Host-Pathogen Interaction (Immunology)
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Lecture: 34
Pattern-Recognition Receptors-TLRs Signalling and MyD88 and TRIF-Dependent Signalling

So, in previous session we have looked at about the TLR and we have seen various kind of PAMPs or I will say the array of PAMPs are sensed by these TDLR which is expressing on variety of innate immune cells such as macrophages, dentritic cells Basophilsspecial and mast cells and B cells as well. So, upon sensing they induce inflammatory cytokine that you are very well aware they are also inducing type 1 and type 3 interferons.

Now let us look at what kind of signalling is operated and in this sessionsession, we will discuss in great length about the TLR signalling.

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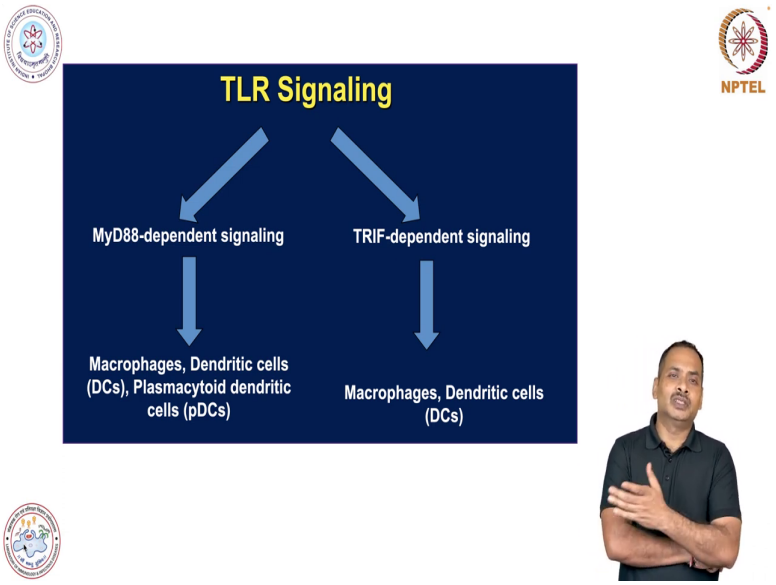

So, this slide you are well remember as I have shown you in previous session that these TLRs are basically localized over cell surface and these TLRs are also expressed in endosomes. And here if you see very carefully there are only few adapters molecules are there which basically play an important role in signalling. So, if you see the TLR4 basically uses four adapter molecule.

The first adapter molecule if you see this uses TIRAPIF which is also known as Mal. In previous session I have discussed this My D88 and Mal which is also known as tirap they have a TIR domain. So, TLR4 uses this My D88 as well as tirap or mal in addition TLR also uses another very important adapter known as tram and the TRIF. So, TLR4 uses all adapters of the of the TLR signalling.

On another hand if you see TLR 2 along with six and 2 along with TLR1 which makes a heterodimer. So, this uses TIRAPIF or Malouse along with My-D88 in order to activate the signalling and rest of other TLRs basically uses My-D88 except TLR3 if you see TLR3 uses only one adapter that is trift it is a quite complicated for you right. But do not worry it is not too complicated if you will see subsequent slide then you will understand very quickly and easily.

Anyway. so upon recruitment of this adapter basically there will be a downstream signalling and this downstream signalling result to the production of pro-inflammatory cytokine and type 1 interferon now here just I want to take your again upstream. So, how this sensing is taking place. So, sensing is as I told you in previous session this leucine rich repeat is playing a very important role in sensing basically this makes a horseshoe like a structure.

And this horseshoe like a structure is basically playing important role. So, what is happening when there is a there is a PTAMP or if there is a or any ligand PTAMP or any AMP or any ligand when it comes in contact with the cell then what happens basically there is a oligomerization or clustering of these receptors are taking place. And this clustering will basically trigger the recruitment of these cytoplasmic adapter molecules.

And once this cytoplasmic adapter molecules are recruited then the cascade of signalling is taking place and we finally result to the transcription of pro-inflammatory cytokine and type 1 interferons.

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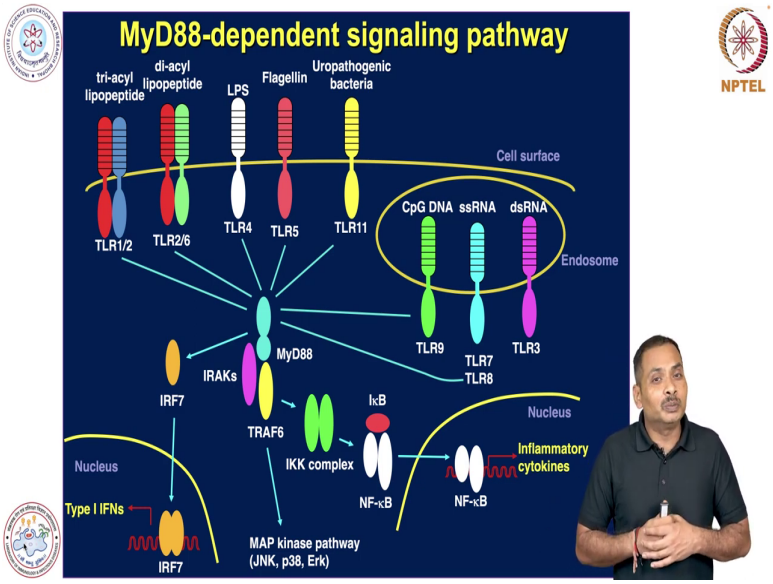

So, since the signalling is little complicated we study this in much more simpler way we study the TLR signalling in two major part one we call it as a My-D88 dependent signalling and another we call it as a TRIF dependent signalling my-d88 is basically myeloid differentiation Primary Response Gene 88 the this is a full form. Anyway, you need not to worry about the full form just remember the My D88.

And basically, TRIFs is a stand for TIR containing adapter inducing interferon beta. So, here you probably you can guess that this adapter is playing important role in induction of interferon beta~~vectors~~ and My D88 dependent signalling is basically operated in these cell types as you can see there is a macrophages, dendritic cells, and plasma-cytoid dendritic cells. TRIF dependent signalling is mainly operated in macrophages and dendritic cells. Now let us take up this My-D88 dependent signalling.

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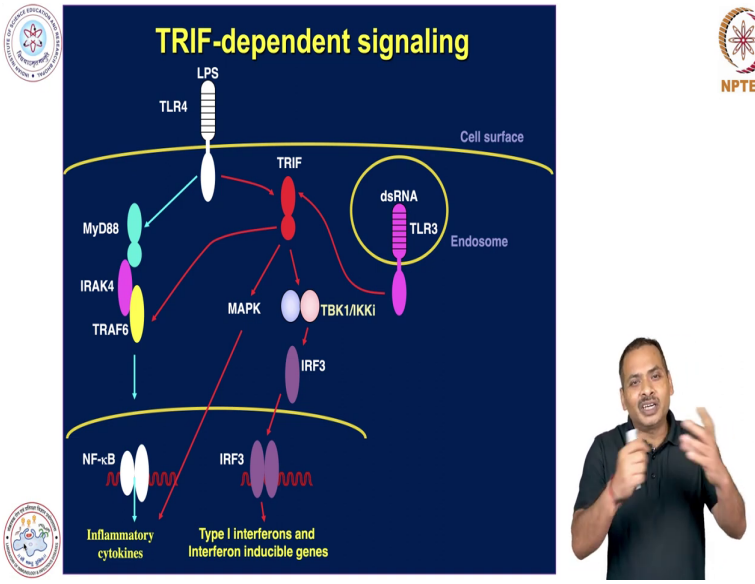

So, here you can see that TLR1 and 2 and 2 and 6 they make a heterodimer and this heterodimer basically activate the signalling. Whatever TLRSs which you are seeing in this image in a monomeric form ideally it is not in monomeric form. And in cellular condition it is either dimer or oligomer. I have told you there will be a clustering of receptor. So, once theise receptors once this ligand bind to the ~~lucsine~~ leucine rich repeat then that will caurse the conformational change in TIR domain.

And this conformational change basically ~~recruit~~recruits the adapter molecule My-D88 but in case of TLR 1 2, 2 6 and 4 there will be a additional adapters will be there that is Mal or TIRAP~~IF~~. And so, upon binding with or recruitment of My-D88 there will be a recruitment of various other signalling molecules which consist of a family of IRAK family protein which is mainly IRAK-1, 4 and there is a some more IRAK are there one of the IRAK is IRAK-M which is basically a kind of negative regulator for this My-D88 dependent signalling anyway.

So, let us move on. So, IRAK will be recruited and then there will be a recruitment of this TRA~~IF~~ 6 and this complex or signalosome basically activate there will be many more proteins are there for simplicity I am showing you in a very simple way. The overall aim of this is signalosome is to activate the IKK complex. And this once this IKK complex get activated then it will phosphorylate that that will activate the kinase activity.

So, this kinase basically activate I Kappa B and once I Kappa B get phosphorylated then what is happening then there will be a ubiquitination of this I Kappa B and this ubiquitinated I Kappa B is is ~~degradated~~ degraded by the proteosome and NF Kappa B will be free and this

NF Kappa B will be translocated into the nucleus and this active transcription Factor transcribes the gene for pro-inflammatory cytokines or inflammatory cytokines.

So, this is a one way by which the signalling is taking place another way is this signalosome this complex of protein can also activate the map kinases as you know you probably studied this map kinase pathways there are various molecules like JN and K, P 38, Erk IRAK all these guys will be activated and that will also induce the synthesis of pro-inflammatory cytokine.

But in PDC's which is a which we call it as a plasma cytoid dendritic cells in those cells there is a there is a little different pathway not little quite different pathway is operated. And this different pathway basically plays a very important role in production of robust amount of type 1 or type 3 interferons. So, what is happening in PDC's the My D88 which is an adapter molecule this directly interact with transcription factor and phosphorylated.

So, here try to understand the whole signalling pathway is kind of cut off. And this My D88 basically directly phosphorylate the transcription Factor known as IRF7 and this IRF7 the phosphorylated IRF7 basically once they get phosphorylated they make a dimer phosphorylated IRF7 make a dimer and then it is translocated into the nucleus where there will be a transcription of a type 1 and type 3 interferons.

So, in that way this whole My D88 dependent pathway is operated. Now let us look at another very important component of the pathways that is TRIF dependent pathway.

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Summary of TLR and it's adaptor

TLR	Adaptor
TLR-2/1	MyD88/MAL
TLR-3	TRIF
TLR-4	MyD88/MAL TRIF/TRAM
TLR-5	MyD88
TLR-2/6	MyD88/MAL
TLR-7	MyD88
TLR-8	MyD88
TLR-9	MyD88
TLR11/12	MyD88
TLR-13	MyD88

So, TRIF is a an adapter molecule which is used by two major TLRs one is TLR4 and another is TLR3. TLR3 exclusively use TRIF in order to induce the pro-inflammatory cytokine as well as the type 1 interferon synthesis. Here you can see so, the upon sensing of this double stranded RNA molecule or poly IC which you have seen in previous session the there is a poly IC. Poly IC it is a basically a ~~polymer of inocin and cytanine~~ polymer of inosine and cytosine.

And this polymer of inosine and cytosine ~~polymer of ionicine and cytodine~~ is we use it as a double stranded RNA and if you introduce this double standard RNA this polyIC ~~inocin~~ inside the cell then it will mimic like a virus infection. And if you stimulate the appropriate cell like macrophages with polyIC ~~inocin~~ then it will induce or activate the TLR3 pathway. So, here you can see the TRIF is used by TLR3 and TLR4. Of course in case of TLR4 and this also recruits another molecule known as TRAM.

And TRAM ~~tram~~ and TRIF is used by the TLR4 and the TRIF can also activate this IRAK and TRAF6 in order to induce the synthesis of pro-inflammatory cytokine via activation of NF kappa B as I have explained you in previous slide. TRIF can also activate the map kinases in order to synthesize the pro-inflammatory cytokine and TRIF basically activate a kinase which we call it as a TBK1 or IKKI, IKKI is also known as IKK Epsilon.

So, once this kinase get activated. So, these kinase is phosphorylate IRF3 and this phosphorylated IRF3 again becomes a dimer and then it is translocated into the nucleus in order to synthesize the type 1 interferon's. So, this is a very simplified view of this whole TLR signalling. Now I will just summarize this TLR signalling with one very simple slide.

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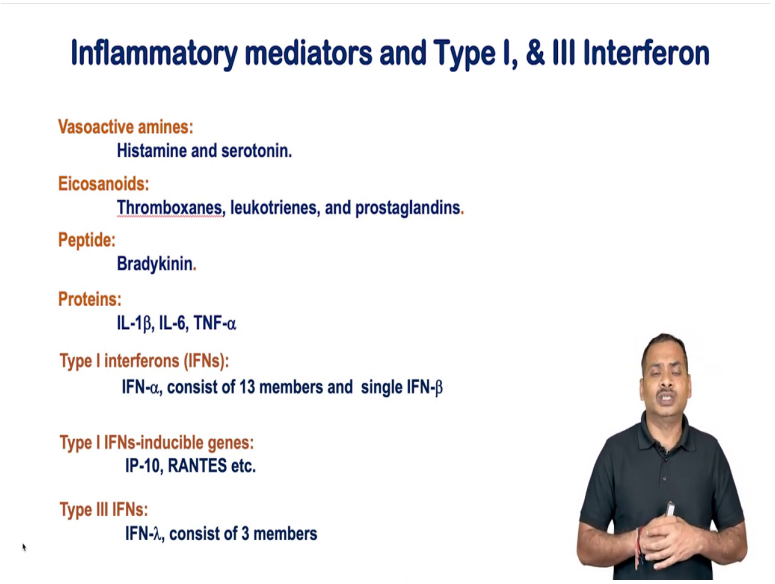

Here in order to remember that what are the adapters used by the various TLR if you if you remember that TLR2 along with 1 and 6 they use My-D88 or TIRAP~~IF~~ or mal and TLR4 uses all adapters that is My-D88, TIRAP~~IF~~ or Mal~~null~~ this is the name of same molecule tirap and Mal and it also uses TRIF~~different~~ and tram and rest of TLR uses only My D88 TLR3 uses a TRIF. So, this is the kind of summary of TLR and its adapter.

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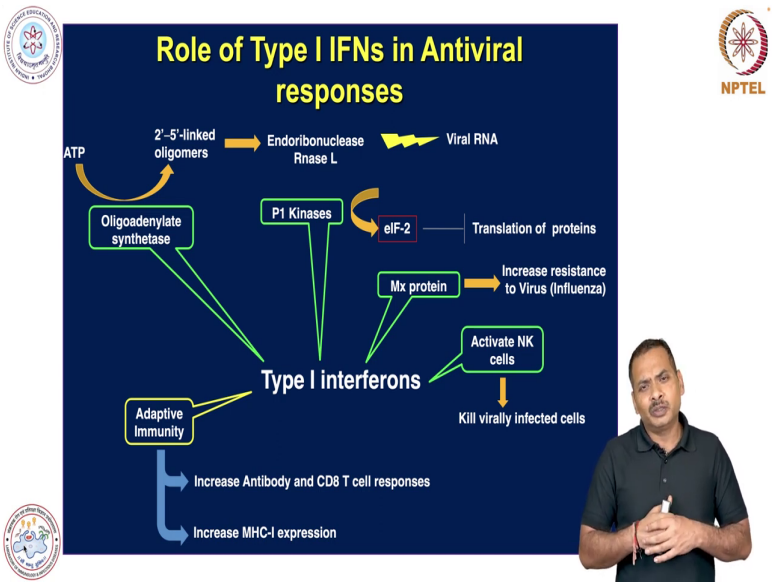

Now let us look at what are the inflammatory mediators and type 1 interferon although I have shown this slide but I will quickly go through. So, this also induces Vaso-active amine. Vaso-active amine I am not very clear about that how this is involved in production of histamine and serotonin but I am sure they are this upon activation of TLR there will be a activation of NF kappa B and that NF kappa B must be triggering some enzyme which is needed for the for the synthesis of histamine or serotonin.

But I am not very well aware and I cannot say how it is operated but I am very well aware about that synthesis of ~~escanard~~ Eicosanoids. So, there is an enzyme if you remember there is a cyclooxygenase which we call it as a Cox 1 and there is a another cyclooxygenase~~cyclooxygen~~ is which we call it as a Cox 2.. So, this cox1 and cox2 is induced upon the stimulation of TLR and in that way this inflammatory, non-proteinaceous inflammatory mediators are synthesized.

Bradykinin most likely it is also derived by a drive through NF Kappa B mediated signalling and there are variety of cytokines like IL1 beta L6 and TNF. So, they are also playing they are synthesized upon activation of NF kappa B. So, these are the inflammatory mediators in addition there is a type 1 and type 3 interferon type 1 interferon which is a basically consists of interferon Alpha and has a 13 member and single interferon beta.

So, they are basically synthesized in presence of transcription Factor IRF-7 and IRF3. There is a set of interferon which we call it as an interferon inducible Gene. So, this is a basically IP10 RANTES~~rentus~~ and there are so many. So, this is also playing a very important role in antiviral immunity into type 3 interferon which is a consists of three members or and it is also known as interferon Lambda it is also induced through these transcription factors.

So, the there is a family of transcription Factor known as interferon regulatory Factor and there are so, many members. Many mainly these two members IRF3 and IRF7 they are playing important role in synthesis of these interferon and some are playing important role in production of inflammatory cytokines.

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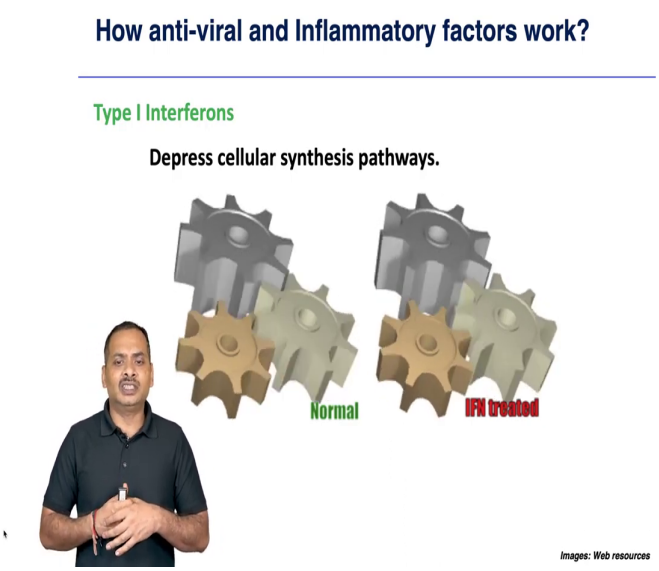

So, what type 1 interferon is doing it is a it is interesting. So, basically it induces hundreds of Gene and all these genes are playing important role in antiviral immunity. Here you can see there is a oligoadenylate synthase basically it induces the synthesis of RNAases L and this RNAs L basically cleaves the viral nucleic acid and in that way viral replication can be checked. This can also induce P1 kinase I am just highlighting few of these molecule but it induces hundreds of molecule.

And this P1 kinase is basically induced in order to shut down the cellular metabolic activity mainly the protein synthesis that is through EIF 2 elongation Factor two. It also induces Max protein in response to the influence in infection of influenza virus and it provides some kind of resistance. In addition, this activates a very important group of cell known as natural killer cells which plays a very important role against virus as well as against transformed cells.

Type 1 interferon activates adaptive immunity and this can basically increase the anti- antibody production by activating B cells and it also activate CD8 T Cell responses. It induces the expression of MHC class 1 molecule and it also a play important role in expression of MHC Class 2 molecule also.

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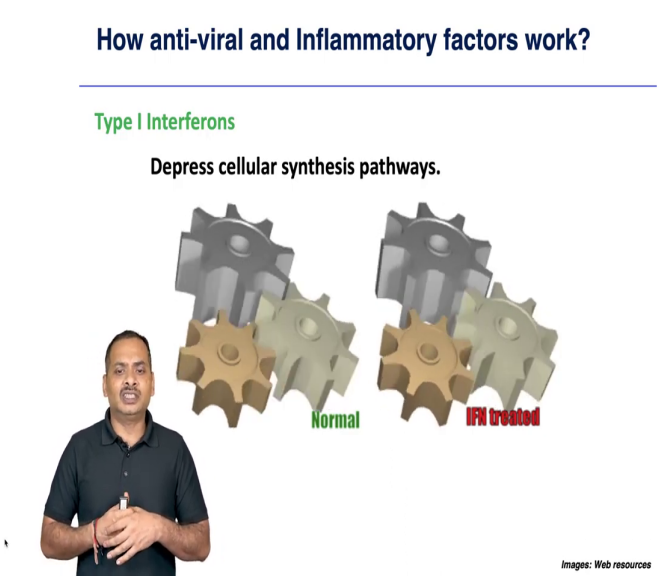

Here I am just trying to give you a more interactive presentation about the role of or how this interferon and type 1 interferon and inflammatory cytokines work. So, basically if you see carefully this induces apoptosis of virally infected cells make healthy cells which is not yet infected with a virus the this interferon make those cells resistant to the virus infection. It depresses the metabolic pathway.

It reduces the as you have seen there is a elongation Factor too2 if you treat the cells with interferon then it will and depresses the metabolic activity in the cell.

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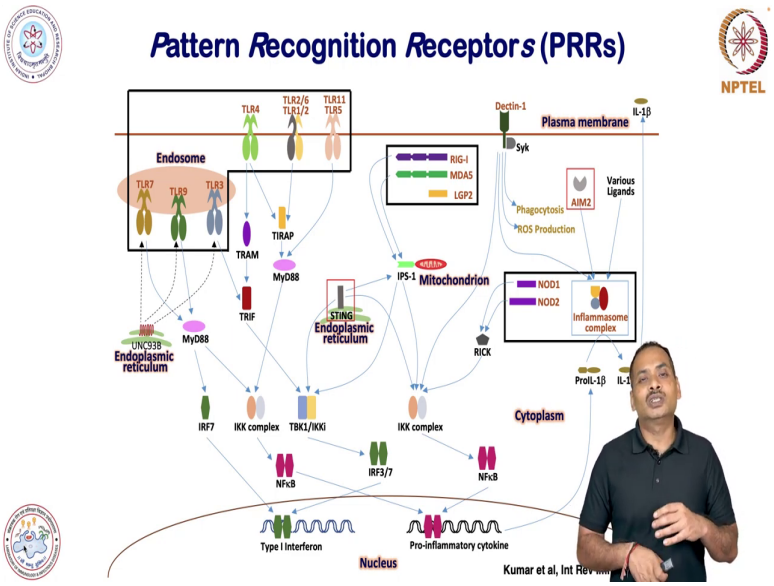

It also prepare and activate the professional immune cells for the for development of viruses specific immunity here you can see in this cartoon and inflammatory cytokines are basically a kind of alert system they activate the whole immunity and make a alert a kind of alarm in the

host. So, in that way this cytokines and type 1 interferons are playing important role in innate immunity.

So, with this I will I will stop here and in next session I will talk about TLR and disease and is there is a way to use this TLR knowledge to use it for the Therapeutics. So, I will cover up these two topics thank you, thank you very much.