Essentials in Immunology

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Lecture No. # 24

The Major Histocompatibility Complex: MHC Class I & II pathway

So, today we will be discussing some remaining aspects about MHC class 1 assembly and then go on to discuss MHC class 2.

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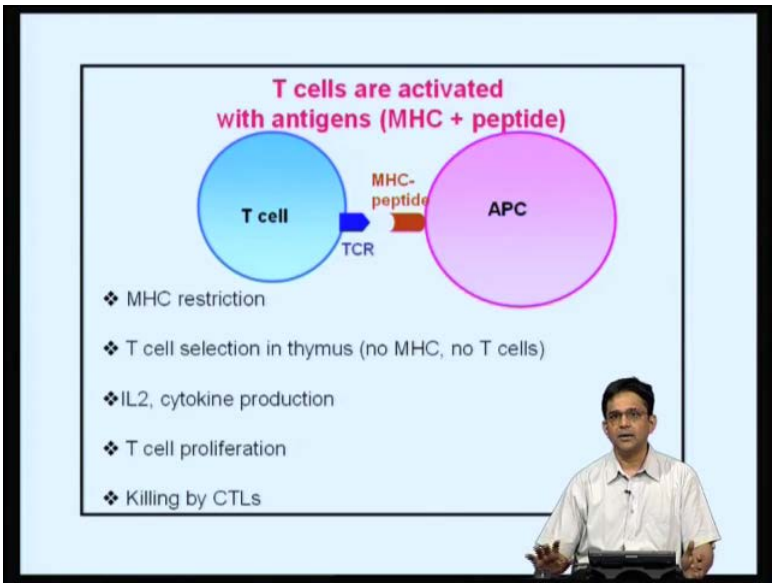

So, this is just to remind us, that you have T cells and they are activated by MHC peptide, which is present on antigen presenting cells. We did discuss this aspect about MHC restriction. What is, MHC restriction, the T cell receptor recognizes MHC, a particular MHC and the peptide complex. So, the MHC is usually, that of the self and so they are restricted by self-MHC, which means, the peptide binds to self-MHC and this is the altered self one that is recognized by the T cell receptor.

Now, this aspect is important in T cell activation, which occurs peripherally, but it is more or it is also important during thymus selection. So, for example, where do T cells learn to recognize the self-MHC? This learning process is done in the thymus and so

therefore, if you do not have MHC, you do not have T cells because the learning itself is not done; so that is a very important aspect, that student should be aware of. This TCR for T cell selection in the thymus, you need MHC. If there is no MHC, you do not have T cell.

So, for example, if there is some problem in, let us say, MHC class 1 expression, you will not get CDAs or for that matter, if there is some problem in MHC class 2 expression, you will not get CD4s. So, it is a very important aspect, that you should be a sort of (( )) of course, in the periphery once the T cells get activated by APCs, then it will result in IL2 and cytokine production, T cell proliferations, primarily by, by CD4 positive cells and this interaction also results in the differentiation of CDA positives into CTLS and then, ultimately killing of the target cells or the, or the ones representing the MHC by the cytotoxic T lymphocytes.

So, this is a, this is a broad over view of what we had discussed in the past few lectures and if you, just to remind you about the structure of the MHC molecules.

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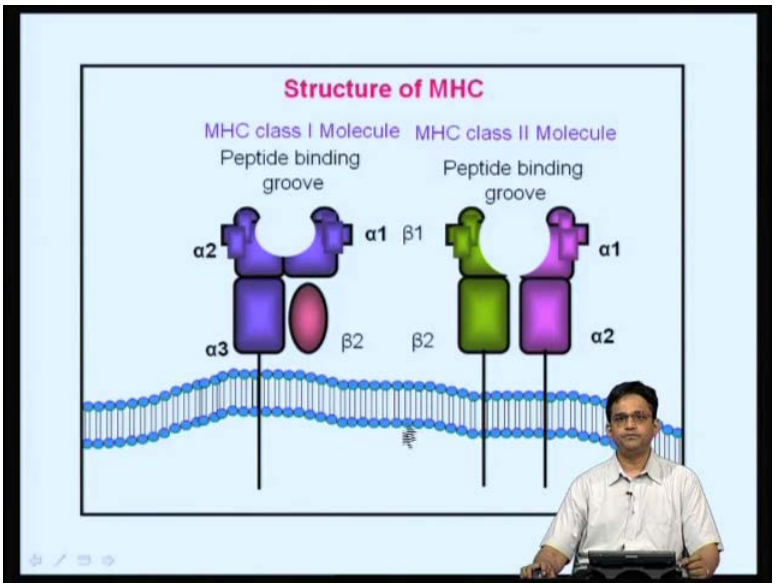

Now, MHC class 1, you have heavy chain and it consists of 3 domains: alpha 1, alpha 2, alpha 3. You can see this, the alpha 3 domain is interacting with beta 2 macroglobulin, which is very important for the assembly of MHC class 1 molecules. So, MHC class 1 molecules needs beta 2 macroglobulin and the peptide is localized, the peptide binding groove is localized in the alpha 1 and alpha 2 domains, which are present over here and

we had talked about some key residues, that are important in binding in peptides. These are known as anchor residues and this is typical of, of MHC class 1 molecule and these anchor residues are located in this groove.

Now, MHC class 1 molecules, you are, consist of 2 MHC class 2 molecules, sorry, consist of 2 chains, the alpha and the beta, and you can see in the MHC class 2, the peptide binding groove consist, is made up of the alpha 1 and the beta 1 domains. So, the, the residues, there are, importance for binding are present in the beta 1 and in the alpha 1 domain of the MHC class 2 molecule. Just to remind you, that the MHC class 1 recognizes smaller peptides, about 8 to 10 amino acid peptides in MHC class 2. There are much longer, 18 to 20, you know, and sometimes even larger, so and as a result of which you can see the MHC class 1.

What is shown over here is that, the ends are closed, that the peptide is closed, that is why, you cannot have longer peptides, whereas the crystal structure of MHC class 2, as shown, that the ends are open. So, that is why you have the peptide that can, actually you, knowing which, which, so, since the ends are open, the peptide can sort of be a, be a lot, lot longer.

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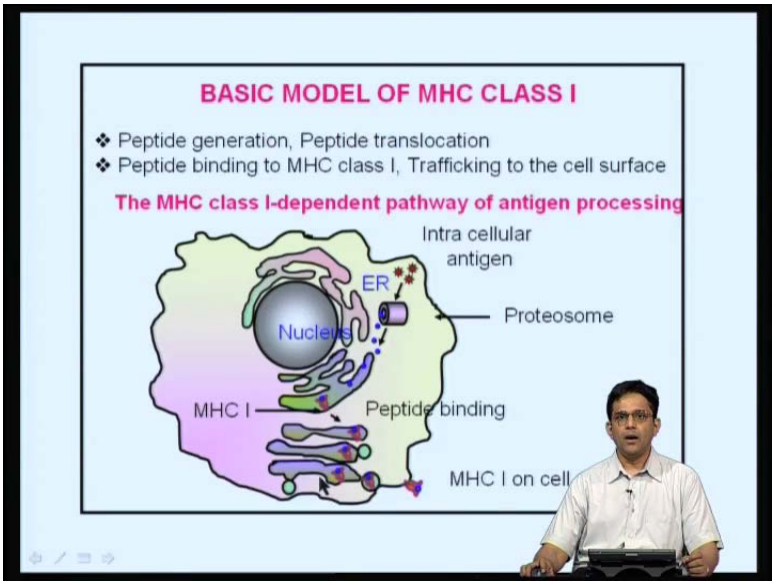

I am just to go over the basic model of MHC class 1. You have the intracellular antigens or proteins, that are, are generated within cells or in case of one infection, you have antigens, viral proteins, for example. So, these are degraded by the proteosome and the

peptides that are generated in the cytosol need to be translocated into the ER. Why ER, because ER is the place where the MHC class 1 molecules assemble. So, this, this translocation from cytosol to the ER is done by transporter associated with antigen presentation and processing, we know as TAP.

So, the TAPs are localized in the ER and these are ATP dependent and so, the peptide ends up over here, and meanwhile, MHC class 1 molecule are synthesized over here. MHC class 1 plus beta to micro ohm, they are synthesized and the 4 over here, what is known as the peptide loading complex, the PLC, and the peptides get loaded on, and then, then the MHC molecules there, there, there is, there is a structural conformation changes in the MHC class 1 molecules. They are more stable and then they traffic on to the cell surface by vesicular trafficking.

So, the pathway is quite clear. So, you have endogenous proteins, that are degraded by proteosomes and then you have peptides, and then you have TAPs and then over here, these are peptides bind to MHC class 1 beta 2 macroglobulin and then traffic on to the cell surface.

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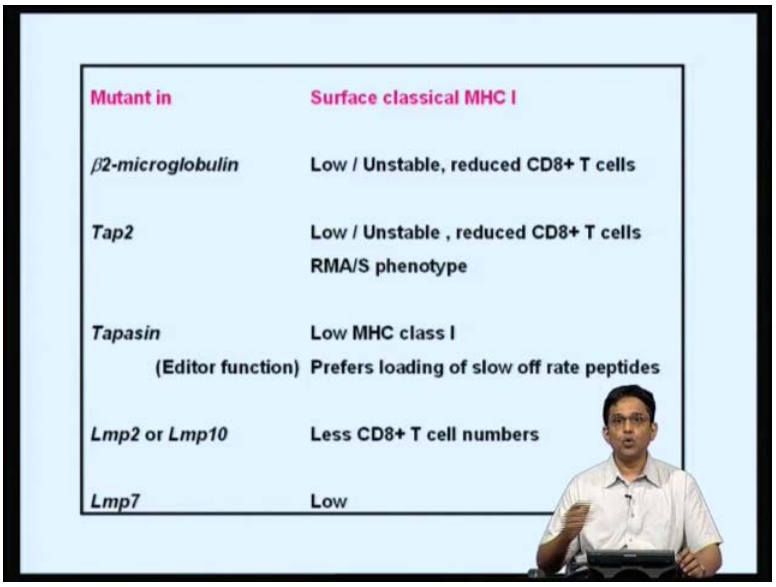

Now, I think, what we should do as an exercise is to try and understand, what would happen if you do not have beta 2 macroglobulin or you do not have TAP? You see, now just to remind you, that the MHC molecules are polygenic; both MHC class 1 and class 2 are polygenic. So, you have different forms. So, even if you lack one, may be, there is,

the other one can be expressed. So, it is difficult to get a complete loss in MHC class 1 genes because there are, there are so many and it is polygenic, and you are inheriting some from the father, some from the mother, but what would happen if you do not have assembly factor; I think, that is an important point.

So, this is what was a, what is (( )) study. So, beta 2 macroglobulin and as was shown previously, beta 2 macroglobulin binds to the heavy chain and its importance gives its, gives its, gives it a stability. So, in fact, the trimer, which the heavy chain, the beta 2 macroglobulin and the peptides are, required for proper structural integrity of the MHC class 1 molecule. So, in the beta 2 macroglobulin knock outs, so if you do not have beta 2 macroglobulin, you know, humans that do not express beta 2 macroglobulins or mice, that do not, are genetically engineers, such that they do not express they are beta 2 macroglobulin, what happens is you generate low, unstable MHC class 1 molecules. Also, because you have such a drastic reduction in MHC class 1 molecules, you have very reduced CD8 positive T cells because the, the, the, the CD8 positive cells are not being selected in the thymus and so, that is a very important, important aspect.

So, similarly, in TAP 2, what would happen is if you do not have the TAP knockouts, you would generate, again, low MHC or unstable MHC, primarily because the trimer is not properly formed. And again, you would have reduced CD8 positive T cells, again for the simple reason, that, that again, selection in the thymus is not occurring because of problem with the MHC peptide complex or, or lack of expression of the surface MHC peptide complexes.

Now, in the previous lecture, we had mentioned about a cell line known as a RMAS and RMAS was critical in, in, in our understanding of the requirements for the surface MHC class 1 expression, but at that time problem in RMAS was discussed and ultimately, what, what, what was found out is, in RMAS there is a problem in TAP 2. So, for some, for, you know, the mutation results in TAP 2 not being expressed, as a result of which you have unstable MHC class 1 molecules; low and unstable MHC class 1 molecules.

We had also discussed another protein, known as tapassin or this is a protein, that is associated with TAP or TAP associated protein. And now, in, in this case, again you have low MHC class 1. Now, tapassin apparently has a sort of editor function. So, it prefers loading of slow off rate peptides, that means, the slow off rate means, that is, it is

allowing for high infinity peptides to be loaded on; so, it has an editor like function. Similarly, in MHC class 2 molecules, we will see a role for DM and that is something we can discuss. So, this is, this is a, so tapassin has an editor like function; it, it, it prefers loading of slow off rate peptide. So, the slow off rate would mean that these peptides have, have, so they come up slowly from slowly off, from the MHC class 1 molecule. That means, they bind fairly strongly and so the structural integrity of these peptide MHC complexes are, are much stronger or, or better. So, this was shown actually in, in mice, that lack tapassin.

So, we will start again and we will start off with tapassin. Now, now, I had mentioned about tapassin and tapassin is a TAP associated glycoprotein and, and what, what it does is, it, it helps, it increases the half-life of, of TAP s and it helps better loading of MHC class 1 molecules with peptides. It turns out it has an editor function, which means, it prefers loading of slow off rate peptides. So, what this means is, that you have peptides that come off slowly from MHC class 1 molecule.

So, in the presence of tapassin, what tapassin does is, it helps MHC class 1 molecules have a, and the peptides have a greater structural integrity. And in fact, this editor function the tapassin has is, sort of, similar to an editor function in MHC class 2 pathway, and that is primarily seen with the DM molecule. So, clearly, you know, it is an, it is an important aspect.

Now, we had also talked about the proteosome subunits Lmp2, Lmp10 and in these single knock outs of Lmp2 or Lmp10 result in less CD8 positive T cell numbers, but MHC class 1 expression is not greatly affected. You just have fewer, some fewer, less number of CD8 positive cells in the Lmp7 knock out, though you have, the MHC class 1 expression is low, but it is not as severely affected as in the TAP 2 knock outs or in the, in the beta 2 macroglobulin knock outs. So, you have different consequences of this.

It is important for students to think in terms of genes and what would happen if there are mutations in these genes and what would be the phenotypes or what would be the function? So, actual function gets shown by these methods. So, it is something, that student should be, should be very well versed with and aware about.

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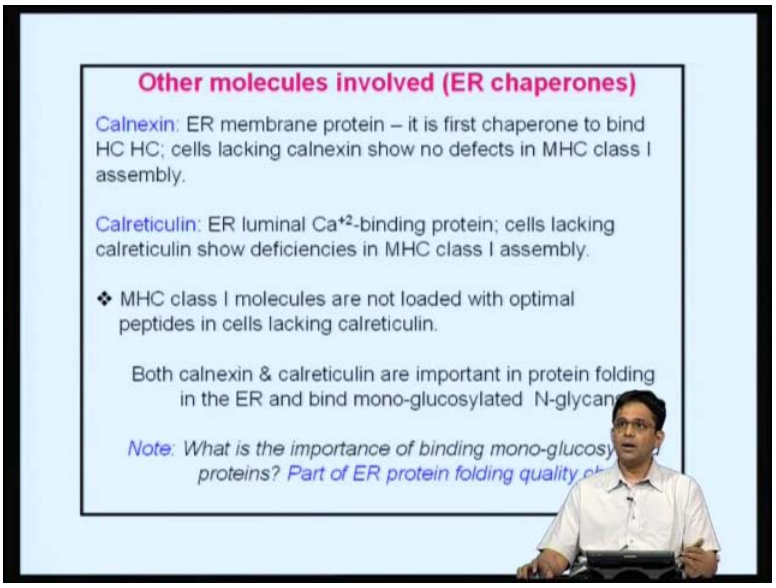

Now, apart from the major molecules, that we discussed, beta 2 macroglobulin, TAP, tapassin, there are other molecules, that play an important role and that is primarily because the assembly of MHC class 1 molecules occurs in the ER and in the ER, there are other machinery, that are also involved in this. So, some of these otherwise are, are 2 proteins, calnexin and calreticulin. Now, calnexin and calreticulin are certainly not primarily associated with the MHC class 1 pathway, but they play a role in, in protein maturation in the ER and as the consequence of that, they are, they are also involved in MHC class 1 assemble pathway.

Calnexin is an ER membrane protein and it was one of the 1st chaperone shown to, bind to, to a free heavy chain. In fact, it is, as, as MHC class 1 is synthesized, free, free MHC class 1 heavy chain binds to a calnexin. However, in cells, that lack calnexin, there is no defect in MHC class 1 assembly. Now, even though calnexin binds to it, it has no obvious functional role.

Now, calreticulin is similar to calnexin except that it is luminary present, it does not membrane bound and in the ER, what was, what was shown is calreticulin, you know, lacking cells, there is some defects in MHC class 1 assembly. Of course, those defects are not as much as shown in the, in the, primarily one that we discussed, which is beta 2 macroglobulin, TAP and so on. What was shown actually, with MHC class 1 molecule are not loaded with optimal peptides, that means, the peptide binding on it is not optimal.

What actually happens is calreticulin is a part of peptide loading complex and so if calreticulin is not there, perhaps the peptide loading is not that efficient and which is why it results in this phenotype. What is important for students to understand from a cell biology perspective is that both calnexin and calreticulin are important protein folding in ER as they bind to mono-glucosylated N-glycans.

So, you have to think, you have to remember, that MHC class 1 molecules, these are glycoproteins and as they are being folded, if the, if in some cases the folding is not proper, you, you have a glucose being put on, on top of the terminal mannose residues and these are, these are recognized and (( )), that the folding is not proper and both calnexin and calreticulin are part of molecules, that bind to these miss folded proteins and helps them fold properly, and that is why, I have said, that you should be, sort of, aware of, of these things. What, what happens is while you are studying immunology, you cannot study immunology in isolation. It is part of, you know, cell biology, physiology, all these are, are integrated in, in, in this molecular biology or it is integrated and you need to understand it and that is why, I have put this, this part in about calnexin, calreticulin and the fact, that they bind to mono-glucosylated N-glycans.

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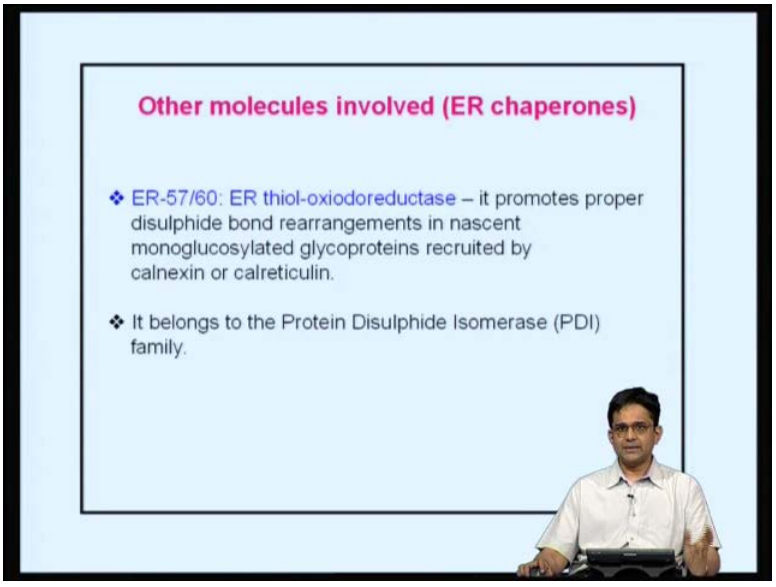

See, some other molecules are also important, for example, the ER 57, which is a thiol-oxiodoreductase and this belongs to the protein disulphide isomerase family. What it helps, what it does is, it, it helps in the proper disulphide bond rearrangements in nascent

glycoproteins as and that are recruited by calnexin or calreticulin. So, it is part of the better folding, ER folding apparatus in the, the protein folding apparatus in the ER, that, that you can, you can appreciate.

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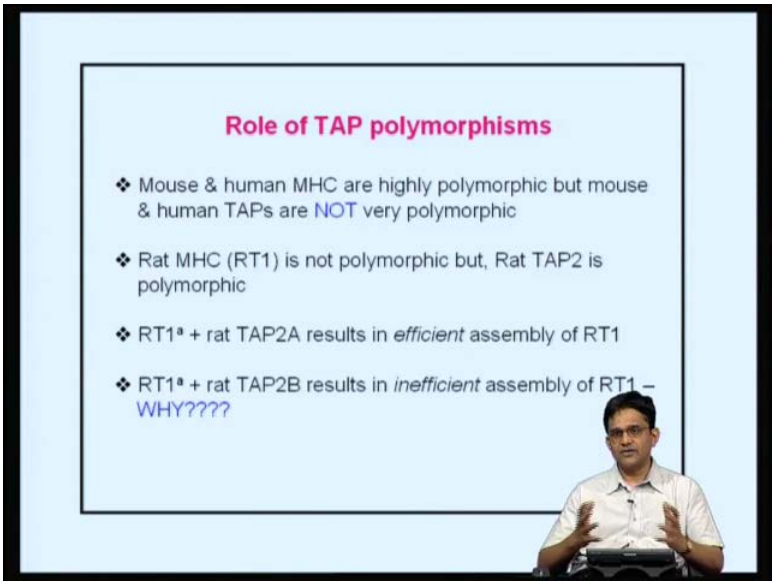

Now, another aspect that I think we should discuss is the role of TAP polymorphisms. Now, I had previously, when we were discussing MHC, I had said, that MHC class 1 molecules are there, are there, are different aspects. What MHC class 1 molecule 1 is the localization, they are H2 or H1 allocated, they are interferon-gamma inducible or cytokine induced because then, you have an amplification of the immune response. The other characteristic is they are polygenic, several genes and polymorphic, your several genes, that are encoding and within the genes, there are variance, within, within these genes in different people. So, it results in polymorphisms and these polymorphisms are useful because it helps them bind to different types of peptides.

Now, what is the role of TAP polymorphisms? Now, what was, what was, what is known is that the mouse and human MHCs are highly polymorphic, but human and mouse TAPs are not, not very polymorphic. There are some polymorphisms, but nothing at the level of, which the MHC molecules are polymorphic. Now, the Rat MHC or the RT1 is the Rat MHC class 1, is not polymorphic, but the Rat TAP 2 is polymorphic and here, here is a very interesting observation. So, RT1A, which is this particular haplotype, the Rat MHC class 1, now this together with Rat1 TAP2A results in efficient assembly of RT1.

That means, you get properly loaded and you have good surface expression of RT1. Now, if RT1A is associated or as you put it in cells, that contain Rat TAP2B, which is a different form of, of TAP, TAP2, it results in inefficient assembly of RT1. Now, why do you think this would be? Can you just think a little bit about it?

Now, it is possible, that, that this particular Rat TAP2 translocates peptides, that are not optimal for binding RT1A. So, you have peptides, that are, that are coming in to the ER, but those peptides are some optimal for binding RT1A and whereas, the peptides, that are translocated by or the preference shown by, by Rat TAP2A is the type of peptides, is more, are more the types, more of the types, that RT1A prefers. So, you can see, as a result of which, so what happens here, in this case RT1A sets in the ER because is not getting properly, proper, is not getting right type of peptides. In the absence of the right type of the peptides, the folding is not proper and since the folding is not proper, the, the, those molecules will not aggress to the cell surface. So, I hope, this aspect is understood and as I said, it, it, it, you know, you understand the cell biology of MHC much better if you understand the cell biology of protein trafficking, vesicular trafficking, so on.

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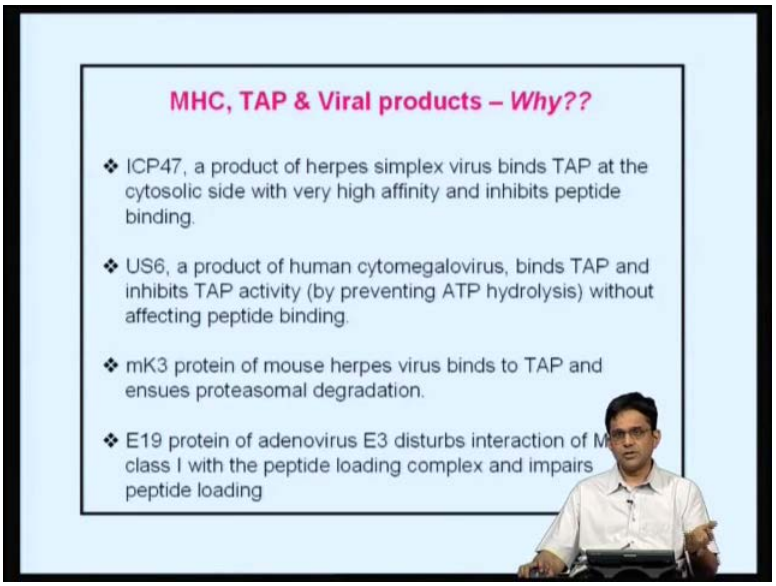

Now, the other aspect that I thought I should cover is, is that MHC, MHC, TAP proteins and viral products. Now, as, as I mentioned previously, viruses are present in cells and they synthesize their polypeptides, you know, in the cytosol, so that they can make more of their numbers. Now, the, the immune system is such that you have mechanism by

which these proteins, that are generated in the cytosol are, are broken down in the peptides and these peptides are presented on MHC class 1 molecule and they are expressed on cell surface. This is a way for T cells to, sort of, peruse and get a feel for what type of, what is happening inside cells.

Now, and you can see, that one of the ways by which and since the CD8 response is, is, is dominant, especially for virally infected cells, viruses that synthesize proteins or have recruited proteins, that will inhibit MHC, may be advantageous for them. It is a double edged zone because on one hand, it might save them from CD8, but is MHC class 1 expression is lower, it make them sensitive to incase cells. But perhaps, there is some sort of a balance in that and from our point of view we need to understand what are some of the factors, that, that play an important role. And over here, what was found is that there are several viruses, which encode proteins, which inhibit the MHC class 1 assembly pathway. And as I said, now, over here, we need to understand if you, if you want to block MHC class 1 surface expression, it is better to target some of the assembly factors because then that will affect a larger number of MHC molecules rather than targeting the heavy chain by themselves alone. So, it is much smarter to, to target the assembly factors. So, again, that is something, that you should try and understand.

So, I will give you some examples. So, ICP47 is a, is actually encoded by the herpes simplex virus and it binds to TAP on the cytosolic side and inhibits peptide binding. On the other hand, US6 is a product of the human cytomegaloviruses and it binds TAP and inhibits TAP activity by preventing TAP hydrolysis, without affecting peptide binding. You can see in these 2 molecules, that these are 2 proteins encoded by 2 distinct viruses, they do the same job, which is inhibit TAP, but the way they do it is different. ICP47, it inhibits peptide binding whereas over here, it inhibits ATP hydrolysis without affecting peptide binding.

So, you should see, different viruses have evolved in different ways and by which they are trying to outsmart the immune system or at least MHC class 1 pathway by adopting various strategies. So, I think, it is an interesting aspect of, for, for students to think about and so the, you have some more examples. MK3 proteins of mouse herpes virus binds to TAP and it, sort of, targets for a, for a degradation. The E1619 protein of adenovirus E3 disturbs interaction of MHC class 1 with the peptide loading complex, the PLC, as I said and impairs peptide loading.

So, you have different mechanisms, where, which these virally encoded proteins, they affect MHC class 1 peptide interactions and loading, and as the result of which, the surface expression of MHC class 1 will be affected.

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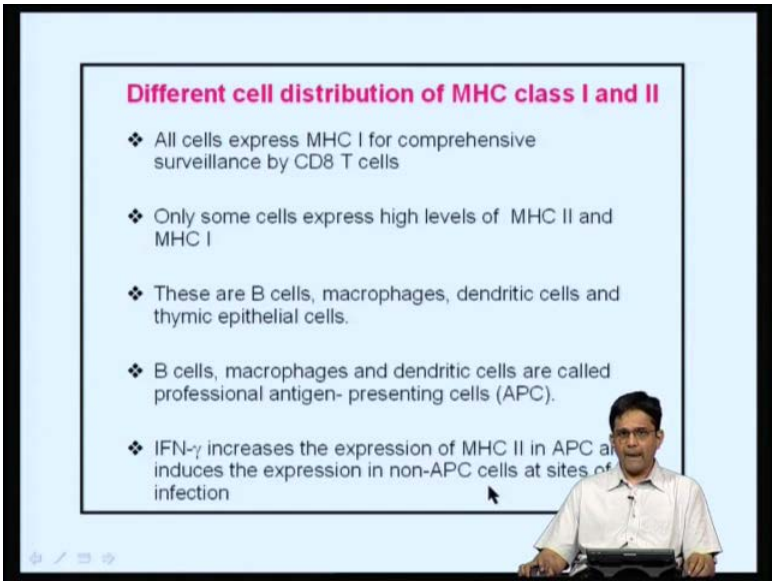

Now, now, one of the other differences between MHC class 1 and this is actually an important difference between MHC class 1 and MHC class 2, is that the MHC class 1 is present on almost all or most, most cells express MHC class 1. However, MHC class 2 expression is restricted, which is present on APCs, on antigen presenting cells and, and these are, you know, B cells macrophages, dendritic cells and thymic epithelial cells, so on, and these are, and this, this, this bunch of cells is called the professional antigen presenting cells and interferon-gamma increases the expression of MHC class 2 in, in antigen in APCs and also in non APC's sites of infection.

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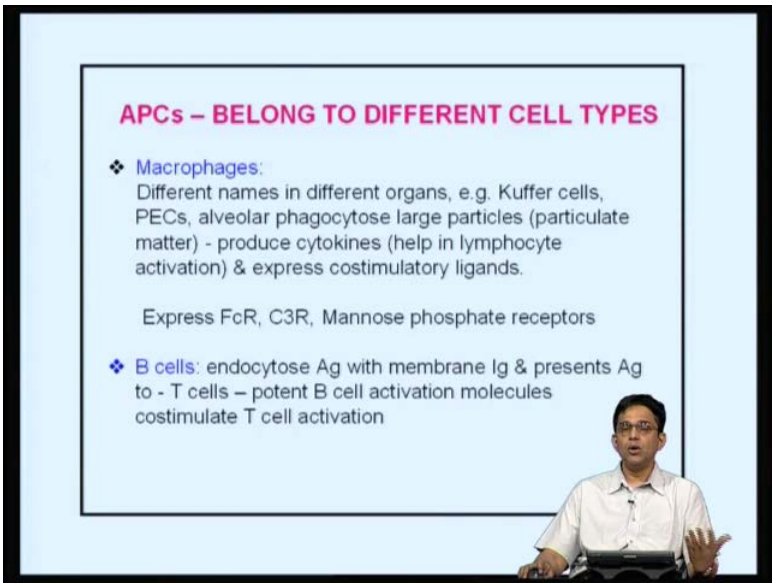

Now, we can discuss a little bit about these antigen presenting cells, primarily because of, because of MHC class 2, which is, shows a restricted cell type expression. So, macrophages for example, they have different type, different organs, so they go by different names. And this is the name given to ones, in the liver are known as Kuffer cells, these are peritoneal PECs, peritoneal exoratecell, which are present in the peritoneal cavity, you have alveolar macrophagocytes, alveolar macrophages are present in the lung and in just large particles because you know, we take in so much dust and so on. And they produce cytokines, they help in lymphocyte activation and they express costimulatory ligands, and these are aspects that we will be discussing in the T cell activation part. The other types of antigen presenting cells are, are, are B cells. Now, B cells are particular in the sense because they can endocytose the antigen with membrane immunoglobulin, that is present and that is what make them distinct from macrophages, in, in that sense, the microphages are going to be nonspecific.

So, they can use FcR or C3R or the complementary receptor or mannose phosphate receptor that is going to be nonspecific; antigen specific endocytosis is done by B cell. So, in, in, in, in this respect, the B cells are somewhat distinct from the other type of APCs and so the, the, so they can process this antigen as an endocytose, and they can activate T cells and they are, and T cells on the other hand, they activate B cells. And so, they express these molecules and you have good interaction between T and B cells,

which leads to a good adoptive immune response. Remember, the 2 major players in the adoptive immune response are B cells and T cells.

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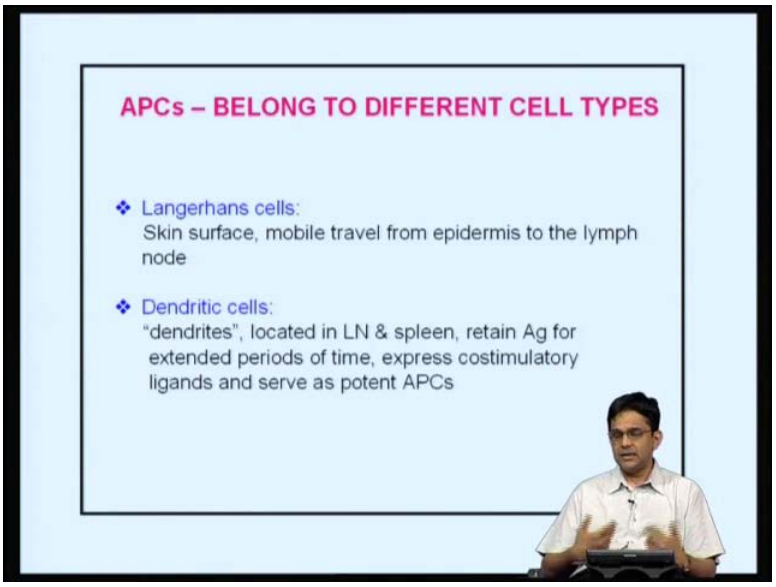

Perhaps, the most physiological antigen presenting cells are your or dendritic cells and these are present in the skin. In the skin they are known as Langerhans cells and basically the, the, once they get the antigen, they enter into the lymph nodes and, and they initiate the T cell activation pathway.

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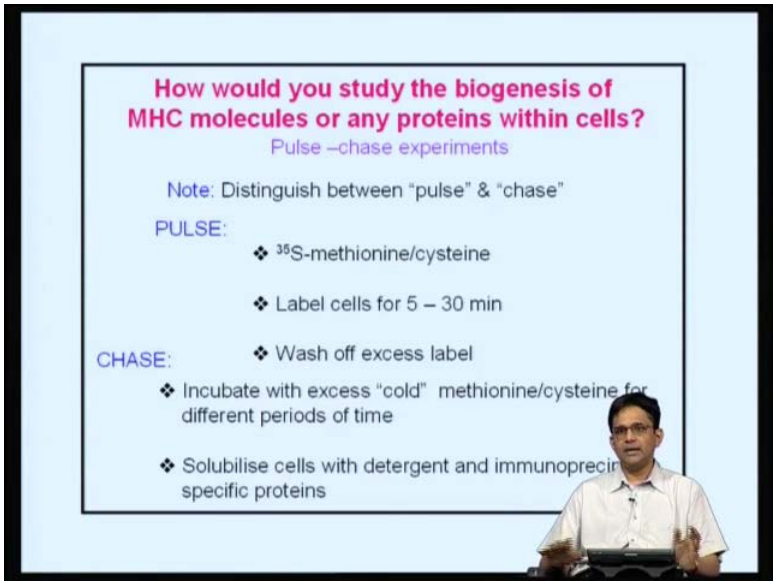

Now, how does one study MHC biogenesis? One critical technique, that has been used in this process is something known as Pulse-Chase experiments. I hope you are somewhat familiar with it. So, what do you mean by pulse; what do you mean by chase? So, what happens in the pulse part is that you pulse with a radioactive tracer and in for the type of experiments, that we discuss of, mainly cell biology experiments, you use a certified methionine or cysteine because these amino acids would get incorporated in most proteins, and at least sulphur containing proteins and then, so, so that would be the pulse. So, what happens, that you would label them for a short pulse, so it is always done in a short this thing, 5 to 30 minute, you wash off the excess label, the label that is not incorporated. So, incubate the label with the cells and so this gets taken up and it is incorporated by, by proteins that are newly synthesized.

So, newly synthesized proteins will incorporate this labeled amino acid in it and then, you remove, wash of the excess label. So, now, these are your cells, that are pulsed. What you need to do is chase and you chase with a cold or excess methionine cysteine. So, that is no fresh incorporation of the radioactive label into your proteins and, and then for, and this can be done at, for different period of times. So, you chase for different periods of time and then you solubilize the cells with the detergent and immunoprecipitate specific proteins. So, that is basically the theory of pulse and chase and this is a very powerful technique.

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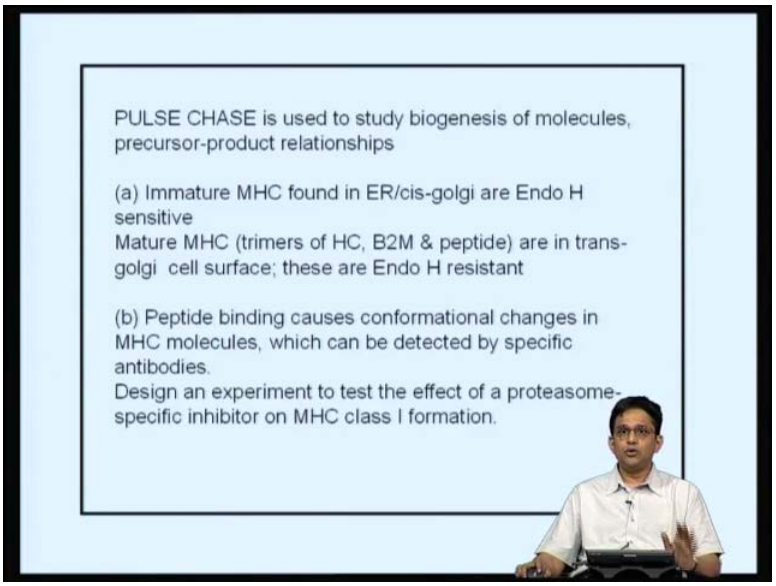

What we will do is we can discuss some, some experiments with you, I think it is very important because by discussing experiments, that is how you are going to be learning, you know, a little bit more about these issues.

So, pulse-chase is used to study biogenesis of molecules, precursor-product relationship. So, for example, immature MHC class 1 molecules are found in the ER/cis-golgi and you can distinguish immature class 1 from the mature class 1 by the sensitivity to Endo H, which is a, which is an enzyme. So, the Endo H ones are, Endo H MHC molecules are, are immature, whereas once they mature, once they have the Salic acids, all sort of put on, on the, on the, on the MHC class 1 molecules, then they become Endo H resistant. So, as a result of which, you can distinguish them immature and the matured one; so, something very important. So, I think, you can think of experiments, that, that are around this particular thought process. In fact, I mean, these are experiments that were actually done to show the, the, the pathway is involved in MHC class 1 and MHC class 2.

Now, so, 2nd example, that I would, that I would come up is, you know, as I said, once if you have that binding to MHC class 1 molecules, then they become more stable and they can be detected with specific antibodies. So, you know, you need to think of or design in experiments, how you would test the effect of, let us say, produce of, specific inhibitor on MHC class 1 formation. Now, what are, what are produced in specific inhibitor? So, this inhibits proteosomes. Now, remember, proteosomes are the ones, that, that generate the peptides in the cytosol. So, if you inhibit, this peptides would not be generated, peptides are not generated, then MHC class 1 molecule, there are no peptides basically.

So, it will be difficult for MHC, for class 1 molecules, to regress to the cell surface or an in, even if they expressed, they would be unstable. So, they would be quickly internalized. So, that is some aspect that you can think about, but you know, pulse-chase experiments are the type of experiments, that people have done to address these issues.

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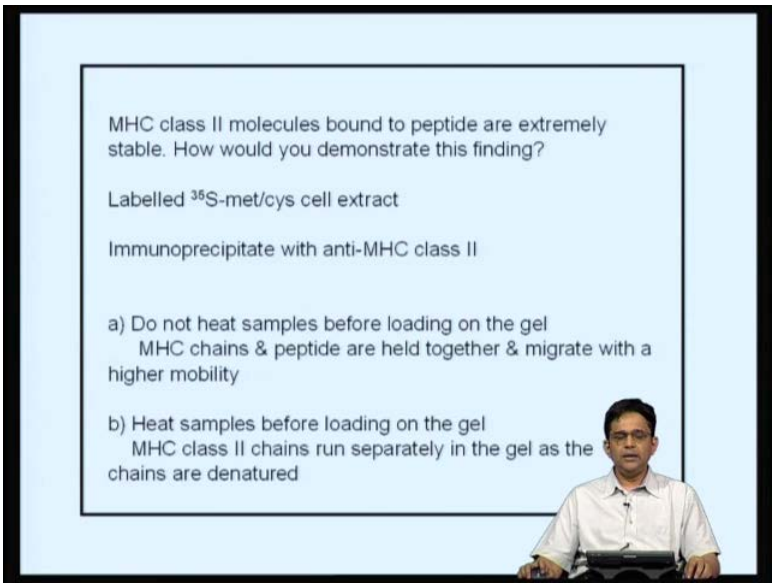

Now, we will, we will start off with MHC class 2 right now. Now, MHC class 2 molecules, once they bind to peptide, you know, they are extremely stable. So, how would you demonstrate this finding? Again, we will, we can use the pulse-chase experiments for this, for this, for this question, to address this question.

So, let us say, you have labeled methionine cysteine cell extract, you can, immunoprecipitate MHC class 2 molecules. Now, what you can do is, after you have immunoprecipitated, so basically you have labeled type of extract, you will have beads; that beads are important because you are, sort of, a matrix and to these beads antibodies are attached, which will recognize the MHC class 2. So, the MHC anti, so these antibodies will recognize MHC class 2 molecules. Remember, once you are labeling your protein, all protein that are being synthesized will be labeled.

So, from this pool of labeled proteins, you need to separate the protein that you are interested in. So, the reagent, that is used, is a particular reagent antibody. So, you can use, you know, specific MHC class 2 antibodies to remove MHC class 2 from the spool of label protein. So, that is, that is what is done. So, once you, you have it, now there are 2 ways by which you can treat, one is you do not heat the sample before loading on the gel. So, and you just, you put it up on a, on a SDSP page and, and sort of, run it. And the other is you heat the samples before you load.

Now, when you heat the sample, what will happen is, remember MHC class 2 molecules consist of alpha and beta chains. They will fall apart and the peptide will be gone because you will not be able to see the peptide in the gel. You will see, you will see the MHC class 2 and MHC class 2-alpha and class 2-beta. Now, if you do not heat, then the MHC class 2 molecules will remain together along with the peptide and so they will run as a complex of MHC class 2-alpha, beta and they are held together. They will migrate with the higher mobility, then the ones where you have heated.

So, it is an important experiment to show, that, that the MHC class 1 molecules, the MHC class 2 molecules are, are, will be held together in the absence of heating. And now, if you, if you do these sort of experiments from cells in which there is a mutation, that FXMHC class 2, what, and if the FXMHC 2 molecules are unstable, then what will happen or they are bound to peptide set, are not very stable, what will happen is, even in the absence of heating, the MHC class 1, class 2-alpha and beta peptides will fall apart. And in fact, these are the sorts of experiments that were done to show, that you know, there are some molecules that are important for MHC class 2 stability. And in fact, if you can translate this cell with some of these assembly factors, then you find, that yes, you know, you have regain back the ability of MHC class 2 molecules to sort of being associated with peptides and migrate as a complex; very important experiments and that is why, I initiated this part of talk with the pulse-chase experiments.

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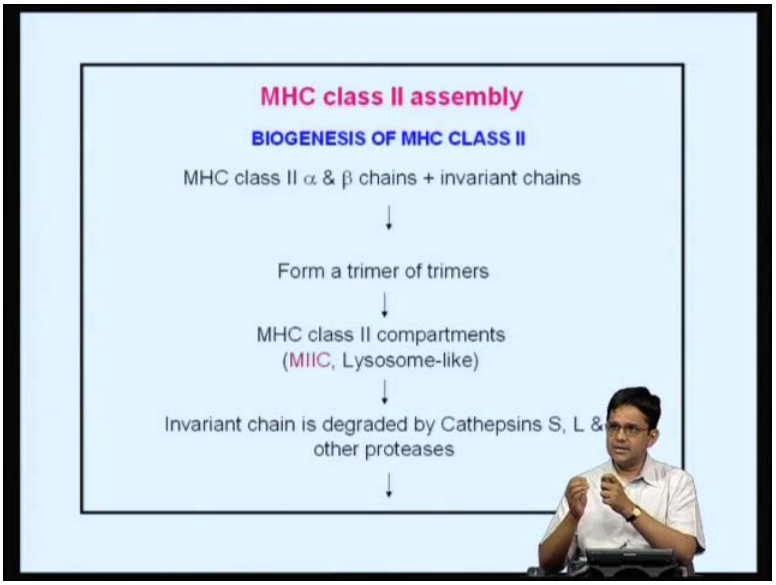

So, we are, we will discuss MHC class 2 assembly. Now, as MHC class 2 is synthesized, the alpha and beta chains, they come together, they bind and they associate with a particular protein molecule, known as invariant chains. Now, why is it called invariant? You see, because for the people in the MHC field, they are so used to polymorphism and polygenic proteins, that MHC class 2 alpha and beta are obviously, polymorphic, polygenic and they associate it with an invariant protein molecules, and then hence, they were named invariant. The other distinguishing aspect about the invariant shape is that it is not encoded in the MHC; it is encoded in a non-MHC location. So, but anyways, once it is synthesized, it comes together, what this invariant chain does is that it forms a trimer of trimers.

So, basically it is a nonomer and you will have 3 MHC class 2 molecule associating with 3 invariant chains and forming this complex. And these, this, what invariant chain is, is a some sort of chaperone and it, it gets the MHC class 1 molecule, class 2 molecule together and then, and escorts this, them to a compartment, known as MHC class 2 compartments or also known as M2C. And M2C is, is the way, usually by which, these are described and these are lysosome like compartments. And so, once it, it goes in there and there invariant chain is then degraded by cathepsins and other proteases.

So, once, so, the way it is, you have MHC class 2 molecules, they associate it with invariant chain and as a deformer, trimer of trimers, and then this complex go to the M2C.

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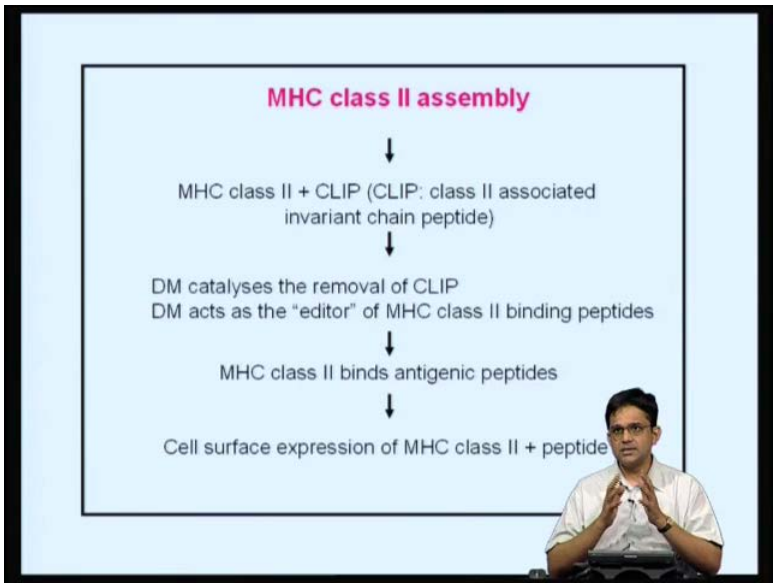

And it is in the M2C, that invariant chain is degraded except for 1 part of it and that is known as the CLIP and that is, the CLIP is the class 2 associated invariant chain peptide.

So, now, you have a complex known as MHC class 2 and associated, that is associated with CLIP and that is a, sort of, remnant of the invariant chain. Now, how does one get rid of this, this peptide that is associated with it? So, there is another molecule known as DM. Now, we had come across DM before. Now, DM is encoded in the, in the, the MHC and it is the genes allocated in the place where the LMPs and the TAPs are also located. Now, what DM does is, it catalyzes the removal of CLIP and in fact, DM acts as an editor of MHC class 2 binding peptides.

So, what DM tries to ensure is that you have very high affinity binding peptides or slow off-rate peptides, that binds MHC class 2 and so MHC class 2 molecule would be stable. And I have I talk to you about the analogous role of tapassin in MHC class 1 that has a similar sort of function. And so, once DM does the catalysis, so, so, if, so here is a question, that I like to ask you, if in cells if there is no DM, what would happen? What would happen is that you would have MHC class 2 molecules associated with CLIP because there is no DM to take it off the, the CLIP molecule. The CLIP association with MHC class 2 is a not a very stable association and, and that is why, the MHC class 2 molecule, that are formed over here are not very stable.

So, what DM does is removes the CLIP of, and, and gets, you know, the peptides, that bind with the slower off rate to MHC class 2 molecules on. So, and then, once you have now properly well-formed MHC class 2 peptide complexes, they sort of egress on and they are expressed on the cell surface. So, the way MHC class 1 molecules are formed and the way MHC class 2 molecules are formed, they are very different and you should be a little bit aware even in terms of cell biology, the localizations and all are very distinct, and that is a very important aspect and you should think about that. So, what would happen is antigens, that are generated, you know, within the cytosols, they are by, by and large, the peptides are generated near the MHC class 1 pathway.

Whereas, pathogens are entire VL isozomes or are endocytose, those, those peptides are generated in M2C and they are found, there, there peptides are expressed by the MHC class 2 molecules. So, what from, from the host point of view or from the cell point of view, it has taken care of both parts, both the endogenous as the exogenous pathway by which proteins are processed and can enter cells.

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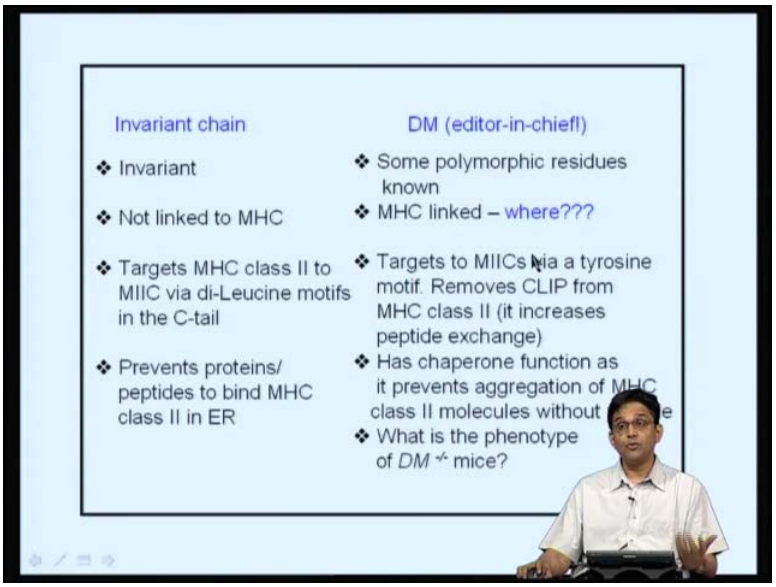

So, a little, some differences between invariant chain and DM, which is known as, also known as editor-in-chief. So, invariant chain is, you know, as the name suggests, is invariant and DM, there are some polymorphic residues known; invariant chain is not linked to MHC, this is MHC linked. Where is it and I think, what we will do is, I will, I would like to just show this.

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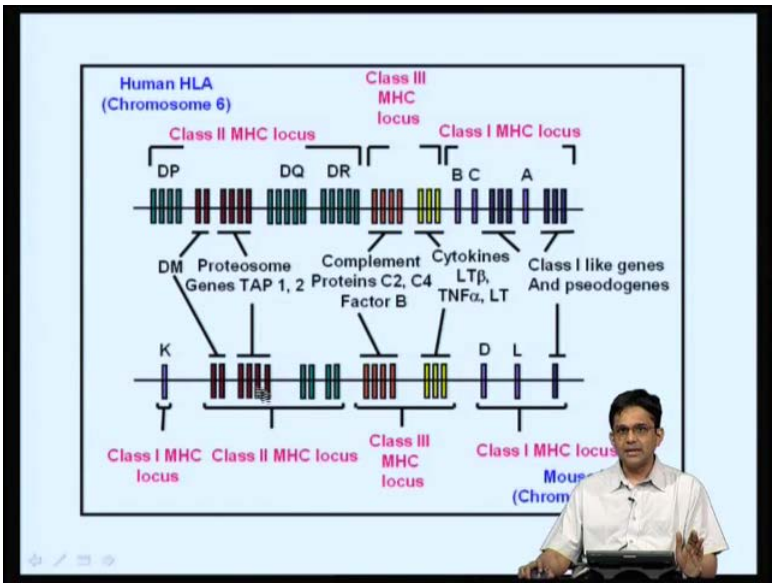

So, this is, this, this is the MHC class 2 region and the, these are the proteosome genes and this is DM, this is where DM and this part is located between DP and DQ, who, and over here, this part is located between the K and the MHC class 2 locus over here, and you can see, this is where DM is and this is where the proteosomes and TAP genes are. So, it is right in the cluster of those 7 genes, that, that were found.

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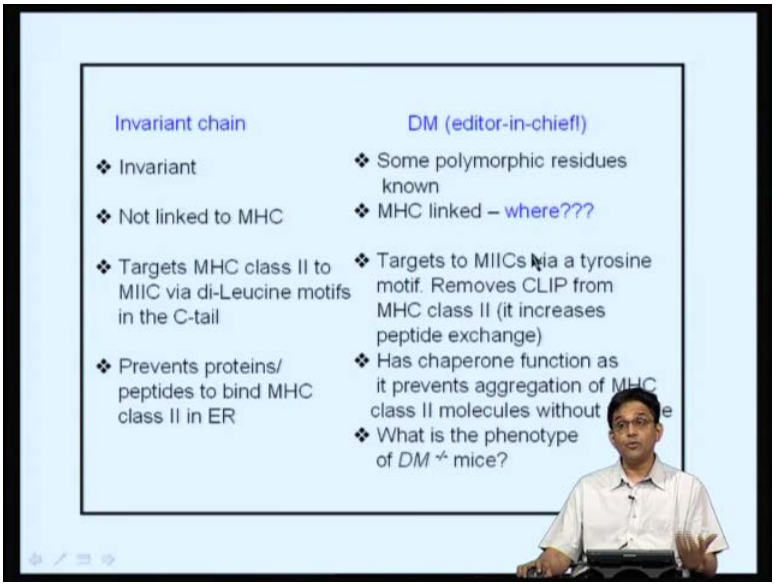

So, the invariant chain, the job of the invariant chain is to take the complex of MHC class 2 molecules and then, chaperon them to the M2C, and it does so by certain

targeting motifs and these are dilusive motifs. DM, on the other hand, goes to the M2C by itself and it targets via a tyrosine motif. Now, its job mainly is to remove CLIP from the MHC class 2 molecules. You see, in invariant chain, once it goes there, its primarily job is done and therefore, you know, and that is why, it is degraded, you have to get rid, the messenger is degraded and the part of the messenger is left on and that part is removed by a DM. The invariant chain, it prevents proteins, peptides to bind MHC class 2 in the ER and you know, but the main job of invariant chain is to chaperone it to the M2C, the main job of, of, the main job of an invariant chain is that. The main job of DM is that it is, is the peptide loading capacity of it.

So, what would be the phenotype of an invariant chain knockout mice and DM knockout mice? So, in the case of invariant chain knockout mice, the MHC class 2 molecules will be stuck in the ER because they would not know how to go to the M2C in the absence of invariant chain. So, there would be no expression of, of MHC class 2 on the surface. Now, if there is no expression of MHC class 2 on the surface or very, very low expression, what would happen is, there would be no selection for CD4 positive T cells. So, no MHC class 2, no CD4 positives; no MHC no T cells.

And the, but in case on DM, what would happen is MHC class 2 would get expressed, but this would be MHC class 2, that is an expressing CLIP, which is an inefficient peptide, but there would be, so there would be some selection for CD4 positive cells. So, you would have MHC class 2 expression, even though it is not the type of MHC class 2, that you would like, but you would have MHC class 2, but the peptides are known to be low infinity peptides, not high infinity peptides. So, the MHC class 2 molecule are not the way, that you would want them to, but you would have MHC class 2 on the surface and you would also have selection of CD4 positives, but, but it would be an altered selection because the proper right of a peptides are not their own MHC class 2. So, you should, students should be able to think a little bit about the differences, about the functional roles of this especially in view.

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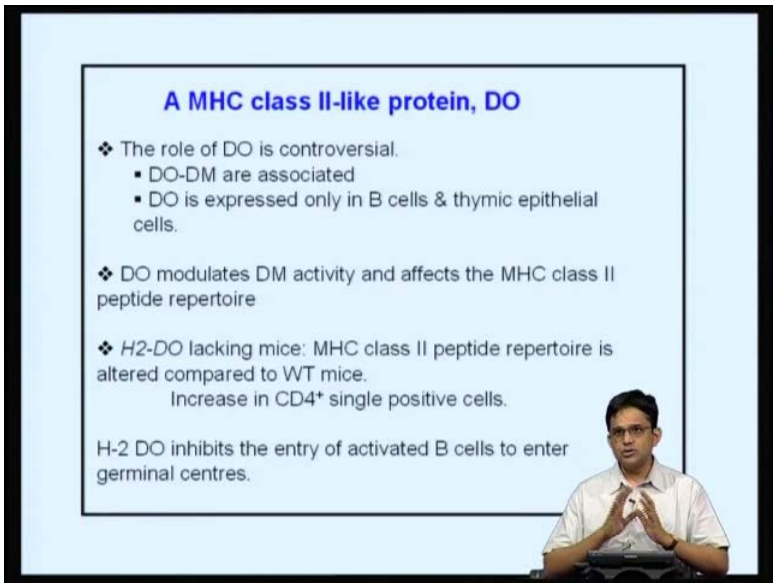

Now, to make matters somewhat more complex, you have MHC, you have what is known as DO. Now, you have DM and DO and both, DM as well as DO, are MHC class 2 like proteins, but they do not bind peptides, but they have other jobs. DM is, as mentioned, was, is some sort of editor in this thing; DO, on the other hand, its role is controversial. But what has been shown is that DO and DM are often associated with each other, but the problem with DO is that it is not expressed, its express, its expression level is, is found only in some type of cell.

So, for, for DM action, you really do not, you know, DO is not required because you have some cells where there is no DO and still, the DM functions and MHC class 2 is expressed. But when DO is there, it modulates DM function. So, and DO for, is expressed only B cells and thymic epithelial cells and so DO modulates DM activity and therefore, effects the repertoire of peptides, that bind to MHC class 2. Now, in the H2 DO lacking mice, the MHC 2 peptide repertoire is altered compared to wild TAP, is not surprising; what is interesting is that you have an increase in number of CD4 positive single positive cells.

So, you have much more single positive cell, perhaps because of alteration in this in peptide repertoire, and may be, you know, in selection of CD4 positive is somehow being effective. Now, in recent days, what has been found is DO inhibits the entry of activated B cells and remember, it is expressed in B cells. So, it inhibits the entry of

activated B cells to enter germinal centers because germinal centers, you have the T and B cells being activated, and that is where actually, the initiation of the immune response takes place, the B cells become responsive to the T cell cytokines, so on. So, DO, DO seems to be affecting that, that also.

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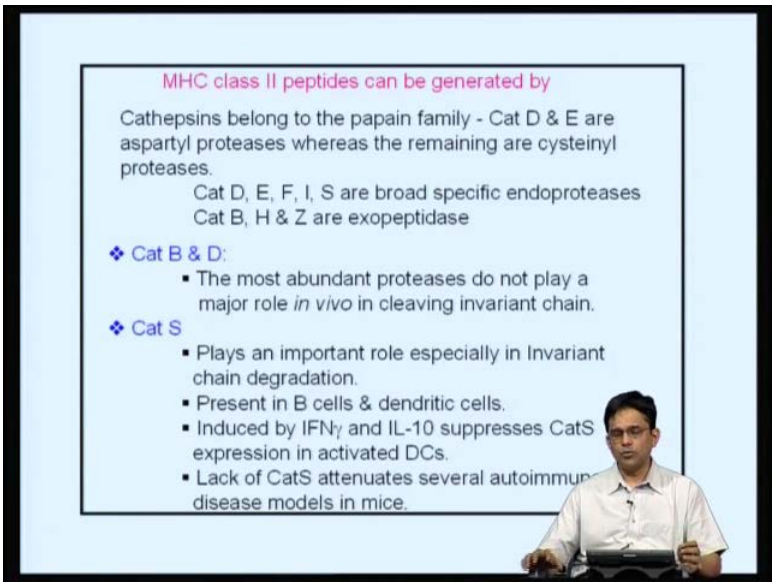

Now, MHC class 1 peptides are generated by proteosomes. So, what are the enzymes, that are, that play an important role for MHC for the generation of MHC class 2 peptides? It turns out it is a family of enzymes belonging to the cathepsins. Now, cathepsins are belonging to the papain family and over here, they are primarily cystein proteases, which means cystein is in the active side except for 2 of the more famous ones, which is, cathepsins in D and E are aspartyl proteases, but other than that, they are mainly cysteinyl proteases. What is also known is cathepsins in D, E, F, I, S, they are broad specific endoproteases, whereas cathepsins in B, H and Z are exopeptidase. Cathepsins are primarily found in lysosome compartments and lysosome or, or MHC 2 compartments. Now, there, you know, for cell biologists there is a difference between lysosomes and the M2C.

So, it appears the, the, the MHC class 2 are present in specialized molecules, which are lysosome like, but not exactly lysosomes and that is why, this name has been given as M2C because the certain markers, that people look for, there are some differences between the markers between the, the, the true lysosomes and the M2C compartments

are, but cathepsins are, are present in both these compartments and they are important for the generation of peptides. Because once you have the proteins over there, they need to be broken down and, and cathepsins are primarily responsible for it.

Now, in this, cathepsin B and D are the most abundant proteases, but they do not play a major role in cleaving invariant chain. Remember, it, invariant chain shepherds in over there and then needs to be clean. Cathepsin S, on the other hand, plays an important role in invariant chain degradation; it is present in B cells and dendritic cells; it is induced by, by interferon-gamma IL-10, which is an immunosuppressive cytokine; it suppresses cathepsin S, expression and lack of cathepsin attenuates several autoimmune disease model in mice. Now, so why would this be? Because there is a co relation over here between MHC class 2 expression and, and autoimmune diseases, it is something that will be discussed.

So, what would be happen is, if you do not have cathepsin S, it might, there might be lesser expression MHC class 2, which might affect the, the autoimmune diseases.

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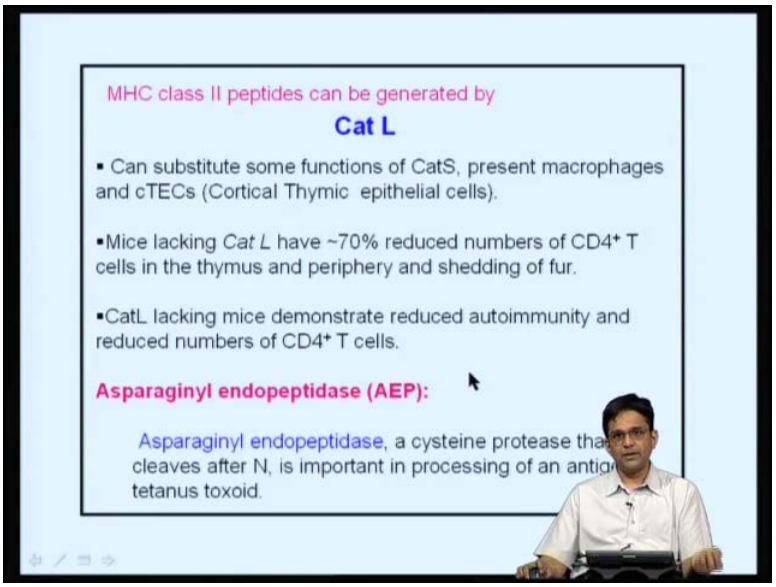

Now, MHC class 2 peptides can also be generated by cathepsin L and cathepsin L can in fact, it can substitute for some functions of cathepsioin S, is present in macrophages and cortical thymic epithelial cells. Now, here mice that lack cathepsin L have 70 percent reduced numbers of CD4 positive T cells in the thymus and periphery; they also shed, therefore.

So, clearly, over here you can see where cathepsin L seems to be playing, you know, you know, a role in MHC class 2 and as a consequence of that it is affecting the selection of CD4 positive T cells. So, not surprisingly, cathepsin L lacking mice demonstrate reduced autoimmunity and reduce number of CD4 positive cells because they are, sort of, linked and that, something that we will see. Now, apart from cathepsins, other proteases are also involved, asparaginyl endopeptidase is shown. It is a cysteine protease that cleaves after asparagine and is important for sync of antigen, especially, for example tetanus toxoid, which is a very well studied antigen.

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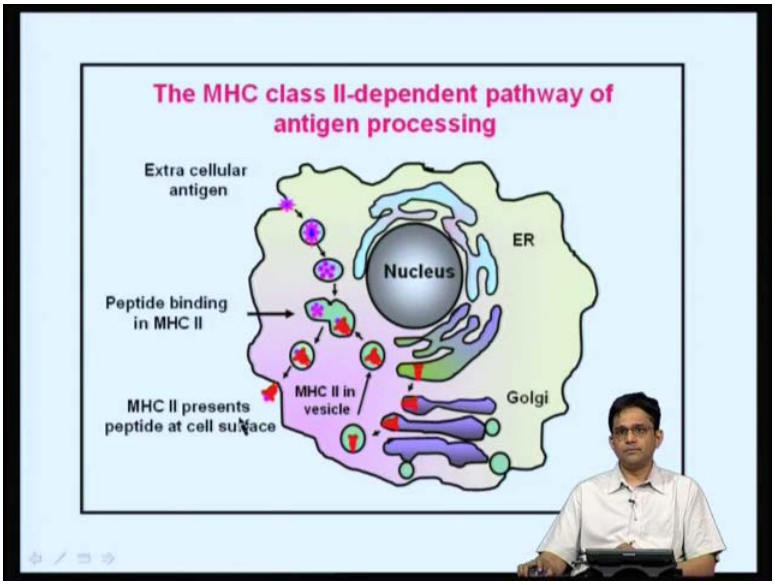

So, we will just briefly describe the MHC class 2 processing pathway. You have extra cellular antigens, that come in over here and so, they meet. The, the peptides are generated in the M2C and what is shown over here is that the MHC class 2 molecules are synthesized, they bind together with invariant chain and invariant, they form a trimer of trimers and they, invariant chain shepherds them to M2C, like M2C compartments, where they meet these extracellular antigens or proteins, and then these are cleaved by cathepsins and part of the peptides are, are bound to. Over here, you will remember, that invariant chain is cleaved and you have MHC 2 molecules along with CLIP and then DM, you know, removes the, the CLIP and then helps the MHC class 2 molecules load on with higher infinity peptides and then, these are expressed on the, on the cell surface.

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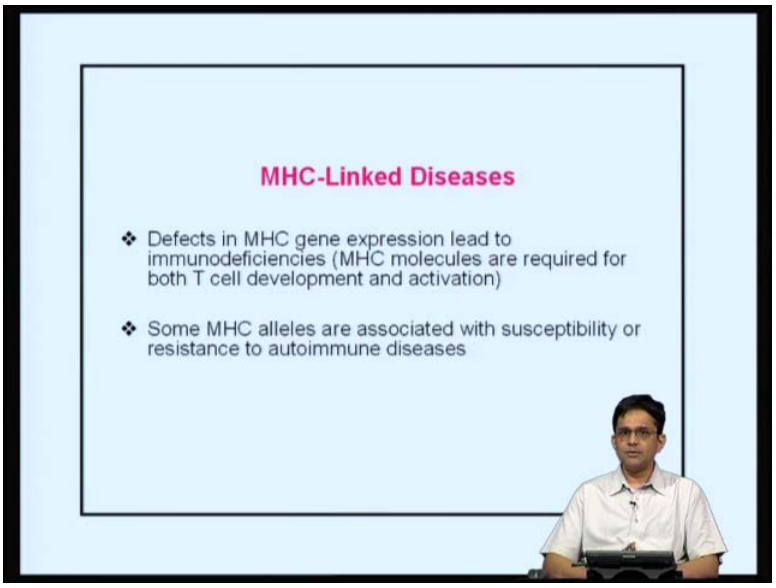

Now, we had discussed this aspect about the link between MHC molecules and disease. Now, defects in MHC gene expression lead to immune deficiencies, so and the reason for that is MHC molecules are, quest, are required for both T cell development and activation. And this is something that we have, we have studied in this part of the lecture. And some MHC alleles are associated with susceptibility or resistance to autoimmune diseases.

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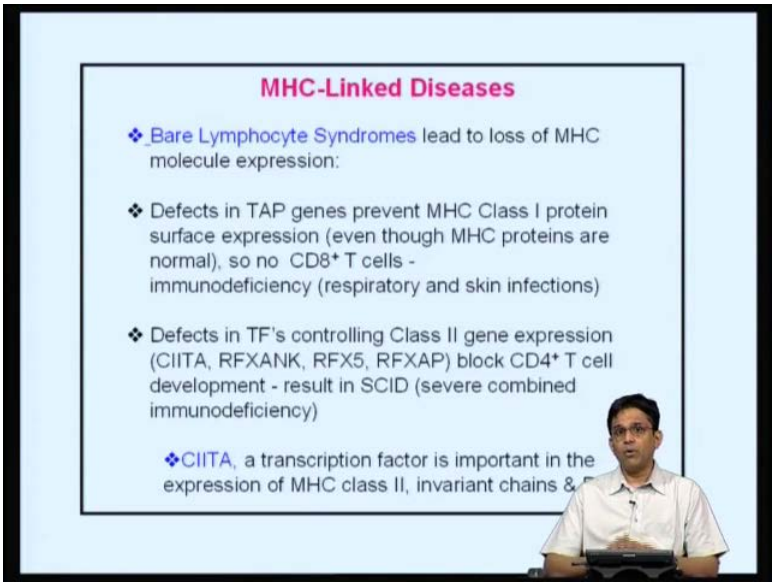

Now, one important disease is known as a bare lymphocyte syndrome. So, there could be several reasons for the bare lymphocyte syndrome. So, what, what this means is, bare lymphocytes means, there are, you know, there are less number of lymphocytes. Now, what, what could that be due to? So, for example, if you have defects in TAP expression, so be a less can, can be due to mutation in several genes, one of them, say, there will be in, in TAP. So, if you do not have TAP, you do not have MHC or in class 1 expression, or even if you have some MHC class 1 expression, those MHC class 1 molecules are unstable and so, because you have reduced number of MHC class 1 molecules, you have less selection in thymic selection, you have less number of CD8 positive of T cells and these lead to immunodeficiency, these are respiratory. So, you will have a respiratory and skin infection.

Now, remember, over here, in this case, you are lacking CD8 positive T cells, your CD4s are there. Now, in some cases you have transcription factors where, which control MHC class 2 expressions. So, for example, very well-known one is C2TA. Now, in, in, in these sort of animals or, or humans, this is important for expression of molecules, that are important in the MHC class 2 gene expression, so MHC class 2 molecules, invariant chain and DM, so on.

So, if you do not have this, what would happen is, there would be no MHC class 2 molecules or very less reduced expression of MHC class 2 molecules. What this would do is, you would have less number of CD4 positive cells. In the absence of CD4 positives, which are important in helping macrophages, helping in B cell help, so on, it results in severe combined immunodeficiency and C2TA is an example of a very important transcription factor that is important in, in MHC class 2. And since MHC class 2 is so important, it results in severe combined immunodeficiency and it is a very important bare lymphocyte syndrome.

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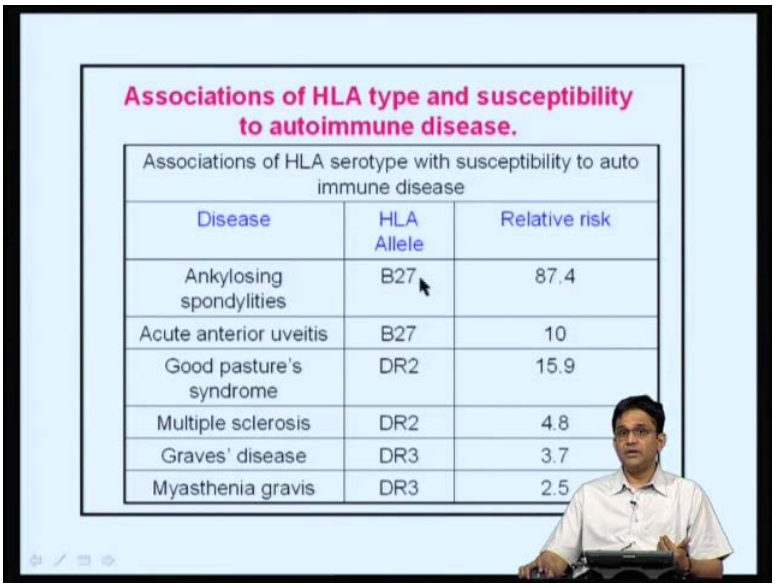

Now, there are some HLA types that are associated with certain autoimmune diseases. So, for example, particular HLA allele, which is the MHC class 1 molecule, HLA B27, there is a very strong association with spondylities; so, very strong association with HLA B27 with spondylities. In fact, the relative, the risk is, is fairly high and you can see some sort of risk along with other autoimmune diseases. So, assemble multiple sclerosis with a DR2 and myasthenia gravis with DR3, there is, even the rate of risk here is not as high as the striking one with HLA B27.

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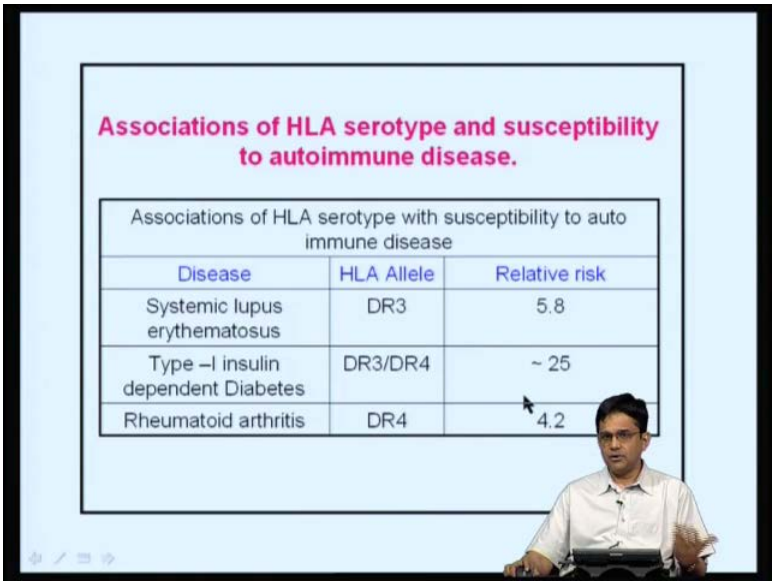

So, the other example of a very high risk is insulin dependent diabetes, where, and where the insulin producing cells are, sort of, are destroyed by T cells and again, there is a big association with a DR3 and DR4 MHC class 2 molecules. So, again, as I said, so you know, having those MHC class 2 molecules, what, what happens is that the chances of a particular autoimmune peptide being presented appears to be high. As a result of which, it triggers off autoimmune CD4 positive cells, which, which kill off, you know, certain, I mean, in this case, there are diabetes are producing, the pancreatic beta cells are being targeted and they are being killed off, and as a result of which you have this, pretty your, your, risk is, is high.

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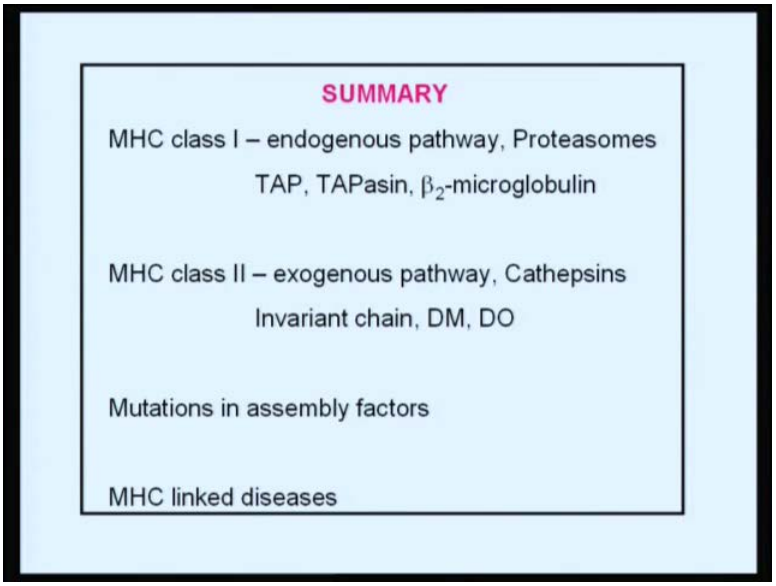

So, we will try and summarize what we had learnt in these classes. So, in MHC class 1 and class 2, the main important point is that the MHC class 1 pathway is the endogenous path, which means, proteins, that are synthesized in the cytosol and peptides are generated from these through proteosomes. Peptides are then translocated from the cytosol into the ER by the transporters, TAP and then, you have this peptide loading complex, where you have the heavy chain and which is bound to, made it to macroglobulin and then, come and meets them, your tapassin, which helps all those. And you have other proteins like caldex and calreticulin, forms a big complex and then, once you have proper peptide loading on, as the confirmation change and MHC class 1 molecules are, now you know, moves to the surface by vesicular trafficking. The MHC class 2 on the other hand, it samples antigens through the exogenous pathway. And over

here, the enzymes, that are important over here are the cathepsins and what cathepsins would do is, you know, degrade these proteins; peptides are generated.

Now, MHC class 2 molecules. Coming to the M2C, with the help of the invariant chain, once they come there, the invariant chain is degraded, but you have a part of it attached on, which is CLIP and this CLIP is removed with the help of DM. And you have DO, which modulates DM activity, but now, and DM helps load on peptides with higher affinity, slow off rate on to the MHC class 2. Once this MHC class 2 molecules are loaded with optimal peptide, this, sort of, move on to the surface. So, one could ask questions, what happens if there is no DM, there is no invariant chain; if there is no TAP; if there is no blended to macroglobulin, and these are aspects, that students should be familiar with. Mutations in assembly factors are important and this is especially true because you have molecules, you know, these molecules play an important role in diseases.

So, for example, bare lymphocytes syndrome, you know, the primary reason for this are, you know, TAP deficiencies or C2TA. We also discussed MHC link diseases. Now, there are certain MHC, that if you express certain MHC molecules, your, there is a high chance of linking with a particular disease with HLA B27. You know, there is a very high risk with ankylosing spondylities with, with particular DR. There is a very high risk with insulin dependent diabetes because the pancreatic beta cells are targeted and they are killed off.

Now, why is, why is this, what happens is, those particular MHC molecules, they express or they have a tendency to express certain peptides and those peptides may, may initiate a CD4 or CD8 response immune response. So, it is, it is, it is a relative, it is a, relative risk is increased if you have some of these MHC molecules. So, MHC molecules are certainly very important for initiation, both for T cell selection as well as T cell activation.

So, so, it is a very important process. So, it is, so, how they are, how they are synthesized, what are the assembly factor, it is very important to understand their pathways and even now, you know, there are better understanding of, this may help us understand the MHC class 1, class 2 pathways more efficiently. It may help us design better ways to boost up the immune response.