Host-Pathogen Interaction (Immunology)
Prof. Himanshu Kumar
Laboratory of Immunology and Infectious Disease Biology
Department of Biological Sciences
Indian Institute of Science Education and Research (IISER) - Bhopal

Lecture: 36
Pattern-recognition receptors-TLRs and Therapeutics

So, in previous several sessions you have learned about the innate immunity and now I will discuss how this innate immunity knowledge or the information which we gained in last about three decades can be translated into therapeutic. And just for your information the philosophy behind the disease or the concept behind the disease. So, any disease basically happen in the host by some perturbation by external environment.

External environment when I say it means it could be environmental factor it could be some pathogenic challenge or some other factors right physical or chemical factors. So, these factors basically perturb the homeostasis. And when this homeostasis is perturbed then that result to the development of disease. In a very simple in words how I can explain that if there is a some hypo situation or hyper situation.

Hypo situation means it could be anything hypo means under activation of signalling pathway under production of some molecules which is produced by this particular signalling pathway under nutrition or malnutrition. So, all those things can result to the development of disease on another hand if it is a too much activated if any production of molecule or any signalling pathway is too much activated then that result to the hyperactivation over nutrition also result to the development of metabolic diseases.

So, the concept is when this homeostatic range is disturbed by any mean then that result to the development of disease. For example, if you remember the previous session I told that there is a LPS and this LPS is sensed by the TLR4 if this ligand is too much then there will be too much production of inflammatory cytokine and if there is a some mutation in this TLR Gene which will not induce appropriately this TLR signalling that is also a problem if you remember there is a disease associated with that that is known as atrophy.

So, overall the concept for the disease is if there is a skewing of this homeostatic range in Lower Side or higher side then that result to the development of disease. And in therapeutic basically what we do we basically try to manage this this hypo response or hyper response by some Agonist or antagonist and that basically bring it to the homeostatic range and those molecules we call it as a therapeutic molecule those procedure we call it as a therapeutic procedure and all those things.

So, let us begin with this TLR and therapeutic and before that I will take you to to a little history of immunotherapy.

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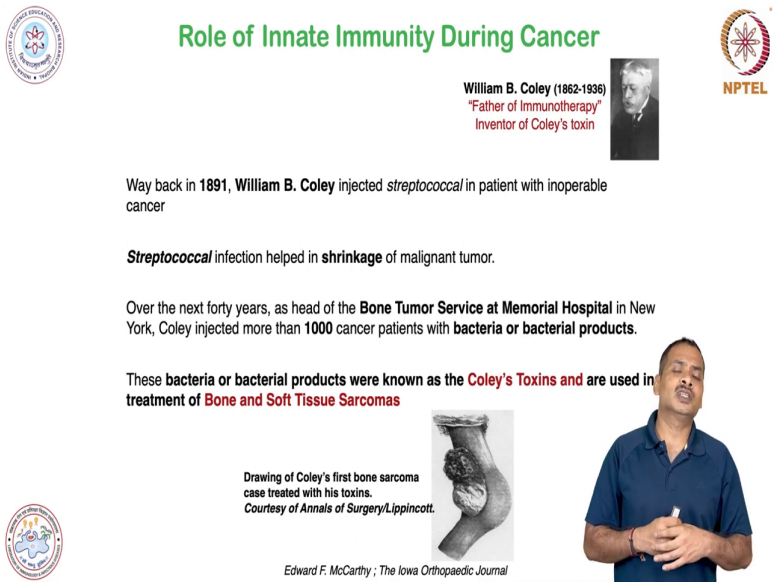

And this immunotherapy is basically started quite early and this was started by one one doctor his name is William B Coley he was there he was a basically a cancer expert or most likely he is a onco surgeon. He injected initially I have learned his little history initially by accident he observed the cancer patient who are infected by some bacterial infection ok those patients are recovered from the cancer or they had a less severity in cancer.

So, this observation was taken further by William Colley and then he intensely injected the streptococcal a cocktail of a Streptococcus in the cancer patient in which the surgery is a little complicated or difficult. And then what he found out that the individual who received this cocktail of a bacteria there tumor was shrinked reduced ok. In some cases this is completely disappear this tumorous growth.

So, this observation was a quite well accepted this is a very very interesting thing that you put some or you in-fact the individual with some bacteria and that bacteria will recover from the cancer that is very very interesting. But unfortunately, he was not able to explain this observation on the basis of science or logic at that time because there was a no knowledge of this innate immunity or PRR, PAMP and all those things which we which was discovered in 1989 right.

So, so this observation was a quite interesting and then this cocktail of these bacteria he named it as a kind of Coley toxins. So, Coleycollate toxin is a toxin which is consist of a cocktail of bacteria and when it is injected in the tumor directly or in patient then there is a recovery from the cancer and his this therapy was a very well practiced at that time but he since he was not able to give the scientific explanation. So, he did not get too much recognition.

So, Coley injected more than thousand cancer patient with this bacteria and bacterial product and many of these patients were recovered or reduced their cancer was reduced. And these bacteria or bacterial products are known as coley toxin and are used in the treatment of bone and soft tissue cancer sarcoma or cancer. So, here there is a picture and why I am giving you this background because there is a very strong basis of the scientific basis which he was not able to explain.

Now we can understand this is a basically the non-specific activation of innate immunity and this non-specific activation of innate immunity result to the activation of specific cells such as NK cell and other cells and that basically occurs the because the shrinkage of this cancerous tissue or elimination of these cancers tissue. So, now we can understand this thing very well. And therefore there is a various molecule or there are various ligands of TLR which is used for the cancer therapy.

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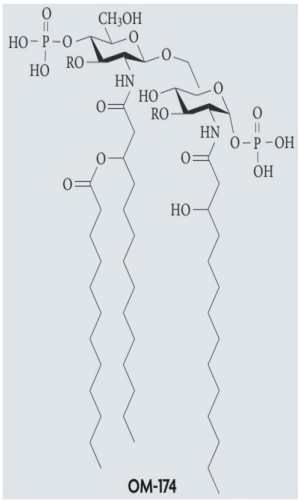

OM-174 is a TLR2 and TLR4 agonist that is in Phase I trails for cancer

Here you can see this is a molecule this is a quite complex molecule ok its name is OM174 and this is a basically a TLR2 and TLR4 Agonist it activates the TLR2 and TLR4 mediated signalling and induced inflammation or all innate immune responses. So, this molecule is used in the in the treatment of cancer and this is in phase one clinical trial.

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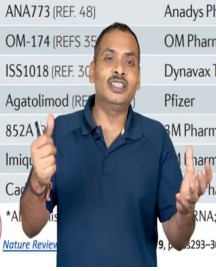
Table 2 | **Development status of compounds that target TLRs for cancer indications**

Compound	Company	Target*	Drug class	Clinical Phase
Rintatolimod (REF. 56)	Hemispherx Biopharma	TLR3	dsRNA molecule	Preclinical
SMP-105 (REFS 39,40)	Dainippon Sumitomo Pharma	TLR2	Autoclaved mycobacteria	Preclinical
IPH-3102 (REF. 34)	Innate Pharma	TLR3	dsRNA mimic	Preclinical
CBLB502 (REFS 41,42)	Cleveland Biolabs Inc.	TLR5	Flagellin	Preclinical
IMO-2055 (REFS 26–28)	Idera Pharmaceuticals	TLR9	CpG oligonucleotide	Phase I
MGN-1706 (REF. 29)	Mologen	TLR9	Non-coding stem-loop DNA	Phase I
ANA773 (REF. 48)	Anadys Pharmaceuticals	TLR7	ssRNA molecule	Phase I
OM-174 (REFS 35	OM Pharma	TLR2, TLR4	Lipid-A derivative	Phase I
ISS1018 (REF. 30	Dynavax Technologies	TLR9	Short DNA oligonucleotide	Phase II
Agatolimod (RE	Pfizer	TLR9	CpG oligonucleotide.	Phase II
852A	3M Pharmaceuticals	TLR7	Small-molecule ssRNA	Phase II
Imiqu	harmaceuticals	TLR7	Small-molecule ssRNA	Phase II
Ca	harmaceuticals	polyTLR	Autoclaved mycobacteria	Phase II

*A RNA; ssRNA, single stranded RNA; TLR, Toll-like receptor.

Nature Review 9, p 293–307 (2010)



There are several molecule as you can see in this table this is these are the basically compound that target TLR for the treatment of cancer here you can see there is a TLR 3 ligand which is double standard RNA molecule there is a TLR 2 ligand which is a autoclave mycobacteria. Try to understand mycobacteria they have a variety of PAMPs a huge it is a heterogeneous complex of a PAMP.

You can have the lipid and sugar complexes like lipo arabino Menon which we call it as a LAM~~lamb~~ there are mycolic acid and so, on and so forth. So, they it is it is a very good mixture of ligand which can non-specifically activate the immunity and just for your information here I would like to say that we use one adjuvant we call it as a ~~fluent~~ freund's complete adjuvant which is basically potentiate the the activity of vaccine.

So, vaccine or if you challenging some antigen in the mice so, this freund's adjuvant ~~fluent adjunct~~ the ~~f~~freund's ~~luent~~ complete adjuvant~~oint~~ is used only in animal or in Mouse it is not used in human please remember this thing. And that fluency complete adjunct consists of heat killed mycobacteria. So, heat kill mycobacteria is a having a tremendous potential in order to activate innate immune response in order to make a appropriate adaptive immune response which is a B cell mediated response which finally result to the production of a high ti~~ghter~~ter antibody.

And it is also important in inducing the T cell mediated immune response. So, here you can see that this autoclaved mycobacteria is also used in or about to use in human and that may that may be used for the treatment of cancer. There is a TLR three ligand which is a basically a double standard RNA mimic. There is a TLR5 ligand which is a Flagellin~~yl~~ in protein Flagellin~~Flagyl~~ in which is present in the flagella of flagellated bacteria.

TLR9 ligand which is basically consists of Cp~~P~~G Motif of DNA it is a basically oligonucleotide which is hypo~~e~~r methylated there is no methylation. So, they also have a good potential to activate the TLR9 mediated signalling. There is some non coding stem Loop DNA which is also used to activate this TLR9 mediated signalling. There are TLR 7 ligand that is single standard RNA molecule and there is a TLR2 and TLR4 ligand which is lipid A~~a~~ if you remember the structure of lipopolysaccharide.

So, you will find out that there is a some o antigen there is a outer core and there is a inner core and there is a lipid which is phosphorylated. So, this lipid is a having a potential to activate the TLR for signalling but it will not induce the pathogenic kind of response it will not activate too much. So, this lipid A is used as a is a it is a quite potential molecule to activate the TLR4 mediated~~itigated~~ signalling and this can be also used as the vaccine adjunct.

Just again I am giving you one very important information in human we use only one adjuvant which is which is a clinically established and that is the alum. There are so, many kinds of adjuvanet but in human or in human vaccine only we use the Alum. So, you can understand we are in in the need of having more vaccine adjuvanet. So, probably lipid a or there is a one derivative of lipid a which we call it as a MPLA can be used as a vaccine adjunct besides this is used for the treatment of cancer.

There is a TLR 9 ligand again this is a short DNA oligonucleotide cpg oligonucleotide. So, these are basically also you they have a potential to use in patient and they are in a very good level in clinical trial this is in phase two clinical trial. Several ligands of TLR7 which is a basically small molecule A single standard RNA this is a this is basically in clinical trial and in it is in phase two trial.

So, most likely in near future we will have a kind of kind of good repertoire of molecules which can be used for the treatment of various cancer there is a poly TLR. So, this autoclaved mycobacteria you can I told you this is a complex mixture is it can consists of all kind of molecule. So, this can be also used for the treatment of cancer.

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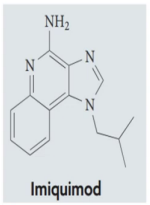

Imiquimod is Toll-like receptor 7 (TLR7) agonist, is launched for papillomavirus infection.

Now I will move to some molecule which is which can be used in some infectious disease it could be a bacterial it could be a viral. Here you can see that there is a one molecule known as Imiqui~~MEQ M~~mod, Imiquimod ~~MEQ Mode~~ this is a basically a TLR 7 ligand and it is a Agonist it potentiates the TLR7 mediated signalling path and this is used against the papilloma Virus Infection.

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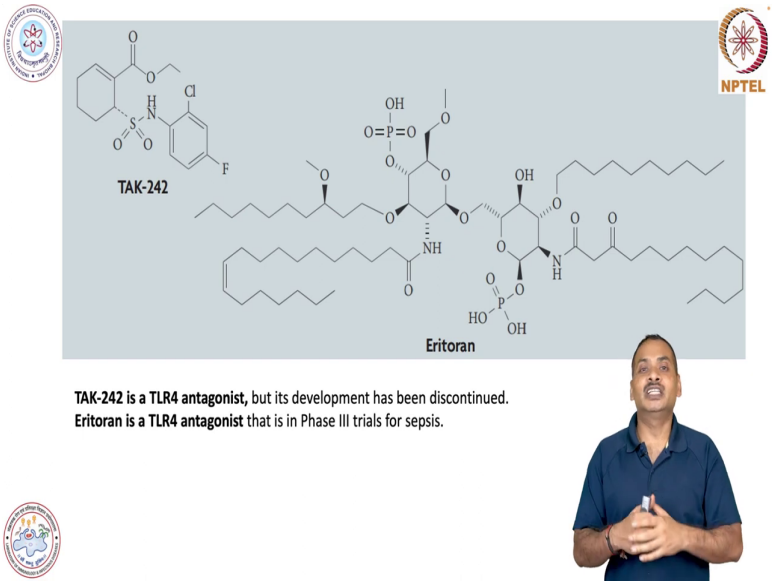

Here there are some molecules one is Take 242 which was proposed to used for or used against success or shock condition LPS mediated substance and shock but somehow most likely this has a some side effects. So, that their development is stopped and it is discontinued however on another hand there is another molecule known as eritoraen. eritoran~~Eritorin~~ is basically also a TLR4 antagonist and it is in phase three clinical trial for sepsis.

So, you can understand if you inject this molecule then this molecule will compete with this LPS molecule and it will it has a more if it has a more Affinity it will bind to the TLR4 ligand and but it will not activate not TLR4 ligand TLR4 receptor and probably it will not activate the signalling it will bind but it will not activate the signalling. So, in that way we can we can treat the sepsis condition this is one way.

Another way is that whatever LPS is there you can sequester by some molecule and then you can remove and in that way you can treat the sepsis LPS mediated or gram-negative bacteria mediated sepsis. And over there in current scenario we use one molecule known as ~~polymix~~polymyxin B ~~in D~~. And this polymyxin B ~~polymerex in B~~ basically is very strongly bind with the LPS. Basically we use in our laboratory in order to remove the LPS in our stimulating samples because you know that LPS is a very strong stimulator. So, if it if your sample is containing LPS then it will give a incorrect results.

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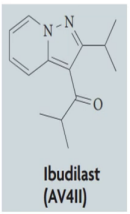

Ibudilast (AV4II) is a TLR4 antagonist and used for ocular inflammation.

This is another molecule which is a again TLR4 antagonist it is a IbudiBU deal luast and this molecule is basically used in ocular inflammation.

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Table 3 | **Development status of compounds that target TLRs for viral and bacterial infections**

Compound	Company	Indication	Target	Drug class	Clinical Phase
IRS-954 (DV-1079) (REF. 72)	Dynavax Technologies	SLE, HIV	√ TLR7 and TLR9 antagonist	Bifunctional inhibitor	Preclinical
SD-101 (REFS 49,50)	Dynavax Technologies	Hepatitis C infection	√ TLR9 agonist	CpG oligonucleotide	Phase I
IMO-2125 (REF. 26)	Idera Pharmaceuticals	Hepatitis C	√ TLR9 agonist	CpG oligonucleotide	Phase I
ANA773 (REF. 48)	Anadys Pharmaceuticals	Cancer, hepatitis C	√ TLR7 agonist	ssRNA molecule	Phase I
VAX-102 (REF. 58)	VaxInnate Corp.	Influenza infection	√ TLR5 agonist	M2e peptide/Flagellin from *Salmonella typhimurium*	Phase I
Biothrax plus CpG-7909 (REFS 51,53)	Coley Pharmaceuticals	Anthrax	√ TLR9 agonist	CpG oligonucleotide	Phase II
Rintatolimod (REF. 56)	Hemispherx Biopharma	Viral infection	√ TLR3 agonist	dsRNA molecule	Phase II
Resiquimod (REFS 45,46)	3M Pharmaceuticals	Hepatitis C infection, herpes	√ TLR7 and TLR8 agonist	ssRNA molecule	Suspended in Phase II and III
HEPLISAV (REFS 49,50)	Dynavax Technologies	Hepatitis B infection	√ TLR9 agonist	CpG DNA plus hepatitis B antigen	Phase III
Eritoran (REFS 75–79)	Eisai Pharmaceuticals	Sepsis	√ TLR4 antagonist	Synthetic lipodisaccharide	Phase III
Cadi-05 (REF. 43)	Cadila Pharmaceuticals	*Mycobacterium tuberculosis* infection	√ PolyTLR agonist	Autoclaved mycobacterium	Phase III
TAK-242 (REF. 83)	Takeda	Sepsis	√ TLR4 antagonist	Small-molecule inhibitor	Suspended in Phase III
Imiquimod (REFS 9–12,20)	3M Pharmaceuticals	Keratosis, papillomavirus infection	√ TLR7 agonist	Small-molecule ssRNA	Approved

dsRNA, double stranded RNA; M2e, ectodomain of matrix protein 2; SLE, systemic lupus erythematosus; ssRNA, single stranded RNA; TLR, Toll-like receptor.

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And there is a list of molecule which is a which is used in in various viral and bacterial infection here you can see that TLR7 TLR9 antagonist it is a bi-functional inhibitor basically this damsp the TLR7 and TLR9 mediated signalling. There is a TLR9 Agonist that is cpg oligonucleotide and there are TLR7 single standedard RNA molecule TLR5 stimulant is Agonist which stimulates this TLR5 mediated signalling.

It is a flagellin from salmonella typhemurium and there is a TLR9 sorry TLR3 ligand that is double standard RNA molecule TLR 7 or TLR8 Agonist that is single standard RNA molecule and like that there are several ligands which is used in which can be used in a in a

treatment of various viral and bacterial infection. And many of these molecules are in Phase three trial and very interesting there is a TLR7 Agonist which is already approved for clinical use. This is me Imiquimod~~MEQ mode~~.

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Table 4 | **Development status of compounds that target TLRs for allergy, asthma and autoimmunity**

Compound	Company	Indications	Target	Drug class	Clinical phase
NI-0101	NovImmune	Acute and chronic inflammation	TLR4 antagonist	Antibody	Preclinical
OPN-305	Opsona Therapeutics	Inflammation, autoimmunity, ischaemia/reperfusion	TLR2 antagonist	Antibody	Preclinical
OPN-401	Opsona Therapeutics	IBD, rheumatoid arthritis	TLR2 antagonist	Viral-derived peptide	Preclinical
IMO-3100 (REF. 74)	Idera Pharmaceuticals	SLE, rheumatoid arthritis, multiple sclerosis	TLR7 and TLR9 antagonist	DNA-based compound	Preclinical
1A6 (REF. 86)	NovImmune	Colitis	TLR4 antagonist	Antibody	Preclinical
AVE0675 (REFS 43,62)	Sanofi-Aventis/ Coley Pharmaceuticals	Asthma, allergic rhinitis	TLR9 agonist	CpG oligonucleotide	Phase I
QAX-935 (REFS 54,61)	Idera Pharmaceuticals/ Novartis	Allergy, asthma	TLR9 agonist	CpG oligonucleotide	Phase I
SAR-21609 (REFS 43,62)	Sanofi-Aventis/ Coley Pharmaceuticals	Asthma	TLR9 agonist	CpG oligonucleotide	Phase I
VTX-1463	VentiRx Pharmaceuticals Inc.	Allergy	TLR8 agonist	ssRNA-based molecule	Phase I
AZD8848 (DSP-3025)	Astra-Zeneca	Allergy, asthma	TLR7 agonist	ssRNA-based molecule	Phase I
CPG-52364 (REFS 43,71)	Pfizer	SLE	PolyTLR antagonist	Quinazoline derivative	Phase I
DIMS0150 (REF 63)	InDex Pharmaceuticals	IBD	TLR9 agonist	CpG oligonucleotide	Phase II
AV411 (REFS 84,85)	Avigen	Pain management, withdrawal	TLR4 antagonist	Small-molecule phosphodiesterase inhibitor	Phase II
Pollinex Quattro (REFS 64,65)	Allergy Therapeutics	Allergy	TLR4 agonist	MPL plus pollen	Phase III

IBD, inflammatory bowel disease; MPL, monophosphoryl lipid-A; SLE, systemic lupus erythematosus; ssRNA, single stranded RNA.

So, some TLR ligands are also used in non-infectious disease that is Allergy, Asthma and autoimmunity disease like TLR4 antagonist antibody or TLR2 antagonist which is a a kind of mono; most likely it is a monoclonal antibody. And it is used in the treatment of this acute and chronic inflammation, autoimmune disease, ischemia. So, TLR2 Agonist sorry antagonist is basically a viral derived peptide it can be used in inflammatory bowel disease, rheumatoid arthritis.

TLR79 antagonist which is DNA based compound it is used in autoimmune disease such as SLA rheumatoid, arthritis, multiple sclerosis I have discussed multiple sclerosis earlier. TLR4 antagonist can be also used as a used in colitis it is a colon inflammation. TLR9 cpg oligonucleotide is also used in asthma allergy rhinitis it is a pollen allergy TLR 9i Agonist is used in allergy and asthma~~rustom~~ again TLR8 Agonist is also used in allergy.

So, here you can see in this list of molecule there are several molecule which can be used in the treatment of various Allergy, Asthma and autoimmunity disease. So, overall what I would like to say that this discovery of this pattern recognition receptor TLR is very important and I hope in future we will see lot of therapeutic in our clinical setup. And with this I will stop and complete the TLR and in next session I will move to another pattern recognition receptor and that will be the Rig-I-like receptor, thank you.