Host-Pathogen Interaction (Immunology)
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Lecture: 32
Concept of Pattern-Recognition Receptors (PRRs)

Hi, so in previous session, ~~W~~we have learned about the importance of innate immunity and we have learned the various component of innate immunity if you remember there is a mechanical physical or ~~biochem~~ biochemical and microbiological barriers. So, we have discussed in a great length all these sections that is mechanical barrier the biochemical barrier and microbiological barrier. So, one more important the most important component of innate immunity was left that is cellular component.

So, cellular component of innate immunity is basically consist of all cells it is very interesting all cells of the host plays a very important role in innate immi~~vity~~unity in addition to the professional innate immune cells. So, the cellular component of immunity is all all cells plus the the macrophages the neutrophils basophil and so on so forth. So, now I will today I will introduce with the concept of this pattern recognition receptor and this pattern recognition receptor is now playing a very important role in not only innat~~ive~~ immunity it is also playing very important role in development of whole adaptive immunity.

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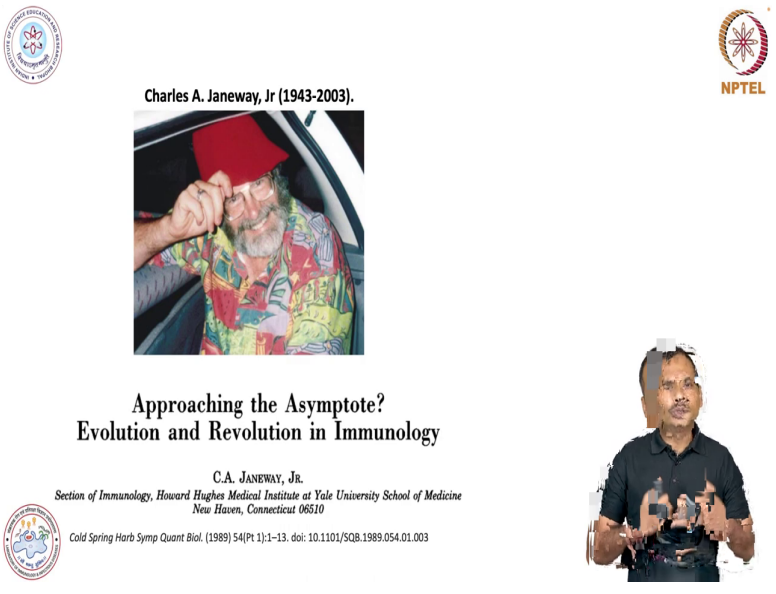

So, this story was begin in 1989 when one professor his name is a Charles A Janeway. So, Professor Janeway gave this concept that the immune system the innate immune system is not non-specific. So, this was before 1989. So, before 1989 people thought that this innate immunity is a completely non-specific thing and this basically consists of macrophages and the cells and these macrophages cell or phagocytic cells take up the antigen and present this antigen to the T cell in order to develop a complete B and T Cell dependent immunity.

But in 1989 this concept was emerged and this concept was given by Janeway and he said that or he suggested that the immunity is not non-specific they do have some broad kind of specificity. And this broad kind of specificity play a very important role in sensing the microbial pathogen and then this is also playing very important role in activation of this innate immunity in order to produce innate immune cytokines.

And that overall plays a very important role in development of adaptive immunity which is mainly dependent on B cells and T cells. So, this concept was basically given in a one of a very ~~reputated~~reputed symposia which we call it as a Cold Spring Harbour symposia in quantitative biology. So, you probably May aware that this Cold Spring Harbour Laboratories are located near New York.

So, over this this concept was given and this the symposia is very reputed symposia over there this discussion about the DNA structure codon and all those things were taken place and there was a good debate among the scientists in order to take it f~~F~~orward. So, over there this this concept was given and this is given by Janeway and the note of his all discussion is given in approaching the Asymptote and this is a title Evolution and revolution in Immunology.

So, this after emerging this concept his Charles Janeway and his group also work toward this direction.

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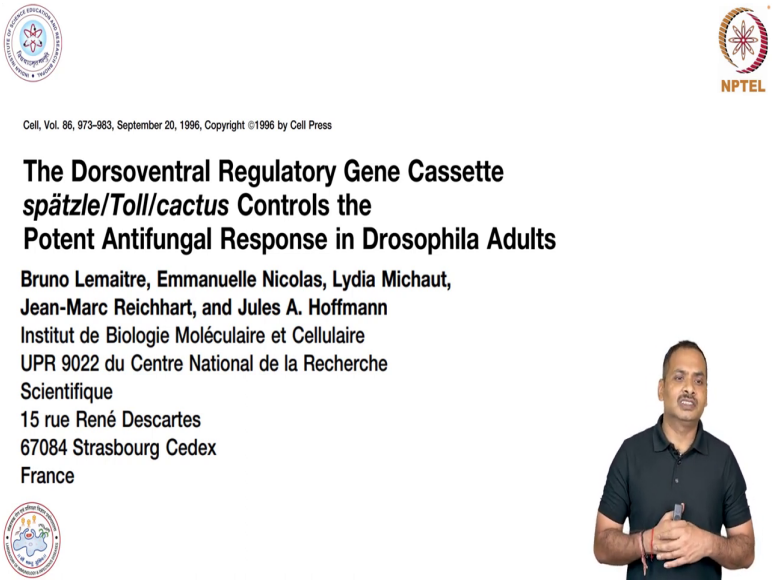

And in 1996 before his work the work of Julie Hoffman who worked on drosophila and he gave this or his findings which is published in cell journal and he very elegantly showed that there is a some group of protein which plays a very important role in dorsal ventral patterning in drosophila embryo plays a very important role in defense against microbes and they are basically anti bacterial they are anti-fungal.

And all this works work was reported in this article as you can see this was published in cell in 1996.

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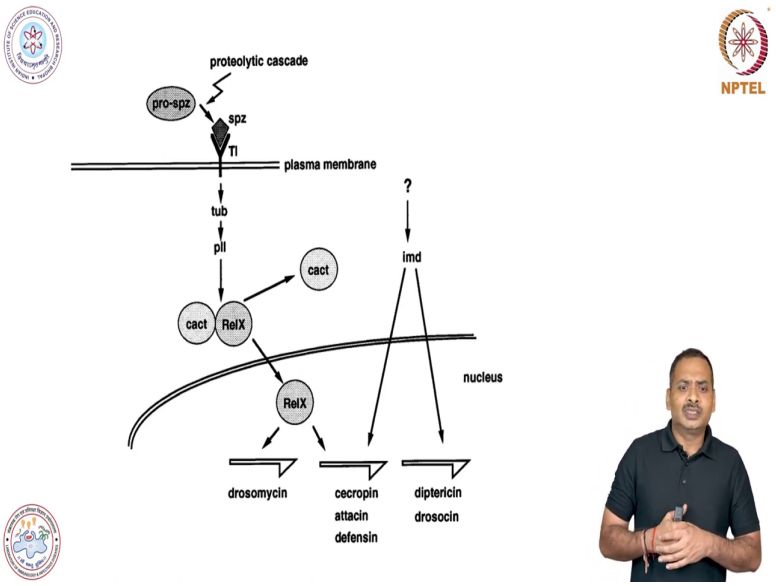

So, basically he suggested that there is a protein known as ~~phasgel~~ spaetzle and there is another protein toll these two proteins play quite important role in development of antimicrobial immune responses in drosophila. Please note that previously this these two

proteins were reported to play important role in dorsal ventral patterning in drosophila embryo. So, this upon upon infection of bacteria and fungi or fungi these protein known as ~~sparsgel~~ spaetzle this was a cleaved by Cascade of proteolytic activity.

And this cleave protein basically bind with a toll protein and then there will be a Cascade of signalling and that result to the activation of a transcription f~~F~~actor diff or dorsal and that result to the expression of antimicrobial peptide known as dorsomycin. He also suggested there are two p~~P~~athways one is this toll pathway and another is IMD pathway. So, IMD pathway uses another transcription f~~F~~actor known as relish and that result to the expression of another set of antimicrobial peptides.

As here you can see that there is a protein known as ~~a~~ attack~~in~~, cecro~~sapro~~pin, defensi~~nve~~ dep~~tericinutyism~~ and also dorsocin~~al seen~~. So, all those protein eventually developer ~~anti wire~~ anti-fungal responses.

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So, after this work this basically established the observation of the Janeways hypothesis and he in his group also they were working towards this and his group identified the homologue of toll protein in human Human Genome and they have characterized this protein in with some very simple and initial experiment basically they first fish out that Gene by most probably by bioinformatic approaches after that these Gene were cloned and then they were characterized and they found out that activation of this protein indeed induced the various innate immune cytokines such as TNF as IL~~R~~6.

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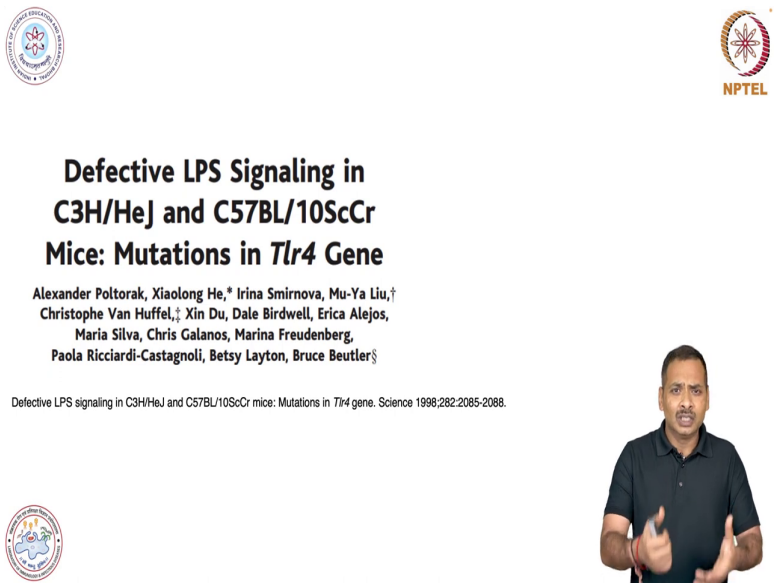

So, this work was published in nature in 1990~~0~~7 and after that one more work was published and this work was given by another group and the group name is this is the group of Bruce Butler. So, they have found out that the one strain of mice is resistant to the LPS or it is hyp~~er~~o responsive to the to the lethal dose of LPS. And subsequent characterization result to the discovery of one very important mutation in TLR4 at that time this name was already in place this is Toll-~~-~~Like Receptor which is present in mammals.

So, this was a further characterized this these mouse gene were characterized and they found out that in the cytoplasmic domain of TLR4 there is a one mutation which changes the amino acid from Proline to histidine and that makes it resistant to the LPS lethal dose. Ideally LPS if you inject the LPS in the mouse or human that will cause a shock that is a very fatal condition.

So, they identified this mutation and they have characterized this mice and they found out that at 712 position there is a change in amino acid and that makes the hyp~~o~~er responsive to the to the LPS and this gene is TLR4. So, with this it is quite well established that TLR are playing a very important role in sensing microbial pathogen or at that time one more term was coined that is a pathogen associated molecular pattern.

So, this is a pathogen associated molecular pattern the LPS or there are. So, many molecules I will discuss in subsequent slides.

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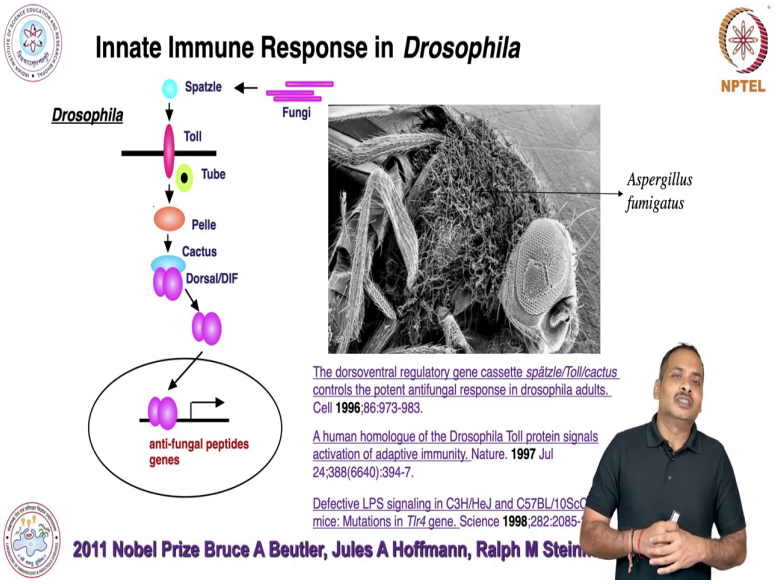

So, after this work received a lot of attention around the world and then and this work also received the Nobel Prize and if you see 2011 Nobel Prize was given for the discovery of this pattern recognition receptor to two person one is a Julie Hoffman who worked in drosophila and another person is the Bruce Butler who characterized this TLR4 and the hypoer responsive of TLR 4 in particular strain of mice.

And in addition the Nobel Prize was also given to the ~~Ralphus Taiman~~ Ralph Steinman you I have discussed earlier in previous session he just passed away three days before the announcement. So, this prize was given to his wife. So, at this time this concept was very well established that innate immunity is not non-specific it is the innate immunity do have a specificity and this is specificity is quite broad.

And this broad specificity is basically playing important role in sensing and then subsequent signalling and then development of innate immune response and which eventually result to the and development of adaptive immune response. So, after this work there are so, many things to complete and finish in TLR field.

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Prof. Shizuo Akira

Most of genetic studies was performed his group.

And the most important contribution was the by Professor Shizuo Akira he is from Osaka University Japan he characterized various TLRs and TLR signalling molecules by creating this genetic by creating knockout mice or by doing the genetic study and this field was a fully established at that time.

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Innate Immune Response in *Drosophila*

Cells for Defense	Fat-body cells and hemocytes are phagocytic cells that act as part of the immune system.
Anti-microbial peptide	Drosomycin (DIF-dependent) Diptericin, Attacin, and Cecropin [Relish-dependent, via Imd (immunodeficiency) signaling pathway]

So, now I would like to talk about the innate immune response in drosophila because this is very important if you see very carefully the original discovery of a phagocytes or phagocytic cells or phagocytosis was given by Emil Merchenkoff which was discovered in a starfish right and after that this discovery was made in various mammals including human. So, similarly this work drosophila work it is a great turning point in innate immunobiology.

And this work results to the discovery of a whole new field of innate immunobiology or there is a pattern recognition receptor and all those things. So, I will quickly tell about the innate immune response in drosophila. And if you remember in early session I gave you the importance about the comparative Immunology study. So, this is a one very good example. So, let us begin with innate immune response in drosophila.

So, basically these this in this drosophila are fruit fly they have a very cells for the defense which is phagocytic in nature and these cells are basically fat body cells and hemocytes. And they take up the take up the microbial pathogen and develop a appropriate immune response and the appropriate immune response is basically mediated by antimicrobial peptide and these are; as I showed you in previous slide in the work of Julie Hoffman now these are basically dorsomycin which is a diff dependent.

So, what is diff. So, diff if you if you know that this is a basically a transcription factor which is similar to the NF Kappa B. So, NF Kappa B plays a very important role in immunity. So, this is just like that and its other name is dorsal. So, diff dependent there is a synthesis of dorsomycin there is a another set of molecule which is a synthesized under the control of transcription Factor relish this is diptericin, attacking and cecrocincyprosyncypropine.

So, and these antimicrobial peptides are generated through this IMD pathway IMD is the immunodeficiency signalling pathway which is operated in drosophila.

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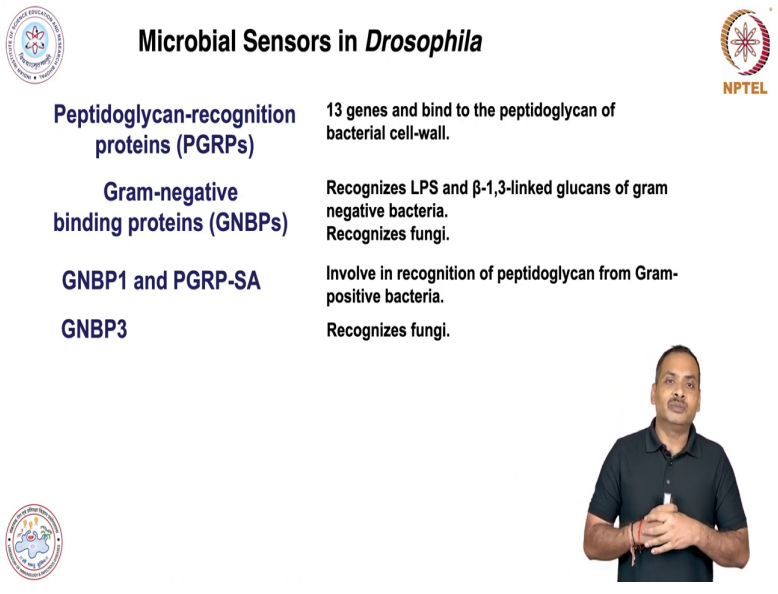

So, but how this these entities are sensed that is bacteria which is consists of gram positive gram negative and there is a also fungi. So, what are the sensors. So, the next question is that. So, the sensors are this there are variety of sensors the two major classes of sensors are peptidoglycan recognition protein. And it is consists of 13 member and basically it binds with a peptidoglycan of bacterial cell wall.

There is another group of sensors which is a we call it as a gram negative binding protein GNBPs ~~and B piece~~ and basically they recognize as LPS which is a signature molecule of a gram-negative bacteria and the they also recognizes a fungi originated molecule that is beta1,3 linked glucan~~ons~~. So, glucan~~on~~ is basically present in fungi. So, in that way they recognizes these GNBP's are basically play important role in recognition of gram negative bacteria and fungi.

So, there are some set of molecules like GNBP1 and PG--RPSA molecule. So, they basically sense the peptidoglycan from gram-positive bacteria and it recognizes and induces the synthesis of anti antimicrobial peptides. GNBP3 basically play a very important role in sensing fungi they recognize as fungi and then that synthesizes the antimicrobial peptide.

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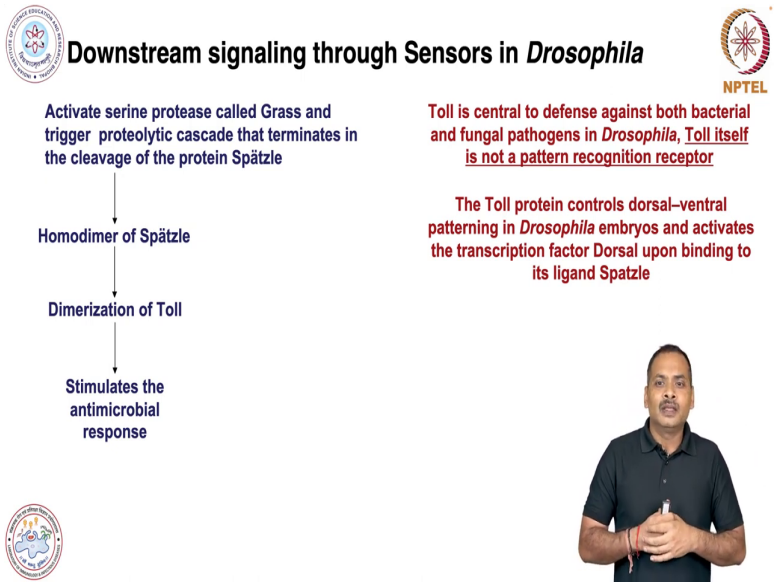

So, how this is basically taking place all this sensing and signalling. So, here I will give you a quick glimpse. So, basically what is happening there is a activation of a serine protease which is one serine protein is known as grass is involved and that triggered the proteolytic cascade~~ket~~ that eventually end up with the cleavage of the inactive form of spaetzle~~sparsgel~~

protein and this is spaetzlesparsgel protein which is generally present in the cell not cell in extracellular space in an inactive form.

So, this is get cleaved and then this is active spaetzlesparsgel interact with a tonell and this basically makes a dimer and this spaetzle spuds will make a dimer and then this interact with or induce the dimerization of toll protein and induces the synthesis is of antimicrobial peptide here you can see it is very nicely shown in this slide. In in here you can see that there is a peptidoglycan and this peptidoglycan is sensed by GN and BP1 and PGRP SA.

And that will upon sensing that will activate the protease Cascade and eventually this activate grass and this grass basically cleave the spaetzle spatial protein and make a dimer and that will make the dimerization of toll protein and then eventually the this will activate the downstream signalling and towards end it will activate the diff protein which is closely associated with a protein known as a cactus.

This Cactus is like I Kappa B in in NF Kappacopper B signaling if you have studied. So, so NF copper B is a transcription factor which is which is generally present inside the cell in Bound form with I Kappa B and once this I kappa B get phosphorylated then this I kappacopper B will undergo proteosome mediated degradation upon after ubic attenationubiqutination and then NF copper free form of NF Kappa B will be translocated into the nucleus where this will trigger the transcription of various relevant genes.

So, this is a quite similar as human or mice there although the molecules are different but they are quite similar. So, if you see very carefully the tollne is a is a molecule which is a very important or pivotal of in defense against these microbial pathogen however they are not direct sensor they are not pattern recognition receptor this is very important note please remember the toll is playing an important role in defense in case of all kinds of microbial pathogen in drosophila.

But it is not the sensor sensors are like as you have seen there are several molecules or family of molecules that they play important role in sensing originally it was discovered as a controller for dorsal ventral patterning in drosophila embryos and basically thise stone toll get activated when spaetzle sparsgel protein binds with the toll.

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Spaetzle

So, what is spaetzle~~special~~ so, this is a little interesting this spaetzle~~spectral~~ name was given due to the appearance of this molecule and this is this is a one of a German food which is made from the wheat it is something like a spaghetti or macaroni or so on so forth.

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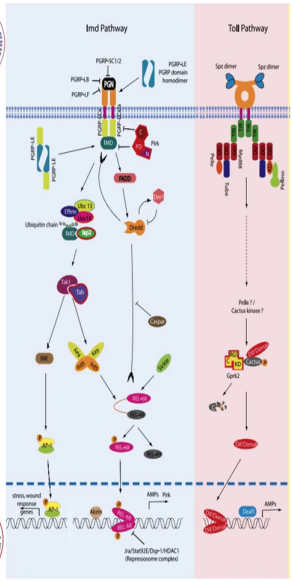

Towards end I would like to just quickly give the glimpse of the toll pathway in drosophila here you can see. This spaetzle dimer~~is dorsal diamonds~~ are bound with a toall protein which eventually activate the dif~~f~~ or dorsal and there is a cactus which is like ~~eye kapavi~~IkappaB and that activates the synthesis of antimicrobial peptides. This is a IMD pathway where if you see towards end there is a activation of a relish and this basically start the synthesis of antimicrobial peptide.

So, in this session I will just stop here. And in next session I will talk about the in talk in more detail about the TLR ligand and TLR signalling pathway and so on so forth, thank you.