Drug Delivery Principles and Engineering
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Lecture – 48
Adaptive Immune Response and Vaccine

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. We are discussing the immune response to materials as well as adaptive immune response. So, let us quickly do a recap of what we learned in the last class.

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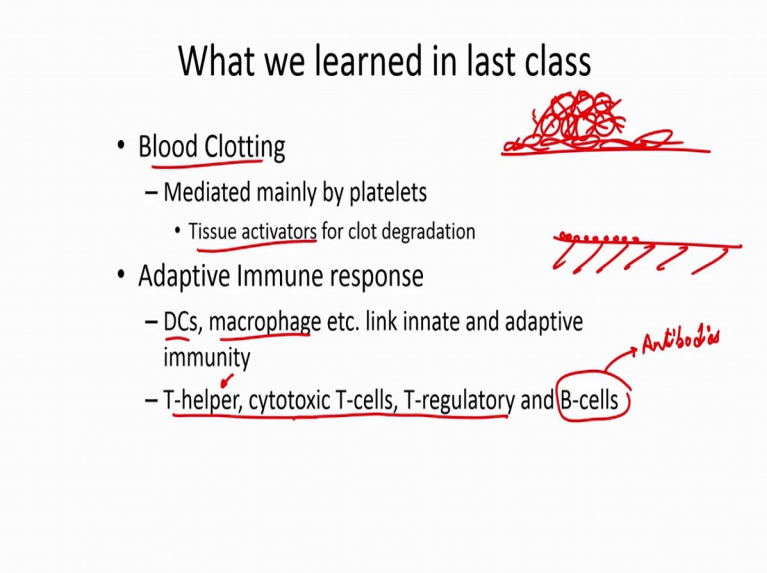

So, in the last class, we finished a discussion on blood clotting. So, any material when it absorbs protein, this can result in binding of platelets and which will then get activated cause a fibrin mesh to form. This is a normal physiological response to any injury so as to prevent bleeding out, mainly mediated by platelets.

And then we also have once the clot has served its purpose, once the healing has started to happen, the area is getting cleared away by the endothelial cells. And then the way this has happens is there are tissue activators that then initiates this clot degradation to happen. And some of these tissue activators then go ahead and start cleaving this fibrin, polymer that is here, so that then causes removal of this obstruction that once the healing has happened, they can go away and then the normal blood vessel can regenerate.

So, these tissue activators are actually good, because what you can do is let us say if you are planting a material, you can coat these tissue activators or have them release out from this material. So, let us say if I coat these tissue activators, then the fibrin can never come in form a polymer here and you can prevent fouling of the surface. But again, you have to be careful; you do not want to give too high amount of these tissue activators, because if you do give too high amount of the tissue activators, you may not even have blood clotting if an injury happens to the patient. So, you just forgot to be careful as a balance between the two.

And then we started talking about adaptive immune response. So, this is the immune response which is very specific, it is called adaptive, because it adapts to whatever the infection is rather than just having a normal generic response which is what innate immune response does. And this is mainly mediated by dendritic cells and macrophages that acts as a link between the innate and adaptive immunity. So, not only these DCs and macrophages secrete, lots of molecules and present some generic response like, let us say bacterial, proteins and lipids. They then also take them up and process them and present them in such a way that the rest of the immune system can see and gets activated to it.

And some of the major cells we talked about, were the T-helper, cytotoxic T-cells and T-regulatory. So, these are leukocytes T-helper is responsible for helping the T-cells getting further activated and killing anything aberrant Cytotoxic T-cells are the ones that are directly responsible for killing the cells that are not functioning well, or the body thinks they are foreign. And T-regulatory is to check these types of cells and make sure that you are not really activating against your self antigen.

So, you know you do not want your immune system will start damaging your own tissues. And then B-cells are the ones that are responsible for the production of antibodies. And these are some of the tags or directly neutralizing, whatever toxin or pathogen and that might be present in the system ok.

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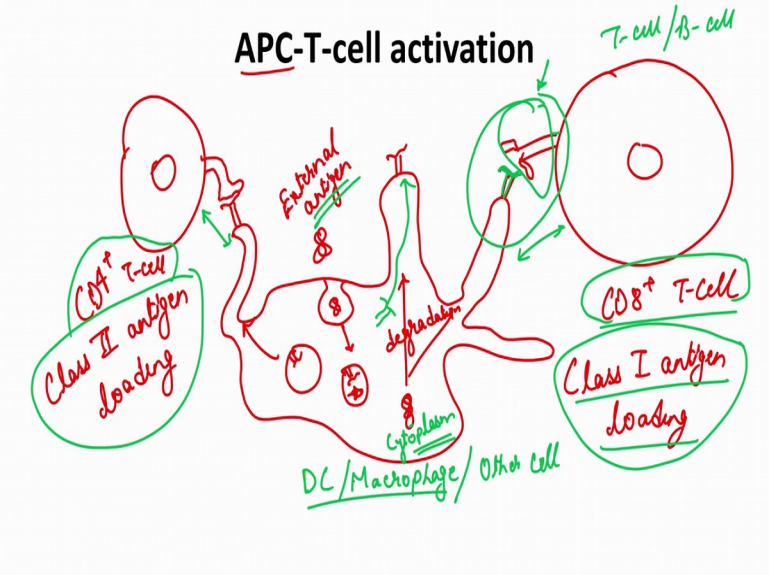

So, let us look at how these antigen presenting cells and T-cell activate the rest of the leukocytes. So, let us say this is an antigen presenting cells. So, let us say this is it changed to brighter color, let us say this is dendritic cell or a macrophage or for that matter let us say this is any other healthy cell, not necessarily this is healthy in fact, let us say that this is some another cell.

So, the way this activation happens is typically these dendritic cells and macrophages have different classes of proteins which class I, class II. So, what is class I? Class I is loading of antigen through an intracellular route. So, let us say if somehow the protein that is an antigen is present in the cytoplasm.

Now, if it is present in the cytoplasm, there is normal functioning machinery of a cell through which it can degrade any protein that is present in the cytoplasm, this gets degraded and this then goes ahead and gets loaded onto the membrane, onto a receptor, which then goes to the membrane and goes and binds to your immune cells. So, this is what now this dendritic cell is presenting. And then an immune cell let us say a T-cell or a B-cell you can come in through its receptor it can see and recognize this antigen. So, these T-cells and B-cells are several types.

So, they have lots of mutation and we will talk about that in future classes, but they have millions and trillions of these receptors. So, this receptor is very diverse in T-cells and B-

cells that are floating in our body. And depending on what this antigen that is being presented here is one or the other that may recognize it. So, once it recognizes it, this gets activated of course, there are some other signals that these APCs need to give to these leukocytes and we will talk about that in the next slide. But, so anything that is happening from the cytoplasm or from internal of the cell is labeled as class I antigen loading.

And typical cells that could activate it through this route is the CD 8 positive T-cell, which we said is responsible for the killing of the cell and its obvious right, I mean if this antigen is somehow externally loaded or externally acquired, then the cell is ok; it is just sampling out the environment, but ever that is something that is coming from the cytoplasm, that is an indication that you have the cell is something that is not supposed to be there or it has become mutated, in some way. So, may it is cancer or maybe it is something else. So, then you need to kill the cell and so that is where the CD 8 T-cells come in, as we talked about these are cytotoxic T-cells.

Then you have another pathway which is the class II antigen loading and that works in a way that this let us say external antigen. These DCs and macrophages then take up this external antigen, they degrade it and again they process it through another class of receptors back onto the surface. Now, these class of receptors bind to the CD 4 T-cells,and they then activated. So, these are two different classes of antigen loading and presentation, which then causes the further activation of these T-cells.

Now, this is of course a big step, because once these things can activate it, they will then go ahead and start to completely go haywire and start to take out whatever wherever they find this antigen. So, it is extremely critical that when the activation here or here is happening; this happens only and only in the case where there is some external antigen and not something that is their own self, because these dendritic cells or other cells will have their own receptors that is something the T-cell or B-cell can recognize too.

So, it is then extremely important that should not happen, and it should only happen, when this is a foreign antigen and so, to do that the body has put some checks and balances. And that is that there are minimum two signals required for activation.

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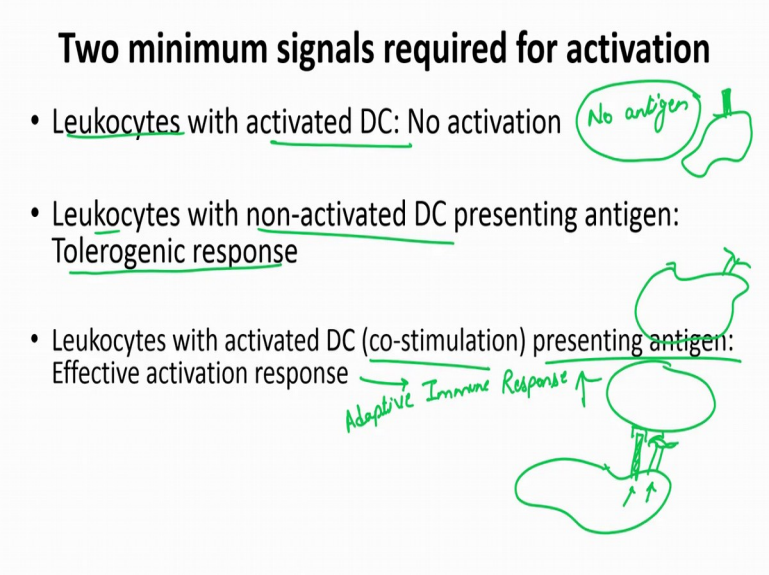

So, just one signal will not be enough, and so what are these two signals? So, let us say if the leukocytes come in contact with an activated DC, so at this point there is no antigen. So, if there is no antigen, but only the dendritic cell is activated due to one of the other reason, maybe there was some inflammation in the surrounding and then this dendritic cell got activated; but does not harbor any foreign antigen, these leukocytes will not get activated, so that is good. Right, because the activation can even happen if I rub my skin, that causes the inflammation and the DC in the surrounding then can get activated, but then I do not really want these leukocytes to get activated against my own antigen, because there is no other foreign antigen.

The other thing can happen is, leukocytes is presented with a non-activated dendritic cell having some antigen. So, not sure why this antigen will be there, but let us say if a DC has an antigen that is being presented, but its giving no other signals its non-activated DC and we will talk about, how does an activity non-activated DC differ, it is not going to give any signal.

And in fact, what will happen is that is going to cause it tolerogenic response to happen, which means that whatever leukocytes that were coming in they are going to become tolerogenic to that antigen, because there is no activation so that means that the antigen has to be self, because non-self antigen will cause activation to happen and so that is why, it will result in a tolerogenic response.

And then finally, you have leukocytes with activated DC which is a co-stimulation presenting a foreign antigen that is going to cause a very enhanced activation response resulting in immune response build up, or adaptive immune response. And so when I say this co-stimulation, what I am saying is just like described above you have a DC that is displaying an antigen, but along with displaying an antigen, it is also displaying another protein, that the leukocyte can come in, and recognize.

So, if there is two signals that are coming in, then you have the immune response that is going to go up. If you have only one signal or with the antigen or just the other signal with the co-stimulation, there is going to be no activation, or tolerogenic response being build up. So that is how the body has put in a check that there it needs to be enough evidence and that there are some problem with the system.

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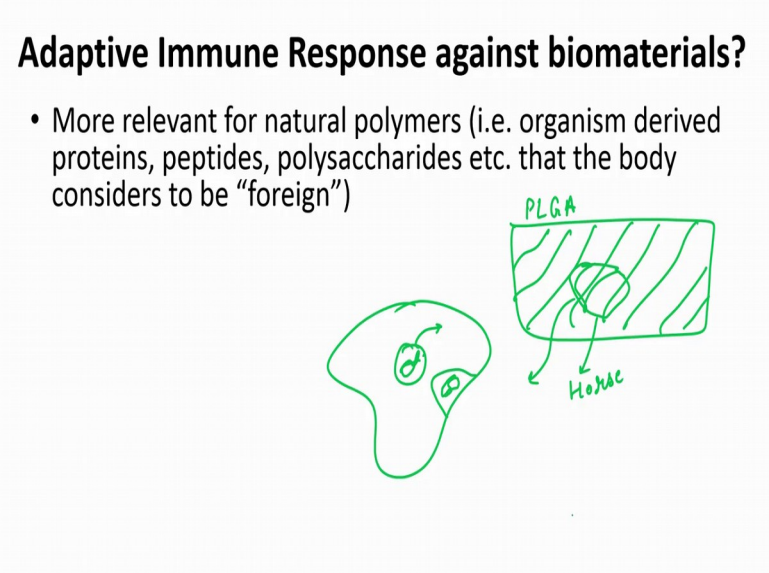

So, what about adaptive immune response against biomaterials, so how does that play around? Now, because obviously we are talking about drug delivery and tissue engineering, how does biomaterial come into the picture here? So, this is first of all more relevant to natural polymers. So, if you have organism derived proteins and peptides and polysaccharides that are present in your body or that you are putting in your body through these materials; obviously, the immune system is going to process those antigens. So, let us say if I have this implant, let us say PLGA again for example and

maybe I am delivering an enzyme to treat a certain disease, which is derived from let us say horse.

Now, this is the protein that body has never seen. So, when this protein gets released out, the dendritic cell will take up this protein and then it goes to the same pathway saying, so this is going to get presented through a class II antigen loading. And then, because this protein has a high concentration in the vicinity of the implant, lots and lots of leukocytes are going to come in, there will be lots of cytokines and that is not going to let this implant to function in a way that was supposed to, so that is one way.

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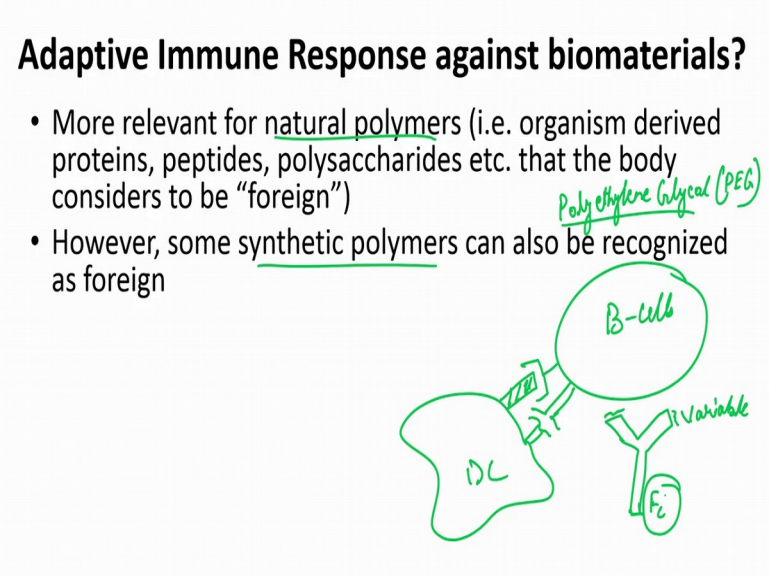

Now, I mention here as natural polymers; however, have already given you guys example in the polymer drug conjugates that even synthetic polymers can be recognized as self. So, I hope you guys remember, so we had talked about Polyethylene Glycol or PEG as one of the polymers that was used to prevent any protein adsorption, enhance circulation, increasing the size, all of those properties; but in this case, because this is a synthetic polymer, we were expecting that the body will not be able to recognize it, but what we started seeing is we started seeing antibodies against PEG.

And so the antibodies are only formed through B-cells. So, you have these B-cells, when they then bind to the DC through their receptors and whatever this receptor might be once it has affinity and recognition and obviously, there is a co-stimulation that also

happens. Once that has happened these B-cells will then start producing these antibodies, and then these antibodies are nothing but these our Y shape structure, I will probably talk about it more in further classes.

And so this is the F C region and this is a variable region. So, these proteins can then go ahead and get recognized by these variable regions. So, there is several of them and this F C is a signal, it is at red flag as saying . So, once it binds to your material or a polymer, it is going to keep on attracting more immune cell; it is going to if its small enough, then the immune cells going to engulfed in and degrade it; if its large, then the immune cells are going to come to the site and try to wall it off. So, all those responses will start to happen.

So, synthetic polymers can also be a candidate to cause a rapid response to happen; however, at least so far the literature has shown that they are not as immunogenic as let us say natural polymers are.

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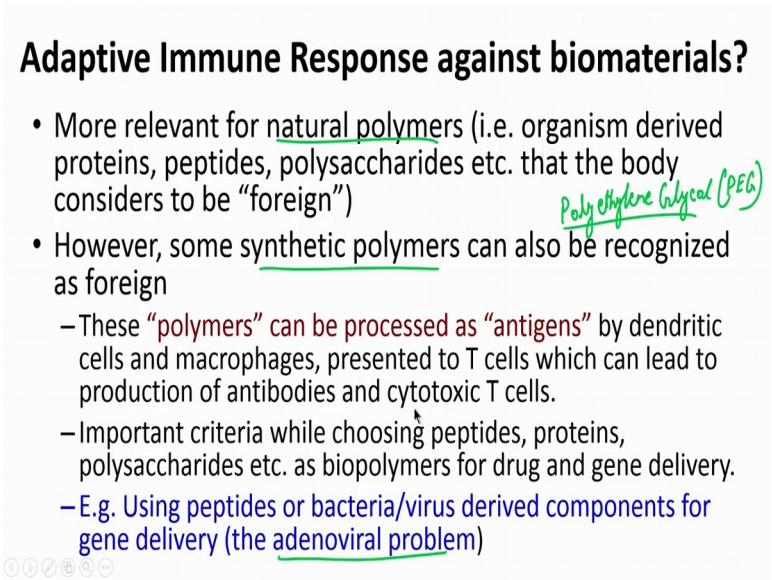

So, these polymers as I said can be processed as antigens, by dendritic cells and macrophages presented to T-cells or B-cells and that can lead to production of antibodies or cytotoxic T-cells against your material. So, this is an important criterion while choosing peptides, proteins and polysaccharides for your biopolymers, so if you if you know that the certain polymer has a history of generation of this, then you may want to

avoid that for any kind of drug engine delivery or any kind of delivery that you are looking at.

So, as an example of adenoviral problem, so when the field started for gene delivery and we will talk about gene delivery in few classes Adenovirus , which is one of the viruses that is very efficient in infecting human cells, was used to deliver these sequence of gene that you wanted, but then this virus gets very easily recognized by your immune system, because it is a viral origin, it has lots of patterns that immune system recognized plus the proteins that this virus has and the core protein at least. All of this is fairly immunogenic, so that became a big problem, because the body was starting to clear these out ok.

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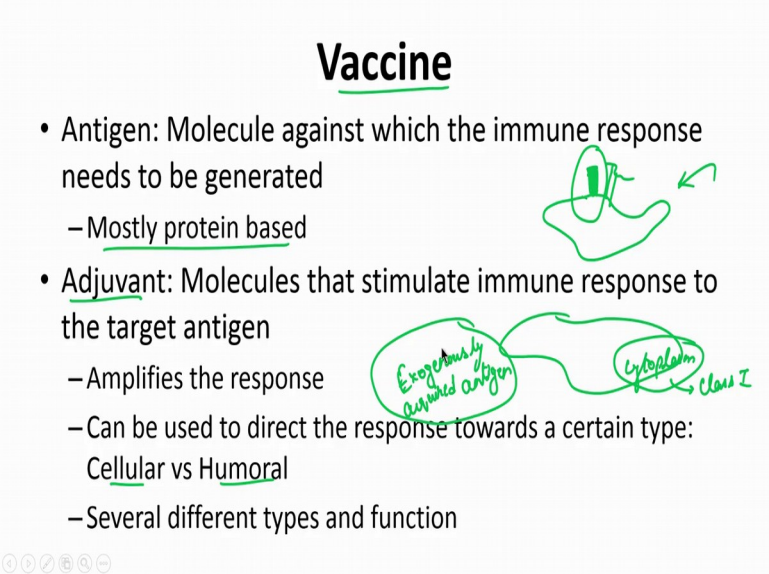

So, the major application of an autoimmune response comes in vaccine. And let us look at what vaccines are, I am sure most of you are familiar what vaccines are, but let us do a quick sort of recap on this vaccine. So, I already defined this, but antigen is a molecule against which an immune response needs to be generated.

And so when we are talking about vaccines, now we want immune response to generate right, because let us say we are saying vaccines against polio. So, we know that polio is a very deadly disease and cause disability to the person it is infecting and we want to make sure that the body is actually prepared, when it sees a polio virus. So, basically we are

generating a vaccine against it, we are generating something that is going to train our body to act whenever it sees a polio virus.

So, typically what you will find is vaccines are protein based, proteins are act as a very nice immunogenic molecules and our bodies are well trained to handle different types of proteins, so most vaccines we will find are protein based. Then you have adjuvants and so what are adjuvants? Adjuvants are molecules that stimulate the immune response.

So, if you remember as I said we require two signals, just the antigen is not enough; we want to make sure that the body also gets activated or the cell that is presenting this antigen is also activated. And it is presenting the co-stimulatory molecule, because if it is only presenting the antigen, it is going to actually cause tolerance against that particular antigen.

And that is going to be complete no in case of any disease, any infectious disease, so you want this to be present as well. So, to cause this to happen, what is done, is an adjuvant is given which is going to boost the immune response or stimulate the immune response. So, these could be something that is commonly seen in certain viruses and bacteria. So, there is nothing but boosting the innate immune response.

So, maybe you put a lipid from a bacteria which is called lipopolysaccharide into the system and when these cells see it they will also get activated along with the antigen that is present, through your protein. So, it amplifies the response. So, not only it is going to actually result in the co-stimulatory molecule being expressed, it will cause more DCs to come in due to chemotaxis.

Again, the type of adjuvant you put can be used to change whether the immunity is going to be cellular or humoral, and this I already defined, so the class I versus the class II. So, class I is something that is derived from the cytoplasm and class II is something that is exogenously acquired antigen. So, depending on the type of co-stimulation you are giving, these are different in cases of different scenarios.

So, let us say it is bacterial derived, which is extracellular you typically get a response which is more humoral or more antibody mediated or if you give a virus, which actually goes into the back into the mammalian cell and will produce its protein into the

cytoplasm and that is going to be more cellular responses needed to kill those cells off that are infected with the virus.

And again they have several different types and function as I said; they are actually quite a lot of these adjuvants that are currently being used both in clinic as well as in research. So, and then they also have different types and functions, some are very immunogenic, some are less, some go humoral, some go cellular, some are even toxic at high doses, so all of that is available and different adjuvants are used depending on what you are trying to achieve.

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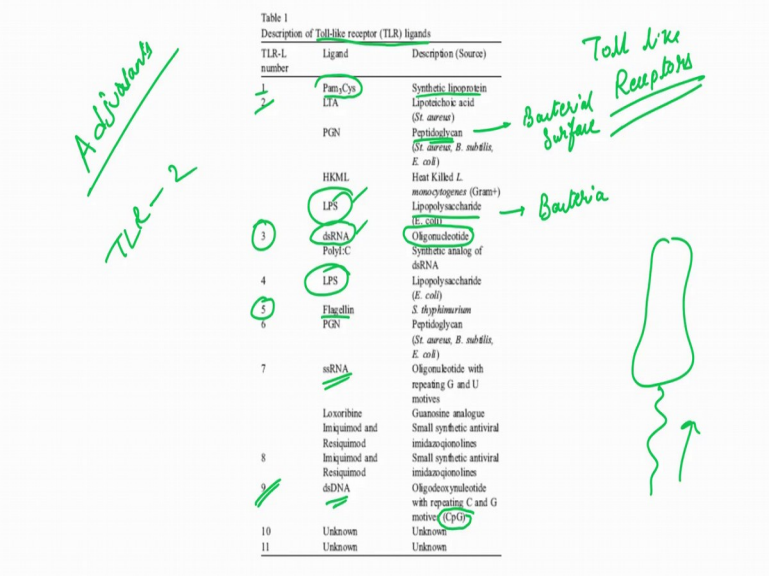

Table 1
Description of Toll-like receptor (TLR) ligands

TLR-L number	Ligand	Description (Source)
1	Pam_3Cys	Synthetic lipoprotein
2	LTA	Lipoteichoic acid (*St. aureus*)
	PGN	Peptidoglycan (*St. aureus, B. subtilis, E. coli*)
	HKML	Heat Killed *L. monocytogenes* (Gram+)
	LPS	Lipopolysaccharide (*E. coli*)
3	dsRNA	Oligonucleotide
	PolyI:C	Synthetic analog of dsRNA
4	LPS	Lipopolysaccharide (*E. coli*)
5	Flagellin	*S. typhimurium*
6	PGN	Peptidoglycan (*St. aureus, B. subtilis, E. coli*)
7	ssRNA	Oligonucleotide with repeating G and U motives
	Loxoribine	Guanosine analogue
	Imiquimod and Resiquimod	Small synthetic antiviral imidazoquinolines
8	Imiquimod and Resiquimod	Small synthetic antiviral imidazoquinolines
9	dsDNA	Oligodeoxynucleotide with repeating C and G motives (CpG)
10	Unknown	Unknown
11	Unknown	Unknown

So, here is more on the adjuvants. So, here are lists of different, so and these adjuvants go and activate and act on something that is present on lots of immune cell which is called toll like receptors. So, there is several of them at least few of them are known and they are listed here and these are receptors that recognize patterns on pathogens.

So, as I said this is more a part of the innate immune response. So, here are some of the examples, so if there is any synthetic lipoprotein that is being recognized by the toll like receptor 1. And then you have TLR 2 that is going to recognize LPS, which I just mentioned is a lipopolysaccharide derived from bacteria. You can have other receptors for this at peptidoglycan is also present on the bacterial membrane, on the bacterial surface.

You can have toll like receptor 3, recognizes a double stranded RNA. So, in our body we do not have double stranded RNA and only have single stranded RNA for the most part and so any kind of double stranded RNA would probably signal that there is virus present, so this toll like receptors are present for that as well. Then you have TLR 4 also recognizing LPS, you have bacterial flagellum. So, flagellum is a protein that is present in flagella and that is causes mobility of the bacteria. So, there is a separate TLR for that.

Similarly, you can have other types of single stranded RNA also, being recognized double stranded DNA, it just depends on what are the different applications. so there could be certain types of patterns within the nucleotides. So, if it is a high in CG content, maybe TLR 9 is going to get activated. There is several of them and you do not need to remember all of these, but some of the major ones you can.

And the major job is to recognize if there is something foreign and something that is a common pattern that is seen right. So, I mean something like double stranded RNA, LPS; these are common patterns with any kind of virus or bacterial proteins. So, the body can immediately start to act on it, while adaptive response can then kick in, later on.

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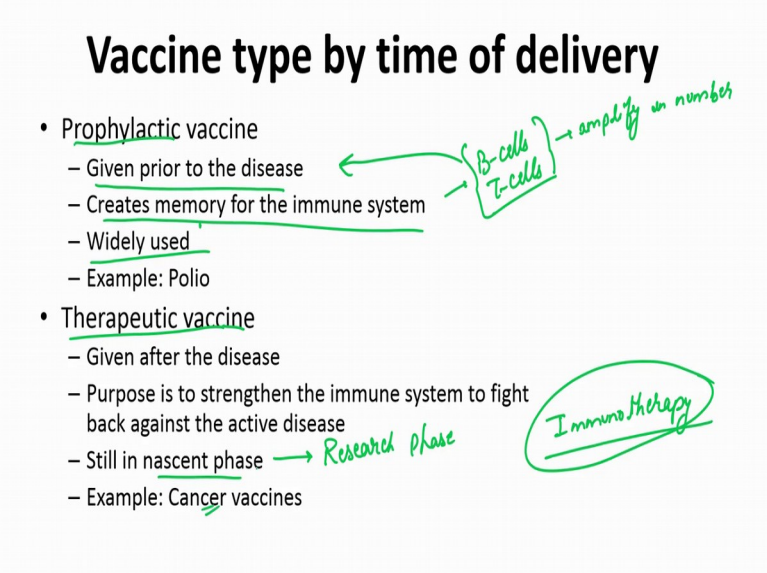

So, vaccine depending on how you are delivering them can be defined into different types. One is called a prophylactic vaccine and I am sure most of you are aware of this and as the name suggests, this is a vaccine that is given prior to the disease. So, we know

that polio is a big problem; we know that it can affect lots of babies. So, why do not we give a vaccine against polio, before the polio has a chance to infect a baby? So, as soon as a baby is born, it is actually mandatory that the vaccine is given to those babies.

So, at this point this is given a priori, the virus has not entered the baby yet, but we are giving the vaccine. So, it is a prophylactic vaccine and what it is doing? It is creating a memory for the immune system. So, the immune system then knows how to handle it, it generates lots and lots of these clones of B-cells and T-cells; that are specific for polio or specific for that particular disease. And this is going to amplify in number, this is because let us say as I said we have trillions of different types of B-cells and T-cells which have different receptors, it will go on and recognize different antigens.

So, let us say polio is an antigen even though we may have 1 or 2 B-cells and T-cells that may recognize polio in our body. Once, the virus infects, we want the amplification to happen very quickly and the virus to be eradicated as soon as possible before it causes any harmful effects. So, for that to happen what we have done is we have pre-exposed these B-cells and T-cells to that particular antigen and for that the body has now amplified these B- cells and T-cells have created actually memory banks, where they have stored that ok; this is a type of infection that we have seen before.

So, a certain type of infection the same type of infection comes back, these cells can then generate in quite a lot of numbers and can then start to kill those viruses away or whatever that is maybe the bacteria, maybe the virus, the body knows how to tackle that. Whereas, let us say if the body was never exposed to it and the bacteria the virus was there introduced into the system and this virus will have quite a lot of time to amplify and maybe it will amplify to such a high level that the body will not be able to handle it anymore.

So that is why it is here, it is actually very widely used, this is one of the most successful stories in the healthcare is the vaccine, we have been able to eradicate quite a lot of deadly diseases, a smallpox for example, polio to a very large extent in at least in most countries have been completely eradicated due to the use of these vaccines and have saved millions of lives.

And then the other is a therapeutic vaccine, you may or may not be aware of this, but this is given after the diseases has happened. So, this may be the disease is fairly diverse and we do not know what type of vaccine to give. So, in this case let us for example, for cancer. So, we do not know what type of cancer will happen, it is a mutation of your own cell, you cannot really immunize against your own cell. So, you then give these vaccines and then the purpose is to strengthen the immune system to fight against the active disease.

So, the body is already fighting against the disease, but you are now strengthening it further, maybe the body is not properly getting activated, maybe there is not enough co-stimulatory molecules that are present and maybe the body is now instead of killing your own cancer cell is going towards toll getting developed tolerogenic immunogenity against this cancer cells. So, you may want in that case to boost the response maybe you want to have more co stimulatory molecules that can then start to kill off these cancer cells.

This is still fairly in the nascent phase, quite a lot in the research phase, but not a whole lot in the clinic. It is still relatively new, and it has shows some success, but more and more needs to be done before it goes to the clinic, some of it has come in. So, you might have heard of immunotherapy and a part of that actually also involved these therapeutic vaccines that are being used in clinic and in patients, but very sparingly at this point of time. And the hope is that this is going to take over it is such a way that these cancers can be tackled up through these cancer vaccines, but that is still to be seen ok. So, we will ll stop here and we will continue further in the next class.

Thank you.