Essentials in Immunology

Prof. Dipankar Nandi

Department of Biochemistry

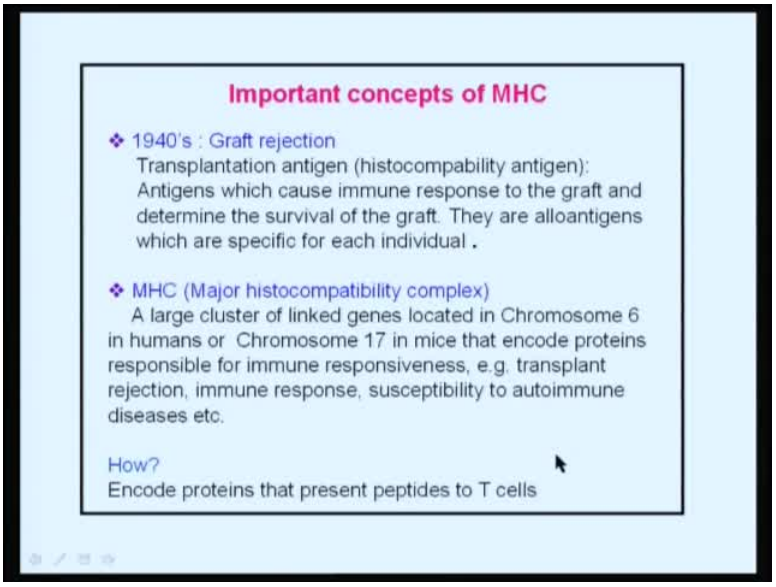
Indian Institute of Science, Bangalore

Module No. # 11

Lecture No. # 22

The Major Histocompatibility Complex

(Refer Slide Time: 00:26)



Today, we will be discussing the major histocompatibility complex, which plays an extremely important role in immune reactions. So, let us try and first understand how this about this scheme about the major role of MHC molecules is in graft rejection. So, basically, in terms of skin transplants and organ transplants, a MHC matching is done and it plays a very important role. So, before any transplant, either skin or organ transplants, MHC matching is absolutely required. And why is that required? That is because our body has developed mechanisms by which we reject other people's skin or tissue, unless there is a close match between MHC, and this point is something that is going to be discussed in this lecture.

The main molecule, or molecules, that are responsible for graft rejection, organ rejection, tissue rejection are MHC molecules, and these are encoded MHC class 1 and MHC class 2. So, we will, we will need to understand these aspects in greater detail.

So, these studies on MHC molecules were, actually, studied as part of transplantation antigens, or molecules that are important in transplantations, and subsequently, what was shown is that there was a particular loci in the human chromosomes, as well as in the mouse chromosomes that were important in rejection of a transplants. And these… and often some of these transplants, at least in the mouse, were done in terms of acceptance or rejection of mouse tumors, and that is something that we will be discussing a little bit later.

But suffice to say that the MHC molecule is a large cluster of linked genes. It is a very gene dense region in chromosome 6 in humans and chromosome 17 in mice that encode proteins responsible for immune responsiveness, and these, as I mentioned, mainly in terms of transplant rejection, immune responses to pathogens, susceptibility autoimmune diseases, etcetera.

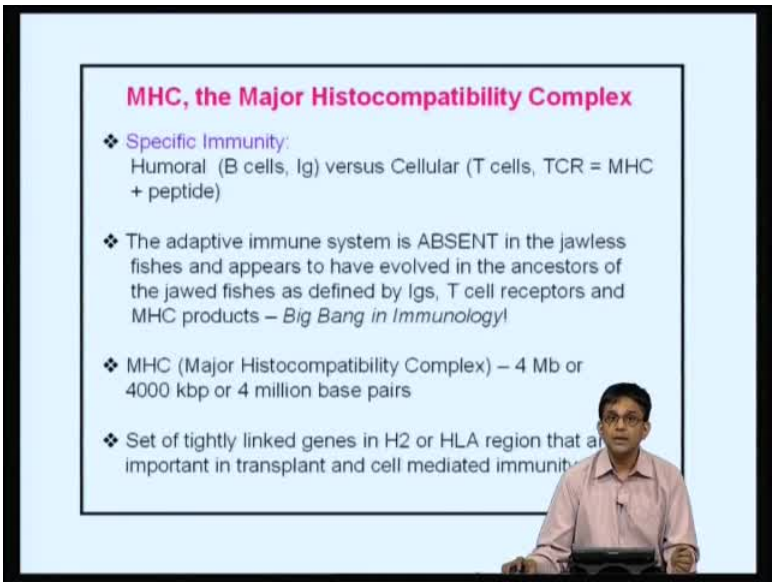
Now, how is it that the MHC molecules will play this role? That is because, you need to think of MHC molecules as, essentially, peptide receptors, so they present peptides that are derived from endogenous proteins within, or intracellular proteins within cells, and these peptides are present up on the surface of cells. And this MHC molecules presents these peptides, and so the T cells then recognize this complex of MHC plus peptide, and either in case upon infection some aberrant microbial proteins or peptides are produced, you have a T cell reaction.

Alternatively, during tumors you have tumor specific peptides derived from tumor specific proteins that are expressed on MHC molecules, and you have a T cell response. So, essentially, what the MHC molecules do is to present peptides, and that is a readout for things that are going on intracellular within cells.

So, that is what this entire lecture is about, and it all started off with transplantation, because our body has the MHC molecules in our body are recognized by our T cell as self, and so they do not respond against it. However, MHC molecules of other peoples are recognized as non-cells and non-self, and T cells mount a vigorous response against

this. This is a very important concept, and it plays… it has ramifications in all sorts of immune responses.

(Refer Slide Time: 04:31)



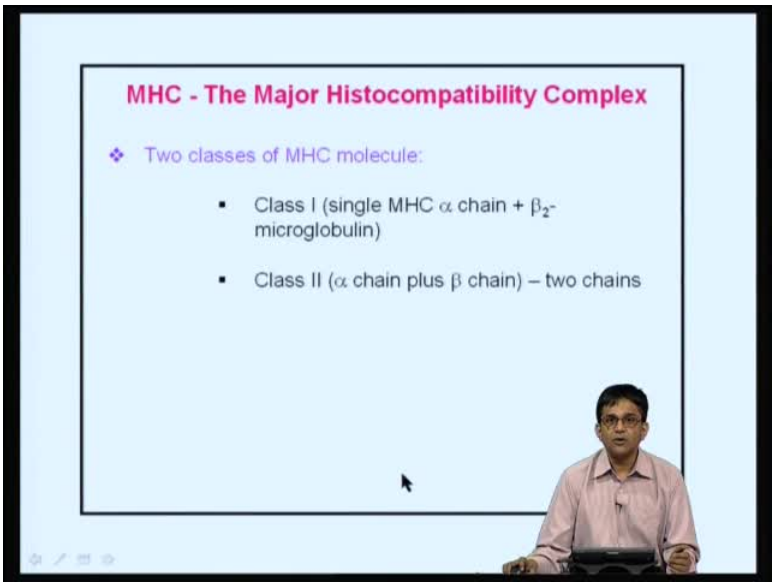
And so, we will move on and see and discuss this a little bit. Now, as you are probably all aware that, in terms of adaptive immunity, there are two types of molecules that are important– one is humoral response, which is primarily mediated by B cells in immunoglobulins, and the other is the cellular response, in which you have T cells. Now, the T cell receptor recognizes the MHC molecules with peptide, and this is where the importance of MHC comes in, because these MHC molecules are encoded by the major histocompatibility complex.

An important point in terms of evolutionary fact is that the adaptive system is absent in jawless fishes and lower organisms. However, they appear to have suddenly arisen in jawed fishes, and so in jawed fishes, you have immunoglobulins; you have the T cell receptors; and you have the MHC products.

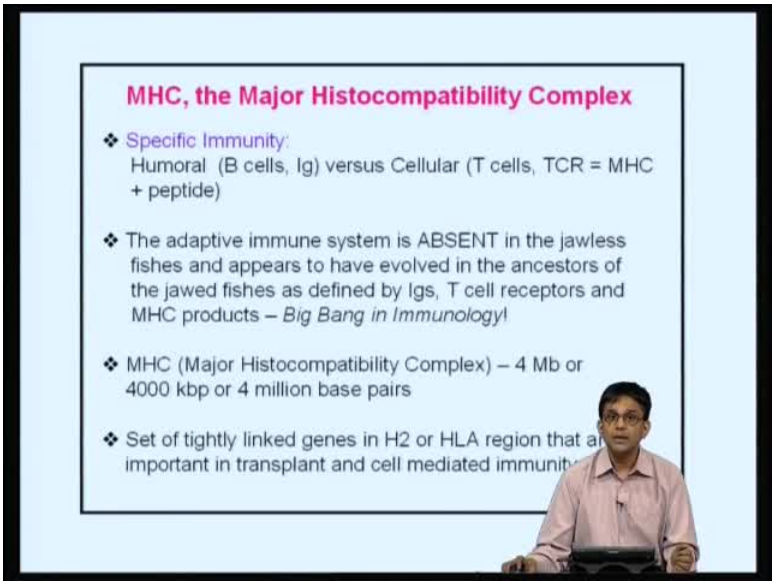
So, all of a sudden, you go from jawless, where you do not have the MHC molecules, and the typical B cell receptor and the T cell receptor with jawed fishes, and suddenly, you see this emergence of the B cell receptors, the T cell receptors, and the MHC molecules, and the sudden occurrence of these molecules is known as the big bang in immunology. This is, again, something important for you to, sort of, remember.

The MHC complex, or this loci, as I mentioned, is a extremely gene-dense region, and it encodes about 4 million base pairs. So, it is a fairly large part, you know, in terms of the genetic localization, because there are so many genes that it encodes, and several of these genes play an important role.

(Refer Slide Time: 06:21)

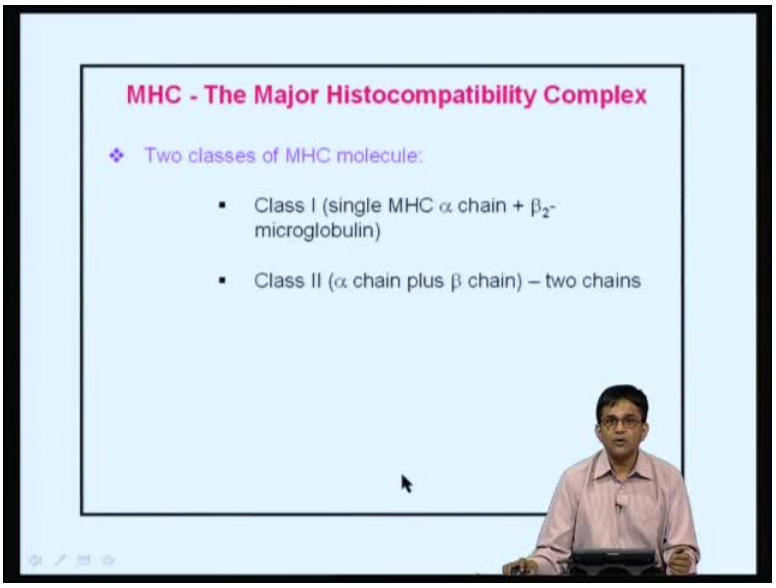


(Refer Slide Time: 06:31)



As previously mentioned, it is a set of tightly linked genes that play an important role in transplant and cell mediated immunity, and primarily because they present the peptides to T cells, and you have T cells that can recognize self versus foreign MHC and so on.

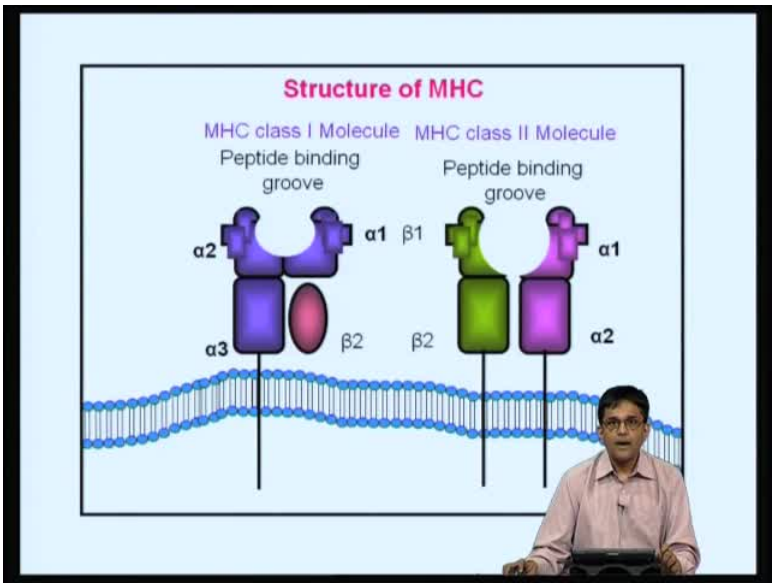
(Refer Slide Time: 06:21)



Now, there are two main types of MHC molecules– one is class 1 and the other is class 2. The structure of these molecules is very distinct, so MHC class 1 has a single MHC alpha chain with beta 2 microglobulin. So, if there is a single alpha, it is non-covalently associated with the beta 2 microglobulin.

And the beta 2 microglobulin– the gene encoding beta 2 microglobulin– is not coded in the MHC. In fact, it is encoded somewhere else, but the protein products comes together to form the MHC class 1 complex. The MHC class 2 molecule, on the other hand, is composed of the alpha chain and the beta chain, and both the alpha and the beta chain are encoded in the MHC.

(Refer Slide Time: 07:27)



And this is a structural depiction of MHC class 1 molecules, and you can see, this is MHC class 1, and it contains that the alpha chain contains three domains– alpha 1, alpha 2, alpha 3, and the alpha 3 is linked non-covalently with the beta 2 microglobulin, and the peptide binding a groove or the region where peptide binds is over here.

One of the interesting aspects about MHC molecules the class 1 and class 2 is that they are highly polymorphic, and the polymorphic residues, in case of MHC class 1, are present in this peptide binding region so, and what polymorphism allows it to bind different sorts of peptides.

So, we will see later that there are two striking features, at least– there are two main striking features of MHC molecules. They are polygenic; that means, you have different types of MHC class 1 class 2 molecule, and secondly, they are polymorphic; that means, there are distinct variations or variants of each of these, and this is important because…. So, the polymorphisms allow different peptides to bind to it, and so this allows for peptides derived from different proteins to bind to these molecules and to be expressed in the cell surface, and this gives an advantage for the T cells to find out what exactly is going on intracellularly.

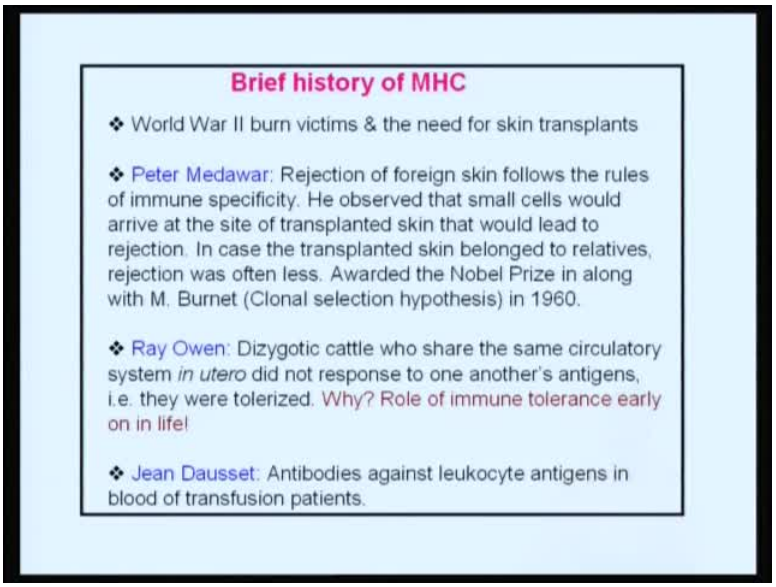
The other MHC molecule is the MHC class 2 molecule, and over here, this is composed of the alpha chain and the beta chain, and you can see here, the peptide binding groove is present over here, and in this case of MHC class 2, the polymorphic residues are present

in the alpha 1 and in the beta 1. And so, this is what forms the polymorphic sites, which allow for peptide binding. So, polymorphisms in MHC are very important, and students should be extremely aware of the role of polymorphisms and the advantage of having such polymorphisms.

If you remember, the ability to bind that the TCRs and the BCRs are variable, and that variability comes from the ability to bind two different segments of gene and from new molecules, and in case of the BCR, subsequently, you have somatic mutation, which drives for selection of higher affinity immunoglobulins.

In case of the T cell receptor too, you have the combination of different genes coming together to form the T cell receptors, and these are diverse and they recognize MHC molecules. In case of MHC molecules, diversity comes in mainly from polymorphism, so this is an important point to differentiate about the mechanisms responsible for diversity between the B cell receptors, T cell receptors, and the MHC molecules, and this is something that students should pay close attention to.

(Refer Slide Time: 10:25)



Before we go into MHC molecules, I would like to take you into a little bit of historical aspects, because I think it is important for students to understand this. Really, the start of MHC molecules comes in with the World War II.

And as you realize that with the World War II, you had a lot of patients who were suffering from burns and skin transplants needed to be done, and certain amount of energy was spent and resources were spent in trying to find ways by which that patients would recover much better from skin transplants, and it is over here that one person's works stands out, and that is Peter Medawar.

Peter Medawar worked on this aspect because that was a pressing need at that point. Sometimes, necessity is the driving force behind discoveries and inventions, and what he observed was that that upon skin transplantation, very often, the rapidity with which the transplant was rejected was directly proportional to the amount of the small cells that would come around the transplant, and he did not know at that time what these small cell were, but he thought the cellular immune response was responsible for it. And these were important for rejection, and based on his observations– he was a very astute scientist– he came up with what is known as the laws of transplantation.

What the important aspect was, he found that if the skin transplants were done with relatives, the chances of rejection were less. So, there was some matching that could be done, and that helped in improving the chances of skin acceptance. Subsequently, and because of his pioneering studies, he was awarded the Nobel prize along with Macfarlane Burnet, and who had propounded the clonal selection hypothesis, and both of them received the Nobel prize in 1960.

And so, Medawar contributions in terms of transplantation laws and his observation about why is it that we accept some grafts versus we do not accept some grafts is really important. A critical observation that helped all this came from Ray Owen, who a lot of people may not have heard about.

Ray Owen had made a striking observation. What he found is that in cattle, that is, in cows, you know if the cattle give birth to dizygotic twins and who shared the same circulatory system in utero, they did not respond to each other's antigens, which, in other words, these cattle were, these twins were tolerized to each other's antigens. Usually, that was not the case.

But in case of dizygotic cattle who shared the same circulatory system, this happened; and this was because of the role of the immune tolerance. So, one gets tolerized very early on during development. So, if our immune system sees something very early on,

then you get tolerized, and this lasts a lifetime, and Ray Owen's work was very important for Peter Medawar to come together, to put together his laws of transplantation, and this is very important in understanding this aspect.

Jean Dausset– he found antibodies against leukocyte antigens in the blood of transfusion patients, so people who had got transfusions, their immune system reacts with… make the antibodies against different MHCs, and you use these as reagents and this was the first demonstration of anti-HLA antibodies.

And these reagents were extremely important because what happened at that time, there were lot of studies that were doing, that were going on in mice and all, and Dausset's studies were important because they were done on humans.

So, we had the first ideas about HLA molecules or the protein level, and the reagents were very extremely important in terms of HLA typing. One of the first starts of HLA typing came from Dausset's studies.

(Refer Slide Time: 15:00)

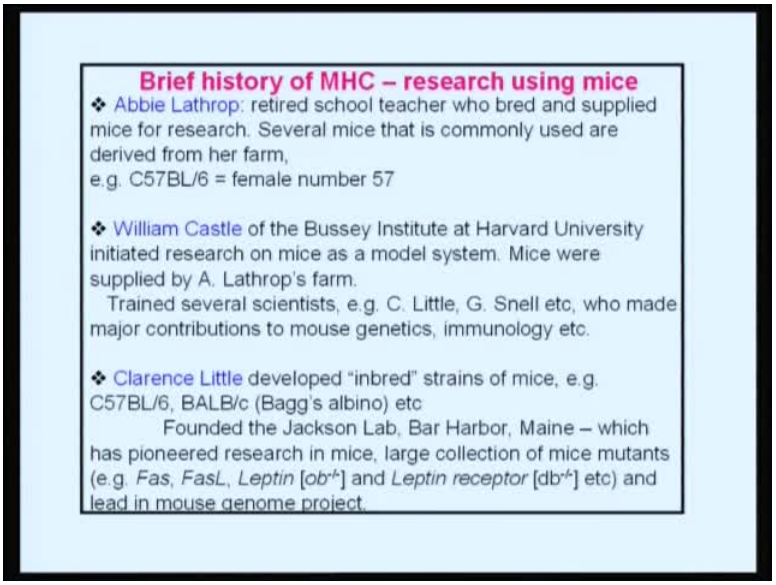
As mentioned, a lot of the studies with HLA have to do with mice, and what are the advantages of using mice for research? First is, they are easy to handle; they have a low gestation; about within four weeks you have the litters, and litters a fairly large. So, you have several of them, so they breed well.

There, you can also time the pregnancies to…. So, this is important for developmental studies and its more relevant, as they are closer to… Studies on mice are, obviously, more relevant because they are closer to humans compared to Drosophila or yeast, like S. cerevisiae, and in this stage it is important to understand about the use of inbred strains of mice.

What are inbred strains of mice? These are genetically homogeneous strains of mice and they have been obtained by brother-sister matings for about almost about 20 generations. What this does is that this allows the genome to be almost identical in mice of a particular strain, and why is that important? This is important in terms of scientific experiments and reproducibility results.

So, for example, if some experiment is done with a particular inbred strain of mice in Bangalore, the same result should be mimicked by that strain in Delhi, or may be abroad in the US or Great Britain, wherever studies are done. So, this allows for reproducibility. So, there are no differences within the genome of these mice. So, inbred strains of mice are critical for the use, and this is especially true in case of MHC, given that the MHC is a polygenic as well as a polymorphic.

(Refer Slide Time: 16:54)



Now, with respect to the using mice as a model, there are several people involved. Perhaps, the person who we all need to thank is Abbie Lathrop. This retired school teacher, she bred and supplied mice for research. In fact, several of the mice strains that

we have in our… or that we use in our laboratories have come from her farm. For example, C57 black 6 is, actually, female number 57.

Now, remember the strain of mice was derived from Abbie Lathrop's farm, and then, subsequently inbred strains were developed much later. But the line came originally from there and that was worked on.

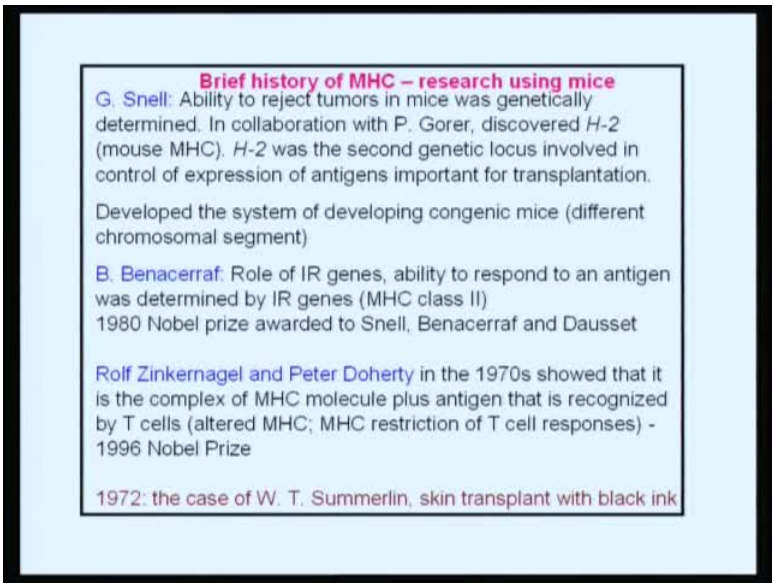
And Abbie Lathrop supplied mice from her farm to William Castle, who was the head of the Bussey Institute at Harvard University. William Castle, or Will Castle, started work on using mouse as a model system. He thought it was a more relevant to use mice, as was mentioned previously. And so, he started serious work on mouse genetics, mouse biology, at the Bussey institute, and what is really important about it is that several scientists, for example, Clarence Little, George Snell, who made major contributions in mouse genetics, immunology, etcetera, were trained at the Bussey Institute.

And one of the members, Clarence Little, have developed the inbred strain of mice, for example, C57 black 6 BALB c, which is Bagg's albino. Bagg was a scientist, and albino because it is white in color, and more importantly, Clarence Little founded the Jackson laboratory in Bar Harbor, Maine.

Those students who are interested in mice research are strongly urged to look up a Jackson Laboratory in Bar Harbor and read up on the role of this laboratory in mouse genetics studies. This… they… The Jackson lab distributes mice strains to different researchers all over the world. They have a large collection of mouse mutants, especially some of the ones like Fas, FasL, Leptin, Leptin receptor, etcetera, were all generated in the Jackson lab, and have been very important in finding the roles of these of these molecules. For example, Fas and FasL are important in apoptosis and in the immune system, because it is a way of getting rid of cells that are activated and cells that we do not want.

Mutation in Fas, FasL, are result in severe problems, and this is something that we will be looking or we will be studying in subsequent classes. Leptin and Leptin receptor are important in terms of satiety or after food feeling of satisfaction and weight control. So, this is… again, these were all discovered in the Jackson Laboratory. They have also played a leadership role in sequencing of the mouse genome, and therefore, the Jackson Lab's contribution to mouse genetics is really critical.

(Refer Slide Time: 20:13)



It was also the place where George Snell worked and discovered the H-2 or the mouse MHC, and how did George Snell do it? So, the readout that he used was the ability to reject tumors in mice, and so, in the tumors in mice that were self were accepted as self and they were not rejected, whereas once they were implanted in mice with the different MHC, they were promptly rejected.

And so, that was the basis of the assay by which H-2 was discovered, and George Snell collaborated with his friend Peter Gorer and discovered this complex, and H-2– why is it 2? H-2 was the second genetic loci involved in control of expressions of antigens important for transplantation that Gorer had thought had found, and so, the collaboration proved to be extremely useful.

George Snell is mainly known for his development of congenic mice. What he… what he developed were strains of mice that were genetically identical except for one loci, and in this case, it was MHC, because again, he used the breedings and the assay system using the tumor acceptance rejection system to develop these congenic lines.

So what this told that there was this, a particular loci, that was controlling the ability to accept or reject transplants, and this loci turned out to be the H-2 or the MHC complex, and so, this was critical in our understanding of the MHC, and along with Baruj Benacerraf and Dausset, they were awarded the Nobel prize in 1980.

Baruj Benacerraf's contributions are mainly in understanding of the role of IR genes, and IR stands immune response genes, that is the ability to respond to an antigen, and the IR genes were subsequently found to be MHC class 2. We need to understand the MHC class 2 is important, because MHC class 2 presents peptides to a CD4 positive cells, and CD4 positive cells produce cytokines, which activate the B cell receptor.

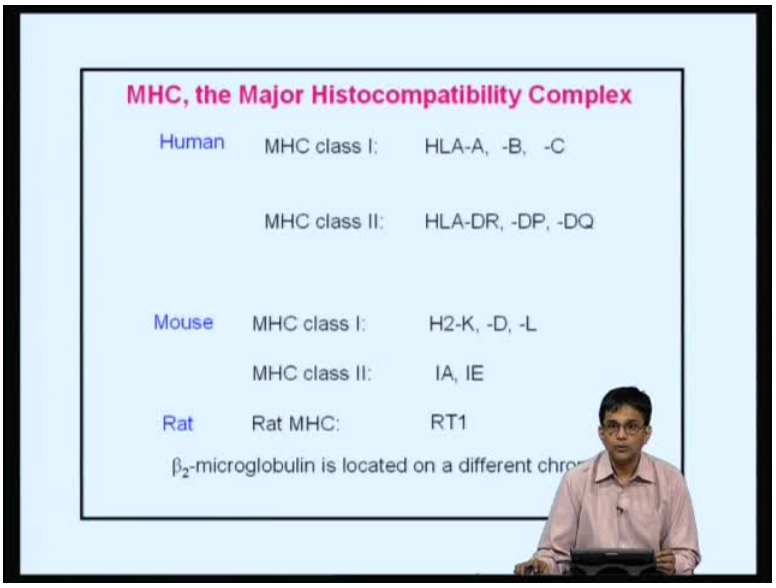
So, what was found is the IR genes control the amount of antibodies that were being produced to certain antigens. If we take the same antigen and give it to mice containing different H-2, you got different responses, and these responses were determined by the IR loci, and IR is nothing but the MHC class 2, as was subsequently found. And I hope you are able to understand this relationship, because the antigens were processed and presented on MHC class 2.

Now, depending on the polygenic and polymorphisms in MHC class 2, these different peptides were presented, and could activate T cell responses or CD4 T cell responses. This differential CD4 response would translate to the amount of different antibodies or different titers of antibody produced. So, you had some strains that were high responders and some strains that were low responders, and this was determined by MHC class 2, and consequently, you got a differential response, and that was the IR loci.

Subsequently, what was found, there were some critical experiments done by Rolf Zinkernagel and Peter Doherty, who showed it is actually the MHC molecules plus antigen that is responsible for recognition by the T cell receptor, and this is known as the altered MHC, and for this they got in the 1996 Nobel prize. So, it is the self MHC that, together with a peptide from a pathogen and this altered self MHC, presents or is now recognized by T cells. That is responsible for T cell activation, and for improving an understanding on MHC restriction, they received the Nobel prize.

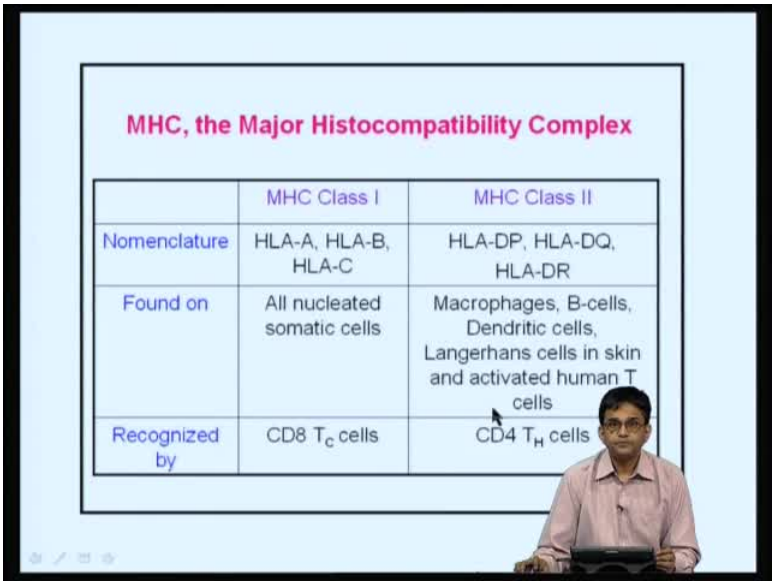
In all these great experiments and contributions of MHC molecules, there has also been a problem. In 1972, you had the case of W. T. Summerlin, who showed that skin transplants that very difficult to do could be done between strains of mice, and what here actually done was, actually, had used ink to fraud his data, and that was not something nice. But, I feel students should be aware of this and it is such a famous case; that is why I have included in my lectures.

(Refer Slide Time: 25:00)



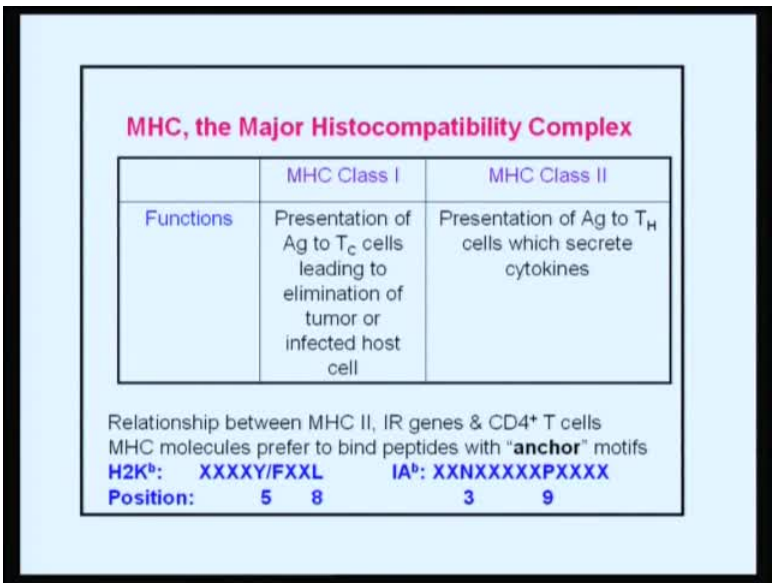
So, the MHC molecule in case of humans –MHC class 1– you have HLA-A, B, and C, and in case of mouse, you have the H2-K, D, and L, and remember, these are the single alpha chains and each of these alpha are in contact with beta 2 microglobulin. The MHC class 2 molecules, in case of humans, you have the HLA-DR, DP, and DQ, whereas in the mouse, you have IA and IE. Now, in other animals, also, there are MHC molecules. They are not as well studied as mouse and humans, but one should be aware of it. So, in rat, for example, is known as the rat RT1 molecule.

(Refer Slide Time: 25:41)



So, the MHC class 1 molecule is present on all nucleated cells and they are recognized by CD8 positive cells, and so, the MHC class 1 is mainly responsible for the cytotoxic T cell response, and the MHC class 2 is expressed only on antigen presenting cells, or primarily on antigen presenting cells, and it is recognized by CD4 response. And this is where the IR loci part comes in, and that something that I discussed in the in the previous slides.

(Refer Slide Time: 26:18)



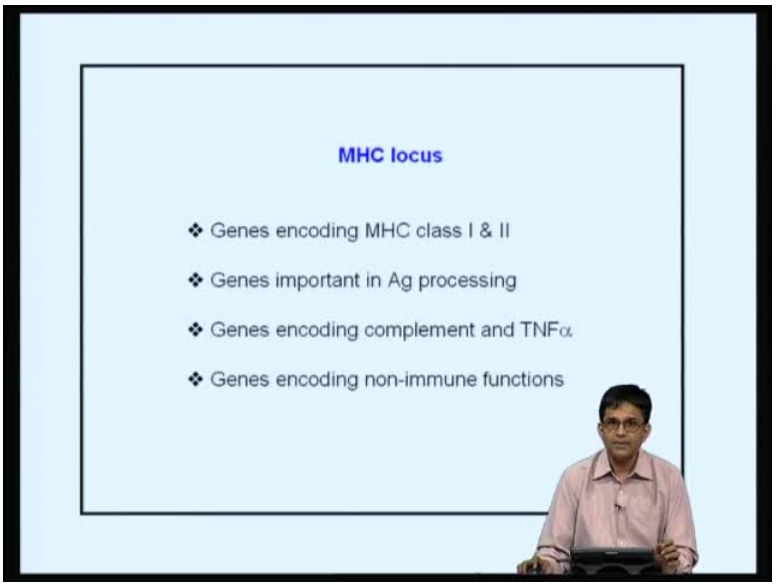
And so, the MHC class 1 presents antigen to CD8 positive, and which leads into generation of cytotoxic T cells, which is important in elimination of tumor in the infected host, and in MHC class 2 is presenting of antigens to helper T cells, which secretes cytokines, and these cytokines, in turn, influence the B cell response.

An important aspect about the binding of these different peptides are the role of anchor residues. If you look at different MHC molecules and because of polymorphisms, you have different polymorphisms, but there are some residues that are fixed. Especially in this, you have this position of the peptide is fixed, and these are known as anchor residues. So, in position 5 in the binding of H2k of b, and the b is referred to the particular allele, you have either a tyrosine or phenylalanine in position 5 and leucine at position 8.

And in case of IA of b, which is MHC class 2, you have a position 3 asparagine, and in position 9, you have proline. So, you have these anchor residues, and these anchor

residues, what they do is they help the binding to MHC molecules much better. So, even though you can have variations in these in the amino acids in these other regions, you have these anchor regions, which, sort of, ensures that the peptide binds strongly to MHC molecules. So, anchor molecules are important in that respect.

(Refer Slide Time: 27:54)



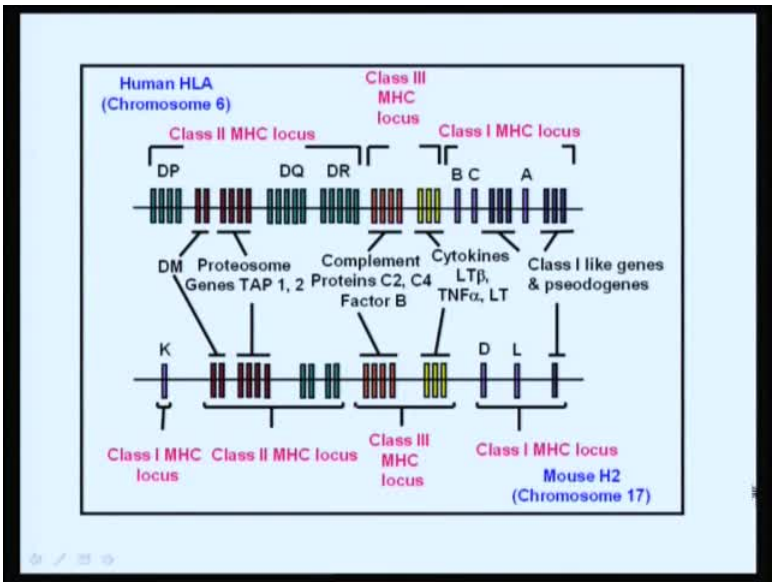
Now, the MHC loci– you have… you have different types of genes. Now, I have been talking mainly about class 1 and class 2 molecules in the MHC, but I do not want to give the impression that these are the only genes that are present in the MHC. So, you have certainly… you have the genes encoding MHC class 1 and class 2. You have genes that are important in antigen processing, and this is something that we will be seeing, in the next two lectures, both for MHC class 1 and class 2, the genes important for antigen processing are present, also, in the MHC.

Now, why do you think that would be? You know, one of the reasons that come up is that both the…, both the structural genes as well as the genes that are important for antigen processing and antigen presentation. If they are together as part of the complex, then they would be, sort of, carried together down, and that could be one possible reason.

But there are…, you know that is certainly one reason, but beta 2 microglobulin, which is so important, is not part of the MHC. So, you have both sides of the argument, but nevertheless, the MHC loci contains different sorts of genes. You have genes encoding

complement proteins and tumor necrosis factor alpha, which is important in cytokine response. You also have genes encoding non-immune functions.

(Refer Slide Time: 29:14)



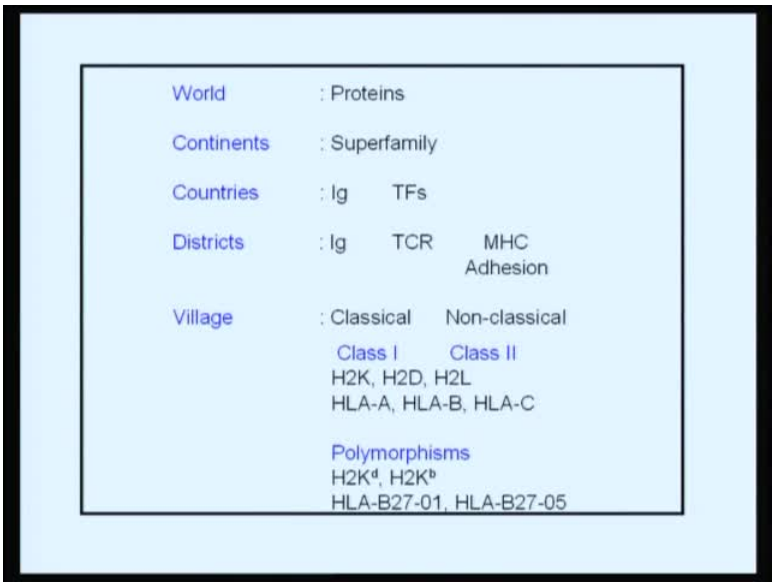
Now, this is a depiction of the MHC loci. This is the human HLA and you can see DP. DP is the MHC class 2– DP, DQ, DR. Now, in between DP and DQ, you can see, DM, proteosome genes, and TAP 1, and this is something that we will be discussing in the next classes. I just want you to focus on the proteosome genes and TAP 1, which are important antigen processing and presentation genes in case of MHC class 1, and whereas the DM is important in antigen processing and presentation in case of class 2.

So, DP, DQ, DR, are important parts in the structural genes important in MHC class 2, and the corresponding genes are shown over here as IA and IE, which are present over here in case of mouse. And what has happened is, in human, the MHC class 2 loci is very straightforward. You have the MHC class 2 loci, then followed by the class 3, which encodes for complement and lymphotoxin tumor necrosis factor, and then you have the MHC class 1, where you have HLA-B, C, A, that are present, and along with it, you also have a class 1-like genes, pseudogenes, etcetera.

Now, in case of the mouse, you have the MHC class 2 loci, you have the MHC class 3, and you have MHC class 1. Now, here, D and L are present; over here, the H-2 D and L. However, H-2 K is actually centromeric. It is present over here.

And so, you can see that there are some differences between the organization of the HLA and the H2 complexes, but you can see, that it is a very gene dense region. You have different sorts of genes being included by these loci, and several of them are very important for the immune response.

(Refer Slide Time: 31:10)



So, one way by which we can differentiate these different genes and the roles of these genes, and the analogy that I use is– you have one world, but that world is divided up into continents, and continents– within a single continent, you have countries, then you have districts, and then, you have villages. So, you have different ways by which you can differentiate this world, which is composed, which is diverse and composed of different constituents.

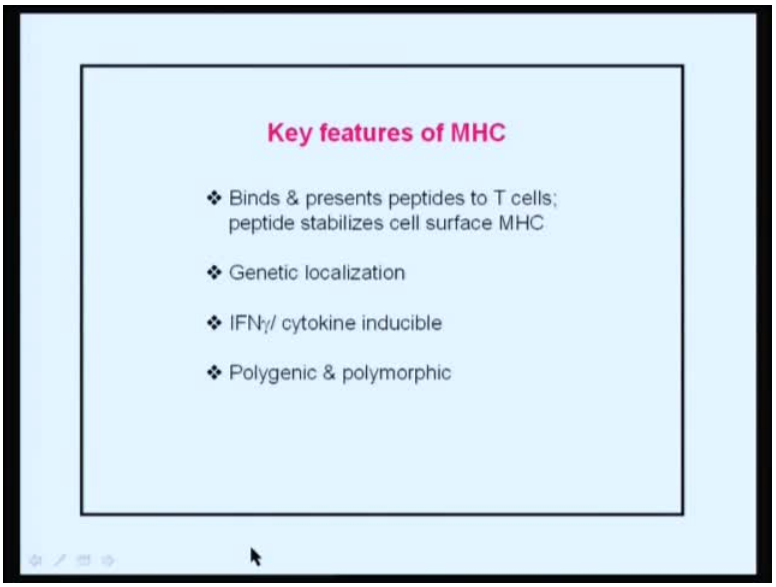
Similarly, in case of proteins, you have proteins; you have superfamilies; you have transcription factors; you have immunoglobulins. Now, the immunoglobulin family is further divided into real immunoglobulins– you have TCRs; you have MHC molecules; you have adhesions, so on.

Now, the MHC molecules can be further distinguish into classical and various non-classical MHC, and in the classical ones or the ones that are primarily responsible for transplant rejection, you have the MHC class 1 and class 2. Now, within class 1, you have HLA– HLA-A, B, C, and that is, these are the different polygenic forms, or in case of mouse, you have K, D, and L. And now, within each of these, you have different

variants, and these are responsible for polymorphisms, and I showed you where the polymorphisms lie, and they lie in and around the peptide binding region, which is an important aspect.

So, you have K, which you have different variants of, in case you have K, of DK of b, so on, and you have different alleles of, in case of HLA molecules, you have HLA-B27-01, HLA-B27-05. These are different variants and these are important, because these variants give us the ability to bind different types of peptides, and they are important in terms of the immune responses and transplant rejections also. So, this is something we need to keep in mind.

(Refer Slide Time: 32:57)



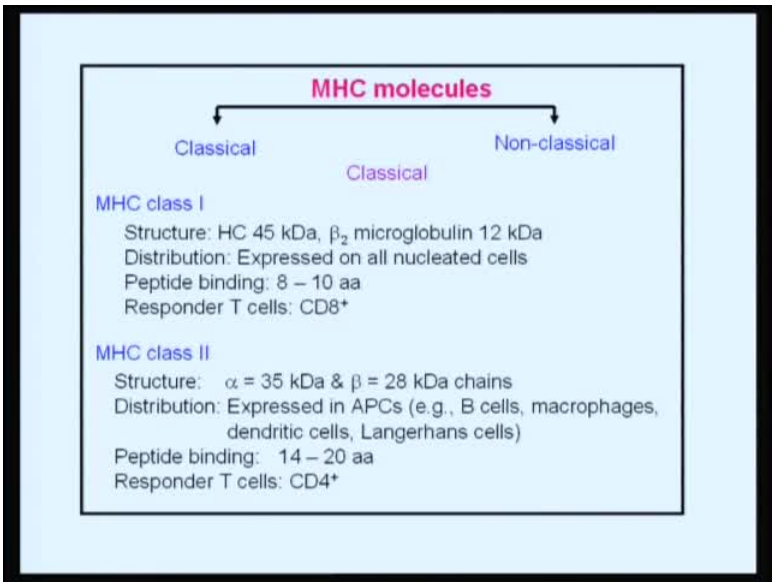
Some of the key features of MHC molecules– overall, these are the important features that we need to understand– their role is in binding and presenting peptides to T cells. This is their primary role, and what is important about the fact that the about the peptide binding is the peptide binding stabilizes cell surface MHC molecules. So, it is very important for peptides to bind to them, because it stabilizes their structure and stability.

Second, is the genetic localization and genetic localization they are present in this loci known as the MHC; it is HLA in case of humans and H-2 in case of mouse. The third important point is that these molecules are interferon gamma inducible or inducible by its certain cytokines. Now, why would that be?

Now, during an immune reaction, you have you have immune responses. So, more the MHC that is produced, more the chances that a T cell will recognize a cognate MHC and TCR complex to which it can respond. So, that is an important aspect that one needs to keep in mind.

The most important aspect about MHC molecules is that MHC molecules are polygenic as well as polymorphic. You have different forms of MHC molecules– K, D, and L, and within K, you have K of b, K of d, K of f, so on.

(Refer Slide Time: 34:19)

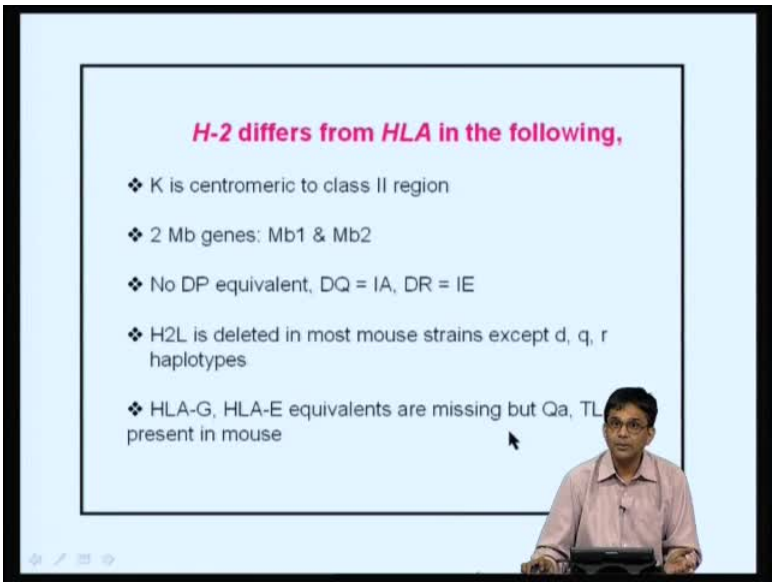


Now, MHC molecules, as I previously mentioned, you have two different types. You have the classical and the non-classical, and MHC class 1, you have the heavy chain, which is 46 kDa, which is linked non-covalently with beta 2 microglobulin, which is the 12 kDa. MHC class 1 is expressed on all nucleated cells, and the peptide bind, and it binds peptides which are about 8 to 10 amino acids long. So, they are much smaller. MHC class 2– you have the alpha chain and the beta chain. The alpha is about 35 kDa; the beta is about 25 kDa.

Now, as mentioned previously, MHC class 2 expression is present on, primarily, antigen presenting cells. So, you have B cells, macrophages, dendritic cells, Langerhans cells in the skin, and the peptide binding over here is much longer. So, it is about 14 to 20 kDa, and the responder T cells are the CD4 responders. So, CD8s primarily recognize class

MHC class 1, CD4s primarily recognize MHC class 2, and this is where the IR genes come into play.

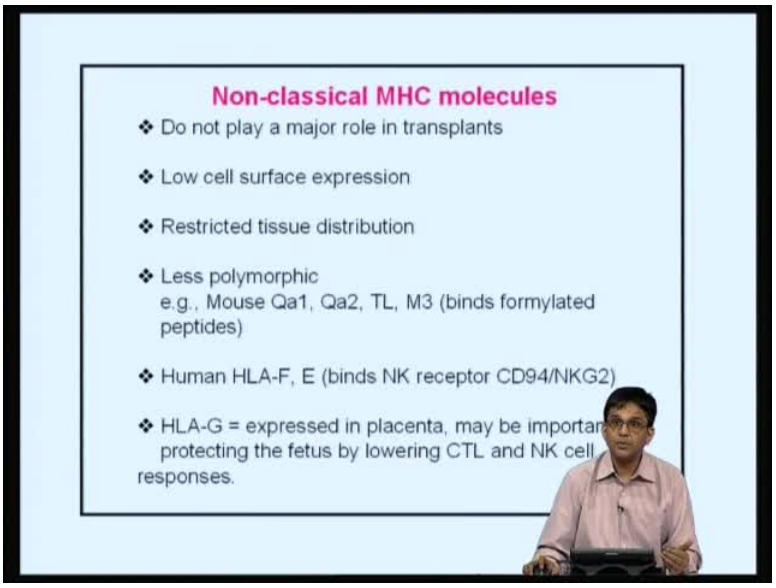
(Refer Slide Time: 35:24)



I also mentioned the differences between the H-2 and HLA, and it is important because I have showed where K is centromeric to the H-2 K is centromeric to the class 2 region. There are two MB regions. MB is important in MHC class 2 antigen processing and presentation. In H-2, you have a two of them, whereas, you have single one in HLA. There is no DP equivalent in the mouse, so the DQ equivalent is IA and DR equivalent is IE, but there is no DP equivalent.

H2L is deleted in most mouse strains except for some haplotypes, which are shown over here, and these are those variants the haplotypes are the H2 d, H2 q, or H2 r. So, that represents the variant form H2 d. Now HLA-G and HLA-E equivalents are missing in H-2, but Q and TL are present. Now, both Q and TL or thymic leukemic antigens are non-classical MHC molecules.

(Refer Slide Time: 36:39)



And coming to non-classical molecules, as I mentioned, the classical ones are the MHC class 1 and class 2, and these are the ones that are really important for transplant or organ rejection, and the… whereas non-classical MHC molecules are do not play a major role in transplants. They have a low cell surface expression. They have a restricted tissue distribution, which means, they play a role only in certain situations, and they are not as polymorphic as the classical MHC molecules.

But their roles, for some of them, have been shown. So, for example, in mouse, you have Qa, Qa1, Qa2, and some of these bind to NK receptors, and the thing that one needs to understand is the NK receptors also recognize MHC molecules, and in case the NK receptor sees the MHC molecules, that inhibits their lysis. However, if the MHC molecule is not present, then it will go ahead and kill. You have certain NK receptors that are responsible for this, and so, Qa is one of the ligands for NK receptors. That is known, and that has some role to play in regulation of immune responses.

Now, M3 is a non-classical MHC molecule, and what is interesting is that it binds formylated peptides. Now, where are formylated peptides produced? Formylated peptides are produced primarily in the mitochondria or by bacteria. So, by recognizing formylated peptides, what happens is during bacterial infection, you have presentation of formylated peptides by M3, which may be important in it. So, it has a very specialized function.

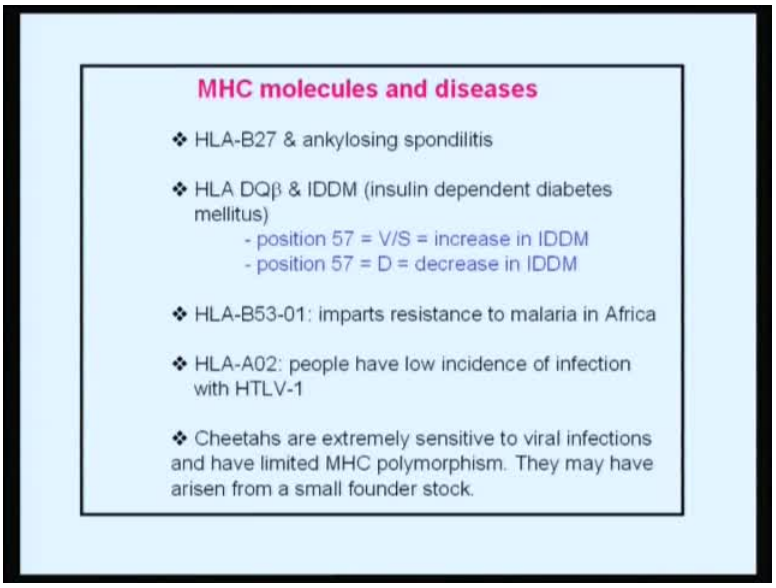
So, human HLA-F, and you have E, as mentioned. Now, E, as mentioned, has been shown to bind to the NK receptor CD94 NKG2, and I discuss the roles of this relationship between binding our NK receptors with non-classical MHC molecules and their role in modulating or regulating immune responses. A very interesting and well-studied non-classical MHC molecule, perhaps, the most classical MHC molecule is HLA-G. Now, HLA-G is expressed in placenta, and it may be important in protecting the fetus because it plays important role in lowering cytotoxic T cell lymphocyte and NK responses.

So, HLA-G is very important because it may be a mechanism by which the fetus is protected, and you can understand the situation that when a mother is carrying a child, that the child has a different MHC. So, it is natural for the mother's immune reaction to, sort of, react against the child's antigens.

But however, this does not happen. There are some protective mechanisms that the fetus has, by which it protects against the immune response, and it is possible that molecules like HLA-G are playing this important role. And, in fact, this expression of HLA-G is that its expressed, primarily, in the placenta, and so it might really be important in protecting the fetus.

So, the non-classical MHC molecules have specialized roles. So, it would be interesting to find what are these roles, and… but the dominant response of MHC molecules comes from classical MHC molecules, which are responsible for organ and transplant rejections.

(Refer Slide Time: 40:34)



Now, MHC molecules are also involved in other responses, and the one that will be discussing is this relationship between MHC molecules and disease. So, there is very strong association with a particular type of HLA molecule, which is the HLA-B27 and spondylitis. So, those people who have HLA-B27 are more prone to this particular disease, and studies have also shown relationship between certain HLA molecules. Here, in this case, DQ beta and insulin dependent diabetes mellitus.

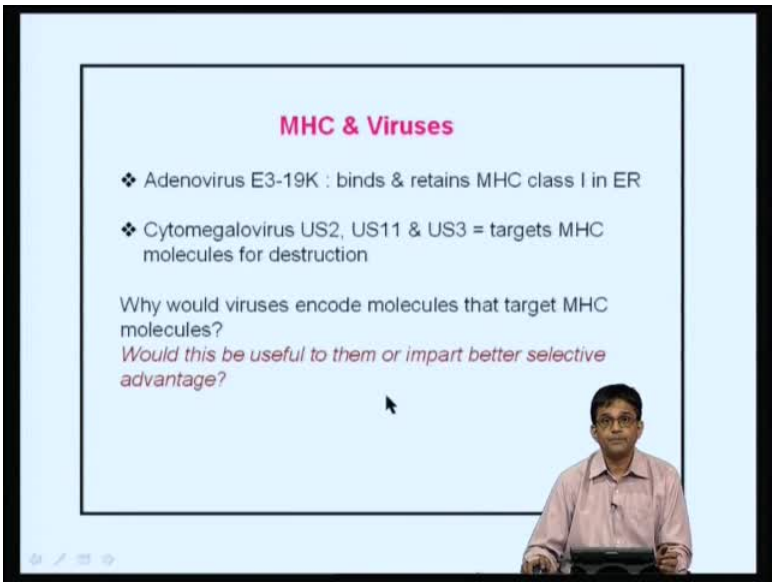
What has been shown is the position 7 is important. So, if you have a valine or a serine, there is an increase in our chance of having insulin dependent diabetes. However, in this case, if you have in position 7 aspartate, then there is a decrease in diabetes.

Now, MHC molecules are important for resistance to pathogens, and this particular MHC molecule– HLA-B53– is important for resistance to malaria in Africa. So, you know these studies have been done. What was shown is people, if you look at the survivors from endemic areas, and if there is a high preponderance of a particular HLA, then there is a link made between this particular HLA and, perhaps, resistance. But how exactly this occurs needs to be studied much further. In again, there is a link between HLA, individuals having HLA-AO2, and there is a low… those individuals have low incidence of infection with HTLV.

Now, cheetahs are extremely sensitive to viral infections, and have limited MHC polymorphisms, and it is possible that they are they are highly susceptible because of

their limited MHC polymorphisms. And one of the possibilities is that they have come from a small founder stock, which means that the initial stock that the cheetahs came from were small in number, and they bred and increased the numbers, but since they came from a small founder stock, their MHC diversity is limited and, perhaps, that makes them highly susceptible to viral infections.

(Refer Slide Time: 42:57)



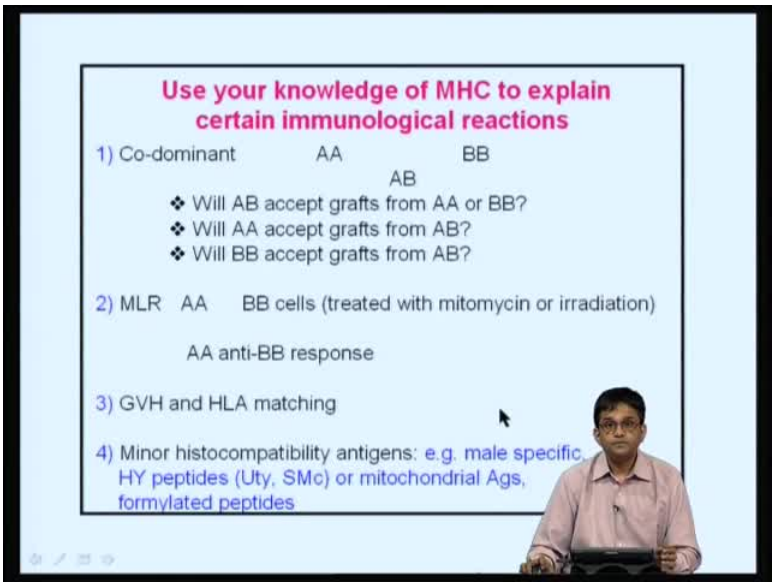
An important aspect that we need to study, especially with respect to host-pathogen interactions is, if the host comes up with molecules, the pathogen also tries to come up with molecules that will evade, and this is seen very well in the interrelationship between MHC and antiviral responses. So, in fact, that you have certain viruses like adenovirus E3-19K, which binds and retains MHC molecules in the ER; you have cytomegalovirus, which encodes molecules US2, US11, US3, that targets MHC molecules for destructions.

Now, I want students to think little about as to why would viruses encode molecules that target MHC molecules? And it is possible that they do so, because if they target MHC molecules, then the presentation of the viral proteins will be hindered, and as a result of which the immune response or the CD8 response will be less, and that gives them a sort of an advantage. So, you need to think of it in those terms that you have the struggle between host and pathogen and both are trying to outwit each other.

So, the host has come up with different sorts of mechanisms and the pathogen is trying to ways by which they target. And one of the ways by which they target is the pathogen is

target is by encoding proteins that targets MHC molecules, which tell you about importance of MHC molecules in pathogen recognition.

(Refer Slide Time: 44:27)



Now, what I would like to do is to go over some terms that are important for MHC studies. So, for example, the first term that we will try and understand is co-dominant, and we will understand it from the point of view of this example. So, let us say, you have a parent which is… which has a two alleles. Remember, we are 2n, which means, we have one from the father, other from the mother. That is what shown over here– you have A and A, and you have another person, which is B and B.

So, and now, you have a F1 AB. I want you to answer the following question– will AB accept grafts from AA or BB? And the answer to that is– AB will accept grafts because from AA or BB, because the A antigen is present in this F1 individual. So, it will see A as well as B as self, and therefore, will not mount a reaction against them.

On the other hand, will AA accept grafts from AB? Not likely, because the B is not present in AA and will be seen as foreign, and therefore, AA will mount, will not accept grafts from AB.

Similarly, with in case of BB, A is not present, and therefore, A will be seen as foreign and it will not accept grafts. So, it is important to understand this concept of grafts with respect to MHC. And the fact is, since we express MHC molecules from one from our

father, one from our mother, both are co-dominantly expressed, and that is the importance of co-dominants.

So, our MHC molecules, we are expressing both from our father's side as well as from our mother's side. So, in terms of MHC class 1, we are in terms of A, B, and C. There are six MHC class 1 molecules being expressed– three from our father, three from our mother, and in case of MHC class 2, you have DP, DQ, DR. Again, six types of molecules that are present– three from our father, three from our mother.

So, we need to understand it from that point of view, and that is why MHC molecules are polygenic and they are co-dominantly expressed. That means, both the father's as well as the mother's MHC molecules are being presented.

So, we will now go to the second term, which is MLR or mix lymphocyte reaction, and now, in this case, we will again use the analogy of AA and BB cells. And now, if you want to check for whether AA will respond to BB, what can be done is you can do it in two ways– one is, you can put AA and BB cells together.

And what will happen is, especially if you take peripheral blood lymphocytes and put them together, the T cells will recognize the MHC molecules. So, AA T cells will recognize MHC molecules and BB will mount a vigorous reaction. The BB, I mean, the BB T cells will recognize the AA MHC molecules and will mount a vigorous reaction.

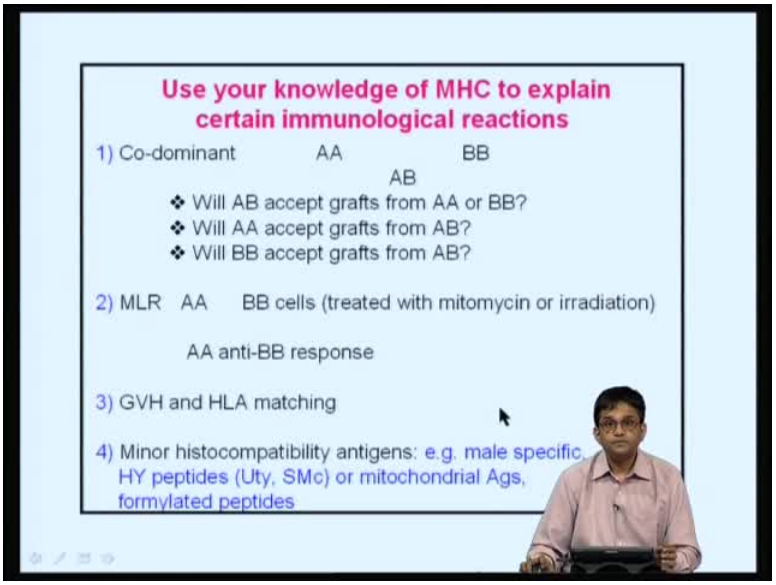
So, you have both types of interactions over here. So, it is two way MLR, so you do not know who is responding against whom. So, in order to understand responses, what can be done is, BB cells can be treated with mitomycin or irradiated, so that they do not proliferate. So, the cells would be fine, but they will not be able to proliferate, but they will able to express MHC class 1 and class 2 molecules. So, if you see this, you will get AA anti BB response, because these cells are not fixed or treated. So, the T cells over here will recognize the BB MHC molecules, and will generate AA anti-BB response. This is called mixed lymphocyte reaction.

So, if you want to check for MHC responses, what can be done is you can take from blood from the donor as well as the recipient, and you can do these sorts of responses because it will give you an indication of the response that is seen. If you see a vigorous response, it is unlikely that the transplant would be accepted. You have now finer studies

by which, following MLR studies, you could do MHC matching studies by looking at specific expression on of specific MHC molecules on the surface using specific antibodies. Further on, further sequencing studies can be done to look at the extent of match, especially, some of the major antigens, which is HLA-A, B, and DP, DQ, and so, those are sorts of things that are important for transplantation, and this is a important that students recognize this.

But before doing finer studies, a simple MLR is good enough to tell you about the vigorousness of the reaction of the anti MHC response, and part of this is because our T cells– about 10 percent of our T cells– have T cell receptor that recognizes allo-MHC, which is MHC different from self. And so, these spontaneously recognize against it, and that is why you have this vigorous T cell response against non self MHC molecules.

(Refer Slide Time: 44:27)



The second one is a GVH and MHC matching. GVH stands for grafts versus hosts reaction and HLA managing. So, what happens is, after HLA matching, once donor cells are injected in, the graft now recognizes, in case, it is possible that some of the T cells from the graft now recognize the host cell as foreign and mount a reaction. So, that is what is known as the graft versus host reaction.

And that is because there are some T cells present in the graft which have been injected, and these cells are now recognizing the host and they are generating this response. You

do not want that. You want to make sure that the T cells from the graft are removed, because otherwise, it results in what is known as GVH.

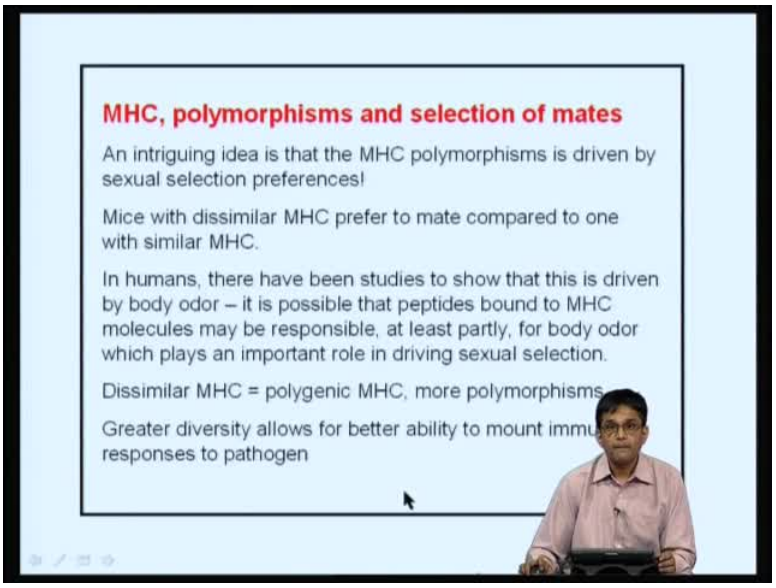
Now, despite the fact that you have…, as I mentioned, the major histocompatibility complex present, which are responsible for organ and tissue transplantations, you have what is known as minor. So, let me give you an example to try and explain this. So, let us say you have two inbred strains of mice; you have inbred strains of mice. So, in theory, the T cells from the inbred strains should not recognize MHC molecules against of the same species, of the same inbred mouse strains.

But sometimes, this happens; and this happens because of the presence of the minor histocompatibility antigen. That means, the T cells over here have not been tolerized to some antigens, and what are these antigens?

So, one would be a male specific antigen, and this could be this difference between male and female. Even though they are inbred, there are some differences in male males and the, for example, males, because the males have proteins that are encoded by the Y chromosome, and so, that may be responsible for it, and you have some of these, or you have some others like mitochondrial antigens which are present. So, these are minor histocompatibility antigens, so again, the response is very small; it is delayed. It takes a much longer time, but these are minor histocompatibility antigens and this needs to be differentiated from the major histocompatibility complex antigen, which are the dominant players.

And the minor histocompatibility antigens are, usually, peptides, for example, that are present or encoded by proteins in the Y chromosome or mitochondrial antigens. Now, if you remember, the mitochondria is transmitted primarily from the mother.

(Refer Slide Time: 52:24)



I am going to discuss, now, an aspect– a newer aspect or newer facet– of the role of MHC, and that has to do with selection of mates, and this is because in a way, what was an interesting experiment was done, and what was found is that MHC– that mice with dissimilar MHC prefer to mate with each other compared to the ones with similar MHC.

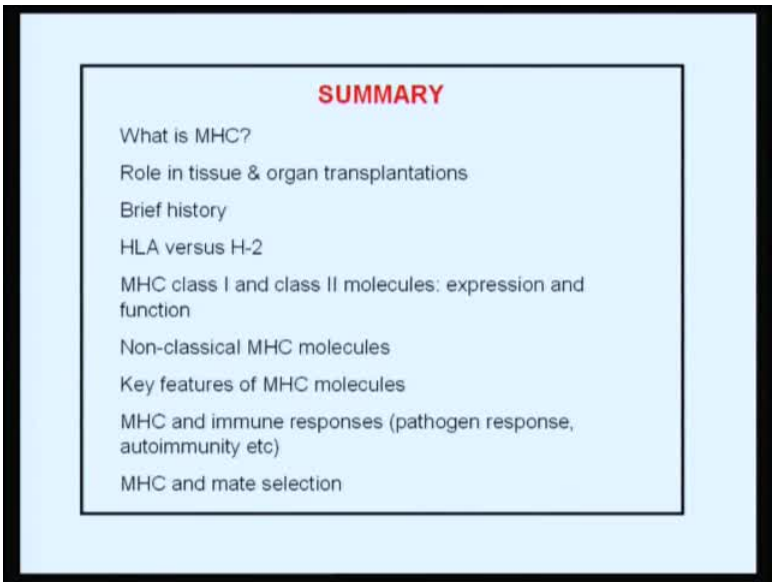
So, this gave rise to the notion that mate selection, that the MHC is also important for mate selection, and how does this occur? And perhaps, body odors play an important role in this. Now, what was shown is in humans that you have a body odors, and this is, primarily, because the part of the odors that is generated by peptides that bound on MHC molecules, and these may be responsible. And this is an important and this drives sexual selection.

This is important if you have dissimilar MHCs. You have, now, if you remember, you have polygenic MHC; you have more polymorphisms and greater diversities. Greater the diversity, it is perhaps better to mount immune response to pathogens. So perhaps, nature has selected for the fact that you try and find partners that are of a different MHC, and that helps in generating a better immune response. And in order to do that, body odors play an important role in, maybe, helping this out, but the mechanisms behind the body odors are, perhaps, peptides that are bound to MHC molecules.

Now remember, most of the peptides that are bound to MHC molecules are derived from self MHC, are derived from self proteins. Majority of the peptides that are bound to

MHC molecules are derived from self proteins, because these are degraded and the peptides that are there are presented on MHC molecules, and so, this is an intriguing thought and idea. And this is a new facet to role of MHC molecules, and I thought it is important for you all to, sort of, understand and appreciate the role of MHC in this process.

(Refer Slide Time: 54:51)



I will now briefly summarize the lecture. So, we had first discovered what is the MHC. MHC is the certain loci; it is this particular loci that present in organisms in, and the ones that are maximally studied are humans and mice. In humans, it is known as HLA, and in mice, it is known as H-2. You have different genes in there, and that primarily dictates organ transplantation, but how it does it? It is, actually, it does so by the production of classical MHC molecules– class 1 and class 2 molecules– which present peptides and which are important.

Now, why is it that you have a vigorous T cell reaction against? It is because during selection, and this is where Ray Owen's discovery turns out to be so important, and that was caught up by Peter Medawar, who realized that we become tolerized as during development and early on, during development.

We discussed the brief history about MHC. I discussed about the role Medawar, Ray Owen, John Dausset, and then the incredible history on mouse biology and mouse research by different workers– Will Castle, Clarence Little, George Snell. It is then

finally coming down to Zinkernagel and Doherty– all extremely classical studies– that students should read up more on and get excited about.

We discussed the differences between HLA and H-2. Remember, in H-2, the K is more centromeric. There are differences in the MB genes; you have two MB genes in H-2, single in HLA. There are differences in non-classical genes.

The most important aspect, of course, is the MHC class 1 and class 2 molecules is that expression and function. MHC class 1 is expressed in virtually all nucleated cells, MHC class 1 on antigen presenting cells. The peptides that bind to MHC class 1 are smaller, about 8 to 10, and in MHC class 2, they are much larger. MHC class 1 stimulates the CD8 response; MHC class 2 stimulates the CD4 response.

We also discussed about the role of non-classical MHC molecules. These do not play a dominant role in tissue transplants, and the most famous non-classical MHC molecule is, of course, HLA-G. There are some common features about MHC molecules– their presence, their genetic localizations, their induction by interferon gamma and certain inflammatory cytokines, so expression is increased, and so, more the chances of recognition of MHC peptide complexes by T cell receptors, and fact that they are polygenic different genes and polymorphic, and this allows to greater diversities, so more the chances of peptides binding and being recognized.

We also went over some examples of MHC molecules and immune responses in terms of pathogen responses, and this, you can see, in terms of population based studies, and this is also important in terms of vaccination regimes and association of MHC molecules with autoimmunity. Finally, I brought forth this important point of MHC and mate selection, and the role of body odor. I hope, overall, you have understood this aspect about MHC, and clearly, MHC is important in terms of immune responses, especially in terms of transplants and immune response to pathogens or to immunity, so on.

But, the overriding feature is really transplant rejections, and especially, its role in acceptance or rejection of grafts. That is how MHC molecules started off with, and that is what we are still following in terms of if you are looking for acceptance or rejection of organ and tissue donors.

But to understand T cell biology, you need to understand MHC molecules, and we will be, in the next class, studying more about the mechanisms by which peptides are generated for MHC class 1 and MHC class 2, and how they are presented. Thank you.