Host-Pathogen Interaction (Immunology)
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Module No # 09
Lecture No # 40
Pattern-recognition Receptors-NLR & NLR Signaling Pathways

Hi so in previous session we have learned about various PRR and among those PRR we have extensively studied Toll like receptor and we have also studied RIG-I like receptor RLR. So these receptors are playing very important role in various compartment of the cell you have seen that toll like receptors are present on cell membrane outside the cell membrane. And it is also localized in the endosome and you have also studied that there are sensors which we call it as a RIG-I like receptor they are present in cytoplasm.

So now this whole cell is fully guarded if you see carefully fully guarded against various invaders or microbial pathogen or its component. Today we will discuss another very big family of pattern recognition receptor and these pattern recognition receptor we call it as a NOD like receptor. We will discuss about NOD like receptor we will discuss about the various microbial pathogens sensed by various member of NOD like receptor and we will also discuss the signaling pathway which is activated by these NLR members.

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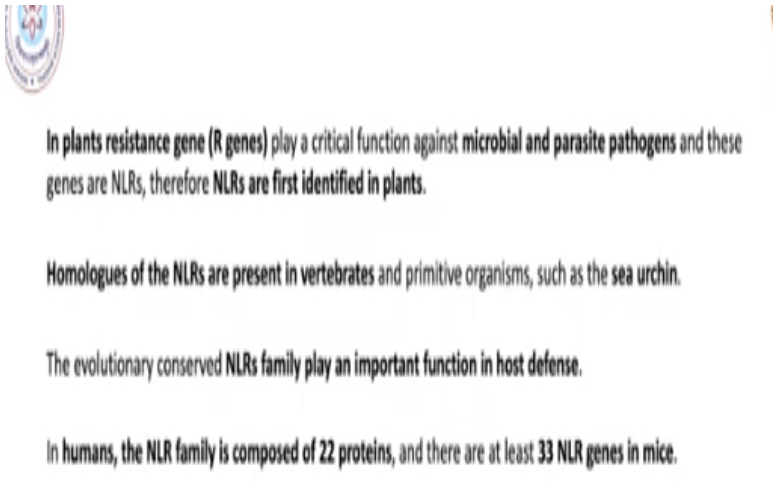

So let us begin with Nod like receptor and NOD like receptor is NLRs or first identified in plants it is very interesting right. It is a first identified in plant and they play a very important role against microbial and parasite infection in plants and these proteins are named as an R genes. So, R genes are basically R is simply resistance gene and this resistance gene is very important in defense in plants. So this is far more interesting you have seen that for example the phagocytosis is originally discovered in the starfish larvae.

And you have seen the toll total protein was originally discovered in drosophila the fruit fly. Here you are seeing that this Nod like receptors they are present or their members are also present in plants and they play a crucial role in defense. So the homologue of this Nod like receptors are present in vertebrates it is present in most of plants and it is present in very primitive or phylogenetically primitive organisms such as sea urchin, zebrafish and in these animals about 203 members are present.

So you can understand that these proteins are kinds of very crucial for defense in these; primitive organism like zebra fish, sea urchin or so and so. And they play a very important role in various vertebrates. It is present in almost all vertebrates so you can understand how primitive this gene is?. This is highly conserved the whole NLR family members are highly conserved in sea urchin and vertebrates, mice, human.

So all these members the Nod like receptor family members they are playing very important role in host defense. And in human about 22 members are there 22 NLR members are there and in mice about 33 and memnumbers are there I will show you in next slide. And these members are having if you categorize these protein then these proteins have a 2 distinct functional feature.

One is that they directly sense and then it induces a activation of pro or NF-Kappa B or MAP kinases in order to produce pro-inflammatory cytokines another group of proteins they make a multi-protein complex and they play important role in production of one very important family of cytokine. Which we call it as IL-1 family cytokine and they are also playing a various role or important role in one or other types of cell death and that includes apoptosis, pyroptosis so and so. So these are the functional type of NOD like receptor there are 2 major categories.

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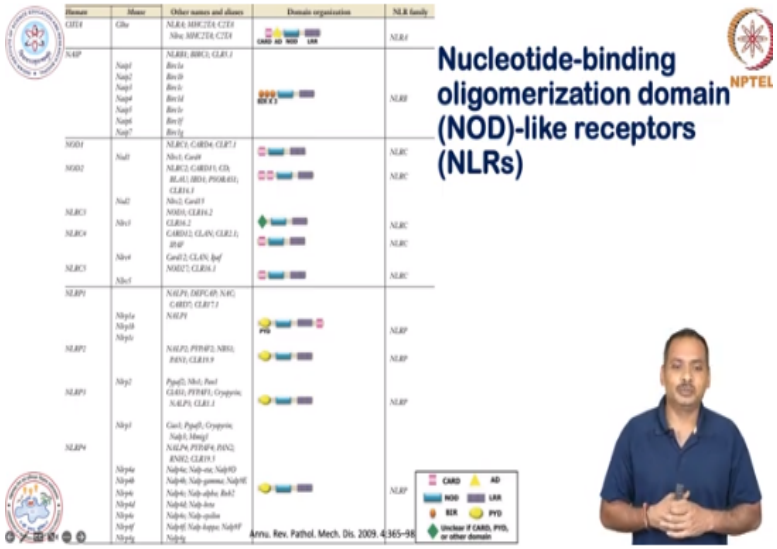

So we will look at all this thing if you see this slide here you can see that in human there are various Nod like receptor members are there. And in mouse theiry are homologues are there and their subtypes are also there in mouse and you can see the domains in these protein. So I will begin with C 2 CIITA it is a class 2 trans activator protein and this class 2 activator protein has a CARD domain you remember the CARD, caspase recruitment domain or caspase recruitment and activation domain have discussed in previous session in RLRs.

So class 2 trans activator protein they have a CARD domain they have activation domain and NOD domain so NOD is basically nucleotide binding oligomerization domain. So basically, it binds with nucleotide and that induces the oligomerization and there is a Leucine rich repeat you probably remember Leucine rich repeat is also present in tolle-like receptors. And over there it is present in ecto-domain which; basically play an important role in sensing the pamps various pamps.

So this is a Class 2 trans activation protein another is NAP and AIP, NAP and they have a BIR domain it is BIR stand for Bakula-Baculovirus virus inhibitor repeat and there is a NODnot domain and Leucine rich repeat domain. So NAIP is also making a some or other kind of multi-protein complex and then it is playing important role in production or induction of mature form of il-1 family cytokine another is NOD 1 and NOD 2.

So NOD1 and NOD2 are basically present in cytoplasm and they sense some key motives of peptidoglycan and basically it activates NF Kappa B and MAPmap kinases. So you can see that NOD 1 and NOD 2 both has a one CARD domain and not domain and leucine Rich repeat domain on another hand NOD2 has a 2 card domain not domain and Leucine rich repeat domain. So here if you notice in this protein NOD1, NOD2, NLRC3, NLRC4 and NLRC5.

so all these proteins they have a CARD domain and this is a based on domain classification these proteins are basically grouped together and they are called it as a NLRC, CARD domain containing. NLRC 3 if you see this has a one domain which is a people are kind of not clear this is either CARD domain or there is a pyriene domain and they have a NOD domain and Leucine rich repeat field domain.

Similarly, in case of an NLRC 4 and 5 there is a card domain not domain and Leucine rich repeat domain. So here just I want to give a note so there are so many members but all members are not very well characterized but few members are extensively characterized. So I will I tell you in upcoming slides another class if you see this has a NLRP 1, 2, 3, 4 and if you see here carefully they have a pyriene domain instead of card domain.

So they have a pyriene domain not domain Lucine rich repeat domain and in case of NLRP 1 there is a both pyrene domain and card domain is present. So NLRP 2 and NLRP 3 they have only pyriene domain in addition NLRP 4 also has only pyrene domain NOD domain and leucine rich repeat domain.

So among all these NLRPs, NLRP 3 is very well characterized it is extensively or I think it is too much characterize it everything we know about the NLRP 3. It is a the mutation associated with that protein the type of ligand on by which this NLRP get activated and how this NLRP get activated? And what are the other components other proteins which interact with NLRP-3 everything is quite well characterized.

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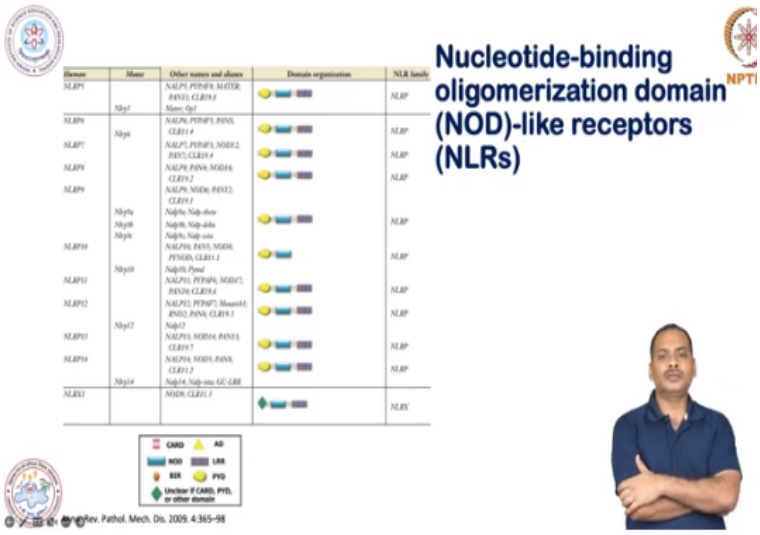

Here there is an another member NLRP file this also has a pyriene domain here you can see that this has a pyrene domain NOD domain and leucine rich repeat domain and rest of another NLRPs are also their NLRP 6, 7, 8, 9, 10, 11, 12, 13 and 14. So all these NLRPs has characteristic pyrinfiring domain NOD domain and leucine rich repeat domain except if you see little carefully NLRP 10 this does not have a leucine rich repeat domain.

So and the last member is NLRX 1 and this has a NOD domain and leucine rich repeat domain and there is a some region which is a not very well defined which will fall either card domain or pyriene domain tt is not very well characterized so NLRX 1 is like that. So now I will show you what are the ligands they these this protein senses I will not talk about all proteins. As I told you all of these proteins are not very well characterized I will talk few of these protein which is a quite well characterized.

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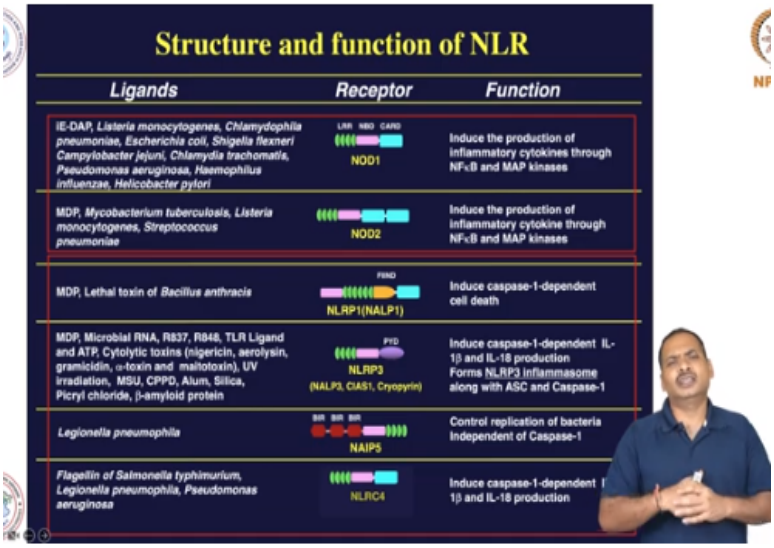

So here you can see there is a NOD1 and NOD2 this has a Leucine rich repeat domain and nuclear nucleotide binding domain or NOD domain or card domain. So NOD1 basically since IE-DAP I will explain you IE-DAP more detail in next slide. So IE-DAP is basically derived it is a component of peptidoglycan IE-DAP I will tell you precisely what is molecularly it is? And this item is derived from Listeria monocytogenes you can understand this is a bacteria Chlamydophila pneumoniae, Escherichia coli, Shigella flexneri, Campylobacter jejuni, Chlamydia trachomatis, Pseudomonas aeruginosa, Haemophilus influenzae and Helicobacter pylori.

So these are the microbes which are sensed by NOD1 and what it is sensing and it is sensing a IE-DAP. IE-DAP is a one ~~moti~~motif~~ve~~ or one region in peptidocyclin on another hand NOD2 which has a two card domain they sense the MDP again I will tell more about the MDP. Basically. it is a Mural dipeptide probably you may aware about that which it senses MDP mycobacterium tuberculosis. It also sen~~d~~se again listeria monocytogen which is also sensed by NOD1.

So once these NOD1 and NOD2 senses these particularly one is IE-DAP and MDP in the cytoplasm it induces the production of inflammatory cytokine through activation of NF Kappa B and MAP kinases. So this is one functional category another functional category this is a basically an NLRP 1, NLRP 3 and NAI~~L~~P 5. So this protein basically senses the ligand and then it will make a multi-protein complex.

This multi-protein complex basically induces a very important cytokine which is our family of cytokine which is we call it as a IL-1 family cytokine. This IL-1 family cytokine basically consists of several cytokine but key cytokines are IL-1 beta, IL-18 and IL-33. Initially these cytokines are synthesized in inactive form and once these protein get activated this makes a multi-protein complex and then they will induce this mature form of IL-1 beta.

I will tell you the signaling in subsequent slidesght so here you can see that NLRP 1 basically senses the MDP and lethal toxin of bacillus anthracis. So bacillus anthracis has a 3 major protein which is a making the exotoxins so there are probably you are aware that there are 2 major kinds of toxins produced by the bacteria one is endotoxin. Endotoxin is like LPsS, exotoxin they are toxin which is synthesized in the bacteria and then it is secreted out.

So bacillus anthracsis makes this lethal toxin along with 2 more proteins which we call it as a protective antigen and there is a edema factor. So this protective antigen basically helps in transportation of lethal factor and edema factor inside the cell in order to kill the target cells. So I will not go in more detail about the bacillus anthracisanthrosis but this is just for your information it is a special note you can remember and this basically induces Caspase 1 dependent cell death.

So NLRP 1 is basically involved in cell death so I have told you in beginning of this session that functionally they have a 2 major category one is activating NF Kappa B and another is inducing IL-1 family and mature form of IL-1 family cytokine and another category also induces cell death. So this basically this NLRP 1 is inducing the cell death and it is Caspase 1 dependent another is a NLRP 3 as I told you this is very well characterized NLRP family member.

We also call it as a NALP 3 and CIAS 1, Cryopyrin. So this Cyropyrin or this protein they sense of ligand it is a huge list I am putting very few not all and they can sense if I see the literature they can sense all kind of like ligand and non-living toxins and so on. So forth they end up our own antigens or our own molecules they can sense and they will induce production of IL-1 family cytokine. Here I am naming few of the ligand here you can see the MDP they can sense microbial RNA R837.

~~This is a r~~ and R848~~37~~ these are the ligand which ~~and it is like~~ activates TLR also they can sense various TLR ligand they can sense the ATP. ATP from the host also they can sense various cytolytic toxin like nigericin, area~~a~~olysin, gramicidin, and Alpha Toxin and mai~~l~~totoxin these NLRPs can also get activated by some physical mean here you can see there is a ultraviolet radiation can activate this NLRP 3 and this is a also activated by some chemical compound.

Which is produced in our body here the best example is a MSU more monosodium urate. This monosodium urate is increased in the individual who has a tendency to have a gout or joint pain so MSU is a one of factor which is responsible for the pathogenesis of gout and another is a CPPD it is a calcium pyrophosphate dehydrate. So this is also produced in our body so basically monosodium urate this is produced more in those individual who has some problem in nitrogen based molecules and you know that amino acids are all nitrogen.

So if there is a problem in metabolism then that may result to the accumulation of monosodium urate and that will be one of a key cause for this gout. This can also NLRP 3 can also activate it by our vaccine adjuvant as I told you in human we use only Alum as a vaccine adjuvant. So this can be activated by LM this can be activated by a silica you know the silica is the people who are working in cement factory or similar kind of situation where there is a generation of lot of silica dust. So those individual may develop some or other disease.

So this silica can also activate NLRP 3 besides this is some chemical compound like a Picryl chloride which we use it in our experiment and beta amyloid protein. So all these things these are very short list of NLRP 3 ligand so all these proteins basically activate the NLRP 3 inflamasome there is a new term here I Am telling inflamasome. So this NLRP 3 makes an Inflammasome not NLRP 3 other NLRPs also makes a inflammation so what is inflammation?

So Inflammasome is nothing it is a simple multi-protein complex and the aim of this multi-protein complex is to activate the enzyme which is present in inactive form and the enzyme is Pro Caspase. It is a Pro Caspase 1 in case of mice it is pro caspase is 1 and 11 so Pro Caspase is 1 is a enzyme which cleaves the Pro IL-1 beta into active form that is IL-1 beta. So try to understand here one simple philosophy that this IL-1 family cytokines they are quite potent.

It's ~~is~~ small doses is quite sufficient in order to develop or induce a significant biological in effects for example IL-1 beta they can induce a fever. If it is produced too much they can induce high fever or they can induce the hypotension and that is a quite a big physiological effect. They can also make condition like a shock condition IL-1 beta is also one of the key factor.

So they make a multi-protein complex and this multi-protein complex the aim is to ~~in~~activate the caspase which is present in inactive form that is Pro Caspase and once this process is activated then caspase will generate and this caspase will make the active IL-1 beta. So this Inflammasome of various kind I will show you in a subsequent slides another is NAI~~L~~P 5 and this NAI~~L~~P 5 which has this distances this Legionella pneumophila. And they also control the application of bacteria independent of casparation. .

Here the caspase one is not involved there is another infamous home which senses the Flagellin protein of salmonella typhimurium, Legionella pneumophilia and Pseudomonas aeruginosa and this inflamasome also induces Caspase 1, IL-1 beta and IL-18 production. So you can see there are 2 distinct category one is inducing or activating the NF Kappa B in MAP kinases and another is basically inducing inflamasome mediated cell death or production of IL-1 family cytokine

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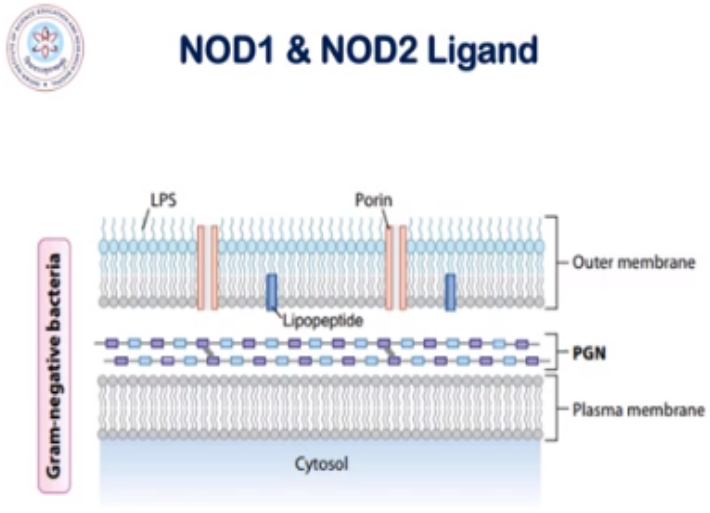

So now I will explain you what is this you have seen previously there is a ligand for the NOD 1 and NOD 2 so here you are very well aware about the structure of gram negative bacteria I am

just showing you for your quick understanding. So there is an outer layer which is consists of LPS outer membrane which contains LPS and there are other things like Porin and Lipopeptide and there is a peptidoglycan. And then there is a membrane plasma membrane in case of gram-negative bacteria

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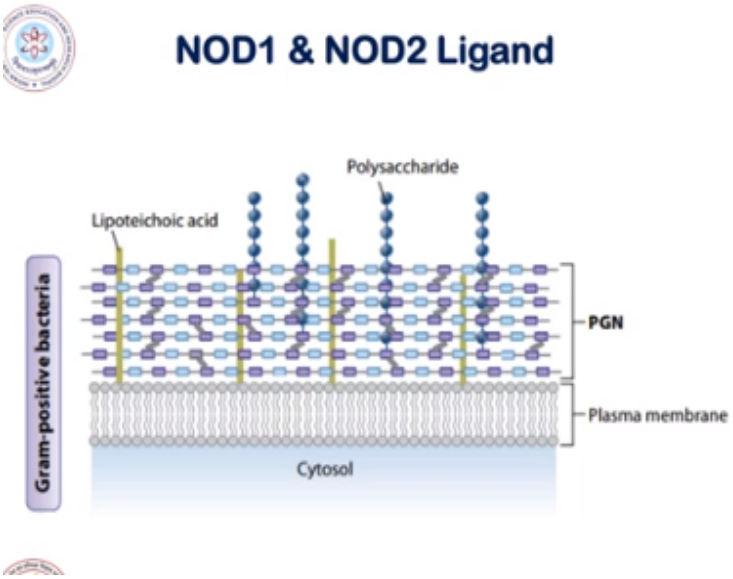

In case of gram-positive bacteria there is a very thick peptide of glycan layer which makes a cell wall and there are some projections of Polysaccharide and of course below the peptidoglycan there will be a cell membrane.

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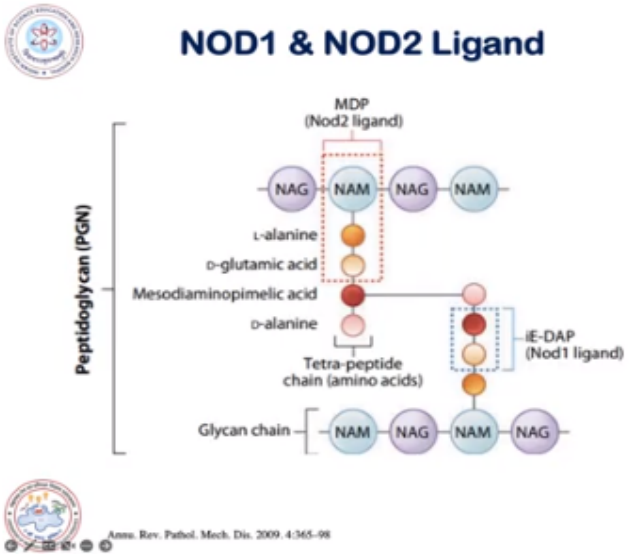

So this is a more detailed structure of a peptidoglycan this is basically consists of or it is a polymer of NAG and acetyl glucosamine if you remember your previous classes or you probably studied in microbiology. So NAG is Nan acetyl glucosamine and this is linked by Nan acetyl muramyic acid and this kind this linkage is basically beta 1 4 glycosidic linkage. So this Nan acetyl glucosamine and Nan acetyl muramyic acid which is linked by glycosidicacidical linkage is basically there is an another molecules which is making a kind of a cross linkage between two to this polymer chain of NAG and NAM.

This is basically linked by L alanine D-glutamic acid Mesodiaminopimelic acid here you can see and there is a D alanine. So this is a molecule where you can see both D and L amino acid that is alanine and D alanine both are present. And this Tetra-peptide chain is again linked with here you can see this is a having a D-alanine and Mesodiaminopimelic acid and then there is a Ll alanine.

So these 2 chains of this NAG and NAM is linked like that and here you can see this MDP is a muramyl dipeptide. So this muramyl dipeptide is basically this component which is consists of NAM and acetyl muramic acid Ll-alanine and D-glutamic acid. So this component is sensed by NOD 2 and there is an another component if you see below then you can see that there is a Diaminopimellic like acid and Dd-glutamic acid.

So this is a D gamma glutamyl Meso Diaminopimellic acid is a IE-DAP and this is a ligand for NOD 1 let us look at how this NOD 1 and not to activate the signaling.

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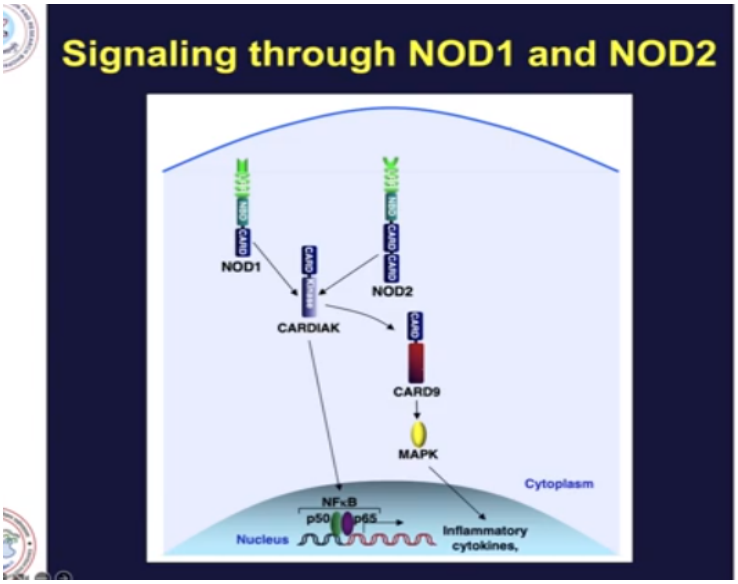

So this is a very simple schematic try to understand I am again saying it is a very same simple but it is far more complex for your easy understanding I made it very simple. So this NOD1 and NOD2 upon sensing this ligand they make a oligomerization they will there will be a conformational change and this conformational change will make it oligomer and then this will recruit another card containing protein which we call it as a CARDIAK.

This CARDIAK is a key adapter kind of molecule and this will recruit another card containing protein known as CARD 9. And then this can activate MAP kinases and CARDIAK directly can activate the NF Kappa B and you know that how NF Kappa B get ~~X activated~~ it is activated via IKK complex which phosphorylate I Kappa B and this phosphorylated I Kappa B undergo degradation proteosomal mediated degradation.

And then this free NF Kappa B will be translocated into the nucleus and then there will be a production of inflammatory cytokine. So this is the way by which NOD1 and NOD2 activates NF Kappa B and MAP kinases.

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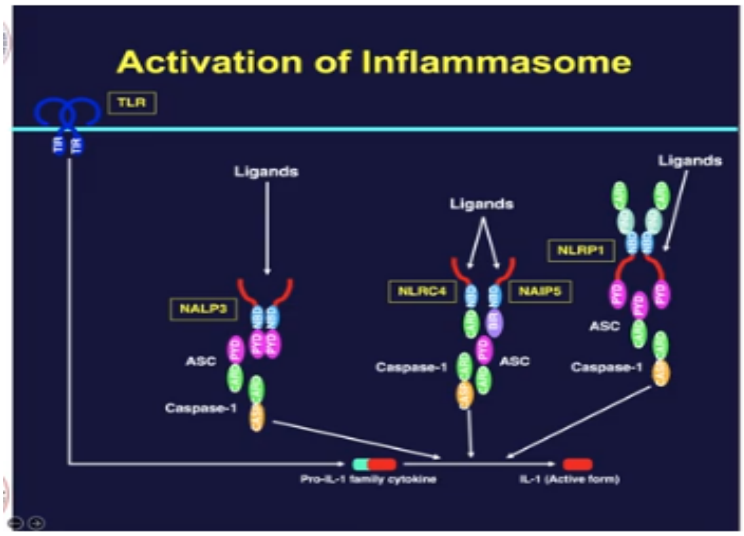

Now I will talk about the activation of Inflammasome here you can see there are various kind of Inflammasome there is a NLRP3 or ~~null~~NALP-3 Inflammasome there is a NLRP 4 NLRP, NALP 5 and NLRP 1 Inflammasome are there and now I will tell the composition of Inflammasome. So basically, this Inflammasome is consists of the NLR family member in case of an NLRP 3 it is a NLRP 3 and there is a one molecule which is also present which we call it as an ASC.

So ASC if you see carefully this ASC has a both pyri~~ene~~ domain as well as CARD domain and it will interact with Pro~~-~~caspase this process is basically having a CARD domain and caspase domain. So here you can see this homotypic interaction is playing very important role in making this multi-protein complex and then this complex this multi-protein complex get activated by the ligand and then it will induce the active form of IL-1 family cytokine.

Here you need to note one very important thing in order to activate this Inflammasome you need a 2 signaling one is that any signaling which will increase the amount of Procaspase 1 as well as Pro IL-1 family cytokine. Which is driven by NF Kappa B here you can see there for your convenience I put a TLR. So there will be a TLR mediated NF-Kappa B activation this NF Kappa B activation will result to the more amount of protein in the cell more amount of procaspase~~ess space~~ IL-1 and ASC.

This is signal 1 and signal 2 basically makes the oligomerization of these NLRP family members and these 2 signaling work together. And then through this Inflammasome multi-protein complex

there will be a processing of process and this Pro caspase will first make a caspase active form of caspase and this active form of caspase will cleave the pro IL-1 family cytokine. Then there will be a generation of IL-1 family and active IL-1 family cytokine which is secretory in nature before cleavage it is not secretory once it is cleaved then this will be a secretory so this is a basic concept of Inflammasome.

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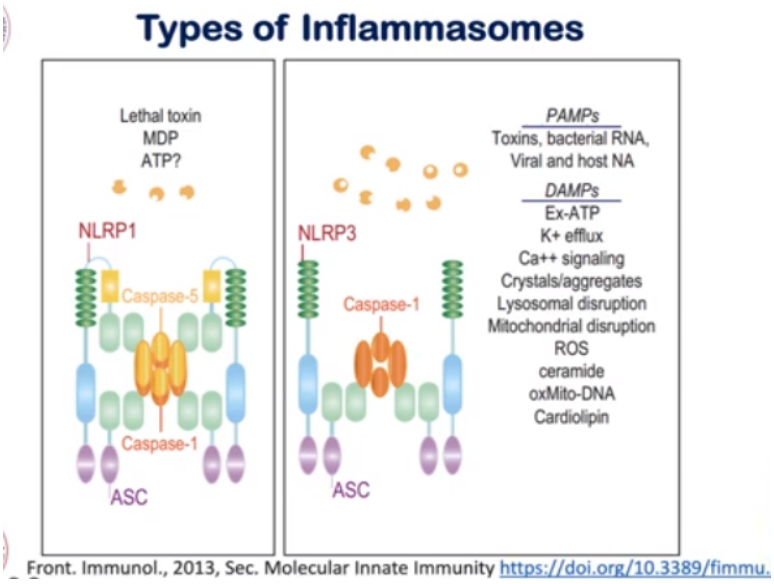

There are various kind of inflammation like NLRP 1 here you can see which is activated by Lethal toxin, MDP, ATP and here NLRP 3 inflammasomet~~ion~~ there is a quite big list and this list is a further very long it is activated by PAMPs as well as DAMPs. PAMP is Pathogen Associated Molecular Pattern which is originated from the pathogen and DAMPs are basically our own molecule Damage Associated Molecular Pattern. So this can be activated by our own molecule also this is about NLRP3 Inflammasome.

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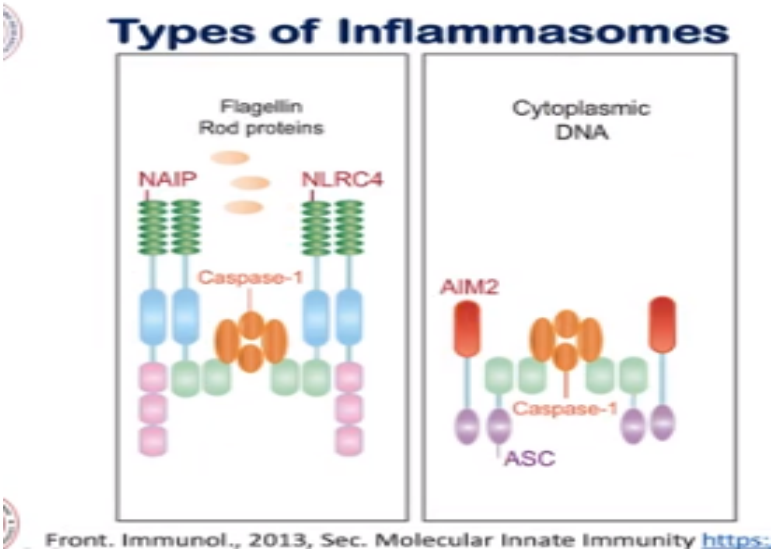

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There is a another Inflammasome which we call it as a NAIP or NLRC 4 and this is also making a multi-protein complex and there is a cytoplasmic DNA sensor which I will talk more in when I will take up sensing of DNA most likely in next session. So this also makes an inflammasome~~tion~~ which we call it as a AIM 2 inflammasome~~tion~~. So now I gave you the overview of NLR family pattern recognition receptor how many members what are the ligands what kind of signaling everything you probably understood.

So in next session I will talk about the disease associated with NLR family members and with this I will stop here and in next session we will discuss about the NLR~~N~~ and disease thank you.