Immunology
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Lecture No -23
The Generation of alpha : beta T - Cell Receptor Ligands

Welcome you all, today's lecture in last lecture we were discussing about the MHC 1 and MHC 2 and there are polymorphism their origin of polymorphism and difference. What is the importance of different polymorphic MHC as well as why we have so many MHC's and how it is important but now the question is how these antigen are going to fit into that MHC 1 and MHC 2 because we already discussed that a antigens will be processed and processed means it should be chopped into small pieces.

So that it can fit into MHC 1 and MHC 2, because MHC 1 can accommodate 8 to 10 approximately the number of a minute residue in the peptide and MHC 2 can accommodate 13 to 20 amino acid containing peptide. So big antigens, antigens may be 100 amino acids to 2000's of amino acids. So it should be processed, so this is today's topic actually the generation of alpha- beta T cell receptor ligands.

Ligands means because whatever any receptor binds with a ligand, so these ligand are basically the processed or chopped antigen which is T-cell receptor going to recognize. So today's lecture again the generation of alpha-beta T-cell receptor ligands.

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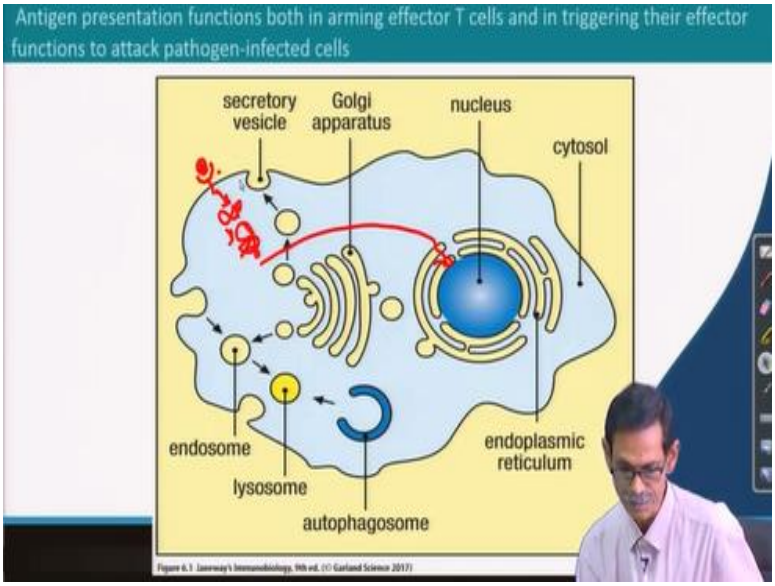

The generation of alpha-beta T cell receptor ligands, before going to that detail let me discuss very simple thing that most of you already know. This is what I am showing in this slide, is one cell which that has a nucleus here and this is the endoplasmic reticulum all a level though this is cytoplasm the bluish part this is Golgi and there are important function of these Golgi's to send the protein to it is membrane or different parts.

So Golgi to trans-golgi network and then secretory vesicle is formed that secretory vesicle goes to the membrane if the protein is membrane bound then it stay there in the membrane. If the protein is secretory then it leaves the cell and coming into the surrounding medium or a fluid inside the body. Similarly if cell wants to take something from outside then we have different mechanism one is pinocytosis another is phagocytosis and normally all cells cannot do phagocytosis.

So what they do is this is endocytosis, this is mostly the receptor mediated endocytosis. So they invasionision happen and this become a vesicles come inside the cell and gradually it comes and fuse with the lysosome then lysosome degrade the particle that taken from the outside. Besides this there is one more thing happen inside the cell, which is called Autophagy. Autophagy many times it misunderstood like that means cells are eating themselves that is one cell is eating other one.

That is not Autophagy that is you can say cannibalism. Autophagy means cell make some vesicle likes thing and those vesicles are basically taking, I am taking the cellular material there are many, many things inside the cell which is not used. So just to recycle them cell makes a vesicle and eat them. So intracellular material they eat and chopped and reused for other parts, so they are many, many times it happened.

So we will come into that, so the thing is why I am saying this particular picture or showing this particular picture is because MHC is synthesized inside the cell. So all protein like any other proteins it will synthesize in the cytosol by the ribosome then it goes to Golgi and then Golgi then through this Golg-trans-golgi network the MHC will go to the signals I mean this membrane.

And one more thing I would like to mention here that normally what happened, like any protein residing in the membrane like any receptor any kind of receptor not only immune system any receptor once one protein made inside the cell there have a life, normally mentioned as a half- life they have a life. One protein is not that that protein synthesized once and it will remain forever. So after certain time depending on the function many some proteins a few seconds some proteins a millisecond, they have lost their activity and some protein may stay for days.

So what happened to the receptor, receptor goes to the membrane stay for some time. So if this is the membrane, suppose mine this hand is membrane a receptor is staying like this so if this receptor stay forever so continuously signal will be there, so it is not happening. So what is happening there is always a recycling of receptor that means the existing receptor will be internalized by endocytosis degraded.

If cell needs the same receptor again they will make that receptor and send it through this channel. So existing receptor inside on top of cell membrane they are internalized, as well as new receptor is making and they are going outside and stay in the membrane. So that is the normal process which almost every are cells doing, because signal transaction or communication between cell to cell or in an outside environment to the cell is very important

and everything is done by this receptor present in mostly receptor present in the membrane.

Except the steroid hormone receptor almost all receptors are staying in the membrane. So any ligand, so if anything is there see for example, one receptor is here. So some ligand will bind here and then some signal will go from one protein to another protein then this protein so that will continue and go and most of the time what happened this signal goes finally to the nucleus, as a transcription factors and new gene synthesis happen, so it should not be continued.

See for example in immune system what is going to happen if as long as antigen present signals should go till then like if the antigen is removed or clear signal should stop. So these particular receptor, if it is MHC it should also recycle. Now today we are going to talk about, how this antigen is processed fit into the MHC and going out to cell membrane. So that T cell can recognize them.

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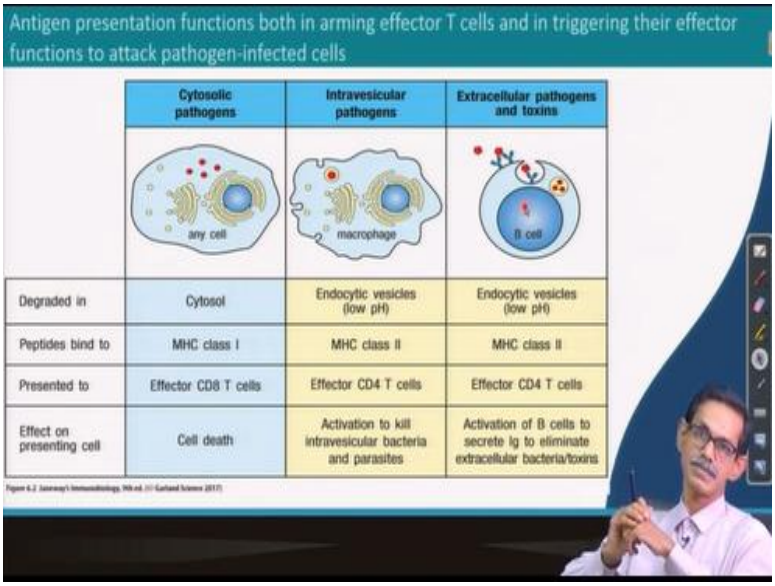

So before I am before going to detail, I am just reminding you again because I talked about this thing, but just to remind you again what is this? This is case of cytosolic pathogen. What are the cytosolic pathogen? mostly the virus, so virus grow inside the cell. So these are the cytosolic pathogen, who is taking care MHC class 1, who is the target cell of this MHC 1 this is the CD8 cells, that we already know and what is the result cell.

Same way, there are some possible pathogens, which grow inside the internal vesicles. So they are intravascular pathogens, like protozoan parasite Leishmania, Mycobacterium tuberculosis, Mycobacterium leprae. So this kind of pathogen, grow inside the vesicle. So red is the pathogen and you see there is inside the vesicle, who is taking care the sender static vesicles, because of the low pH as there is one more thing happening in the cell system.

That more this these vesicles when they are going they become acidic and this acid activates some proteolytic enzyme which cleaves that. So after that this cleavage it is going to be presented by MHC class 2 and MHC class 2 is presenting to which kind of T cells CD4 T cells, all you know we already discussed all this thing. Just this is recapitulation I am going to dip it. I told you many times that, I am going to repeat things because many times it get confused.

So just to going in detail of the antigen processing and presentation I am just reminding you again and these CD protease also what they are doing. They are activating these cells having the macrophage or something and you know the TH1 response, they kill intravesicular bacteria and parasites. Another thing is happening, like in B cell this can happen in macrophage or dendritic cell also.

But mostly B cell is doing that extracellular pathogen or toxin, it is attached to the B cell receptor then endocytic vesicles is formed. So receptor mediated endocytosis bring them inside, then you know what is going to happen. Lysosomal fuse degrade them and then it will be presented by again what MHC class 2, this MHC class 2 is going to activate the effector CD cells, a CD4 T cells.

And this is going to do two things one this interaction like this B cell receptor and antigen interaction will activate the B cell as well as the CD4 T cells which is activated by this B cell presentation. It will in turn help the B cell to understand what is happening and convert them to plasma cell. That signal B cell is going to get from T helper cells. So here two things actually happening activation of B cell to secrete immunoglobulin that is happened by CD4 cells and as well as the B cell is also eliminating the toxin and things from the system.

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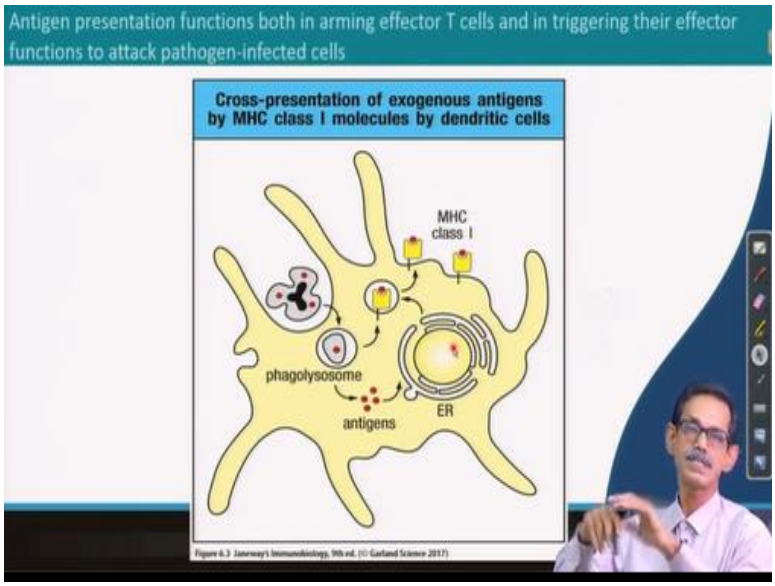

These; whatever I said so far it is called direct presentation. This is the direct presentation, because you are directly presenting yourself but there are something which will come into later in little more detail which is called cross presentation of exogenous antigen. So now a question can come, if it is if you were in the in front of me; some student definitely will ask I am sure, because I am receiving this question.

So CD4, CD8 T-cells are activating the activated by macrophage or dendritic cells, mostly dendritic cells. So CD8 T-cells get activated, to kill the virus infected cells or tumor cells. Now how this is going to be presented. Because if dendritic cells because CD4, CD8 T cells can be activated only by Antigen presenting cells, so either dendritic cells or macrophages should activate the CD8 T cells.

So that it can kill virus infected cells. So how it will be activated; two possibility majority of what is happening; most of the time dendritic cells itself infected by virus dendritic cells itself infected by virus. So that because you remember the MHC 1 is present or in three kinds of cells mostly, three kinds of cells: B cell, another is macrophage and other is dendritic cells. So other cell MHC 1 is presents everywhere, but it cannot activate the t-cells.

Activation of T cytotoxic, T cell is only possible by these 3 antigen presenting cells. So if any

other cells are infected by virus they cannot activate the T cells. If T cells or cytotoxic T cell is not activated, it cannot kill the virus infected cells. So who will activate and how it will be presented. So that time one is direct that means dendritic cell is infected by the virus and in that case it will be presented by MHC 1, but if dendritic cell is not activated by the virus or macrophage is not activated by the virus what will happen then the virus infected cells are taken up by the macrophage and dendritic cells.

Because they can also do phagocytosis and micro pinocytosis. So these phagocytosis or micro pinocytosis, they internalize the virus infected cell. Which already has this red dot means they are the virus, so when they take our infected dead cells they will take and this particular antigen will come through this phagocytosis or endocytosis of this phagolysosome fusion and these will release, I mean this is probable hypothesis I mean it is not exactly clear what exactly happened.

So this phagolysosome sometimes mixed with the different vesicles and during that mixing they load this antigen process to the MHC 1 which is expressed or this phagolysosome released the antigen and these antigen is going normal process because this antigen when coming from outside normally they are supposed to I mean if you remember the previous slide external antigen is supposed to be presented by MHC 2 but here it is presenting the MHC class 1.

So what is happening; these and even come into the cytosol and then it is as usual like just another I mean antigen like the antigen expressed in cytosol is equal to that. So pre antigen present in the cytosol will be processed as before I am in the last slide, so this is the cytosolic pathogen. So cytosolic protection growing inside the cytosol and if this particular same thing is coming again in the cytosol it will be treated as same way.

So it will take the normal path, so that is called cross presentation. So I am just repeating it again when virus is infecting regular other cells non immunological or non antigen presenting cells they cannot activate cytotoxic T cells. Cytotoxic T cell or CD8 cell need to be activated by antigen presenting cell only eat the virus most of the time virus infects the amniotic cell also but if the virus there are some viruses which does not infect dendritic cells.

So then the activation of cytotoxic T cell is not going to be possible by the regular other cells or other tissues. So what will happen that particular antigen will be escaped from the immune system? No it is not happening that way. Then, there is cross presentation which is very active cross presentation is very active in the immune system, what is happening they internalized the dead cells which take the viral antigen by regular other (())(17:03)).

Or outside antigen like bacteria or toxin say they come inside the Phagolysosome directly go to MHC 1 or the antigen released to the cytosol which is just going to be treated as other cytosolic pathogen and presented by MHC 1. Otherwise these kind of viral infections cannot activate the cytotoxic T cell but it happens and it happened very efficiently in the immune system.

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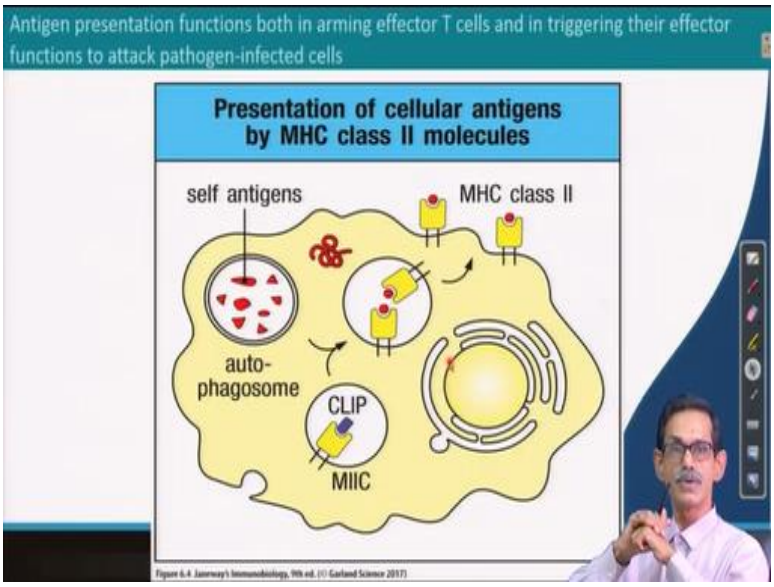

So presentation of cellular antigen, so during this process normal cellular antigen you will see something here like this clip I will come later when we will explain the cellular antigen which is present what is happening I just told you auto Phagie. So when MHC is presenting the foreign antigen same process I as the very beginning of today's class I said this is the normal process is that all the protein is recycled in our inside the cell in our body.

So our own antigen is also going to be presented by MHC 2. So this Auto phagosome, Auto phagosome means which kills our own cellular machinery which is not used or do not need normally what happened when cells are under stress it happened in even the protozoan parasite

also when cells are under stress they just stop many of their regular activity, they realize like many of you know that if bacteria is under stress they make endospore.

So they just suddenly stop all the metabolic activity and stay idle and waiting for better environment or the favourable environment, so that they can multiply again. Same way make all our cells if they are under stress oxidative stress or heat stress they stop many of their regular activity and wait for some times like when the favourable condition will come that time what happened they have lot of ribosomes lot of proteins and other RNAs.

What is going to happen? Because the cell will not keep them because stress means suppose there is no food so if there is no food how they are going to get energy how they are going to make their minimum protein, so that they can survive for that period stress period. So what all the extra material of the cell they just collect in a phagosome or auto phagie and these material they reuse.

So while processing all the cellular material they also normally treat as the exogenous antigen and will be presented by MHC 2 but that will not cause any problem, presentation of self antigen by MHC 1 or MHC 2 should not cause any problem, why? Because all self-reacting B cell receptor and t-cell receptor are eliminated during the development that is the clonal deletion happen.

If you remember the clonal selection that all the self-reacting B cell and T cell at two different places in bone marrow as in thymus all the self reacting or self antigen reacting B cell receptor containing B cell or T cell receptor containing T cell are dying. So even our own protein is presented by MHC 1 or MHC 2 it will not make any difference in the immune system or we will not face any problem unless there is a defect and if there is a defect that is caused autoimmune disease.

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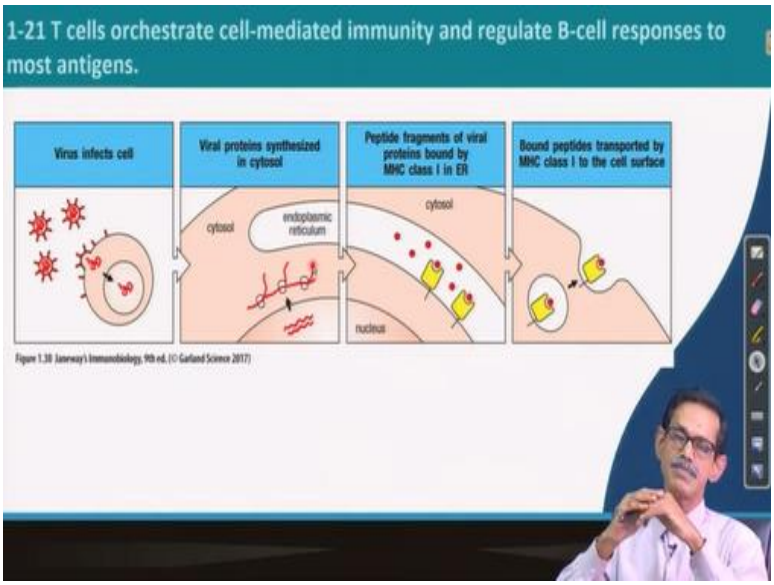

So this picture you know why we have seen this before I am just going repeating again, so virus infected cell virus come inside that viral protein is synthesized inside the cell and they go to endoplasmic reticulum then endoplasmic reticulum as a process form they fit into the MHC 1 going out that is how the T cells or cytotoxic T cell can see them this which I mean, the same slide I showed you in the very introductory lecture of immunology or in this course.

And, now we will see what we will see just we will enlarge what exactly happening here. So what happened to normal protein all the protein I said that when the job is over we do not need that protein anymore even if you need the protein every protein has a turnover number like one protein can function say 100 times some protein can function say 200 times after that they will degrade if cell needs that protein then it will cell will make again if they do not need they will stop the production.

So all once the protein is synthesized what is happened to that protein when the job I mean when their function is over or the purpose is over that will be degraded. So the during protein synthesis what happened during this ribosome process many things happen well pricing may not be right, some protein may be incomplete, some protein may not be pull properly, so this is called defective ribosomal product.

So defective ribosomal product finally the protein is not complete or not folded properly so non-functional peptide. So this non-functional peptide as well as the protein which is with the cell does not require anymore all these proteins are degraded and there is a system and this system is called ubquitin proteasome system. Proteasome is machinery or the system which dictates the protein.

I mean, I will come to that part very quickly but that proteasome does not degrade everything, what happened if this is the protein so some labelling is there. So somebody is sitting inside the cell which will label this cell that they we do not need this, so this is just oscillate. So these protein we do not need we have to kill so there is a tag this is called ubiquitination. So this ubquitin protein are evacuated and this poly ubiquitin that not one the multiple ubiquitin protein is attached with it and that is recognized by that degrading system, so that it will degrade.

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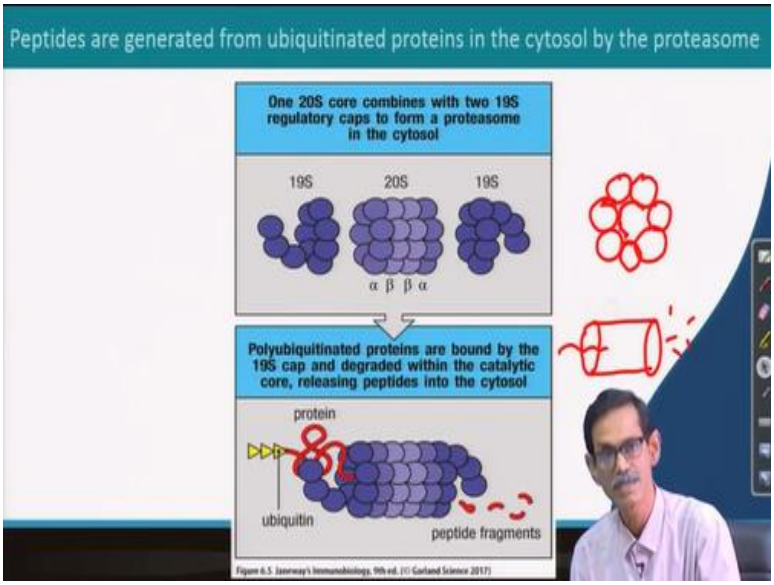

So what is this proteasome? Proteasome is a complex structure, complex means lot of proteins are there if you see this there are two cap like structure which is 19S and there is a 20S core structure. So if you see if you can count there in each colour there are 7 such block that means 7 protein, so 7, 7, 7, 7 four 7 for alpha, 7 alpha then 7 beta then 7 beta then 7 alpha total 28 proteins are there total 28 proteins are there and these 28 proteins how they are located?

So I am sure that all of you know how the carom board looks like or how the beads are how to play carom, so if you just remember how we just arranged the beads of during before playing carom board, so there is in the center there is a bead called red and along with the surface of the red what we do is we put one like these beads there are 6, so instead of 6 if I draw 7 what will happen? So there will be inside there will be gap, so these there is nothing here.

So inside there will be a gap if you put one after another such bead, so this is here in this case this is the 7, so what is going to happen we will see later? So these all 28 proteins are proteolytic enzyme all 28 proteins are proteolytic enzyme. So what is happening if you see this you know is a ubiquitin, so all the proteins supposed to be degraded are tagged with this ubiquitin this 19S subunit of this cap is hold this protein ubiquitin, ubiquitinated protein unfold this and pass through this channel.

It will be a channel like, so it will be like, it will be like this channel. So inside the protein will go and when it will come it will be pieces, so this is the proteasome, proteasome is a complex structure of 28 proteolytic peptide, proteolytic enzyme and which is cap it is role is to bind the pubic ubiquitinated protein unfold it and channelized through this channel.

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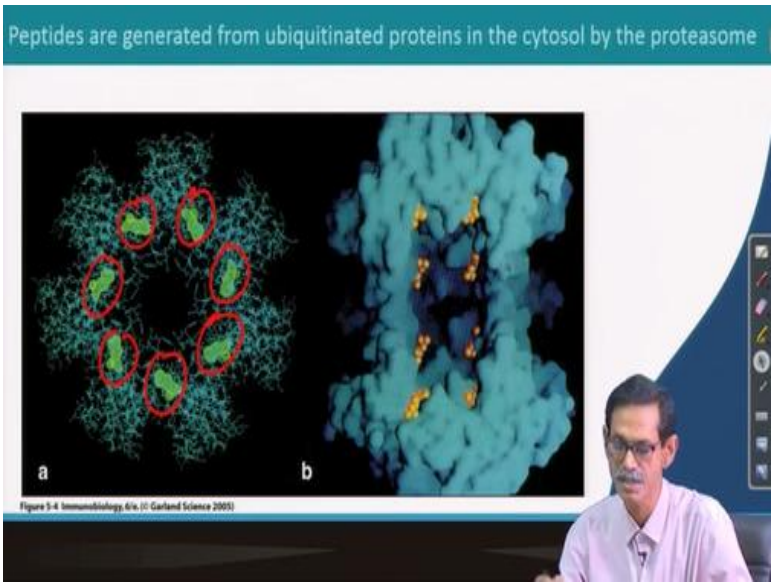

If you see the cross-section that what I was trying to draw it is much better way it is drawing it, so these 7 are this 1, then 2, 3, 4, 5, 6, 7 so here is the hole. So if there are same thing are 4, so

you will see just like a barrel. The protein will enter through this and come as a job this is same crystal structure with bigger.

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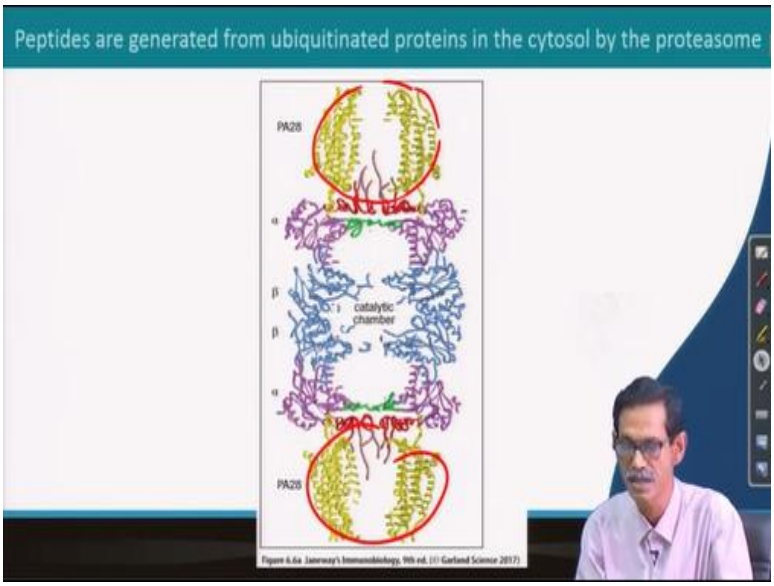

And if you see the transverse section it looks like that so it is the 19S part and this is 20S part, where their protein is located.

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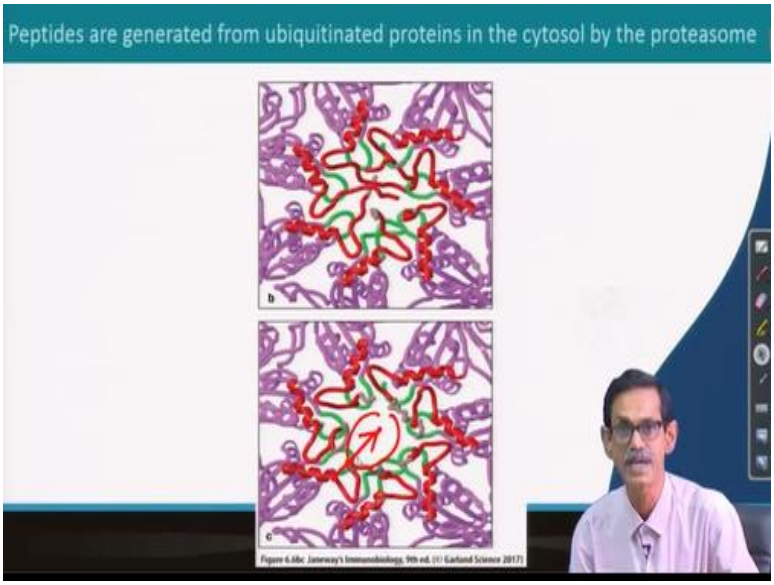

And, again little more detail structure this is the inside where all the protease is sitting here, so protein when any protein cross that either of the protease will cut it and make it small pieces and this is the center space through which the protein will go. So through this the protein will enter and go.

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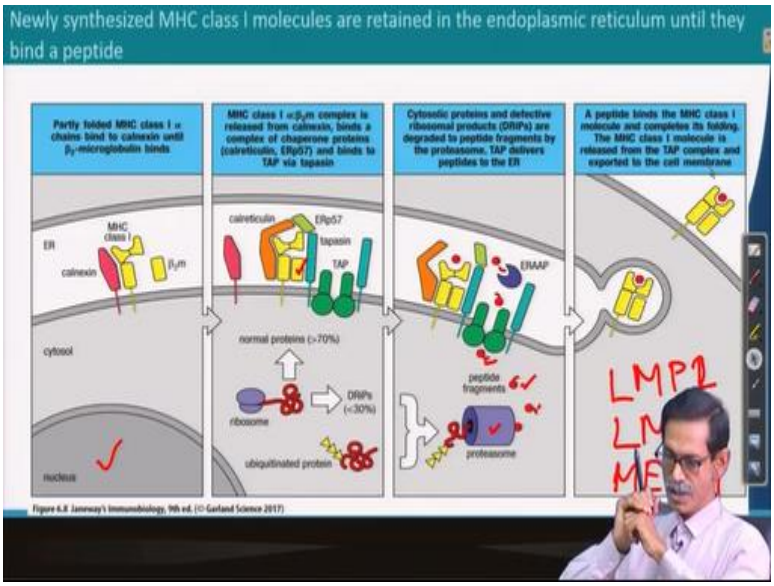

Now do not be scared this is very simple and straightforward, what is happening? So it is actually if you see the whole screen is a cell if you think that whole screen is a cell and this is the nucleus, this is proteome what I was showing that barrel-like structure, this is endoplasmic reticulum, this is considered as single segment, so what is happening? Normal protein is 70% of the ribosome activity is normal.

So when any cytosolic pathogen like virus they are going to produce their protein most of the protein will be fine but 30% of the protein will be defective ribosomal product which is called as DRIP defective ribosomal products that 30% protein like unfolded or incomplete will be ubiquitinated and after ubiquitination the proteasome will identify them and you see the big protein is going unfolded protein and small pieces are coming.

These small pieces will be produced in the cytoplasm, so if I stop here and then go in this channel at the same time MHC also synthesized I am talking about MHC 1 and I told in previous lecture the MHC molecule is very unstable even complete MHC 1 is unstable until unless it is attached to it peptide and you know that MHC 1 is two protein combination one is alpha, another is beta 2 microglobulin, alpha 1, alpha 2, alpha 3 by one gene and beta 2 microglobulin by other gene.

So they will synthesize in two different places, so initially what happened only alpha chain is synthesized which has a transmembrane domain but this protein is unstable any protein unstable inside the cell or as long as the folding is not proper what is happening there is a another set of protein called chaperone they are helping them to hold their structure as long as they are not getting the final confirmation.

So here calnexin, calnexin is a chaperone which support this MHC as long as beta microglobulin is not coming and help it. This is a general chaperone it is not a specific immune system so it helps, after that what happened there are many other chaperone like ERP 57, calreticulin so all of them cover it, so that MHC class 1 even the beta microglobulin is attached to it, it is not stable anymore you see there, there is a gap it is just cartoons to explain that it is not complete, complete will look like this very compact.

So it is even it is not very compact, so this calreticulin, ERp57 they help this is another chaperone as soon as beta microglobulin attached to it, it calnexin will separate and two other chaperone on will come into the picture. So they will help another protein tapasin, tapasin I will come later. So before going to tapasin what one more protein this TAP, TAP is you see this green one, this is type 1 and type 2 this is a transporter it is atp-binding or active transporter.

Which has atp-binding cassette the transporter which has atp-binding cassette is also called a ABC transporter atp-binding cassette containing transporter or ABC transporter that means active. So this role of TAP here is all the product produced here all the product produced here these product all the product produce here these product will transport from cytosol to in the endoplasmic reticulum.

But you know any membrane whether it is endoplasmic reticulum membrane or cell membrane they are fluid they are not rigid nothing is sitting very fixed there all are floating, so this MHC 1 molecule all this complex is floating somewhere tap is present here, so tapasin what going to do is tapasin will make a bridge between them. So it will get the complex of that MHC 1 and all other chaperone and tap they will bring them together.

So what will happen? So these complex will all together, so tapasin will help to bring them together or make them closer so all the proteins which is a ubiquitinated clipped by proteasome immediately it will be transported by TAP it may be little bigger you see there is there is one hinge like of structure here. So there is another Protease endoplasmic reticulum amino peptide is associated with antigen processing it is a big name.

So you just remember ERAAP it is another proteolytic enzyme that is enough those who can remember the name is fine otherwise you can just remember that this is a protease which will cut this big piece into small because MHC 1 cannot fit bigger one it should fit small. So even any big piece is there, there is protease sitting in the endoplasmic reticulum which will make them smaller and as soon as it will be ready to fit it will go there fit it and after this MHC bind with the peptide that will be more stable so all chaperone will be separated.

All chaperone are separated and this molecule will make a vesicular structure and then gradually through Golgi and trans-golgi network it will go to membrane and display there is that clear? So this is the thing happening one more important thing I should remember inform or tell you that these proteasome system is common proteasome systems continuously working for regular cellular activity so they have a standard protease.

But to fit into a MHC you need some specific cut you if you remember the peptide that I showed you that certain amino acid are fixed say hydrophobic amino acid at the tail region so to do that some specific protease is required, so that specific protease this are lmp1 they are they are LMP 2, LMP 7 these two Proteus these two Proteus are replacing the proteolytic enzyme of proteasome that means out of that 7 proteolytic enzyme two will be replaced by 2 and 7 this is in the beta chain actually.

And this is the beta subunit I mean bit of alpha and beta was there in beta another was MECL 1 LMP 2, LMP 7, MECL 1, so three different Proteus will replace the existing Proteus of proteosome which will make the peptide such a way so that it can fit into MHC 1, so this normal process is their protease a change and all the internal peptides are or cytosolic peptides are

presented by MHC 1 this is called antigen processing for cytosolic antigen. So, this is today or I mean this is enough for this lecture, then bye.