

Now, this H 2 complex which was different, referring to the difference presence of difference between congenic resistant inbred strains of mice, also was represented as having two types of MHC as I alluded to earlier and that is the presence of the class 1 antigens being the K and D and the class 2 antigens being the I region. Now, these 2

assays were instrumental in discovering this sort of a difference in the type of MHCs that were present.

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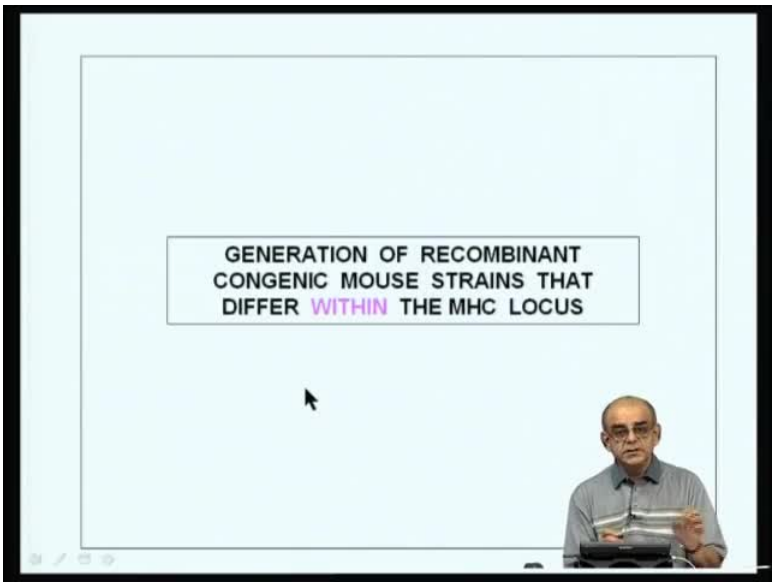

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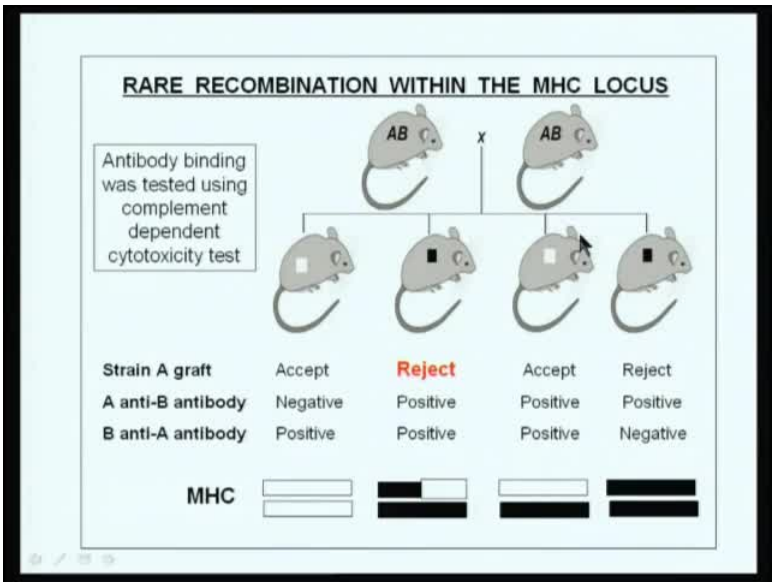

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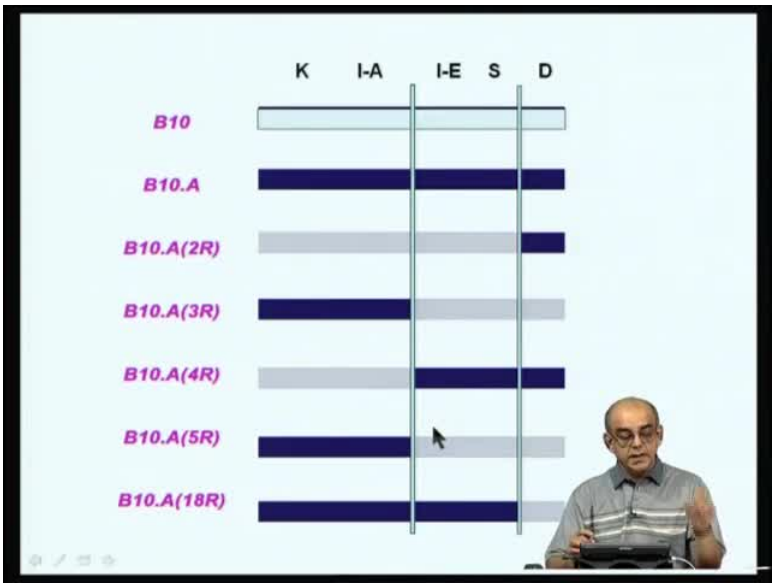

Now, to see how these how these were discovered again, one has to go into how these different inbred strains of mouse mice were being mated and during this inbreeding process, they found out that these mice had actually undergone an intra H 2 recombination, although this locus is very closely linked. So, these were rare events and therefore, the generation of these strains was also very rare. These could be distinguished by their ability to reject versus accept the particular skin graft in the heterozygous mice. We will not go into the details of this for lack of time.

Suffice it to say that these are intra H 2 recombinants and these are different strains of mice that were generated. 2R, 3R, 4R, 5R and 18R refers to the cycle - second cycle, third cycle, fourth cycle and so on, during which the extremely meticulous record keeping by those experimenters demonstrated that these mice were actually different, when they were subjected to these different assays of CTL as well as graft rejection and proliferation assays.

So, you will see that the CTL reaction is associated between a difference in the class 1 locus. For example, the D antigen being different in these 2 strains caused a difference or a CTL activation phenomena, cytotoxic T lymphocyte or lytic acid becoming positive as opposed to the proliferation acid being positive in the strains of mice, where the class 2 locus or the I E locus was different between the 2 different strains of mice.

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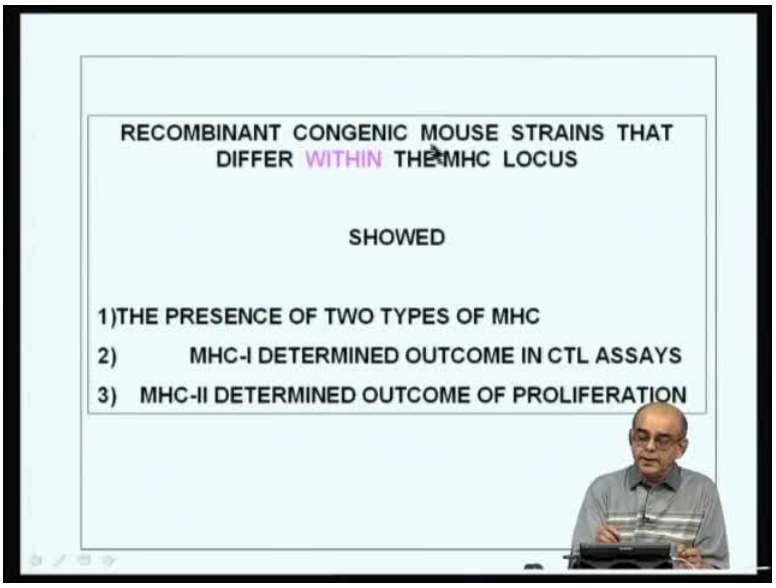

So, these are just examples, where an intra H 2 recombination had occurred and how these strains of mice were actually used to demonstrate that there were two types of MHC called as the MHC 1 and the MHC 2; MHC 1 always determined the outcome in CTL assays and the MHC 2 always determined the outcome in proliferation assays. In other words, a difference in the MHC locus led to a higher degree of proliferation, whereas a difference in the MHC 1 locus led to a higher degree of CTL generation or killer cell generation.

Therefore, the importance of recombinant inbred mouse strains that differ within the MHC locus showed the presence of these two different MHC types called as the MHC 1 and MHC 2. So, all these experiments that involved the CTL assays, the proliferation assays and especially the generation of a mouse sera, which had public specificities and private specificities enabled the use of these antisera to show the presence of specific determinant or specific protein antigens that were present on the cell surface derived from these different inbred strains of mice expressing different MHC molecules or MHC molecules that differed in their primary sequence.

So, these antisera then led to experiments that involved the immunoprecipitation of these molecules from the different inbred strains of mice and the immunoprecipitation of these molecules actually help in the purification or the affinity purification of these different MHC antigens that were different in different inbred strains of mice and these purified

antigens were then subjected to an amino acid sequence, in order to find out the primary amino acid sequence of these MHC antigens. And as I told you earlier, these amino acid sequences demonstrated that in fact these MHC antigens were very polymorphic in nature. This polymorphism is different from the gene recombination that is seen in antibody molecules or immunoglobulin genes, where the variable region genes are taken out and juxtaposed to the constant region genes.

There it is a phenomena of gene recombination, which results in different variable region genes, but not the whole molecule being different in different cases and that the variable region genes had to be associated with the binding of the antigen and therefore, different variable region genes had to be used during this immunoglobulin gene rearrangement.

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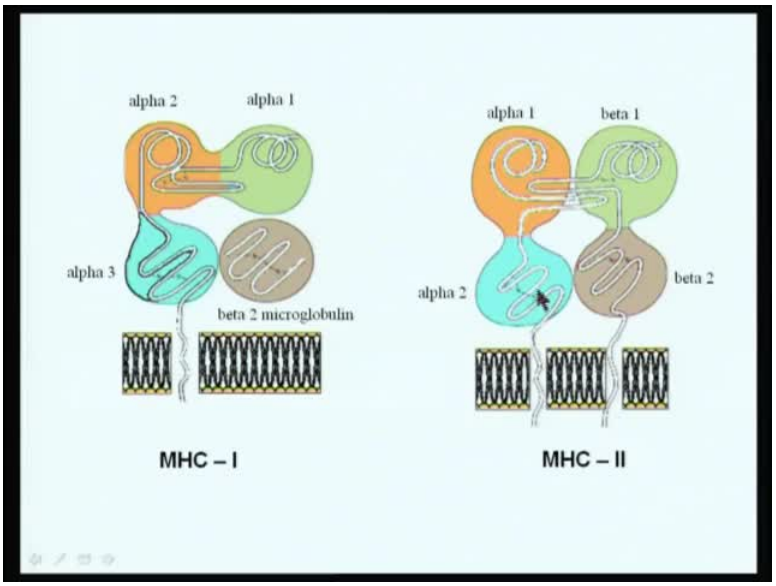

The MHC region being polymorphic, the experiments demonstrated the structure of these MHC antigens was then elucidated as being of the two types, called as MHC 1 and MHC 2. So, the antisera that were used to show this by immunoprecipitation experiment showed that MHC 1 specific antisera actually brought down a heavy chain; that is shown in this particular slide.

This heavy chain can be divided into 3 domains called as the alpha 1, the alpha 2, as well as the alpha 3 domains. These domains, the alpha 2 and the alpha 3 domains are linked by disulfide bonds and there is a trans membrane region and a very short cytoplasmic region. This is the C-terminal end of the molecule and this is the N-terminal end of the

molecule. This, so called MHC or class one heavy chain is stabilized on the cell surface by another protein subunit called as a beta 2 microglobulin, which is coded for by a different gene. This beta 2 microglobulin is bound to these domains by hydrophobic interactions and is itself not anchored within the membrane.

Now, these are also disulphide linked and as opposed to the polymorphic nature of the class one heavy chain, the beta 2 microglobulin is in fact, conserved across species. As opposed to the class 1 molecule, you see that the class 2 molecule has got a different kind of a structure in that it has 2 proteins sub units as the class 1 has, but the difference is that both the sub units are anchored into the membrane; that is both the subunits have trans membrane region and similar to the class 1 heavy chain, both the subunits of the MHC 2 molecule have a short intra cytoplasmic tail. These 2 protein subunits of the class 2 molecule have again 2 domains called as the alpha 1 and alpha 2, for the alpha sub unit and the beta 1 and the beta 2, for the beta sub unit of the class 2 molecule. These two, the alpha 2 and the beta 2 are linked by disulphide bonds and you see a disulphide bond here in the beta 1 subunit.

As you will see in this figure, this is represented in this particular fashion as having 2 alpha helices and beta pleated floor and then having this particular structure because this is what is indicated by crystallography study crystallographic studies involving the purified molecule. In fact, early experiments involving the purification of these molecules involved the use of the enzyme trip papain, where there is a cleavage site over here because the papain, release this molecule reminding us of the immunoglobulin molecules being cleaved by proteases, in order to generate the different fragments.

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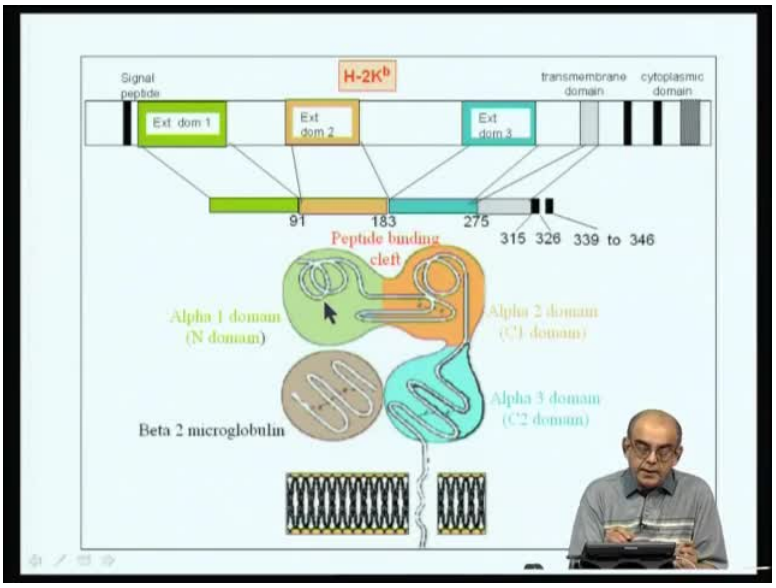

So, this particular structure has actually been written in hindsight after seeing the X ray crystallographic modeling studies and how this particular antigen is actually modeled. Now, looking at this particular structure, you will see that this domain structure of alpha 2, alpha 1 and alpha 3 actually corresponds to the various exons that are present within the H2 class 1 gene. This is an example of the H 2K b locus and you will find as represented by the corresponding colours, you find that the alpha 1 domain, this is the protein representation and this is the gene representation.

So, you have the signal peptide followed by the external domain 1, which codes for the alpha 1 domain and then you have an intron, and then the external domain 2 exon codes for this orange section, which is the alpha 2 domain. Then you have the external domain three which is coding for the alpha 3 domain. These domains are variously referred to as the N domain, the C1 domain and C2 domain in humans. As I told you earlier, that the beta 2 micro globulin comes from a different gene. Then you have the trans membrane domain coding for this trans membrane section. Then you have the cytoplasmic and an untranslated 3 prime region and these are the various amino acids represented over here; the numbers that are represented over here to define the different domains over here.

So, 91, 183, 275, 315 into 326 is actually the trans membrane domain. Then you have the 339 to 346 representing the cytoplasmic domain. So, you will see here that when you look at these alpha helices we have represented this particular distance between these 2

alpha helices as a peptide binding cleft. If you were to remind yourself of lecture number 3, where we looked at T cell activation in response to various antigen presenting cells or macrophages, we will remember that these macrophages actually took up an antigen either by endocytosis or by phagocytosis and they would proteolyse them within the lysosomal compartment and they would bring them back onto the cell surface which would then activate T cells to proliferate.

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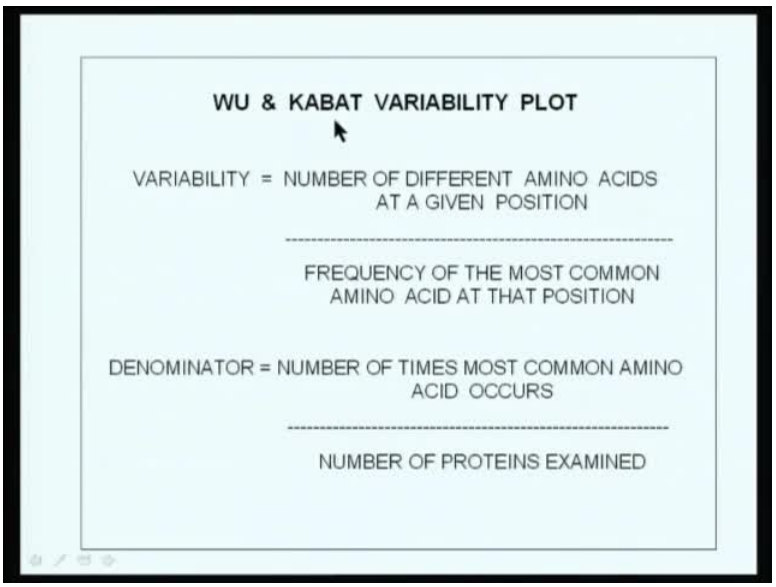

So, it was known earlier that this sort of proteolytic processing of the antigen had to occur before T cells could be activated and proliferation could result in a mixture of T cells that would recognize a particular antigen from a mouse that was that was immunized with the same antigen. So, these sequences were actually compared from different molecules of MHC and this was done not only for MHC molecule, but in the beginning, they were done for immunoglobulin molecules to look at how the variable regions were variable.

So, you had a variability plot that was actually designed by WU and KABAT, the famous immunologists who looked at the immunoglobulin structure and they defined the variability as being the number of different amino acids at a given position divided by the frequency of the most common amino acid at that position. So, you had looked at several protein molecules, many, many of them and then looked at the number of different amino acids at a particular position and you divide that by the frequency of the

most common amino acid at that position. This denominator being the number of times the most common amino acid occurred divided by the total number proteins that were examined because the immunoglobulin that were being generated and being purified in those experiments were so many, they could have a kind of an overall view of how these immunoglobulins varied in their variable locus or that was that was how they found out that a particular region in the immunoglobulin molecule was actually very, very variable and therefore, they were termed as variables regions as opposed to the constant regions, which did not vary so much.

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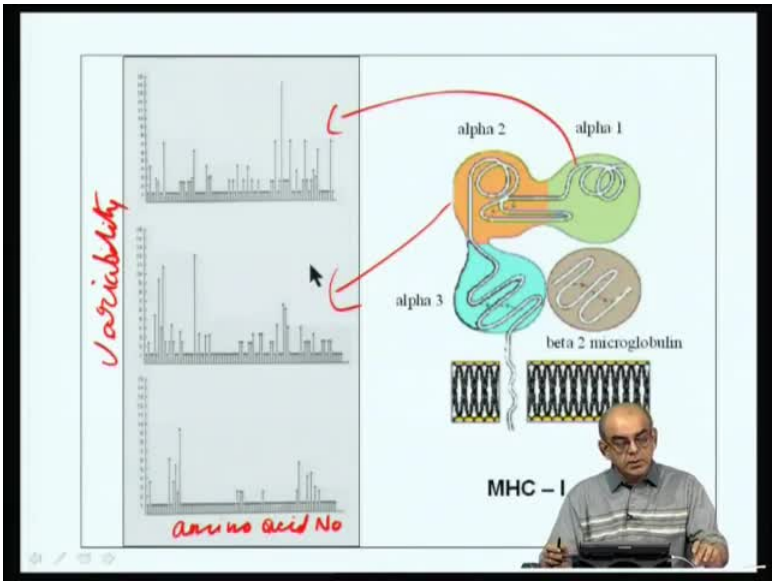

Now, the same exercise was done for the MHC molecules and you will find that the surface exposed domains, the alpha 1 and alpha 2 domain, which are external, most external so far as the membrane is concerned, which are alpha allies over here, these were the ones that were most variable. Now, this is the WU and KABAT variability plot and this represents variability over here on the x axis on the y axis as derived by that formula and the amino acid number or the residue number on the x axis.

So, you will see here that the alpha 1 domain had the extremely variable nature to it when you looked at the different amino acid residues. Every one of these lines which showed a higher number of variability or the amino acid that were being variable from inbred strain of mouse to inbred strain of mouse. In other words, MHC antigens of

different haplotype or different allotypes would be very different in terms of variability when you compared the primary amino acid sequences.

So, the amino acid residues being much more different than the alpha 2, especially compared to the alpha 3, which was not surface exposed, but closer to the membrane had variability much less than those of the alpha 1 and alpha 2; the alpha 1 being represented over here in this graph; the alpha 2 being represented over here in this graph and therefore, this gave the clue that in fact just like immunoglobulin molecules, these 2 domains had actually something to do with binding of antigens because the variable residues, the variable domains of immunoglobulin molecules were found or were known at that time to bind to the different antigens of the immunoglobulin had to recognize. So, the reason that these particular regions of the alpha 1 and alpha 2 domains or these regions of the protein had actually something to do with the antigen binding.

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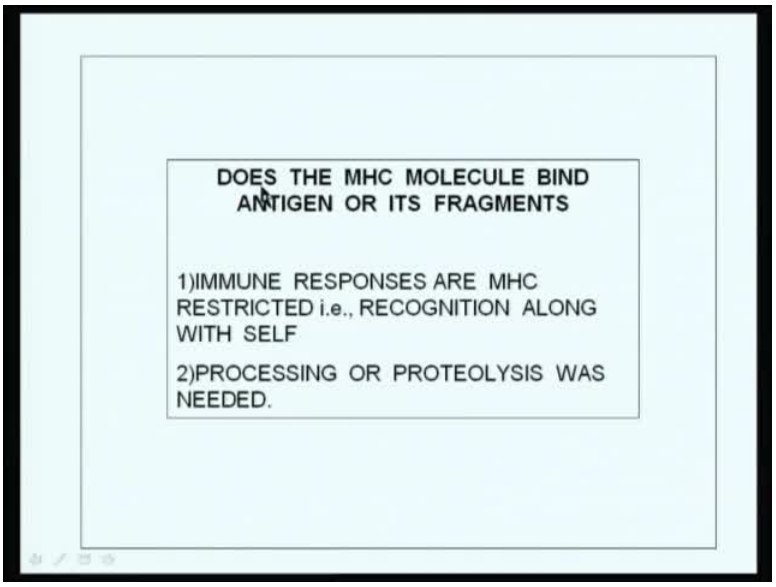

So, in order to further go into these studies, one has to ask the question how does this MHC antigen have primary amino acid sequences that were variable and did they actually have anything to do with antigen binding or in other words, ask the question whether does the MHC molecule bind the antigen or its fragments and this came about because they knew that the immune responses and the skin graft rejections associated with these immune responses are MHC restricted; that means that these immune cells always recognized self.

So, the antigen had to be recognized with the self MHC molecule and as I told you earlier, that this recognition of antigen or the final result of T cell proliferation had to do with something that involved the proteolytic fragmentation of the antigen that was endocytosed by antigen presenting cells or macrophages. So, in other words, it needed a processing of the particular antigen in question as opposed to an immunoglobulin molecule, which would recognize free floating antigen or unprocessed antigen.

So, if you mixed a purified protein molecule with the specific antibody that was to it in a test tube they would combine and make a complex. In fact, you will remember that this complex was what was precipitated as a immune precipitated, which resulted in the classical antigen antibody precipitation curve. Now, as opposed to this, it would mean that the T cell could see the antigen not in it is free floating form, but had to be proteolysed and this proteolysed fragment had to be associated with the self-molecule in some fashion, in order for it to be recognized by T cells because the whole purified antigen in question could not stimulate T cells to proliferate.

But they could stimulate T cells to proliferate, only after it was exposed to antigen presenting cells and these antigen presenting cells had to be present along with the T cells that that they could stimulate. So, in other words, T cells and antigen presenting cells as well as the antigen had to be present in the same tube, in order for the T cell to proliferate. So, to ask to understand this question about MHC antigen bind MHC antigen, you see the confusion between MHC antigen and an antigen that is going to bind. Therefore, this sort of nomenclature or terminology, one has to be clear that MHC 1 is referred to as an MHC antigen because it itself was used as an antigen in previous experiments involved in crossed immunization.

So, let us say that the MHC molecule has to bind to a fragmented antigen. How was it demonstrated? In order to understand this, one has to understand certain events in immunological history, which found out that these inbred strains of mice were being used not only to generate antibodies, but they were also used to see how T cells could proliferate after immunizing with the particular antigen. In other words, one would immunize series of inbred strains of mice. That means these inbred strains of a mice expressing different MHC antigens were immunized with a model antigens like for example, hen egg lysozyme or other types of antigens.

This hen egg lysozyme was then being added on into a culture tube, which would have a self-antigen presenting cells derived from the same inbred strains of mice that were being immunized, as well as the T cells that had already been activated from the same inbred strains and it was then that they found out that if one added different antigen present antigen presenting cell that were that was derived from a different inbred strains of mice, it could not present the same antigen to the T cells that were that were derived from a particular inbred strain of mice; so, the importance of the self-molecule.

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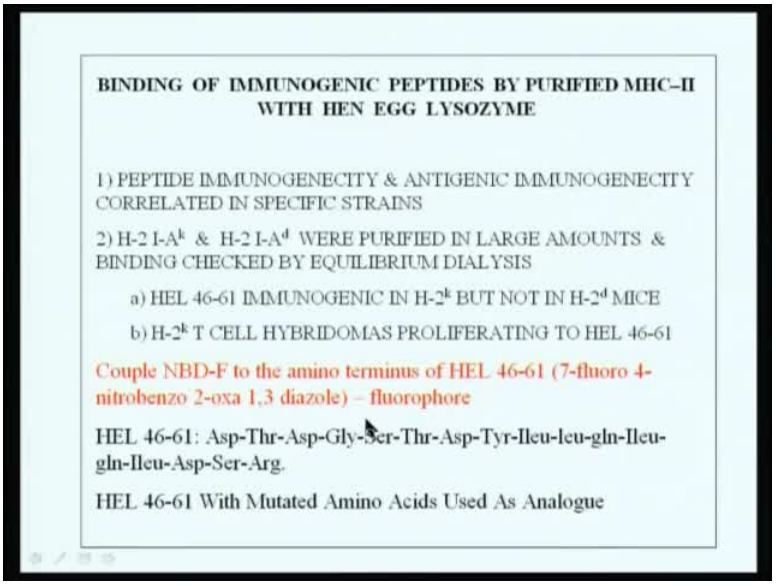

So, in these experiments, they found that in certain in certain strains of mice, they found that the immunogenicity and antigenicity co-related and these were specific strains of mice. In other words, what they were doing is to take a take an antigen, which was let us say, had a structure as a primary immune acid sequence. So, they took this whole antigen or whole hen egg lysozyme and immunized it into inbred strains of mice including H 2 k as well as H 2 d, and then they fragmented this lysozyme by proteases. In other words, take protein fragments, which were shorter and shorter in size and they immunized the same strain of mouse with these shortened fragments and they found that there was a correlation, when they put these shorter strain, shorter fragments of hen egg lysozyme or the proteolytic fragment of hen egg lysozyme would co-relate with its immunogenicity in terms of T cell activation, when the self antigen presenting cells were present.

So, in other words, they had a particular fragment called as the HEL stands for hen egg lysozyme. Hen egg lysozyme, 46 to 61 was a minimal fragment, which they could use not only to immunize mice to generate antibodies and result in the activation or proliferation of T cells, but they could also be used as an antigen that would bind to antibodies.

So, apart from this, another event that one has to know was they had generated T cell Hybridomas. Now, what are T cell Hybridomas? T cell Hybridomas or T cells that were specific or they would proliferate in response to a particular antigenic fragment being presented by the self MHC molecule. So, in other words, T cell hybridomas derived from H 2 k strain would proliferate in response to that particular HEL fragment or hen egg lysozyme fragment and in this case, 46 to 61, that was being presented along with H 2 k antigen.

So, these T cells were cloned. So, a clone of cells or they would be the same, having the same type of genes and therefore, having the same structure in terms of the receptor that would recognize the antigen, which is called as the T cell receptor. So, these clones were then made immortal and that is why it is called as the Hybridomas, by using certain immortal cell lines called as BW5147, which we will not go into this class. just like Suffice it to say, similar to the generation of antibody secreting Hybridomas, these T cell Hybridomas had the ability to recognize HEL 46 to 61 in association with the self-restricting MHC molecule and in this case, being the H 2 k.

So, one had a reagent here, in order to find out whether these T cell clones would proliferate in response to the processing and presentation of this HEL 46 to 61 by H 2 k antigen presenting cells and this activation of T cells which results in proliferation could be assay by the thymidine incorporation experiment or for that matter, by another more sensitive event called as the IL2 release or the secretion of lymphokine IL2, which happens during the T cell activation studies.

So, in other words, T cells T cell Hybridomas, when they recognize that they are specific antigens, they would release IL 2 into the medium which could then be quantitated. So, summarize it to say for now, that these inbred strains of mice, that is the generation of inbred strains of mice, the congenic resistance which was a difference only at the H 2 locus and the strains of mice which differ within the MHC complex resulted in a series

of experiments giving knowledge about how the self-antigen was represented by the MHC antigen, which came in two types called as the MHC 1 as well as the MHC 2 and that this MHC molecule had something to do with the binding of the antigen because they were the antigens that were that had to be present during the processing in presentation or the T cell or the consequent T cell activation during this whole reaction process or immune reaction process.

So, in the next class, what we will do is to look at how this immunogenicity and peptide antigenicity helped to demonstrate by experiments involving equilibrium dialysis and purified class 2 MHC molecules, how these experiments could demonstrate the binding of the antigen by the MHC 2 molecules and then we would go on to look at some more interesting properties of the MHC molecules and from there on to the T cell receptor. Thank you very much.