Immunology
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Lecture No -24
The Generation of alpha : beta T - Cell Receptor Ligands (Contd.)

We are discussing about the antigen processing. Hope you remember the antigen processing of cytosolic pathogen to MHC1. I am sure that you might have several questions so my suggestion will be I hope you already read that so if you have any question you read the book first. Because in book there are many things you may find which is not discussed in the class as well as you will find something which your which will make your conception very clear.

We have one more MHC right, MHC2 which is presenting the intravesicular pathogen or the antigen coming from outside by phagocytosis or macropinocytosis. The two complete channel they are two different pathway because MHC2 is helping I mean or activating the helper cells MHC1 is activating the cytotoxic T-cells so they should not cross react. They cross react their cross reactions should not hamper their activity because if MHC2 to somehow interact with the cytotoxic T cell that will be a real havoc in immune system.

So there is that is why evolution made two distinct channel so again I mean today's lecture I mean this is again we are going to discuss about the generation of alpha beta T cell receptor but today or in this lecture we are going to talk about the processing for what is happening in MHC2 loading or MHC2 presentation of antibody antigen.

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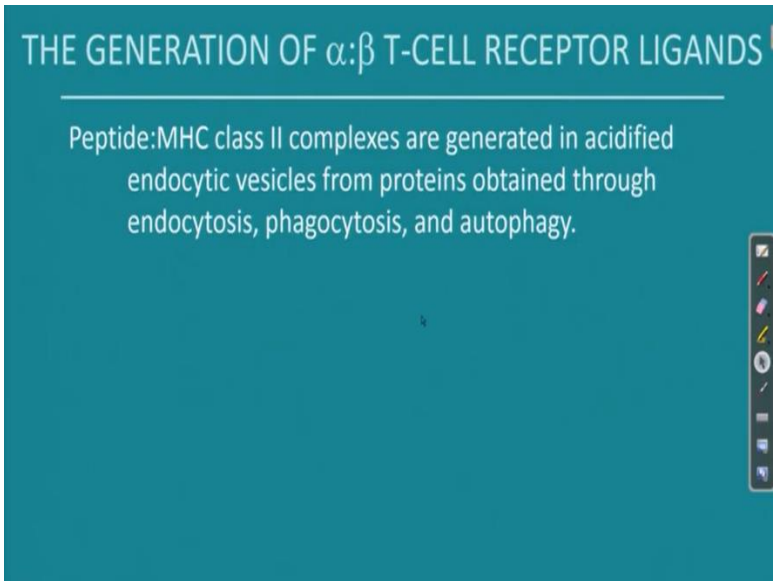

Next slide so is MHC class II complex are generated in acidified endocytic vesicles that proteins obtained through endocytosis, phagocytosis or autophagy either one because what is happening that vesicle is having the protein lysosome is fusing and these two vesicles that phagosome or endosome or autophagosome they fuse with lysosome and this become together I mean this of single vesicles and that is going to degrade all the protein and we already know that we talk during the early classes

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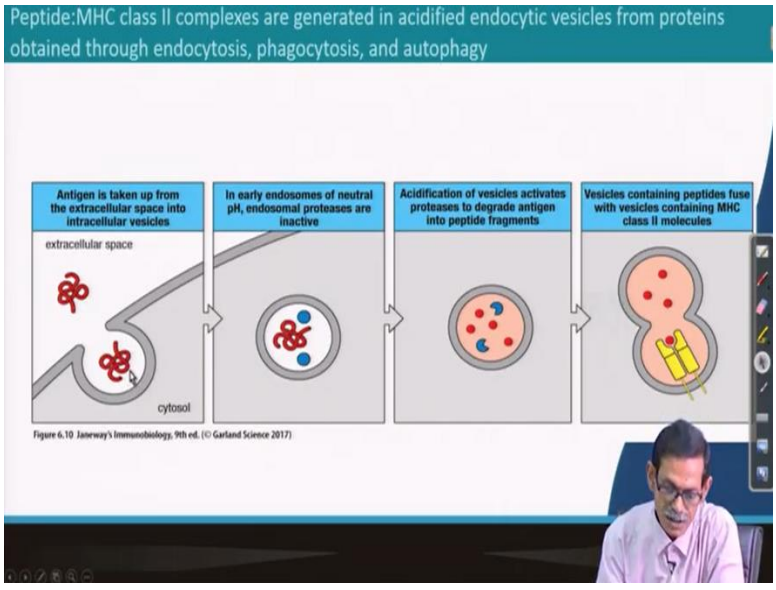

This picture is very straight forward representation of what is happening so this one, this one this is the cytosol there is a vacuole kind of thing so this endosome or phagosome the protein is coming here they will make a vesicle. This vesicle fuse with lysosome it will be degraded so this whole protein you see this filamentous protein is into small pieces all the red circles and here actually what happened this is fused with the vesicle where the MHC2 is there.

And this processed peptide is fit into MHC2 and going out so vesicle containing peptide fuse with the vesicle which has MHC, so what is happening that if you remember in the last lecture in the early part of the last lecture we were discussing like any protein coming I mean staying in the membrane they are going inside when their job is over so there are always in cell if you see the cell there are always some incoming endosome.

And there are some vesicle which is formed like this picture so vesicle form taking outside material coming inside there are some endosome coming inside, what is happening the vesicle which has this MHC2 or MHC class II containing vesicles they are seeing both of them so one which is in like this slide which is coming from outside from extracellular space through endocytosis or phagocytosis and there are some MHC sitting outside with different proteins or internal proteins.

They are also coming because some receptor mediated endocytosis or endocytosis is happening. These vesicles with MHC2 are moving here and there so they meet each other and they fuse and it will be fitted with this processed peptide and after that this vesicle will go and present the anti present the antigen via MHC2. MHC2 is very very stable I mean like with peptide these fitting is very important MHC2 or MHC1 both of them their turnover time is few days.

Because you know what is happening when there is a infection inside by virus or there is a bacterial infection what is happening the material or the antigen from tissue site of infection they are going to nearest lymph node and they stay there which is presented to T cell that takes few days, two to four days so if MHC is not stable with that if there is anything happen that they are degraded or something happen then they cannot serve the purpose.

Because one infection happen here it will taken up by the antigen presenting cell they will carry it go to nearest lymph node then it will present two T cell if it takes two to four days the MHC with antigen processed antigen should be there outside the surface of the dendritic cells as well as macrophage till then when a T cell see them and activate. So this process should not be hampered by anything right otherwise immune system will not work.

If it lost its structure or if it lose its antigen by I mean antigen by any chance suppose this is MHC all protein-protein interaction is what all protein-protein interaction is non covalent, so it is reversible it should not stay for long time so to chop them into pieces such a way the affinity should be very very high. If affinity is low if they just fall apart in very short period of time. So what is going to happen is suppose some I give you something to say you are taking something from a shop to your home.

And if you cannot hold this material till you reach home and drop it on the way what will happen you cannot take that material to home most of the time right. So here is that so you have to hold this material till you reach home then you drop it. So that within that period you have to make sure that no way you just drop it or miss it or putting somewhere. Same way MHC should hold that after holding the antigen in the site of infection it should bring to the lymph node.

So and stay so long that it activate the T cell, that is why this fitting is very important this processing is very important and that is why all part of the antigen is not epitope. So whole protein will be chopped into multiple pieces very few of them are going to fit into MHC that is why all protein are not all part of the protein is not epitope all small pieces are not epitope. Some of them which will really satisfy this can fit into MHC and their association will stay for 3 to 4 days only can serve the purpose of T cell activation in immune system.

So that is very, very important that we need to this is just to clear your concept otherwise it will not take more than 5 minutes to say what is happening its just few things protein is coming it is chopped fit into MHC2 going out. But what is the background what is happening actually just to understand how immune system work I am telling all this thing. So these until unless you understand that so whole concept or whole immune system you cannot imagine.

It is just mere few facts and that will not explain you like how immune system works. Here dendritic cell is also present in antigen by MHC2 macrophage and B cell is also present in antigen by MHC2. They have slight difference in their final work. Dendritic cell is bringing the antigen from site of infection to lymph node and what they are doing they mostly activate the T cell. In this case MHC2 they mostly activate the T helper cells which in turn is going to help either cytotoxic T cell or B cell to produce antibody.

But dendritic but macrophage and B cell also doing the same thing or similar thing but they do one more thing. So in along with the activation of T helper cell to help cytotoxic T cell or B cell to produce antibody they also activate the Th 1 response. Th 1 response means it is also telling one kind of T cell to activate on macrophage so that it can realize that something is growing inside like in case of intracellular pathogen, which is a very important job for macrophage only or mostly macrophage.

Exactly dendritic cell is not doing anything I cannot say but mostly macrophage is doing that helping or activating the Th 1 response so that it can save itself also because leishmania, micro bacterium they are growing inside the macrophage. So they are activating a specific set of T-cells which help macrophage to kill the intracellular pathogen. So this thing I hope you understand this is very straight forward right.

So it is coming, chopped, fit into MHC2 and going to membrane. But now as it happen in case of MHC1 as long as this peptide is not fit into it MHC2 is also unstable because what I said that MHC2 containing vesicle will fuse with this endosome so before that before this peptide come into picture MHC2 how it was stable that something must be there right.

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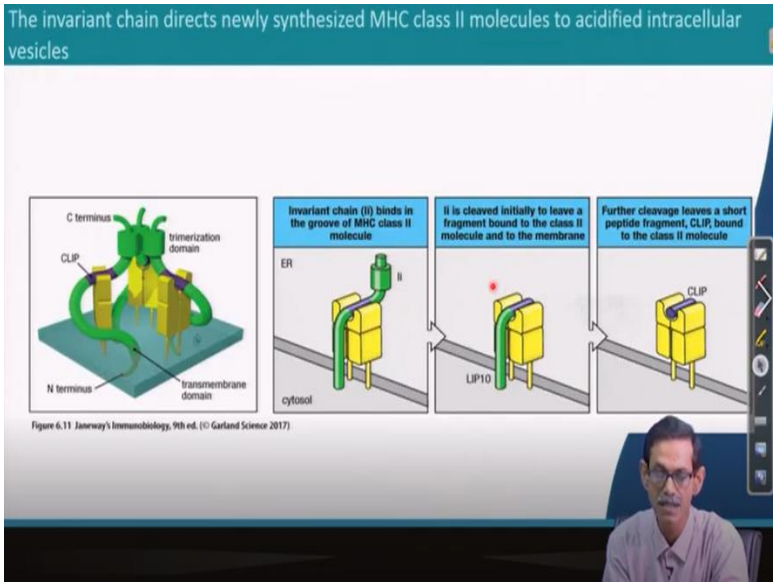

So this is actually there is one more thing, what is happening so this green platform is a cell membrane, there are three MHC2 you can see the yellow one, three MHC2 they are already anchored in the membrane so if you see this part there is one part is very shadow kind of thing this is actually below the membrane. So what is happening if this is the membrane this part is what is in the cytosolic tail of this MHC2 is shaded which is below which we cannot see here.

So there are another protein you can see the green kind of snake kind of structure another protein, so just assume one instead of three there is another protein this protein has a this violet domain this specific name is there it is called CLIP. So this green protein is also another membrane bound protein which will if you see this picture it will be much better to understand see this picture, this protein some part is in the bottom and which will fit into this peptide binding cleft which makes it stable.

Any MHC without binding to peptide is very unstable so to keep it stable so there is another protein which will fit like that and there is a dumbbell like structure or globular structure, so what happened they do not stay together I mean alone so three MHC and three such green protein that CLIP containing protein are stay together, actually trimalyze. So these complexes are stably present in the vesicle so that MSH will not be degraded or not destabilize or make separated.

Because two protein alpha and beta chain are two different fully anchored they should stay together, by any chance if they go away then the whole purpose is lost. So just to keep them together three of them again stay together where this I mean this domain the trimerization domain in the C terminus, this is a type one type of membrane protein where N terminus is in the cytosolic domain and C terminus is in the outside.

So this they are this they trimerize actually this domain this verbal set domain is actually trimerize and what if you take three together all will come together, that is how they stabilize inside the vesicle but if they stay like this how the antibody or antigen process antigen will fit. So they start their life like this and I already told when any vesicle formed inside the cell gradually they become acidic.

Inside that vesicle there are some peptide this or proteolytic enzyme which will these called actually invariant chain, this green one is called invariant chain. So, as soon as this vesicle will be acidic and proteolytic enzyme was there which was not active in neutral ph, as soon as they become acidic this proteolytic enzyme will be activated. And this proteolytic enzyme activation what it will do is this invariant chain ii actually it is not li this invariant chain is cleaved.

So you can see next picture this upper part or the C terminal part is cleaved, so now only this CLIP part CLIP the violet part CLIP part is here and its N terminal part which is LIP10 is still attached to the membrane. This is the first cut further cleavage is going to happen it will cut the other side C terminal end of this N terminal end of the CLIP, so first C terminal end of the CLIP is digested by or chopped by the proteolytic enzyme.

And next step N terminal end of the CLIP will be chopped so ultimately what will happen you see it is just like the MHC2 and if you consider that CLIP is an antigen piece of antigen which is process antigen it is fine MHC2 will be stable. These in this form they maintain when we see this so before they fuse with this vesicle MHC2 had that CLIP with it so one vesicle with MHC and CLIP and another vesicle with peptide will come.

I am repeating again so the MHC2 containing vesicle is actually having MHC2 with this CLIP molecule, another vesicle is having chop or processed antigen which is coming from outside or external or internal protein but coming from outside. Different vesicle with peptide another vesicle with CLIP. What will happen next?

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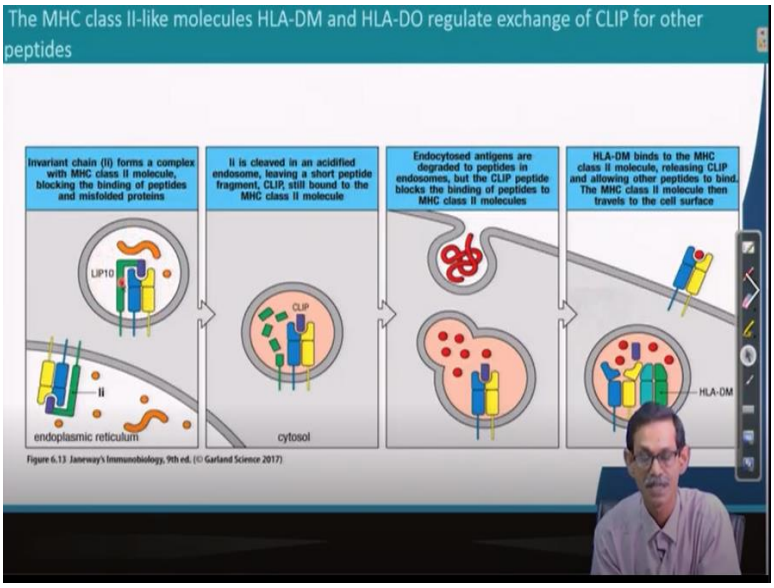

What happened next so this is what I was talking actually so this was initially like this and then it chopped only CLIP is there so CLIP containing MHC2 and this vesicle fused together clear, then one thing should happen. Then CLIP should go and antigen should fit, so one big protein so this is one big protein and this protein after I mean proteolytic cleavage I am talking about this one after proteolytic cleavage it chopped into different pieces.

So now each piece, each such piece is each circle here. I just told you all the pieces are not good to fit into the MHC to give it a stable structure so the best fit candidate should be there so that MHC antigen complex should stable for at least two to four days till the T cell get activated after that it is fine. So now what happened this CLIP molecule should be replaced by this peptide who helped that there is another protein very similar to MHC which is called HLA-DM.

Which also has a alpha and beta sub unit, very similar only difference straight there are many difference but straight forward difference they do not have any peptide binding to it. This is also

present so it is present here also but we are gradually increasing in the slide just to understand, so it is not alone there is one more protein over side which is not shown here but it is shown here. This DM is actually this HLA-DM which is another immunoglobulin type of protein and very similar to the MHC molecule this is a kind of chaperone activity.

What it is doing this MHC is here so as soon as DM attached to it MHC become unstable so what happened as soon as it unstable the CLIP fall apart so CLIP was like this, CLIP was like attached as soon as this space or the space between this finger increase the CLIP fall apart and so the space is now vacant, so this space backhand space will not vacant remain vacant what will happen there are so many pieces of peptides as soon as it CLIP fall apart one of the peptide will come and take this position, clear.

So CLIP fall apart and one peptide will come in this position. How long it will stay? If the binding is perfect and this is the best fit it will be stable. Otherwise what will happen, one will come if it is not good it will be replaced again. Because DM is still attached, DM protein HLA-DM will keep this open till there will lot of exchange of peptides so there are hundreds of peptides of variety of size variety of sequence and DM is attached to MHC.

So they will one by one although it is a random process one by one they will come and go, until unless it become or the MHC get the best peptide to fit into it, as soon as it become best fit peptide if the binding is so strong then DM cannot open it anymore. The HLA-DM cannot open it anymore, so there is one more protein which is not shown here called HLA-DO. The another protein is HLA-DO.

This HLA-DO sorry I cannot see that HLA-DO, this HLA-DO is the negative regulator of HLA-DM, because if it continuous HLA-DM keeps it open then the exchange will never end. It will continuously exchange but immune system will not work that way so HLA-DM is going to attach as long as best fit is happening. As soon as best fit happen HLA-DM and MHC interaction will be little loose and HLA-DO will come into picture and take this HLA-DM away.

And as soon as it detached it will be cleaved by proteolytic enzyme and HLA-DO will help that so after that this complex if you can see this it is a stable one and again it will migrate to the membrane and stably stay as long as MHC I mean the helper T cell is activated, so this is very very important. This is very simple and straight forward, if you consider that there is a protein I mean think simple way forget all this thing there is a protein which stabilize MHC2.

As long as there is no peptide and this is big protein two processing is there two chopping is happening in endocytic vesicle only CLIP part is attached to it. So how all this thing is discovered because there are some patient or there are some mutation happen or forceful mutation has been done in mouse or some patient where antigen processing is not good and it was found say the DM protein is mutated.

They have they found that some patient which antigen processing is not happening properly that is why immune system is not acting properly it was found like DM protein I mean when the genome was analyzed like there is a protein called HLA-DM is not working perfectly then people started doing research and seeing what is happening. But HLA-DO same way HLA-DM is so important at the same time HLA-DO working as a negative regulator.

But if HLA-DO is not working there or there is a malfunctioning of HLA-DO antigen processing is not hampered that much so its function is also not very clear how exactly what is happening but it is there, as long as HLA-DO is there it is doing its job but when it is not there processing is not that much hampered. I told you in the beginning also many part of the immune system is still not clear, many thing we just assume and it is like it is not like immunologist not I mean any those who are doing research active research on all this thing.

They also cannot tell many things because what is happening actually immune system is working its own way so it is not depending on what we understood or not. It has its own function it is working all possible way what the scientists are trying to understand what is happening. It is not always possible to answer why. It is not always possible to answer why this thing is happening but what we can say is that this thing is happening, what is the advantage of this?

We can say many times many cases like if it is not there what will be the problem but if you say why it is there how it is developed is not known. It is evolutionary process. Everything is doing working together scientists or the researchers are trying to understand what is happening and how it is beneficial that is our job and our job for this particular class is what is so far discovered minimum way how much we can understand in the basic immunology course.

Everything we cannot discuss here is not scope of this course as well as it will be I mean one part will take the whole course will cover the whole just this antigen processing part. So many things are there but the outlining of all this thing what is happening you have to just remember to understand the immune system.

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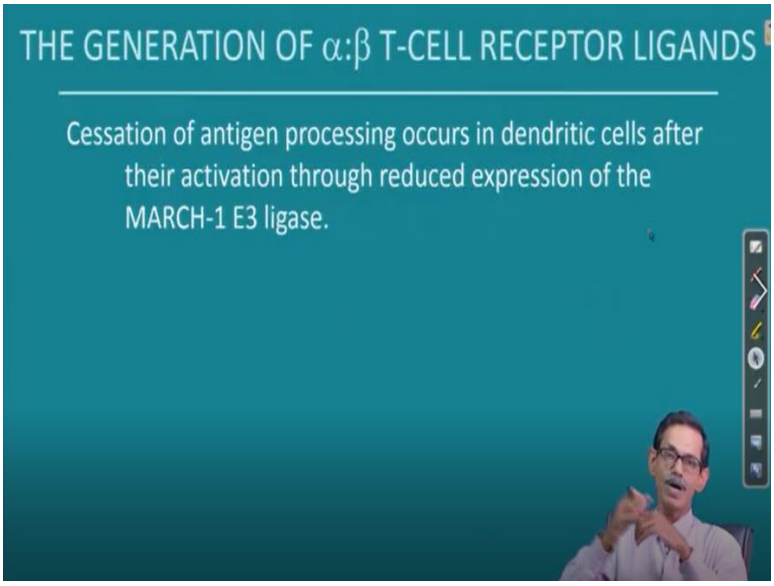

But what happen normally, normally when there is no infection normal means a normal individual when there is no infection, I told once in the last two few lectures back that number of MHC on the surface is not as much before infection so they are very few just few in number. I just gave an example like if you culture macrophage and see how many suppose there is a device how many MHC molecules are there in the surface.

You count and give some bacteria to macrophage to eat them and after few hours you see how many MHCs are there what you will see is the number of MHC molecule on the surface

increased. So, why it is not I mean why how because the protein is synthesizing why they are not expressing.

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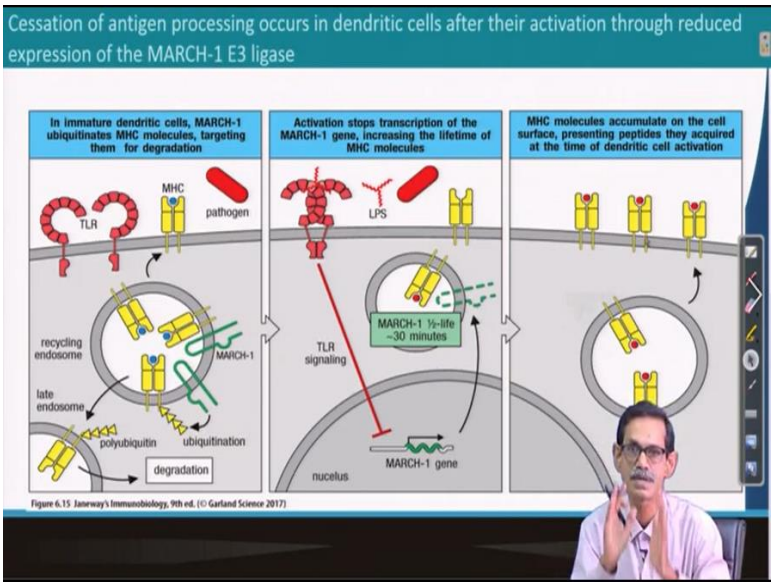

So some very brief I mean just glimpse to you that normally what happen normally this is a basically where lot of MHCs are there say three MHC molecules are there with CLIP molecule so there is another protein called MARCH-1. So when there is no infection this MARCH-1 protein is helping to ubiquitinate this MHC this MARCH-1 is expressing normally suppose there is no infection you see infection means TLR will involve if there is a bacteria infection.

So what is happening this MARCH ubiquitinated this MHC is producing but it is ubiquitinated by this protein and you know what is going to happen what is the fate of the ubiquitinated protein any protein ubiquitinated means it will be degraded by proteasome, so the number even it was initially three two of them degraded one go in the membrane. But what happen after infection as soon as infection is there the lipopolysaccharide the LPS of bacteria crosslink this TLR this cross linking of the TLR give the signal to the nucleus do not make any more MARCH protein.

No I do not express any MARCH protein so if you do not make any MARCH protein what will happen the number of MARCH protein will be less and it is written in the slide also see the MARCH protein half life is only 30 minutes. So up to 30 minutes all the existing MARCH will be there so it will destroy most of the MHC but after 30 minutes of this signal no more MARCH-

1 gene will be expressed so if there is no MARCH-1 gene expressed all the MHC expressed will be active.

So as a result what we will see more amount of MHC is going, so it is not that MHC1 is not synthesizing without infection it is cell is always preparing it. So no risk is I mean there is no risk I mean all the antigen presenting cells are always expressing MHC2 because they I mean body does not know when the infection will happen, so it is they have a backup preparation but when we do not need it is degraded as soon as we need them that degradation is stopped. Why?

Because the protein involved the MARCH-1 which is degrading this MHC or helping the degradation of MHC2 is no more there inside the cell as soon as it disappear as well I mean as usual the MHC2 was expressing they will make more and more MHC2 and cell will make more and more presentation so that immune system can work better way. So this is actually how the antigen is presented.

So MHC1 antigen processing is totally proteasome based and there are a lot of protein is involved there are few chaperone is there, calreticulin, calnexin, ERP-57 they are helping tapsin is bridging the tap, tap is the transporter which transport all the peptides and fit into the MHC-1 going, in that case also that exchange of the peptide is going on the best fit peptide is fitting into the MHC1.

Here the protein coming outside chopped into vesicles MHC2 is in another vesicle they fused DM is destabilizing the MHC2 so that CLIP can be replaced by the peptide and it is the process is going on as long as the best peptide is fitting into MHC2 and make the strong bond so that they can survive 3 to 4 days, both of them going to the membrane present the antigen one is MHC1 and there is MHC2.

But there target is different because MHC1 interact with the CD8 activate the cytotoxic T cell it makes you to activate the CD4 that we already shown that which sub unit is interacting how it is helping so that tcr can interact. So this is what happens MHC then we process antigen processing

and these small dots are really the epitope which is called B cell sorry T cell epitope. So thank you for your patience, so next class will discuss new thing.