Host-Pathogen Interaction (Immunology)
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Lecture: 33
Pattern-recognition receptors-TLRs and TLR Ligand

In this session we will discuss about the pattern recognition receptor and we will take up in more detail about the toll like receptor. In previous session we have discussed the origin of pattern recognition receptor concept and that was a quite interesting and after the discovery of pattern recognition receptor the view of immunologist is completely changed. It is previously we thought that it is a non-specific thing and it is a not so, important but now we know it is very important.

There is a some broad sensors and these broad sensors eventually since the microbial pathogen and they play a pivotal role in development of adaptive immunity. So, in this session I will talk about TLR.

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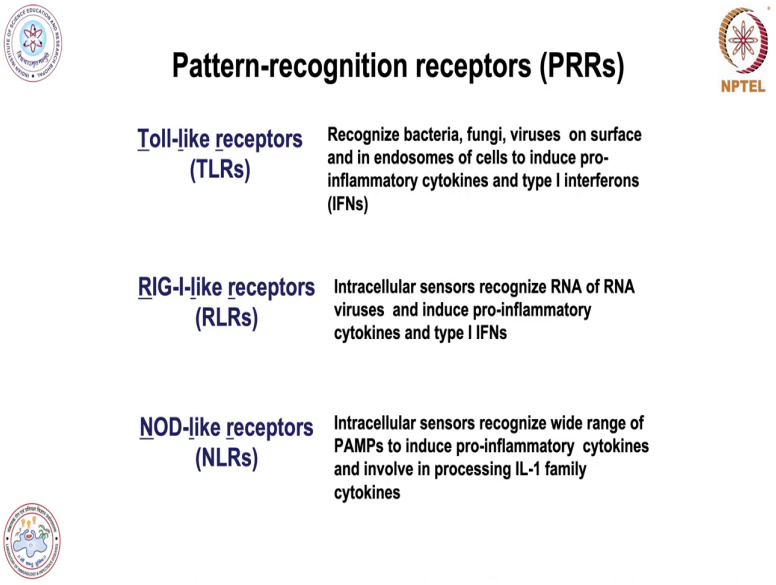

So, what is TLR, TLR is basically the toll like receptor the toll word is came from if you if you remember my previous session you can understand that toll protein was discovered in the drosophila and protein similar to the toll~~tar~~ drosophila toll~~e~~ in human and in mammals was discovered and we call it as a toll like receptors. So, these toll-like receptors are basically

recognizing all kinds of microbial pathogen that includes bacteria, fungi, viruses and the most interesting thing is that they recognize these entities on cell surface.

As well as it also recognizes inside the vesicles inside which is present inside the cells. We call it as the endosome and it depends on the stage of ~~the center zone~~ endosome we can call it as a phagosome, phagolyisosome. So, and upon recognition they activate of course the cascade of signalling and eventually it result to the production of pro-inflammatory cytokines and in addition to pro-inflammatory cytokine, they also induce a antiviral factor or its antiviral cytokine you can you can say that and this we call it as a broadly we call it as a type 1 interferon.

Besides this recent in recent past it was also reported that there is a tType 3 interferon's which is also playing very important role in antiviral immunity. So, this is about the totall like receptors which is quite brief and I will talk extensively about a toll like receptor in subsequent slide. But before going to that I just want to introduce there are some more pattern recognition receptor or there is more families of pattern recognition receptor this is much more appropriate to say that families of pattern recognition receptor.

Another very important family of pattern recognition receptor is Rig-I-like receptor here you can see this Rig-I-like receptor is derived from Rig-I and this is a retinoic acid induced gene and there is a family there is a family of molecule which is induced by written retinoic acid. These sensors are very important against RNA viruses because they sense the RNA molecule and therefore they play a crucial role in sensing RNA from RNA viruses.

In addition, they may also play important role in sensing DNA virus as you can see you see that when DNA virus replicates then there is a some RNA intermediate and that RNA can be sensed and then that will induce the response. And the responses are again this induces a pro-inflammatory cytokine and type 1 and type 3 interferon's and they are playing very important role in antiviral immunity this Rig-I-Like receptors.

Another member is a the Nnod-like receptor and this is also an intracellular sensor Rig I like a Rig I is also intracellular sensor not like receptors are also intracellular sensors and they sense the signature from bacteria in the cytoplasm and then that will basically induce a

pro-inflammatory cytokines and they play a very important role in production of one very potent family of cytokine which we call it as a IL1 family cytokine.

And this is basically mediated by some multi-protein complex are formed in the cell upon sensing of some microbial PAMP is basically pathogen Associated molecular pattern. So, upon sensing these molecules these sensors basically induce either inflammatory cytokine and or they will make some multi-protein complex and then it will play important role in production of IL1 family cytokine.

There are some more sensors which plays a very important role in DNA sensing here I have not shown that those sensor I will talk in more detail about the DNA sensors in subsequent sessions. So, DNA sensors are also there which senses the DNA molecule from pathogen and then it induces the appropriate immune response.

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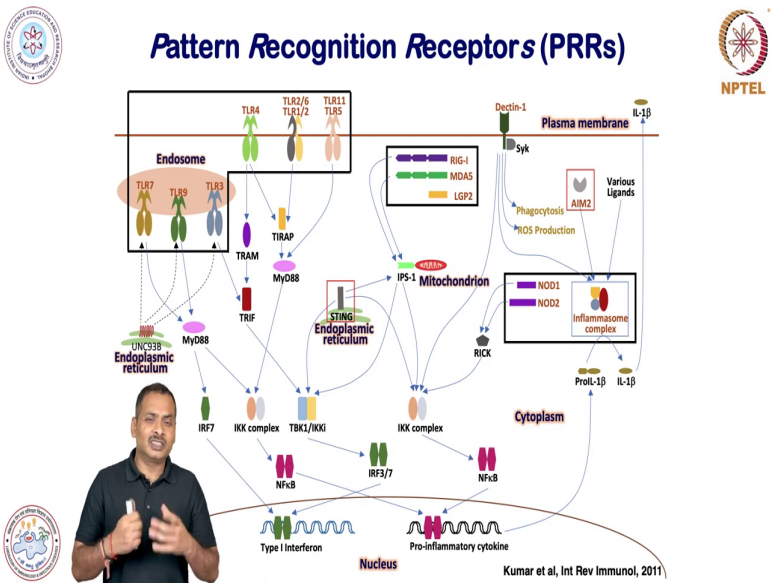

So, let us let us move on to to take a look how these sensors are located. So, here you can see as I have explained you in previous slide this TLR4, TLR2 which is making a heterodimer with a 6 and TLR1 also make a heterodimer with two they are located on the cell membrane and TLR11 and TLR5 is also located over the cell membrane and this sensor their respective PAMPs. I will discuss about the PAMPs and their expression in subsequent slide and here you can see that TLR 7, 9 and 3 and 8 they are expressing an endosome here I did not show it.

So, here you can see that these sensor basically sense the PAMPs in endosome and this is very interesting right many viruses if you if you see carefully many of these viruses are

basically endocytose through this phagosome. So, over there they get and the the protein or coat protein are dislodged and then the nucleic acid come in picture. So, this nucleic acid can be sensed and this is very appropriately located ok the sensors are very appropriately located.

And they sense and then this will induce the immune response. Another family of molecule is a RLRs here you can see that members of RLR are located in cytoplasm and some of this virus infection they are basically come in the cytoplasm the nucleic acid comes in the cytoplasm and over there this can be sensed and then that there will be a appropriate immune response.

Another is a NOD~~not~~-like receptor. So, basically nod like receptors there are two major categories of not like receptor one is a Nod 1~~c~~ and Nod 2 which one category is made of nod 1 and nod 2 they basically senses the bacterial signature molecules such as some component of peptidoglycan. And there is another group of sensor which basically oligomerize and then they make some multi-protein complex and then they will induce I~~A~~L1 family cytokine.

So, this will study in great detail when we will take up the nod like receptor here although I have given in a schematic there is a production of IL1 beta which is synthesized in inactive form and this inactive form is a basically converted into the active form by this multi-protein complex known as inflammasome~~tion~~ we will discuss in great detailing and NLRs~~analogs~~.

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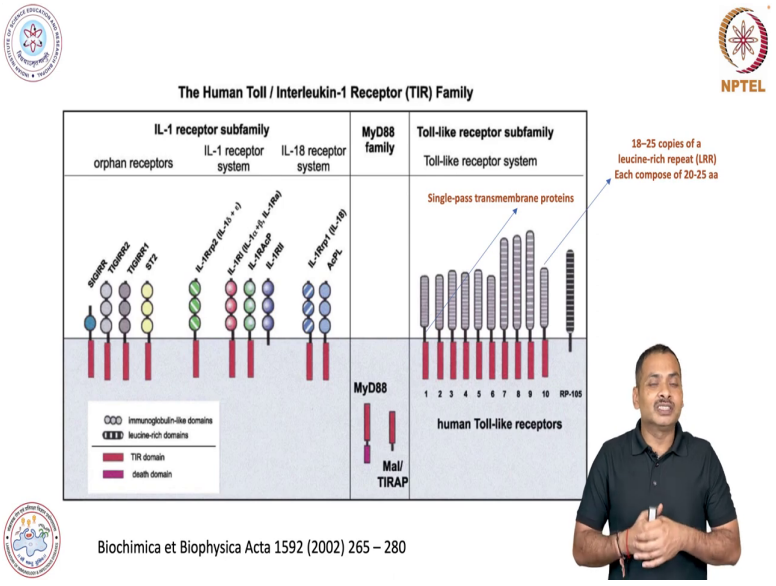

Now let us come back to the toll like receptor. So, toll-like receptors they have a three distinct region if you see very carefully the first region which we call it as a leucine rich repeat. This

leucine rich repeat is playing a important role in sensing the PAMP and basically these TLRs are consists of 18 to 25 copies of leucine Rich repeat and each leucine Rich repeat is consists of about 20 to 25 amino acids.

And they make a one unique structure which we call it as a horseshoe like a structure and then they sense the PAMP. In some cases in some TLR this leucine rich repeat is undergo cleavage in order to sense the sense the PAMP and generally these these TLRs are basically expressed in the endosome. So, all most of these nucleic acid sensors they undergo cleavage and over there, there will be a generation of active sensor which will sense the nucleic acid and then it will transduce the signal.

And this cleavage is basically mediated by various proteases like cathepsin family protein. The another reason is the transmembrane region which we also call it as a type 1 transmembrane region and this is a basically consists of mainly you can understand this is consists of a hydrophobic amino acid and this plays a important role in inserting in the membrane. And another most important reason is this the TIR region here you can see that toll IL1 receptor region.

And this toll IL-will one receptor region is the cytoplasmic region and this playee the most important role in signalling. So, if you eliminate this domain then there will be no signalling. And this name the TIR name came from IL1 family receptor here you can see. So, there are various IL1 receptor sub families and you can see that all these receptors they have TIR domain.

This is basically IL1 receptor domain which is which is playing uh a very important role in downstream signalling upon binding with the respective ligand that is IL1 or IL18. So, this TIR domain is playing very important role in downstream signalling and this TIR domain is also present in sum of adapter molecule of a TLR. As you can see there is a My-D88. This My-D88 is also having this TIR domain and there is another adapter molecule known as Mal or trap. So, these proteins or these adapter proteins has a this TIR domain.

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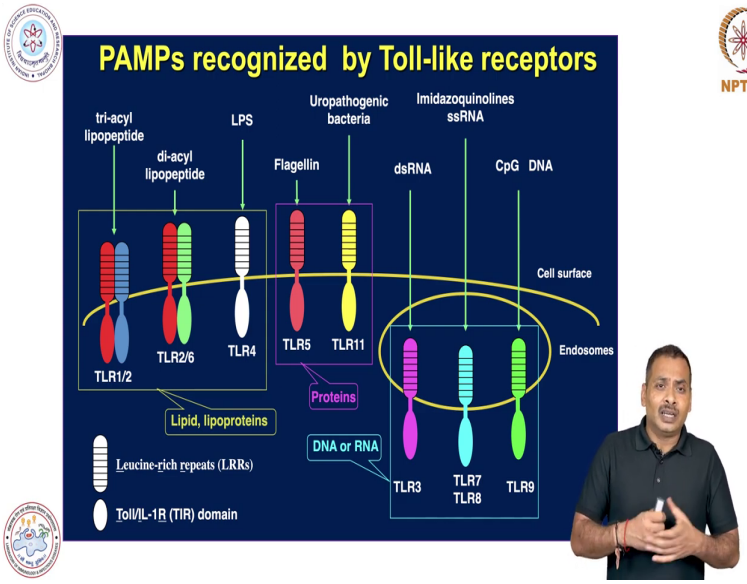

Now I will talk about the PAMPs recognized by TLR. So, there are there are if you see the TLRs then you can find out that this TLRs can sense various biomolecule derived from the microbial pathogen. They can sense lipid or lipid derivatives, they can sense protein, they can sense nucleic acid. So, these are the major biomolecule in living system. Here you can see that the heterodimer of TLR-1-2 and TLR2-6 basically they since tri-acyl lipopeptide and diazcyl lipopeptide, respectively.

And TLR4 which sends can sense the LPS and this LPS is the sensing of LPS is not so, simple and you can understand in previous session I have talked about the work of Bruce Butler. So, so the TLR field basically discovered through the discovery of TLR4 and sensing of LPS is little complicated. For sensing of LPS you need various molecule as you can see in subsequent slide you need a CD14 you need MD2 and there is a one more protein known as LBP, LPS minbinding protein.

So, all these things are needed in order to induce the LPS or TLR4 mediated signalling. TLR can sense the protein if you see carefully that TLR5 senses flagellin and this flagellin is derived from a flagellated bacteria in addition they can sense Euro-pathogenic bacteria and TLR3, 7, 8 and 9 they can sense nucleic acid. Like TLR3 basically senses double stranded RNA molecule TLR7 and 8 sansesince A single standard RNA molecule and TLR7 arend nine senses CPpG Motiff DNA which is hypeor methylated.

So, here I just want to give a one important message that that this pattern recognition receptor or germline encoded receptor which is not the case in case of in case of adaptive immunity

over there the receptors like B cell receptors and T cell receptors they are generated after genetic rearrangement. There is a recombination is taking place where the gene segments are joining and then they are they are making a receptor.

Here in case of innate immune receptor they are germline and coded receptors. And if you see very carefully then you can see that all those molecules which are come in category of PAMPs they are very important for the for the for these microbial pathogen like peptidoglycan, LPS, nucleic acid but they these sensors basically sense that distinguishing feature like hypoermethylated nucleic acid single standard RNA phosphorylated RNA so on and so, forth and like a human cell they do not have a peptidoglycan.

So, they sense the peptidoglycan. So, here I just gave you the overview about the various PAMP.

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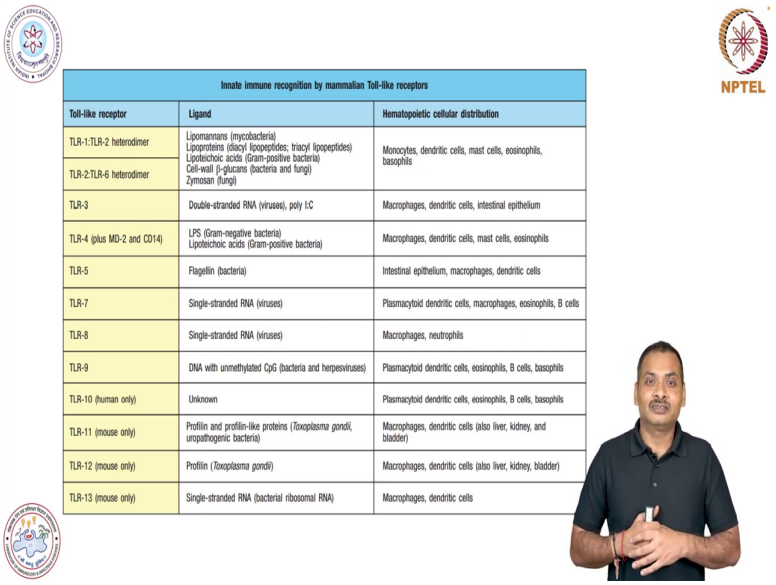

Innate immune recognition by mammalian Toll-like receptors

Toll-like receptor	Ligand	Hematopoietic cellular distribution
TLR-1:TLR-2 heterodimer	Lipomannans (mycobacteria) Lipoproteins (diacyl lipopeptides; triacyl lipopeptides) Lipoteichoic acids (Gram-positive bacteria) Cell-wall β-glucans (bacteria and fungi) Zymosan (fungi)	Monocytes, dendritic cells, mast cells, eosinophils, basophils
TLR-2:TLR-6 heterodimer		
TLR-3	Double-stranded RNA (viruses), poly I:C	Macrophages, dendritic cells, intestinal epithelium
TLR-4 (plus MD-2 and CD14)	LPS (Gram-negative bacteria) Lipoteichoic acids (Gram-positive bacteria)	Macrophages, dendritic cells, mast cells, eosinophils
TLR-5	Flagellin (bacteria)	Intestinal epithelium, macrophages, dendritic cells
TLR-7	Single-stranded RNA (viruses)	Plasmacytoid dendritic cells, macrophages, eosinophils, B cells
TLR-8	Single-stranded RNA (viruses)	Macrophages, neutrophils
TLR-9	DNA with unmethylated CpG (bacteria and herpesviruses)	Plasmacytoid dendritic cells, eosinophils, B cells, basophils
TLR-10 (human only)	Unknown	Plasmacytoid dendritic cells, eosinophils, B cells, basophils
TLR-11 (mouse only)	Profilin and profilin-like proteins (*Toxoplasma gondii*, uropathogenic bacteria)	Macrophages, dendritic cells (also liver, kidney, and bladder)
TLR-12 (mouse only)	Profilin (*Toxoplasma gondii*)	Macrophages, dendritic cells (also liver, kidney, bladder)
TLR-13 (mouse only)	Single-stranded RNA (bacterial ribosomal RNA)	Macrophages, dendritic cells

And in next slide I will show you the great detail of these these these PAMPs what are the PAMPs and where these sensors express. These sensors express here you can see that a TLR 2 in association with one and TLR2 in association with 6 they sense lipo-arabino-Maennoan lipop lipoproteins, lipotechoicoic acid cell wall from fungi which is consists of beta glucoan and zymosain which is derived from fungi.

And they are expressing in variety of cells like monocyte, DC's, mast cells, eosinophil, basophil. So, if you see this table very carefully then you will notice that most of TLRs are expressed in macrophages and dendritic cells. Except few if you see TLR7 the it is expressed

it is not expressing in in DC's rather it is expressing in PDCs which basically produce ~~10~~tone amount of type 1 interferon upon viral infection.

So, you can go through this table and you try to understand and try to remember that these TLR are playing a very important role in various compartment of the cell. And here you can see that TLR 10 is only expressing in human whereas TLR 11 12 and 13 only expressing in Mouse. So, with this I will stop here and in next session I will talk about the TLR signalling, thank you.