Drug Delivery Principles and Engineering
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Lecture – 49
Vaccines

Hello everyone. Welcome to another lecture of Drug Delivery Engineering and Principles. We are talking about Vaccines and how drug delivery plays a role in vaccines. So, let us jump into this.

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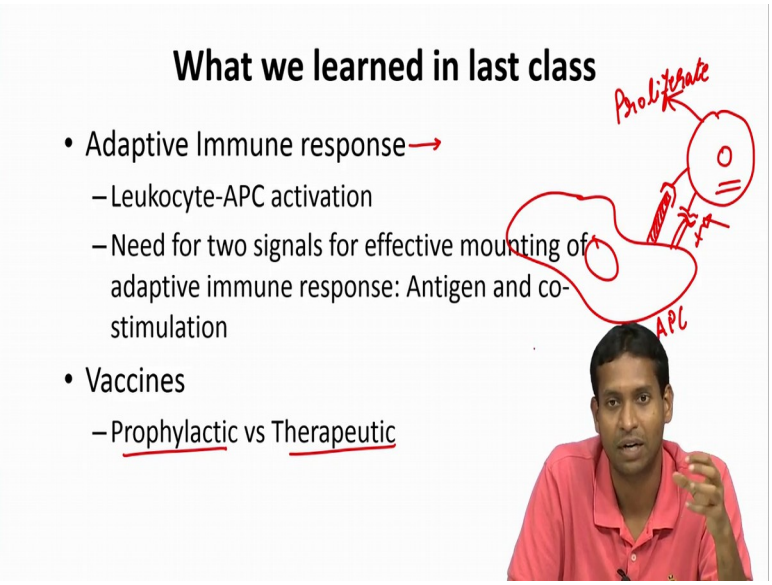

Just a quick recap what we did in the last class. So, we were talking about adaptive immune response. This is an immune response which the body kicks in once the innate immune response is not able to eradicate the infection. The body then uses some adaptive immune response where we have B-cells and T-cells as the leukocytes that start to learn about the antigens, there are lot of mutations in their receptors, and then they adapt to whatever the challenge has been presented to the body. And once they do find a match for let us say a T-cell that can react with a particular antigen, then they replicate in quite high numbers and build up the numbers at the site and eventually able to kill the pathogen.

And then we learned how does these APC activate the leukocytes. So, you will have let us say an antigen presenting cell. It will have receptors through which it will present the

small fragments of these peptides which are derived from a bacterial or viral source or whatever pathogen source it might be. And then you have leukocytes which also has receptors with several different mutations, so we have billions and trillions of types of leukocytes which all have different slightly mutated receptors and so, those get sampled out till one of them has high affinity binding to this particular antigen. And once its born, this then goes on and proliferate generating quite a high number of these leukocytes. And then these leukocytes then go and directly either they kill the pathogen or the infected cell and then somehow is able to protect us from these pathogens.

And then we discussed that this activation actually requires two signals. So, just the antigen signal is not enough, if you only give antigen signal what will happen, that if the APC, which is the Antigen Presenting Cell is not activated then these cells will think that this is a self antigen and this is maybe an antigen from the dendritic cell itself. So, that will lead to a tolerogenic response. So, instead of getting an enhanced immune response you will actually have a suppressed immune response.

If a co-stimulatory molecule is present which gets expressed when these APC's gets activated due to some innate response, this could be bacterial proteins and LPS or some other patterns, then that is get signal to these leukocytes and once both the signal come in only then there is an activation of leukocyte that is going to lead to proliferation of the leukocytes and further enhancement of immunity. And if there is only co-stimulatory molecule no antigen then it is a normal scenario and then nothing really happens to the immune system in terms of adaptive immune response.

So, this is kind of a check that the body has put in to ensure that the immune system is not going haywire for things that may not be pathogenic or it is not really starting to kill its own cells and all of those checks and balances have been put in through millions of years of evolution.

Then we started our discussion on vaccines. Basically, vaccines are a small units of pathogens or it would be the full pathogen we will talk about this today, but these are something that - are given to teach the body that this is a pathogen and if the pathogen comes in future or if this pathogen is already existing you how does the body can handle that. So, it creates a memory bank of these effecter cells leukocytes and all which if required can be then called upon very quickly, so that when the threat actually arrives the

body is already prepared for it ,it has clones of these cells ready to fight that particular pathogen or the condition.

So, in vaccines we discussed vaccine content then type on the basis of its timing. So, we discuss a prophylactic vaccine is something that is given before the disease has taken place. So, which is the most common scenario, we take polio vaccines, we take some other BCG vaccines, and these are vaccines which are given in anticipation that a baby may get a virus or a bacteria in future.

So, these are given ahead of the time. Their main purpose is to generate an immune response, have that immune response tabulated in some memory bank, - and that bank is nothing, but the memory cells. Then these cells are ready, so when the actual pathogen comes in these memory bank will release all these cells and these cells are going to continuously ensure that the pathogen or the infection is cleared.

And then the other therapeutic response or therapeutic vaccines and in this case the disease is already there, but the immune system is not able to handle the disease very well, maybe it is not getting activated to the amount it needs to, maybe it is going in a different immune response then what is required for that particular condition. So, this is given after a person has been suffering from a disease and this is to boost immune response more directed towards the disease.

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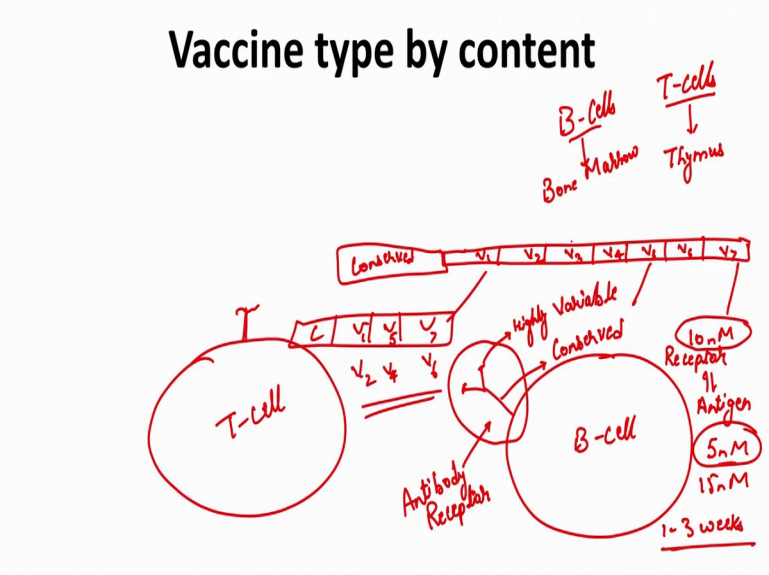

So, we are going to learn more about vaccines and type of content. Before that I just wanted to point out a couple of things, which is that these B-cells and T-cells, which I am classifying as leukocytes, are the ones that are mainly responsible for your adaptive immune response. So, if we go back into how these B-cells and T-cells are able to adapt and tackle different infections, that is what we are going to discuss briefly now. So, T-cells are of course, generated in thymus and B-cells are generated in the bone marrow.

And so, what are these cells? It has several mutations that happen. So, I am not going to do a lot more detail of this, but what you can appreciate here is that let us say these are the gene sequences. So, if let us say this is a receptor that is present on a B-cell and so, this is an antibody receptor that is what the B-cells do. So, the gene that codes for it is, let us say this gene. And of course, when we talk about these regions and this is true with T-cells also and so, if I also draw a T-cell. Let us say it has a receptor.

So, there is always some part of this particular receptor that are fairly conserved and then there is some part that is highly variable. So, typically the head portions here and here, these portions are highly variable and the tail portion or where it connects to the membrane these are conserved. And so, when this protein synthesis is happening what usually happens is there are several segments in the gene and these segments can also be of multiple types and so what usually happens, the conserved region is maybe constant. So, let us say this is conserved.

And then when the expression of this particular protein is happening, there is option for the body to choose from different segments. So, maybe it needs and again I have only drawn 5 or 6 here, but there are 100's of these and maybe it wants to choose 3 out of those 100's. So, maybe it picks out one from this section, one from this section, one from this section and that then combines to form this conserved region and then these 3 variable regions and that is what lead to this expression.

So, now this can change. And so, in another scenario, so, let us say this was v1, v2, v3, v4, v5, v6 and v7. So, let us say in this says what I have drawn is this is v1, v5 and v7; in another case it could be v2, v4, v6. Of course, this is I am simplifying this and there are actually names to these, called VDJ region. I am not going to a lot more detail, but at the time of the generation of these cells these things can pick out different segments and that is how the variability arises.

So, that is why we have millions and trillions of these T-cells and B-cells floating in our body. And not only that once they have found out one of the proteins that may be responsible for binding to an antigen let us say, then then there is a hyper mutation that happens in these regions further, to let us say if a protein is binding with an affinity of 10 nanomolar. So, if this is an affinity for a receptor binding to an antigen that is presented by the antigen presenting cell.

So, then there will be further hyper mutation that is going to happen in these segments from that particular cell and that can result in again millions of B-cells and T-cells being generated. And from that particular segment, so now, they all will have let us say this was v1, v5, v7. So, they will all have even v1, v5, v7, but they will be a hyper mutation in these segments, and that can then result in this 10 Nano molar can either go up or down.

So, maybe a few of the clones go and have an affinity of 5 Nano molar. And then some have 15 and there will be of course, all the ranges here, but then the more the binding affinity, so lower it is basically means that it is more effectively binding. So, it is going to then promote these B-cells and T-cells to then interact further with their antigen and be able to kill it.

So, that is how this amplification happens and that is how our body already has quite a bit of immune response. But once you have; so, all of this takes time. So, from all the way down to here it may take anywhere between 7 to 14 days or let us say 1 to 3 weeks. And well then these are generated these can go into memory bank. So, they some of the numbers, so, most of these numbers when they amplify when the infection is gone and they die out, but some of them gets conserved and are sort of just circulating or remaining stationary in some lymph nodes where then when the same infection comes back again, these cells are now ready.

So, they are not going to take 1 to 3 weeks, they will generate these numbers within a couple of days. And so, that is how you can then prevent the disease from happening because if you wait 1, 2, 3 weeks then maybe the bacteria or the virus has enough time to expand the infection so much that the person can die, but then if they only get one or two days and then an immune response is very effective and clearing them before any threat to the human being is faced. So, this is in very brief I mean obviously this is a huge topic

you can read about it in various books or on the internet, but I just wanted to expose you of how this variability is already present in the body and then how it can further mature into getting a very effective response against any particular pathogen ok.

So, now having this background let us get back to vaccines. So, vaccines can be divided on the basis of their content. So, we have previously seen they can be divided on the basis of the timing. So, let us see what content means here.

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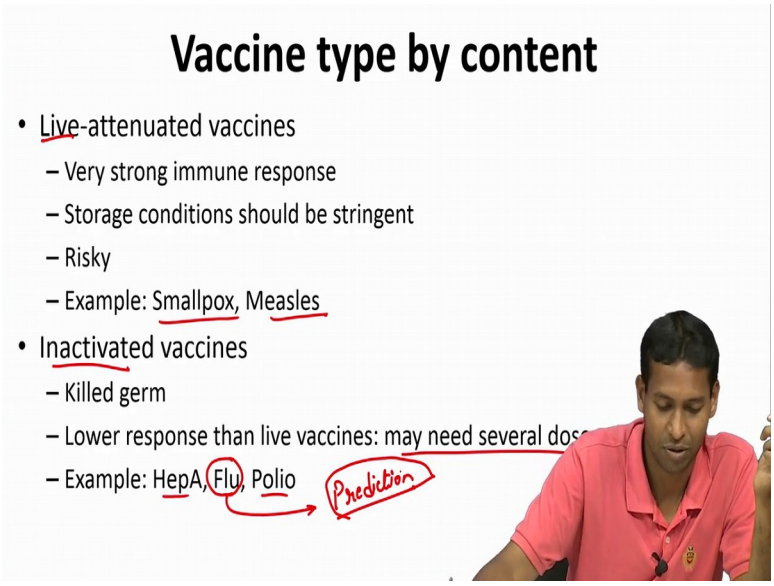

So, we can use a live attenuated vaccine. And what these are is basically these are let us say if you are trying to deliver a vaccine against poliovirus. You can take a poliovirus, you can somehow attenuate it which basically means you somehow decrease its efficiency or virulence and that could be done either by heating or by treating it with some reagents that may cause it to be slightly subdued then what it normally is, in nature.

So, these generate then very strong immune response, just because they have basically all the units that are needed to be there and they can then really activate all parts of immune response which are responsible for eradication of this particular pathogen in our body. The problem with this is the storage condition should be fairly stringent because let us say if you have a live attenuated virus or you have a live attenuated bacteria you want to make sure that first of all it is alive because only then it can generate the immune response that is required.

And then secondly, not only that, but you also want to make sure that it does not get its virulence back. Because what happens if in case your storage conditions are not right and the virus or the bacteria whatever it might be, is able to get the virulence back, Then it can when you inject it as a vaccine it can actually act as a driver to cause the disease and that is the last thing you want - because that will essentially mean that we are giving the disease to the patients themselves or the healthy person in that case.

So, the storage conditions need to be very stringent. It is fairly risky because of course, you have said that you have attenuated it, but then there is a chance that maybe the patient or the healthy person that you are injecting this in may itself be not very healthy at that point, and they may be suffering from some condition which the immune response may not be able to mount enough activity to clear these attenuated vaccines. In that case the virus or the bacteria will have chance to then adapt to this environment and become virulent again. And so, that is why they are fairly risky because if that happens then like I said again you are giving the disease to a healthy person or to a patient which is a complete no, you do not want to do that.

Some of the examples here are smallpox and measles, both of them are very successful. Small box of course, we have eradicated already, it was a big menace in the early nineteenth century where actually, nearly one-fourth, one-third of the population was getting wiped out whenever these outbreaks are happening. So, this live attenuated vaccine were used to treat smallpox and they were fairly effective, in fact, they were very effective, that to an extent that it got eradicated by this time. Measles still exist, but it is a fairly milder disease, but even then, you can get vaccines against measles and this is a virus based disease and you get vaccines against it and eventually these work well to not cause the measles again.

That is why something I do not know if you guys have ever heard about it or ever experienced this yourself, but once you get measles you never get it again. And even though this is a more extreme form where you instead of, so that is by nature you are getting infected with measles the body is now learning about the measles that is why you are getting infection and you are suffering from that disease for the first couple of weeks, but the when the body adapts to this measle virus it is not able to mount a response to first of all clear the existing disease and then also have a memory bank ready. If whenever the next measles virus comes in the body can then handle it.

So, there is a classic example of how does the vaccine work. Obviously, this is an extreme case where you first are getting infected with the disease and then learning about it. So, that is what vaccines are doing instead of having you suffer for the 2 weeks or sometimes it could be life threatening as well, you are basically giving and teaching the body how to handle an attenuated vaccine which will not cause the disease and then when the actual virus comes, the body is already ready. So, most of these things are typically given when we are babies and so, that we are already aware of all these diseases that are there, or immune system is already aware of all these diseases and we are protected from most of them.

The other example is an inactivated vaccine. So, as the name suggests in this case it is not alive anymore it is inactivated, which means that you are either killed the germ or maybe you heated it to such an amount that they have all died or you maybe have treated with certain reagents that they have died they cannot really come back alive.

So, of course, they have a lower response than live vaccines. Sometimes they made may need even several doses. So, you might have heard about this or might have even gotten injections several times for a particular vaccine. So, the doctor may ask you to come 7 days after the injection again to get another injection or another booster dose, is what it is referred to in the literature. Sometimes these booster dose could be months apart, some its 1 month or 6 months, a lot of hepatitis vaccines are like that.

So, and that is basically and is needed because unlike these live attenuated vaccines, these vaccines are not as effective in generating immune response as you are live attenuated vaccine. And the whole reason for that is the because it is not alive, it is not replicating, it is not performing various functions in which the immune system can see what all different functions its performing and target all those different functions. These are killed germs, so they are just lying there. So, body does get exposed to some antigens, but maybe not all the antigens, maybe not the antigens that these viruses and bacteria produce.

So, they are not as strong as the live attenuated vaccines. But they are still fairly strong and with some booster doses, these work very well. As an example, for that as I said is a hepatitis vaccine, you have polio vaccines and flu as well. So, all of these work very well with this.

Flu is of course, a little tricky in this regards, because the flu virus can actually mutate and so and mutate quite rapidly actually all virus can mutate, but flu virus we know is fairly notorious with this mutation. So, it can mutate several times it happens like within a year itself it can mutate several times. So, you get flu multiple times even though you would expect that once you get the flu at least for a few years you should be protected, but it is mutating at such a high rate that even though your body learned about whatever flu you got, maybe 4 months down the line when this mutated flu comes in, this is so different from the original flu, that it is almost a new entity that the body will have to relearn everything again and to be able to tackle this flu virus.

So, these are some of the challenges with all this, but inactivated vaccines are used for this they will take the current flu and then they will inactivate it and then finally, inject it. Sometimes, actually for the flu virus what is also done is there is some prediction involved as well. And what do you mean by that, they will start predicting as to how would the flu mutate?

So, what are the different segments, what are the different proteins, what are the different receptors with the flu can mutate too and then they will try to immunize against all of those by designing those are synthetic flu viruses which are already mutated in what the thing is through these predictions. And because of that then even in the flu virus mutate and let us say if you already got it gotten very similar synthetic virus or mutated virus already, then the body is ready for it. So, and those things can also be taken into account.

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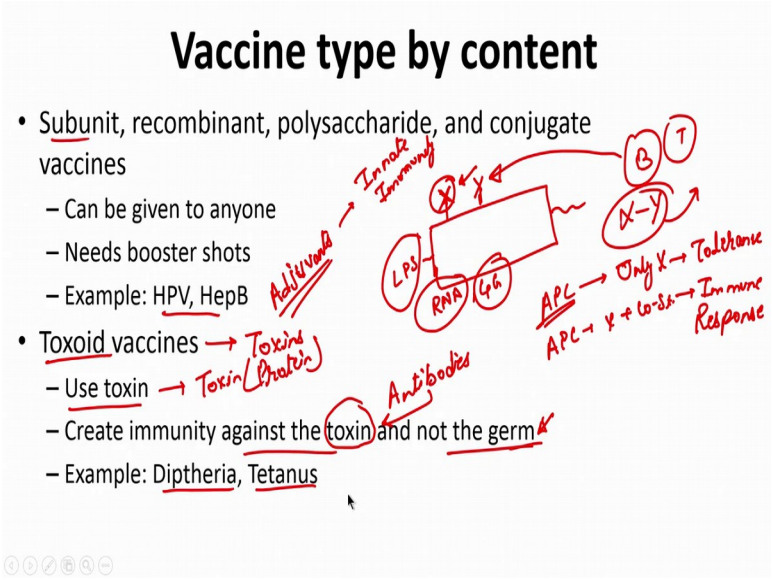

So, let us look at another type of vaccine by content and this is subunit vaccines, also sometimes could be recombinant or a polysaccharide or some conjugate vaccines. So, in the previous two cases, the bioengineering was not really involved much, right if it is a live attenuated vaccine all you are doing is taking the pathogen or the germ you are just heating it up or treating with some reagent and this injecting it. So, this is really no bioengineering approach there.

The delivery also does not as critical at that point. And the same thing with the inactivated vaccines you are basically killing the germ, then injecting it back in the body several times, if it is a booster dose requirement. And so, you are not really talking about any engineering aspects of it although with flu there is some engineering aspects of prediction and all, but then these vaccines which are subunit and recombinant vaccines these have quite a lot of bioengineering involved, and we will talk about that.

So, first of all they can be given to anyone. It does not have to be somebody whose completely healthy even if the immune system is weak you can give this, this will never cause the disease. And even in that inactivated vaccine there is a chance that maybe you did not inactivate all the bacteria or all the virus that may cause disease, but in this one there is absolutely 0 percent chance that this will cause the disease. So, because of that it is not as immunogenic its actually even lesser immunogenic then let us say your inactivated vaccine.

So, it may require booster doses for certain applications. And some of the examples for this are HPV and hepatitis B and basically what we are talking about the subunit vaccine is let us say this is a bacteria. We know that bacteria has lots of protein and some proteins may be conserved in a pathogenic bacteria let us say there is a protein x that is fairly conserved on this pathogen.

So, what you can do is you can take this x, you can produce it as recombinantly and what that does mean that you produce it using some bacteria some other bacteria in mass quantity. So, you generate grams and grams of this. Then you take this protein inject it back in the body. And so, what will happen is now that the body sees this protein x which it identifies as foreign because it is not a part of the mammalian system. So, it will generate an immune response those same thing and the B-cells and T-cells will get generated against this protein x and once we have high affinity binding of these things they can go into memory bind.

So, now let us say now actually this bacteria comes in and because this is a fairly conserved proteins. So, whatever that bacteria is we will still have this protein x, this concept of the body is already ready. So, those T-cells and B-cells can then quickly amplify in numbers, since they already are in the memory bank go and attack this protein x containing bacteria and hence cause the clearance of this. So, you are protected. So, that is one way. The another could be subunit; so, maybe you do not even want the whole protein x maybe there is a small segment of protein x that you can work with or instead of protein maybe it is some polysaccharide on the surface. Maybe some sugar moiety or some conjugate vaccine, maybe there are two proteins x and y and you just want to combine x and y using some protein engineering and then inject it back in the body.

So, all of this is within this class of subunit vaccines and its fairly effective at least in the animal models and some examples have you have already seen. So, these are these have been successful, although they are not as immunogenic as what do you see with the attenuated the live attenuated as well as the inactivated vaccines. One thing to know to know here is, so when he was injecting the inactivated and live attenuated vaccine you are actually giving adjuvant along with it, right.

So, I mean if it is let us say inactivated bacteria it has all these LPS or some other patterns there, RNA is there DNA, CPG islands. So, all of those things are present

already in those vaccines. So, you do not need to give the adjuvant. So, what they will ensure is even if the antigen presenting cell is presenting this protein x, its actually presenting it along with a co-stimulatory molecule because these molecules will also enhance the innate immunity and these APCs will be then presenting the co-stimulatory molecule.

However, now you only inject the protein x. This protein x may not be one of those molecules that can trigger an innate immunity to upregulate. And because of that what will happen is that the x by itself, even if it is presented through an APC. So, let us say if an antigen presenting cell is presenting, it is not displaying the costimulatory molecules ,and this is the last thing that you want. Because if you do that then what will happen is the immune system will think it is a self-protein because there is no co-stimulatory molecule and it may actually go into tolerance mode. So, if you only do this with only x ,you may go into tolerance.

So, to overcome this what is done is along with the x there are some adjuvant that are also given. So, to some adjuvants such as LPS or the CPG or there are several others are also given to ensure that the innate immunity is also hit at the same time, and that would mean that APC has both x plus co-stimulatory molecule and that could lead to good immune response ok.

So, then there is another type of vaccine which is called toxoid vaccines and so, what are toxoid vaccine? As the name suggests, this is against some of the toxins that are being produced. And let us say the bacteria acts by producing a particular toxin, bacteria by itself is not really the main cause of the disease, but these toxins that maybe its producing maybe it is causing the lysis of your blood cells or maybe it is causing lysis of endothelial cells.

So, maybe these toxins are the one that are mainly responsible for any toxicity that is seen. And the bacteria by itself the body may be able to handle it even if it takes 3 weeks it is not a problem as long as these toxins are not present. So if that is the case, these toxoid vaccines can then be used. And so, this is basically nothing, but you are still injecting a protein. So, instead of this protein x, you are now injecting the toxin which is of course, also a protein in most cases. So, this is to create immunity against the toxin, but not the germ.

So, the body then only sees the toxin along with some adjuvant of course. It never really sees the germ. So, although this toxin is a part of this job it may be secreted it may be intercellular, or on the surface. So, this germ when it comes the body is not very directly attacking the germ, but the toxin that is produced. So, maybe it has generated antibodies against this toxin. And because of that, so what will happen? If the germ is producing this toxin the antibodies are constantly neutralizing this toxin and not letting it cause any harmful toxic effect that it was causing in case the vaccines were not given.

So, if it was killing endothelial cells now it cannot kill because already an antibody is bound to it and got cleared, it is not able to reach its target. The same thing if it was causing lysis of red blood cells, then it cannot access red blood cells because these antibodies are first neutralizing it already before it can go and bind to red blood cells. So, those things then can neutralize this toxin and very common examples are diphtheria and tetanus which are again widely given when we are babies and they are able to target the toxins produced by each of them by giving those toxins without at a modulated dose to generate immunity against it ok.

We will stop here and we will continue rest in the next class.