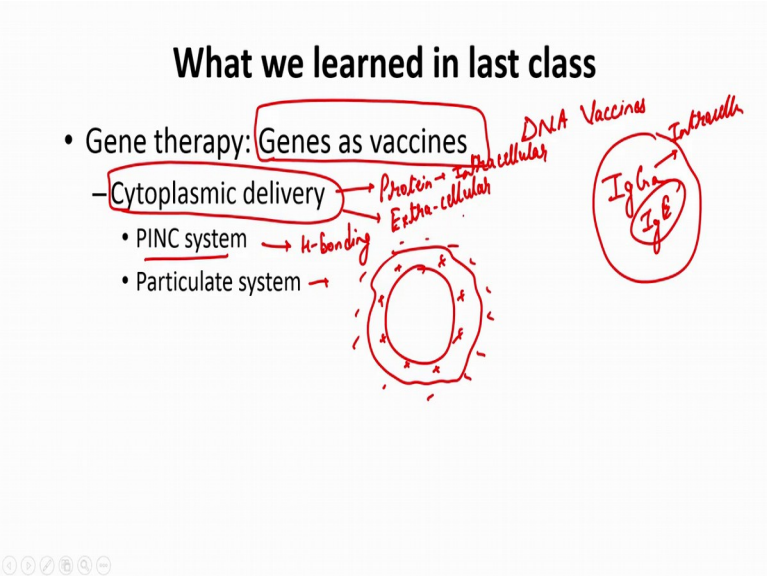
Drug Delivery Principles and Engineering
Prof. Rachit Agarwal
Department of BioSystems Science and Engineering
Indian Institute of Science, Bangalore

Lecture – 56
Vaccines Gene Delivery and Other Variants

Hello everyone. Welcome to another lecture of Drug Delivery Engineering and Principles.

(Refer Slide Time: 00:33)



We have been talking about Gene therapy for the last few classes now. So, in the last class, we particularly started looking into an aspect of gene therapy which is using genes as vaccines, also sometimes termed as DNA vaccines. And the major thing here is to be able to deliver your gene cytoplasmically or your protein cytoplasmically; however, having said that, you can still deliver your gene or protein of interest in the extracellular environment.

So, the protein is something that is secreted out from the cell, then it is going to come out too. So, you can target both pathways - both extracellular as well as intracellular and depending on what pathway you are targeting, you will get different type of human response; it could be cytotoxic, it could be a IgGa mediated or it could be IgE mediated all depends on which pathway has been targeted. As I as previously described, IgGa is more towards the intracellular response and IgE is more towards your anaphylactic and

more allergic type of response. So, all of this can be changed and out and we will we will go a little more detail into this in today's class, but before that we also discussed PINC system, which is not forming complexes, but just forming hydrogen bonding with the polymer, acts as bulking agent.

So, very simple system then we talked about particulate system we had only talked about cationic polymers and then this case we talked about particulate system where you make positively charged particles. And then you can put your DNA which is negatively charge on the surface which can then be used to deliver it in your target cell.

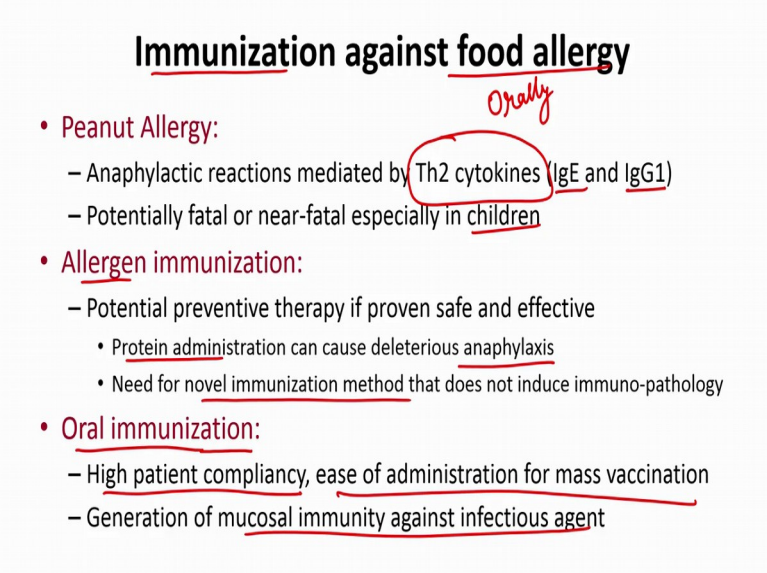
(Refer Slide Time: 02:45)

So, let us look at one example of how this was used in one of the paper. So, this is we are talking about an immunization against food allergy. So, food allergy is a fairly common. You find that lot of people have certain restrictions that they may or may not be able to eat any type of food especially something like crabs, fish. These things illicit quite a bit of allergies and not only the it does not have to be a non-veg food, it can also be something as simple as peanuts.

This is a big problem actually, in western countries, where quite a bit of people; if the intake peanuts, they get a huge anaphylactic shock. They are unable to breathe sometimes and a lot of people actually die because of that. So, we will look into how this food allergy can be tackled; obviously, since its allergies; it is some response of the

immune system. So, can we then train our immune system, do not cause allergies when we intake these food material.

(Refer Slide Time: 03:56)



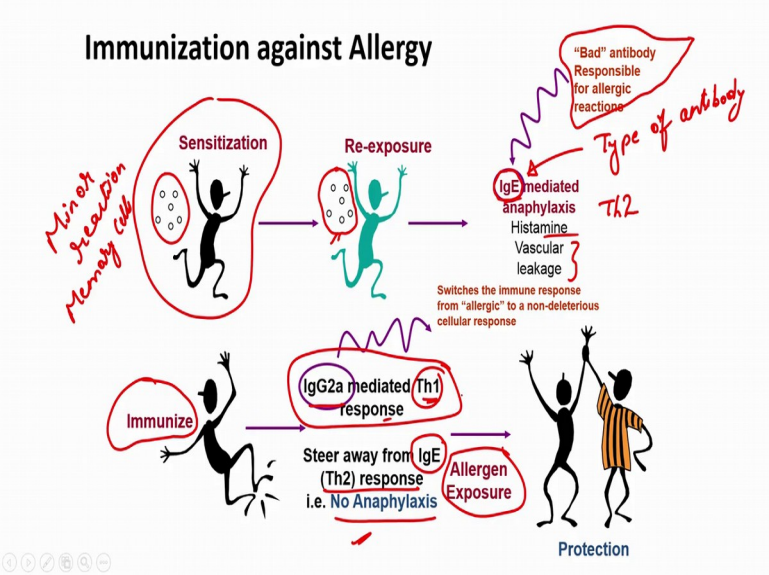
And so, we will focus on peanut allergies for this example now. So, peanut allergies, they cause anaphylactic reactions and that is because you get this Th2 based cytokine response; immune response which results in IgE and IgG1. And this is potentially fatal or near fatal especially in children because lot of the time these babies and children are given food that may contain peanut without knowing that they may have allergies. And it may then result in quite a bit of immune response and they may not be close to a hospital setting and sometimes that even causes death unfortunately.

And the major allergen is a protein in peanuts that is present and we will talk about that in a moment. But how about we immunize a body against this allergen present in the peanut? Would that prevent the immune response to mount in a certain way to cause this anaphylactic shock? That was the purpose of this study. And so, we are saying that this protein administration can cause this anaphylaxis. So, how can we develop some normal immunization method so that we do not cause this anaphylaxis.

And then the oral immunization can be done with this because; obviously, all of this is taken orally. So, when we are taking food, we are taking it orally so, can we use oral immunization, because that will be fairly compatible. So, high patient compliance and ease of administration to mass vaccination, so, you can at a time as the babies are born,

you can easily administer this and it is not a problem and you can generate mucosal immunity against infectious agent also through oral immunization, but in this case we are mostly focusing on food allergy.

(Refer Slide Time: 06:05)



So, here is what a typical patient lifecycle with this allergic response would look like. So, maybe the first time they eat it, they get sensitized; they get some small immune reaction. So, you can say minor reaction. The first time they eat it, depends on the quantity. So, minor reaction and now because of this minor reaction, the body is now exposed to this antigen which then means that the body is now getting trained. So, it is acting as a vaccine, but a bad vaccine at this point where the body is getting trained to tackle this in a much more serious manner when it comes again.

So, the body has generated memory cells for this. It is now able to bind to it very strongly; it is able to produce lots of cytokines in presence of this. So, activate the immune response in a big way. And now the second time or the third time the patients or the folks suffering from this peanut allergy, take this peanut; the body is actually ready for it.

And unfortunately in this case, the body mounts such a high immune response that there are so many cytokines, there are so many other immune responses that are generated that the body goes into anaphylactic shock. And what that is, the blood vessels dilate, that

causes the blood flow to decrease everywhere, you may not be able to get enough oxygen in your brain, in your heart and suddenly it may cause death.

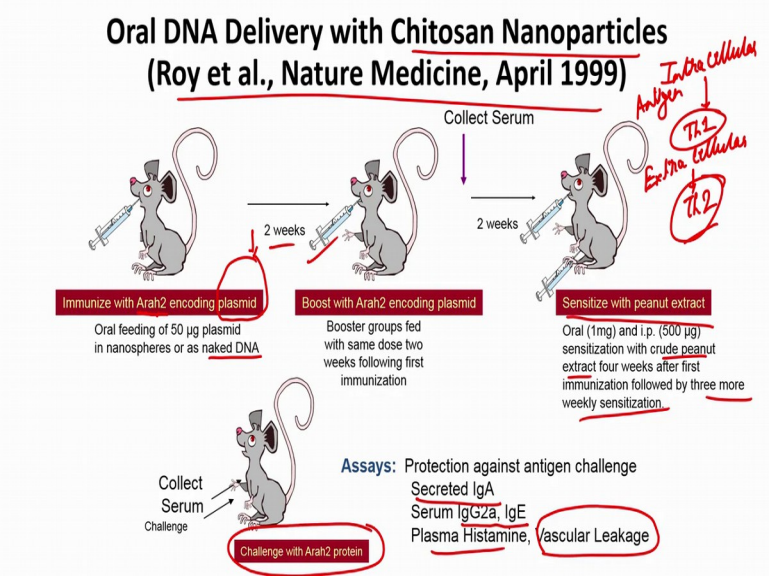
And then the major reason for that is, in this particular case, is that the IgE mediated shock is given. So, IgE is a type of an antibody and in this particular case, this is a bad antibody.

So, if it is a pathogen, it is actually a good antibody, but in this case it is a bad antibody because this will cause anaphylactic shock in presence of high amount of peanuts. And it causes all this histamine to be circulated, vascular leakage, I was talking about and that is not ideal. So, the whole concept here is - let us immunize these folk suffering from peanut allergy.

Once we have immunized them, let us switch from an IgE mediated response to IgG2a mediated response which is a more Th1 response. We want to steer away from this IgE response which is a Th2. And since IgE is the major reason for causing the anaphylaxis in this patient, if the immune system, even though it still is acting against the peanut, if it acts through a Th1 pathway, then there is a chance that we may be able to protect the patient from any kind of harmful immune response effects.

So, once you have done this and you have shifted the memory to this direction. So, earlier the memory was here, but because in a certain way that you have immunized instead of just taking peanuts, we have these authors have done something different and we will talk about that in a moment that has caused a more Th1 response. The memory bank of Th1 has increased and then now if the allergen is exposed, what you will have is a protection. Even though there is some immune response still being generated, it is not going towards the IgE and it is not going towards towards the anaphylactic response. So, that is the whole concept behind it. So, how do we do this?

(Refer Slide Time: 09:53)



So, in this particular paper, they are using DNA delivery with chitosan nanoparticles. So, we have already discussed chitosan being a natural polymer and also containing primary and tertiary amines, it is used quite a bit with DNA delivery and it is a mouse model that they have used. So, what they have done is they have now used the antigen which is the Arah2 and they have put a plasmid that codes for this and they have immunized it with either these chitosan nanoparticles or just the naked DNA.

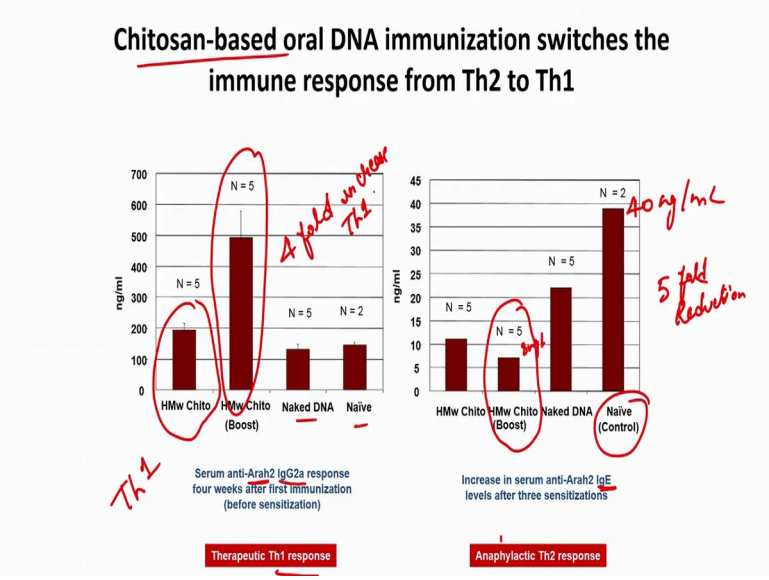
So, the whole concept here is, why do not we instead of having this allergen in an extracellular environment put it in an intracellular environment. So, this antigen because now it is intracellular, it is going to go more towards the Th1 pathway. So, as we already discussed a few times, intracellular will go towards Th1 and the extracellular is more towards Th2.

So, right now it was going towards Th2 because; obviously, we do not have this particular antigen in our body being produced by ourselves in the cytoplasm. So, it is all extracellular when we eat it. So, that was causing the Th2 response. So, if we now deliver it onto a plasmid; that means, now the cells are going to produce this and this is also going to be present in your cytoplasm and that may shift it to Th1. And so, once you do this, you boost it again at 2 weeks. So, you make sure that there is enough immune response that is being generated against this Arah 2 through Th1. So, there is enough memory of it as well.

So, in this case, the authors after two weeks gave the same dose again and then they actually sensitize it with peanut extracts. So, now, they essentially just challenged it to see what happens if you take peanuts, a crude extract of it and you then inject it three more times weekly to sensitize the animal. And then once your sensitize the animal, then you come in and start challenging with the Arah2 protein and see what is the outcome of all this process that you had done on the mouse earlier.

So, you can collect the serum and then once you have the serum, you can then look for whether there is a IgA response whether there is a IG2a, IgE response, whether some of the vascular leakage is happening or not. So, anaphylaxis is happening or not or and similarly the plasma histamine which is another marker for anaphylaxis.

(Refer Slide Time: 12:55)

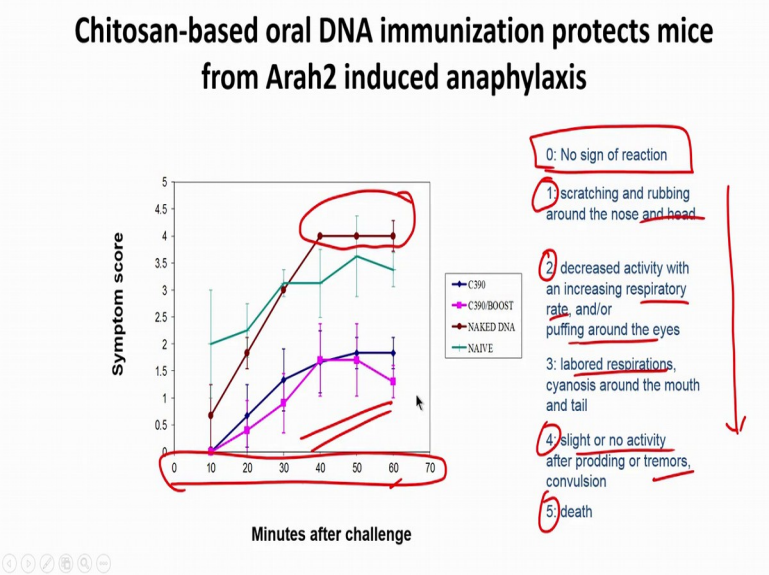


So, let us see what happens so; obviously, the title says here that this chitosan based oral DNA immunization is able to switch the immune response from Th2 to Th1 and here is some data to support it. So, they used a high molecular weight chitosan. So, if they give it only once and they are measuring for IG2a against this antigen which is the response and we wanted. So, this is the Th1 response as it is also written here.

So, what you see is you get a lot more IG2a when you do the booster dose compared to if you just give a naked DNA or naive animals. So, naive animals are obviously not treated with anything, they were directly challenged and so, that is what the Th1 measured through the IG2a response. But if you do the same thing and measure for IgE which is

the anaphylactic response which you do not want so, again you see that naive animals; you see quite a bit of the bad antibody in this case. So, more towards anaphylactic ratio, but in the cases where you given a booster dose, you see quite a bit reduced amount of that. Almost I mean if this is let us say, close to 40 nano gram per mL. Here you talking about let us say 8. So, we are talking about a 5 fold reduction. So, 5 fold reduction in a bad response in this particular case and similarly about 4 fold increase in a mode Th1 response.

(Refer Slide Time: 14:42)



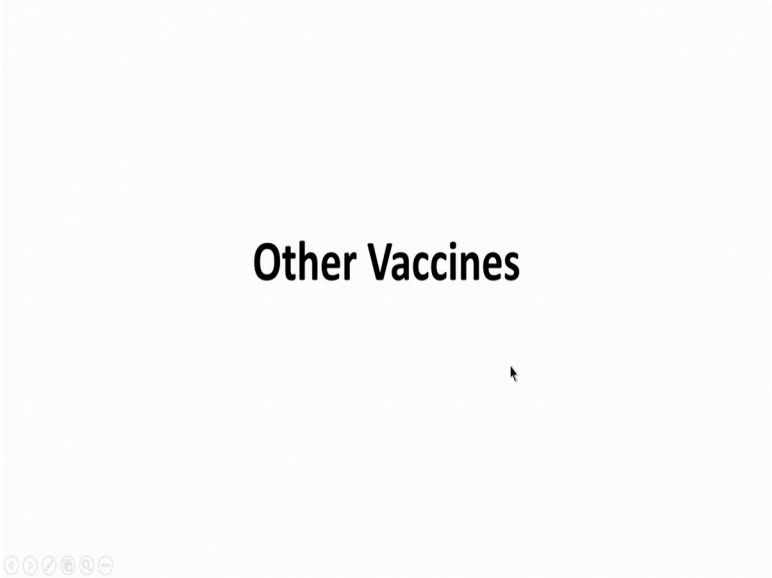
So, here are some more examples of that. So, then they looked at the mouse health. So, they looked how the mouse is behaving. So, they made scale in which they looked at this any sign of reactions. They do not see anything abnormal, then the mouse gets 0 as a score. If they are starting to scratch and rub their nose and head that means there is some toxicity that they are feeling with this so, then the score is given 1.

Then you can also have if the mouse is actually struggling to breathe and trying to breathe quite heavily, it is puffing around its eyes then you give a certain score and then similarly as the severity increases. So, it cannot breathe very well its laboring with that or if it is not even moving around, it seems fairly sick and just tremoring. Then you give a certain score and if it dies then you give; obviously, the worst score and in their study, they measure what are the different scores they get. So, what you see is naive animal is

of course, the worst where they are getting quite a bad response whereas, the animals that were given the chitosan with a booster dose, they do the best in this scenario.

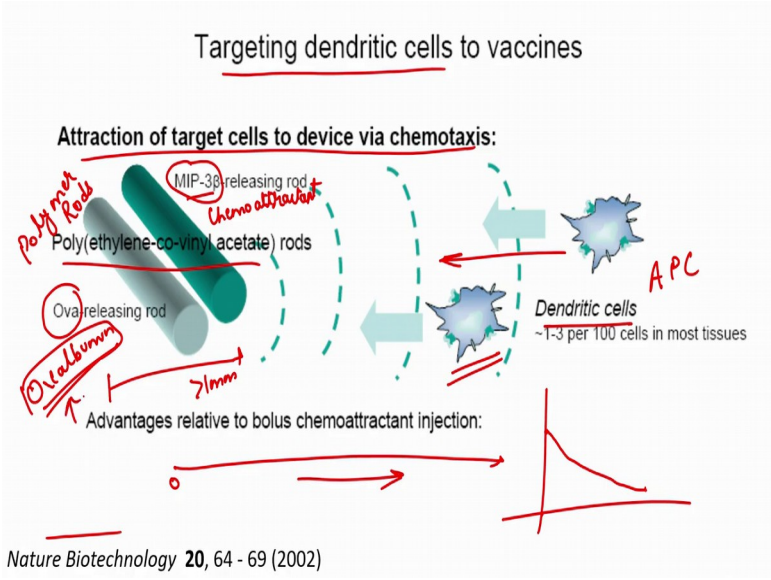
So, if this could be translated to humans. You can have protection of quite a lot of humans from this fatal based allergic toxicity and this is fairly quick. As you can see this is within the 70 minutes of or even in fact, 60 minutes of the administration of the antigen.

(Refer Slide Time: 16:24)



So, we have talked about some of these. Let us look at some other types of vaccines that cannot really be classified in one or the other, but can be used for various applications.

(Refer Slide Time: 16:36)



So, here is a vaccine that was being used to target dendritic cells and in this case, what they are doing is they are making a depot of the vaccine and so, what the authors have done in this paper is they are trying to attract the target cell to the site that they are injecting.

So, far what we were doing is we were injecting particles or even injecting some naked DNA and relying it to move and translocate to wherever we want it to target. But what if we create a depot and have the immune cells come to the site themselves. So, what they have done is they have created these polymer rods. These rods are in this case, they made it out of polyethylene copolymer and they are releasing an antigen which in this case is ovalbumin.

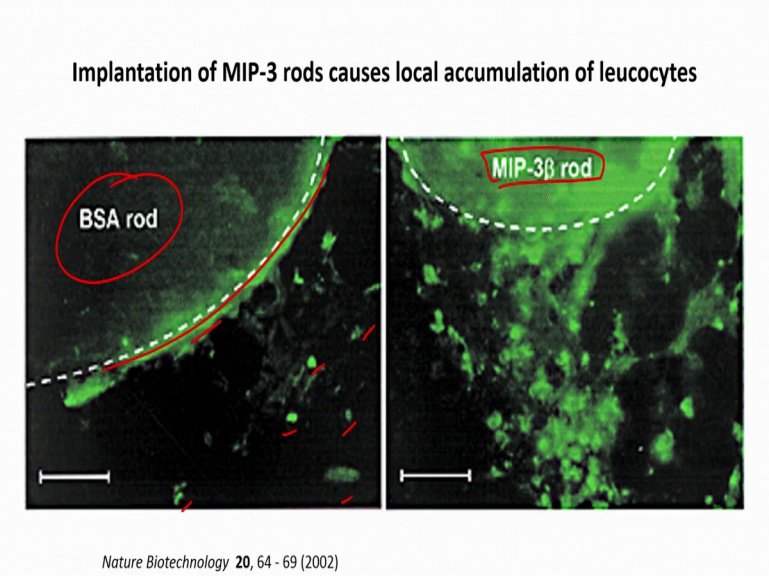
And along with that, what they have done is, they have also put another rod which is releasing a protein called MIP-3 alpha and this is a chemo attractant. And what does it do? It actually attracts the immune cells to the site especially the dendritic cells which are major antigen presenting cells.

So, because of this now there is a gradient of MIP-3 alpha that is created wherever you implant this and these rods are big; I mean these we are talking about greater than millimeter ranges. So, these cannot really move around. They are too big to move in the human body. So, what will happen is they will create a depot wherever they were injected or wherever they were implanted and because this MIP-3 alpha was being

released, these concentration of MIP-3 alpha, if I say that this is my axis, if this is 0 and this is increasing x. So, what you will find is the concentration is highest near the rods as the rods are the one that are producing it and then MIP-3 alpha was diffusing out into the system.

So, as you further move away from it you will see that the concentration of MIP-3 alpha is decreasing away from the site. So, these dendritic cells will sense this gradient and they will start to move towards the MIP-3 alpha rods. Now it is not only the MIP-3 alpha, the Ovalbumin is also being released. So, you have now this protein that is also being released and then this can be uptaken by these APCs, macrophages, dendritic cells whatever is called to the site. And can activate them and generate them immune response against that particular antigen, in this case, ovalbumin.

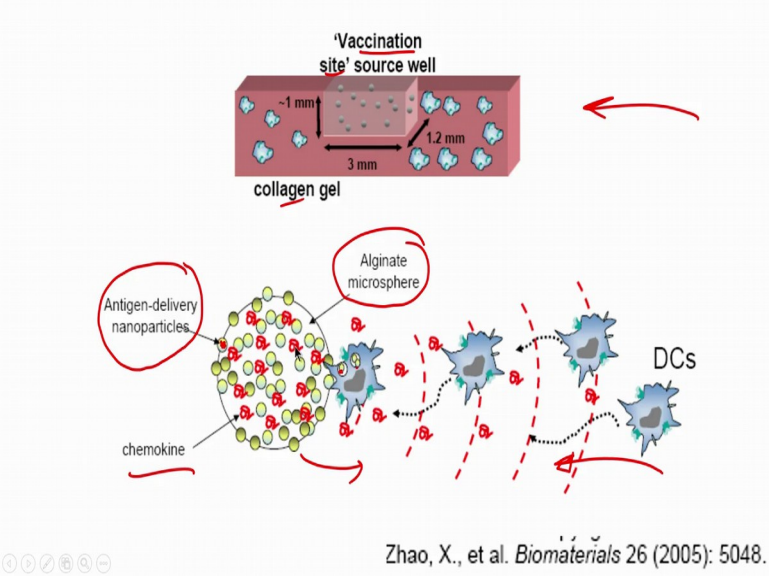
(Refer Slide Time: 19:37)



So, here is just some data from an animal study. So, if they only put BSA rod, this is a zoomed in image. Then green is your dendritic cells that are in the surrounding.

You see that some dendritic cells have come in and started interacting with the surface whereas, and then and so, green is actually leucocytes. So, its dendritic cells as well as some other immune cells and if they have a MIP-3 alpha rod, you see quite a large number of influx of your leukocytes and immune cells coming to the site. So, just reinforcing that yeah, you can actually create a depot and have immune cells come to that depot if you release these chemoattractants.

(Refer Slide Time: 20:25)

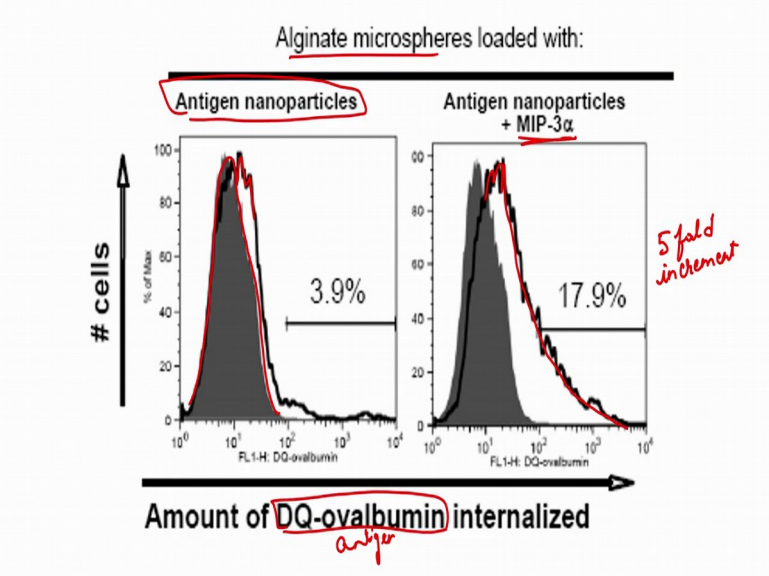


So, very similar concept here; so, in this case again you are injecting a collagen gel and you are creating a vaccination site. So, this is a big gel as you can see, it is about a millimeter by 3 millimeter, and what you are doing here is you are encapsulating whatever antigen and whatever chemoattractants you want and that will cause the immune cells to come to the site. Here is another example. So, in this case, you are putting in alginate microspheres. These alginate microspheres are encapsulating both, first of all, antigen carrying nano particles. So, you have these nano particles which are encapsulating the antigen and then you have also encapsulated chemokines which are the chemo attractants.

So, like your rod in the previous case you will have these things release out. They will attract more and more immune cells, dendritic cells to these alginate microspheres. And when these come in, they will take up your particles containing the antigen and then mount an effective immune response against whatever antigen that you are delivering.

So, basically what you are doing is you are creating a depot where all this interaction is happening. So, rather than moving around and finding your target through the whole body, what do you have done is you have given that job to the immune system that this is where you got to come and get activated and depending on how large and how faster degrading, this can stay there for quite a bit of time and continue to activate the immunity against the antigen that you are using.

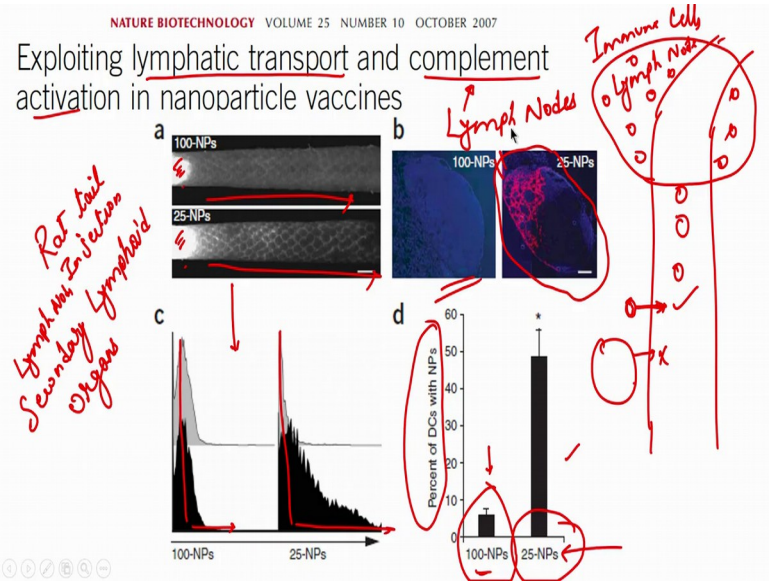
(Refer Slide Time: 22:01)



So, here is some more data on the alginate microspheres that were used. So, if you use just the antigen nano particle, you and you look for the cells. What do you find is if any look for cells that have encapsulated or taken up the ovalbumin, which was your antigen which is labeled by a fluorophore.

What you find is compared to your control; you see a very minor increment in your uptake of your antigen. So, this is antigen whereas, if you compare this with the microspheres, they were also releasing MIP-3 alpha. Then you see that in vivo you have quite a bit high almost 6 fold or 5 fold increment in the amount of cells that are positive for your antigen. And the more cells that are positive for antigen; that means, the more immune and response is going to get activated against this.

(Refer Slide Time: 23:13)



So, here is another type of vaccine. So, in this case they are utilizing the lymphatic transport and they are also using the complement activation as one of the innate pathways to trigger the immunity and so, these are nano particles vaccines. So, what these authors have done is they have made two sizes of particle. One is the 100 nanometer particle and another is the 25 nanometer particle and then what they are showing here is this is a injection in the rat tail. So, these are the base of the tail. So, once it is taken in the rat tail what is happening is then they are imaging and these particles are of course, fluorescent.

So here is your injection sites and then what they are looking for is, then they are starting to image the rest of the tail. And so, what they see is if you have 100 nanometer particle, those particles are not able to move around quite a bit and they rely on some cells to come in and take them up and they have also form a depot. However, if you have particles in the size in your 25 nanometer, what you see is a very organized flow of these 25 nanometer particles through the tail. And this is nothing, but this is the lymphatic system that these particles are now small enough to enter the lymphatic system. These are lymphatic vessels which is slightly more leaky compared to your blood vessels.

So, 100 nanometer particles are potentially too big to be able to go through this, but 25 nanometer particle are able to go through and that is what you are seeing. So, their transport through the lymphatics has increased dramatically. Now all the lymphatics are

directly connected to lymph nodes which are the secondary lymphoid organs. So, this is lymph nodes.

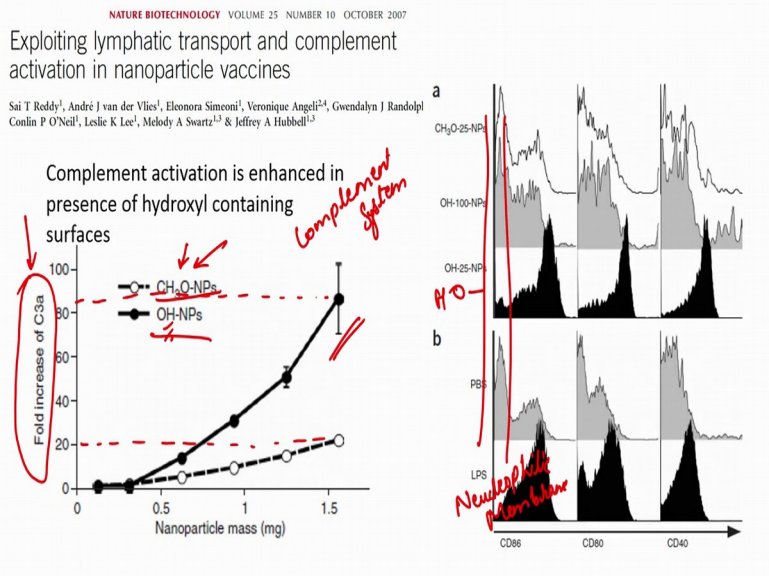
So, these lymphatics are flowing and then they flow into a depot which you call lymph node, this is this is where lots of immune cells reside and this sample out whatever is coming and flowing through the lymphatics and so, now, what you ensure is because you have 25 nanometer entering in, you are ensuring much higher delivery of these particles into the lymph node which is a site of quite a bit immune cells.

So, if you are looking to generate immunity this side is very attractive for your targeting because all the immune cells are there already. So, and that is what they have seen. So, they have image the lymph nodes and you can see how if they do not see any 100 nanometer particles in the lymph node which is closest to the tail vein, but what they see is in the 25 nanometer case, they see a very nice flow of these particles into the lymph node through the lymphatics. And then whatever these particles are carrying and then release at the site and activate the immune response.

So, here again further quantification. So, this is the site of injection and you do not see any much movement away from the site of injection whereas, in these smaller particles you do see that in these lymphatics. And then you also quantify how much dendritic cells have taken up these nano particles at the lymph node and you find that hundred nanometer is fairly low. Whereas, 25 nanometer particles have a much higher amount, in fact, almost 50 percent half of your dendritic cells and now positive for your particles in that site.

So, that is quite a bit a bit of achievement compared to the 100 nanometer particle. So, that is fairly straightforward and intuitive that of course, the smaller particle will be able to translocate to the lymphatics much more easily compared to the bigger particle. Now what these authors have done is they have also targeted the complement system which is not very well studied much in terms of targeting using bioengineering approaches. So, that is what causes this paper to stand out in that scenario.

(Refer Slide Time: 27:47)



So, what they did is, they took this 25 nanometer particle and they put different functional groups on the surface. So, they have put hydroxyl or they have put methyl group on this. And the whole idea here was that, if you remember our initial classes of lymphatics and the complement system, what we find is on nucleophilic membranes, those that contain OH, the complement activation is much higher.

So, one of the strategies we said if we want to prevent complement activation is to not have your membranes or your implants to be nucleophilic whereas, in this particular case, they want to activate the complement system as one of the pathways to cause the immune response to go up against their antigen. So, what they have done is they have now played around with different functional groups.

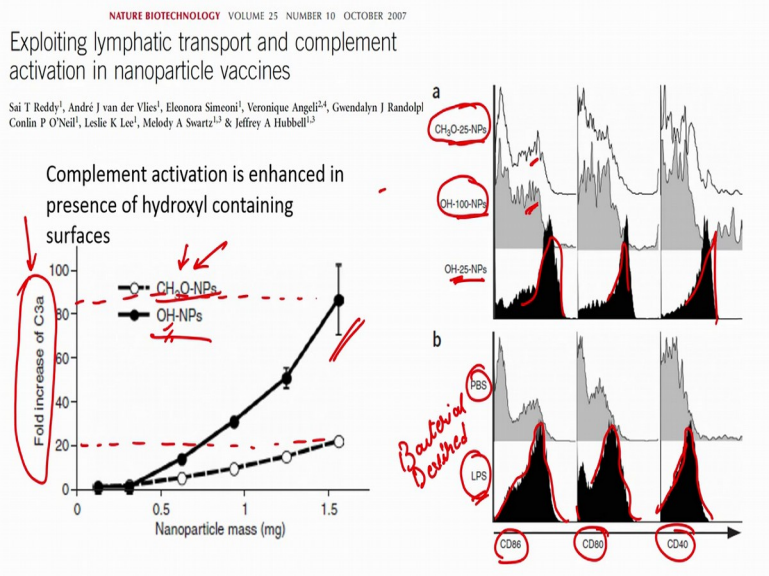
So, this is not nucleophilic whereas this is and what they see is the amount of C3a that they measure and you can clearly see that, if you have particles which contain these hydroxyl groups, you see quite fold quite a much fold increase compared to the basal level. So, with the with the OCH3 particle, they get about twenty percent increment, but with the hydroxyl particle they get all the way up to 80-85 percent increment in the amount of C3a.

So, this is going to act as a good activation for lots of immune cells and then if you now present antigen, it is going to generate much more immunogenic response than without this complement activation. And this is then here further looked into. So, what you are

seeing here is you have 100 nanometer particles with hydroxyl, you have 25 nanometer particles with hydroxyl and you have 25 nanometer particles with this CH3O. And what you are looking at is the activation of your cells, in this case, your antigen presenting cells and so, these are activation markers.

So, CD 86, CD 80 and CD 40 - these are all activation markers that get up regulated if the immune response is activated through various PAMPS, the pathogen derived factors and all.

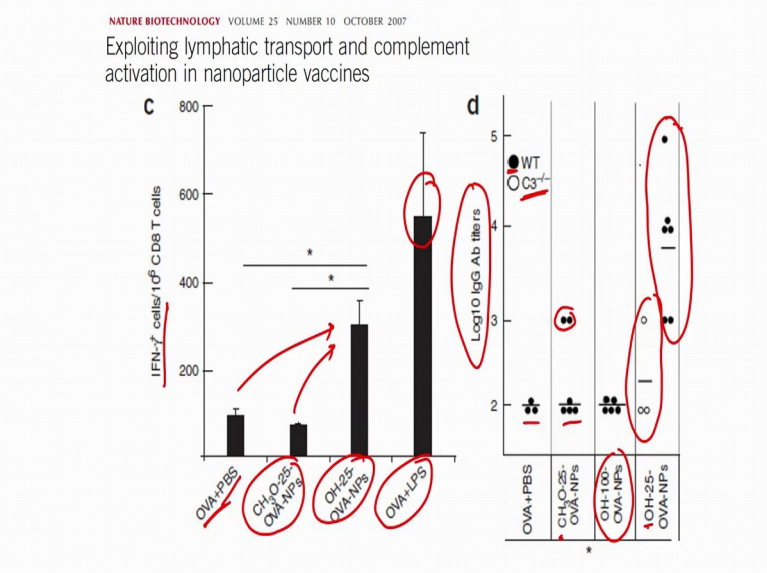
(Refer Slide Time: 30:45)



So, complement is one of the way to do this and what they find and they have a positive control, which is LPS is a bacterial derived lipid. Remember we talked about that LPS is very good in activating your cells and so, you do see that actually. So, antigen presenting cell express a lot more receptor on their surface when they have LPS in the surrounding.

So, this is your PBS. So, this is the basal level and this is after activation in positive control and now if you then compare it with what happens in the scenario, where you are delivering either a bigger particle or a particle which is not as nucleophilic. So, you see that it is very similar to PBS in this case. However, if you do deliver it with hydroxyl particles, you get very similar activation. In fact, in some cases a slightly higher activation compared to your positive control which was LPS in this case.

(Refer Slide Time: 31:47)



And then you can then exploit this to see if you then deliver antigens in this case ovalbumin along with PBS or ovalbumin along with these particles or the positive control in this case is LPS now. You see that you can get quite a lot of IFN gamma cells which is showing that functional increase in that. If you do not get it as much as LPS in this case, but you do get compared to naive and compared to non nucleophilic particles, you get much higher response of this and similarly the antibody titers can also be measured.

And you see that if you have a wild type so, let us look at the filled circles first. And so, you get a certain response; if you just give the antigen. In some cases you get a better response, if you put some methyl terminated particles and you do not really get a whole lot of response with a bigger particle.

But if you use this 25 nanometer particle with hydroxyl, you see quite a bit of response which gets completely knocked down in a knockout animal which does not have complement. So, this shows that this activation is mainly through the complement system that is causing the upregulation of all these markers as well as functional benefits. So, that is where we will stop now and we will talk about more vaccines in future classes.

Thank you.