Drug Delivery Principles and Engineering
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Lecture – 50
Vaccines and Immuno isolated Cell Therapy

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. We are continuing a discussion in the Immune system module of this course and we are talking about Vaccines. So, let us do a quick recap of what we learned in the last class.

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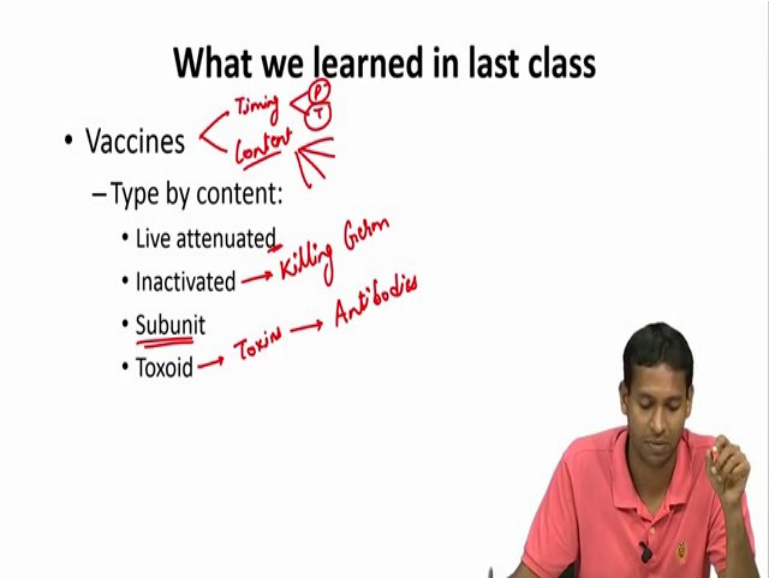

So, in the last class we talked about vaccines as I just mentioned. We had previously talked about dividing vaccines into two types; one through timing and another through content. So, in timing we had said there are prophylactic and therapeutic vaccines depending on when they are given. If they are given before the disease that is prophylactic vaccine; if they are given after the disease that is therapeutic vaccine.

Then in the last class we talked about content and from content, we divided them into four different classes and one was live attenuated in which you take a bacteria or a virus or whatever the germ is. You attenuate it basically decrease its virulence; so, it cannot immediately cause a disease, but it can potentially cause a disease if it persists. So, but you are basically giving enough time to the body to adapt to it before it can cause a

disease. These are highly immunogenic the body reacts to them like it is an actual disease. So, it is very good in that regards, but; obviously, there is a big risk that what if these attenuated vaccines are able to get the virulence back either by some mutation or its allowed enough time and that may be fairly harmful.

So, that is one class; obviously, its being used quite a lot for various things, but there is obviously, some inherent dangers and the practices that are followed in manufacturing of these vaccines need to be absolutely spot on in what they are trying to do. Then we talked about inactivated vaccines. So, just to overcome this instead of using an attenuated germ, we are basically killing the germ.

And they are also fairly managed immunogenic because they have every component of the germ, but just that the germ is not alive. So, there is slightly less immunogenic in the live attenuated and because of that they may sometimes require booster doses; that means, you may have to inject these vaccines several times for the body to develop effective immune response against it, but there again widely used. There are several examples that are given to humans and which we discussed in the last class.

Then there are subunit vaccines, which is where the major engineering lies in and we are going to see how this happens in future classes. But what we are doing here is we are completely taking the bacteria or the germ out of the scenario; we are just homing in on some conserved proteins that we know most pathogen will have when they actually try to cause a disease. And we are training our immune system against that. So that the immune system is now well versed as to how to handle it when, the actual thing arrives; it can directly attack those conserved proteins.

And that because fairly quick clearance of the disease from the body rather than actually causing the disease. And then, there were toxoid vaccines which are mainly directed against the toxins only and not the bacteria itself. So, these may be security toxins. So, if they are secreted then, we are looking at antibodies as the major defense. So, like diphtheria and tetanus is some of these where, you are ensuring that the toxin is not able to cause harmful effect because the body is already ready for it. It has several antibodies that are able to bind to it and neutralize it and hence, there is no toxicity seen in humans ok.

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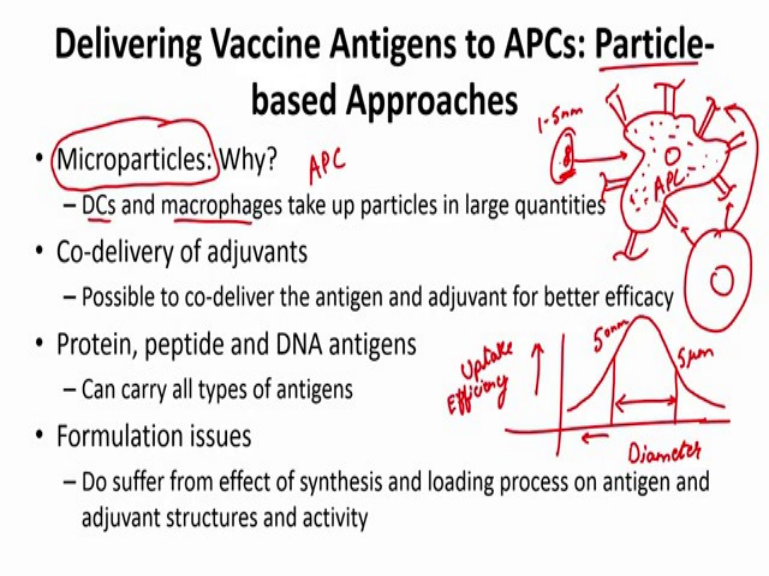

So, now having done this, we are going to; go to how we can use particles to deliver these vaccines. And so, delivering vaccines can be done through particle based approaches and why do we want particle based approaches? So, first of all micro particles or micro nano particles are widely used. And why they are used, is because these APC's, Antigen Presenting Cell like genetic cells and macrophages; they take a particles in large quantities. So, to give you an example, let us say this is an indexed cell and we are trying to deliver a subunit vaccine. So, in this case we were trying to deliver a protein.

Now, the size of this protein typically would be anywhere between 1 to 5 nanometer. At that size range, there is not much uptake of this protein into the cell because first of all even though it is a small, it cannot diffuse through the cell membrane because it is still fairly large and to diffuse as well as it may be hydrophilic, it may have some charged moieties and may not be able to go through the membrane; so, that is one problem. The other problem is that the uptake of these small particles or small proteins into the APC's follow the trends. So, if I plot this what do I find is? So, that let us say this is the size or diameter let us say. And on the y axis let us say it is a uptake efficiency.

So, now if I want to deliver subunit vaccine, what I am finding is as the diameter is decreasing the amount of uptake in the antigen presenting cell is also getting lower and lower. So, now this is a problem because these cells are the one that are going to present

this protein they are going to internalize, this they are going to degrade it and then, present this protein.

So, if their uptake efficiency is going to be low then; that means, that out of let us say hundreds of the receptor that these APC's may have only some of them will contain this peptide. So; that means, that if now a leukocyte comes, it may not even see a peptide unless it goes to one of those receptors that are having it; the rest of them may not even activate it. So, the chances for your immune system to kick in at the high efficiency are low.

So, that is why it is beneficial to work in this range where, we know that the uptake for these particles carrying these proteins are fairly high. So, what will that mean; that means, that intracellularly now, you have quite a bit amount of this protein present in the APC and that means, more and more receptors will be filled in with this peptide from this particular pathogenic protein. So, that is the whole concept here and what we typically find is this range is fairly good from 100 nanometer to about 5 micron. And these are just some approximate numbers, they can vary a little bit, but between 100 nanometer to 5 micron. In fact, actually edit it and then, say about 50 nanometer to 100 5 micron; this is a uptake is fairly high.

So, now if I want to make sure that the uptake efficiency is good. I cannot just inject the protein by itself. So, what I can do is we have already learned through the course that I can use bio module based strategies. I can take a particle with the certain size; I can encapsulate this protein in that particular either during manufacturing or just absorb it on the surface. And that would mean that I have lot more chance to enhance the immune response; so, that is one advantage. What is the other advantage? So, now as I said, the subunit vaccines will also require adjuvants to be delivered.

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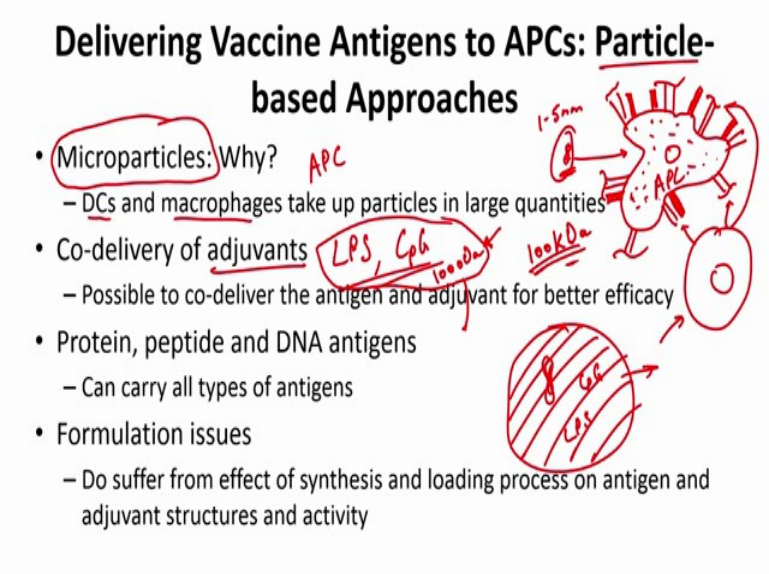

Now, the same thing can happen with adjuvants that; let us say if in a human I am injecting adjuvants intravenously or intramuscularly. Now, these adjuvants are such as LPS or small peptide small DNA such as C p G containing nucleotides; they are fairly small. So, they will diffuse out on the system very quickly. So, now, let us say my protein is huge. So, these we are talking about in the range of let us say 1000 Daltons.

But my protein could be a 100 in kilo Dalton; so, almost hundred times bigger. So, the diffusion of these will be fairly high and they will disperse all through the body fairly quickly whereas, this protein may not be as quickly dispersed through the body as these particular adjuvants. So, what can happen now is even though I have protein present in high concentration at the side, the adjuvants have distributed throughout the body and they are at low concentrations everywhere.

So, now the innate immune response is not getting kicked in as you would have liked because these are the ones that are going to kick in the innate immune response. So, now same problem becomes at the co stimulatory molecules are at a lower expression if you are doing this, but what happens if I also encapsulate this adjuvants along with these particles then; that means, wherever there is a high concentration of your protein there is the high concentration of your adjuvants as well.

So, that would mean that for the dendritic cells that I have taken this or for the macrophages that have taken the protein , there is a high chance that you also have co

stimulatory molecules present to trigger a enhanced immunity or basically, to trigger these leukocytes too and go towards the immune response rather than going towards the tolerogenic response.

So, that is the advantage with the particle because I can take a particle and I can encapsulate both the protein and these LPS or CpG in these particles ensuring that as the particle degrades there is both controlled release of these adjuvants along with the antigen. The other thing is these particles can get all types of antigens. So, it could be protein; it could be peptide; it could be DNA all of those could be carried; so, it is better. However, there is a bit of limitation. So, there are formulation issues that these particle base adjuvant and antigen delivery may suffer with. So, what can happen is at the time of synthesis let us say I am making this particle using an emulsion based method.

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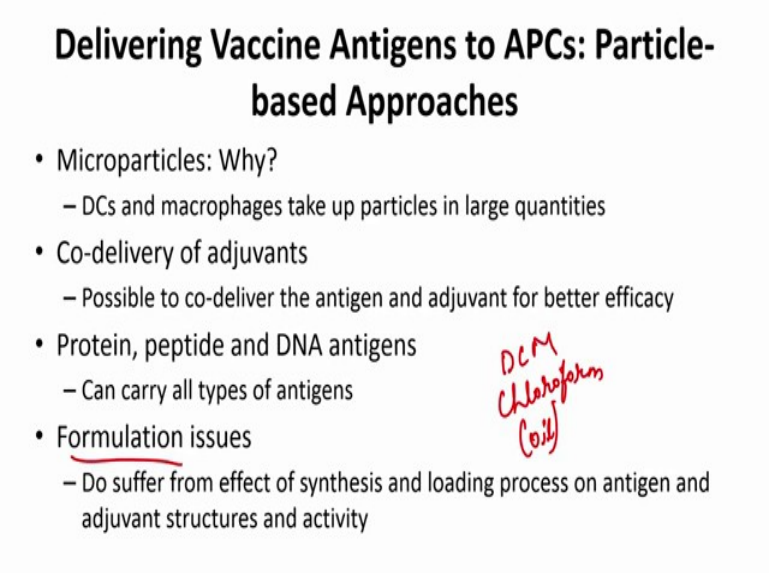

So, I am now exposing this protein and adjuvants to several organic solvents like DCM, chloroform or some oil phase. Some oil based solvents and that can naturally cause denaturation or complete inactivation of these antigens. The structures may change; maybe there is a pattern that we want to deliver in cases of adjuvant specially and those patterns may get completely disrupted. So, those are some of the challenges that are associated with formulating these particles, but then, given the results that these particles have shown the better pharmacokinetics that controlled release. These are some things

that people are working on to decrease formulation issues, but this still is very promising as far as translational of any of these vaccines is concerned ok.

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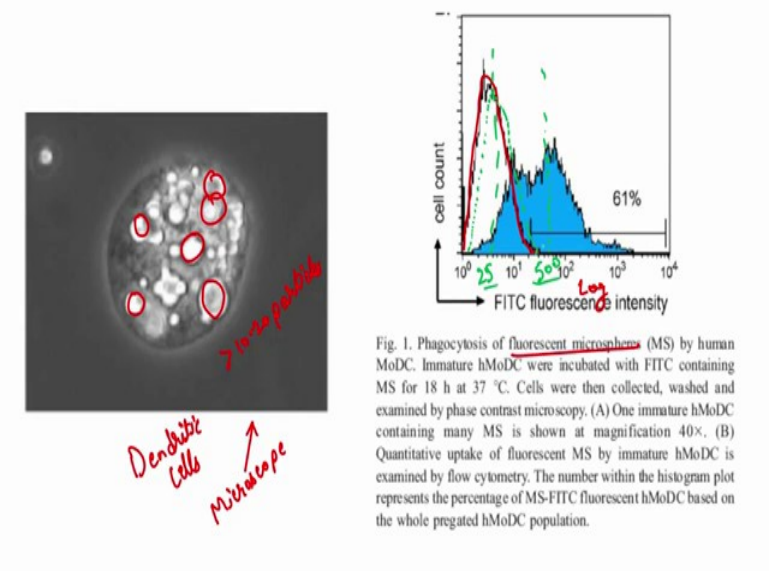

Fig. 1. Phagocytosis of fluorescent microspheres (MS) by human MoDC. Immature hMoDC were incubated with FITC containing MS for 18 h at 37 °C. Cells were then collected, washed and examined by phase contrast microscopy. (A) One immature hMoDC containing many MS is shown at magnification 40×. (B) Quantitative uptake of fluorescent MS by immature hMoDC is examined by flow cytometry. The number within the histogram plot represents the percentage of MS-FITC fluorescent hMoDC based on the whole pregated hMoDC population.

So, here is an example. So, here what you are seeing is phagocytosis by a dendritic cell. So, you have dendritic cells, which have been incubated with the fluorescent microspheres. So, all of these are fluorescent microspheres and what you are seeing here is the; on the a on this particular figure you are seeing fluorescent microscope image and it is showing look at how much amount of particles is in one cell.

So, this one cell; just by briefly looking at it you can easily say it contains more than 10 to 20 particles and all of these particles carry a heavy dose of whatever indigent that me you may require. So, that is there and then, this further depicted by the fluorescence intensity. So, maybe your; in this case the particles are fluorescent level. So, you can see if this is before particle treatment or the one not treated with particles. You see, quite a bit of shift then, remember this is in the log scale. So, you see quite a bit enhancement of your fluorescent molecule. So, instead of fluorescent molecule the same will also apply for your antigen.

So, fluorescent molecule is essentially an antigen in this case and if you only give free drug what is scene is not plotted here, but I will just plot it, if you only get free drug, you get very little uptake. So, maybe the curve might shift something like this; so, much much lower. So, if you compare MFI for this versus the mean for this. So, MFI is mean

fluorescence index. So, the mean for this some would be lying somewhere around here or the median would be lying somewhere around here.

And what you will find that this; we are saying is only let us say 10 20, this is only let us say 25 where, this is already 100, 200, 300, 400, 500. So, this is almost 500. So, you can see quite a bit enhancement, but this is something that I have hypothetically true, but if you read enough papers you will find that the free antigen and does not really penetrate through the dendritic cells as efficiently as particle based antigen.

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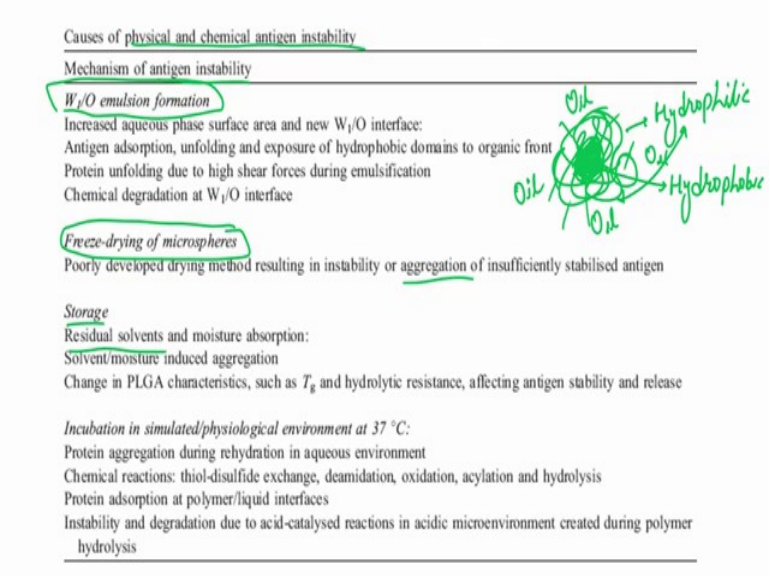

So, here some more table and this is looking at what are the different factors that causes antigen instability in particle formulations? So, there could be both chemical as well as physical instability that is being caused. So, one is of course, I briefly discussed is the water or oil emulsion. So, because you have that oil present, this can expose these hydrophobic domains; it can really cause the structure to completely fall apart .

So, again as we had discussed previously in the protein adsorption case; let us say I have a protein that is folded and of course, this protein is folded in water. So, all the inner domain will be hydrophobic, but once it comes in and all of these will be hydrophilic. So, once at this point there is water outside and this is the native structure of the protein. So, it is fairly happy because all the hydrophilic domains are outside; the hydrophilic domains are outside; they may be pockets here, which may be the active site or maybe

some other active site. So, this protein is functional, but let us say it comes in contact with oil now.

So, now we have removed this water and put oil outside. Now, these domains as I said is hydrophilic. So, they do not really want to interact with the soil because oil is hydrophobic and similarly, these domains which are hydrophobic, they would like to interact with the soil. So, what will happen? The structure will completely change. So, this is going to untangle and this interaction will start these hydrophilic domains will start to burry inside and hydrophobic domains will start to come outside.

So, you are now completely change the structure. The other thing that can happen is maybe in a certain solvent there is more liability for things to degrade. So, maybe in presence of water or in presence of some solvent these bonds may cleave in or some chemical direction may happen maybe this will start cross linking,

So, if that happens then, you can have suddenly change structure; your size is changed, it may not be; may be the immunogenic antigen that is being present is now cross linked and cannot be presented the cells cannot degrade its. So, all of those problems are there. So, this is something that the water in oil emulsion can cause instability in your system. Then, what is done is once these particles are formed for storage purposes freeze drying is widely used.

And what it is? You are taking these particles and you are going putting them through a life lization or a spray drying process in which you are ensuring that all the water in the system is gone. And the reason you do that is we have really discussed before is presence of water is a big problem for long term storage because that may contain some contaminants and may degrade the drug or may degrade in this case the proteins that you have maybe there are some basal contamination of some enzymes some proteases that are cleaving it.

So, to improve a stability these freeze drying methods are used; however, again these freeze drying methods can themselves introduce instability. So, they may again cause aggregation, denaturation for some antigens. So, for most antigen these processes work, but for some antigens they may not work. And again the storage is an issue over there. So, there is residual solvents, moisture absorption from the atmosphere; all of these can cause instability in the system. And then, you before you want to deliver, you want to

ensure that maybe these things need to be in a certain solvent rather than freeze drying. So, all of those factors can also play a role in changing the formulation of the drug and in changing how the drug is perceived in the body .

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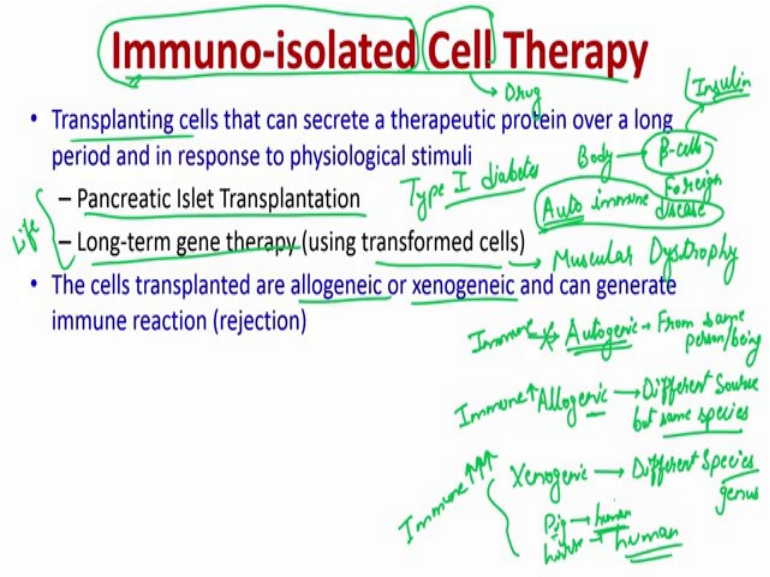

So, we will now move on to a next class. In this same module, which is called immuno isolated cell therapy and what is immune isolated cell therapy is; we are now looking to deliver cells instead of many drugs. So, in this case, the cell is a drug. So, again this can be classified as drug delivery, but in your; in this particular case the cell is a drug and maybe the cell is producing a certain enzyme which is then acting.

So, effectively that particular enzyme is a drug, but since cell is producing it, you have to ensure that the cell is stable as well as a cell can form whatever function you may require and when I say immuno isolated, what does this term mean? This term means that I want to isolate the cell that I am delivering from the immune system of the host and this could be due to several reasons. Typically, if I am putting in cells from an external source, which means from some other patient into my body my immune system is going to recognize this as not being a self antigen and it will start adapting to that particular cell and start killing it. So, to prevent that we want to make sure that this is immuno isolated.

So, far we were talking about how to generate an immune response against a vaccine. Now, we are just doing a reverse in this case. We are trying to make sure that the immune response is not generated against whatever you are delivering. And in this case,

this is cell. So, it could be transplanting cells that can secrete therapeutic proteins over a long period of time in response to some physiological stimuli. So, this could be for pancreatic islet transplantation.

So, let us say if a person is suffering from type I diabetes and what is type I diabetes? Type I diabetes is nothing, but it is that your body for some reason considers your pancreatic cells or beta cells is what they are called, that produce insulin, for some reason the body is now considering your beta cells has to be something foreign and this is of course a disease condition in which also classified as autoimmune diseases. So, this is an autoimmune disease, which means that your own; your own cells are being detected by the immune system as being foreign.

And so, when that happens the body is going to kill all the beta cells; the immune system is going to kill all the beta cells, they will home in and try to find wherever, they find the beta cells and they will kill it and it could be because maybe the insulin is the one that they are recognizing as foreign or maybe some other receptor. On these beta cells, that for some reason the body thinks is not cells and for these patients is actually the life is very difficult because all the beta cells are dead. So, they do not have insulin, which means that the blood glucose level which is maintained by the insulin is not maintained.

So, the blood glucose level can go extremely high and the high blood glucose level. Then, causes infections to happen because the bacteria likes that environment, it can cause a several malfunctioning of signaling through our body due to these various functions in heart and brain in everywhere. So, what is done for these patients is you want to then, there are two routes either you keep on taking insulin regularly. So, every time you have to take an insulin dose to make sure that glucose that you are taking in and gets metabolized or you can either put cells in them in such a way that because; obviously, the original cells have been destroyed.

Now, you can try to put some other cells or maybe the original cells. If you are able to retrieve some of them you can put them back in, but then, the body is going to react again against those cells because the body is already on getting an autoimmune disease. So, what do you have to do is you have to make sure that when you put these pancreatic islet cells into those patients and these are isolated somehow on the immune system, they could also be long term gene therapy. So, I mean, this was one it could be maybe I want

to put some transform cells. So, let us take another case of muscular dystrophy. So, in this disease what is happening is one of the proteins that is responsible for your function of your muscle is either not getting produced or getting produced in some mutated form.

So, one of the protein that is widely involved in this is called dystrophin and let us say during your birth or at the time of your embryo genesis, there was some mutation. In this gene and now because this gene is now mutated it cannot really function very well. So, your muscle starts to degenerate and it basically starts from bottom to up. So, your first thing that defects is your leg muscles, which are large muscles.

And as more and more dystrophin inactivity happens; it eventually may reach your lungs and heart and that will eventually cause death because if the heart stops beating or if the lungs are not breathing then, the patient cannot survive. So, in these patients what you want is you want to give cells, which are producing the correct dystrophin molecule, but now this dystrophin is going to be recognized there is a foreign antigen.

Because this was not the part of the body for that particular patient; the immune system had never seen the correct dystrophin. So, even if you give the correct dystrophin. Now, your immune system may recognize this as a foreign dystrophin or it could be from some other source of cells and they may itself immunogenic. So, you want to make sure that these cells do survive for long duration because this is a therapy for life both of these therapies, these are as long as the patient is alive; you want these cells to be alive. So, in that regards you want to immune isolate these particular cell therapies from your immune system.

So, the cells transplanted are often as I said is allogeneic or xenogeneic and can generate immune reaction. So, what are allogeneic? So, let me define these terms. So, it could be autogenic which means from same person or being. So, it could be that my own cells have been taken up; they have been expanded. And then they are putting in back that will be an autogenic implant it could be let us say if I am suffering from some bone disease and I want some bone to be put in one of my fracture may be that bone has been taken out from some extra bone from my hip region where, those cells are then being put in. So, this is an autogenic implant.

So, it is very well tolerated; of course, the immune system has no role to play in this because for immune system all cells are yours, there is no foreign antigen. Then there is

allogenic and what that means, is from a different source, but same species. So that means, that maybe I am suffering from disease; I do not have enough cells for that particular disease. So, I take cells from any other human being or the same could be applied for animals as well maybe one horse gets cells from another horse or any other species for that matter as long as is between the same species it is called allergenic.

So, even though it is the same species; so, it is almost 99 percent same. There is still a little bit of differences between each of us, that is why we are all different and the immune system will act on it. Immune system will go up and start to reject this and then finally, the other one is the xenogenic. And so, this is from what different species altogether; sometimes it will be even different genus and what that means, is for us let us say if I want an implant; maybe these implants are being or these cells have been taken from uptake.

So, it is let us say pig to human or this could be horse to human; whatever it might be the source be and because of because they are so different from your original species. So, the pig cells have lot more differences then, let us say a human cell. So, even though they are still from not the same person, this is still very close to what you have in your body, but this is so far away that the immune system goes much much higher and this is the most difficult to get accepted in the body. So, that is what allogenic and xenogenic means.

So, anytime you are doing immuno isolated therapy, you have to think about all these terms and all these factors. And most cases, you will find that the patient may themselves not have any source of these cells. So, most of the time you will have to rely on allogenic and xenogenic sources.

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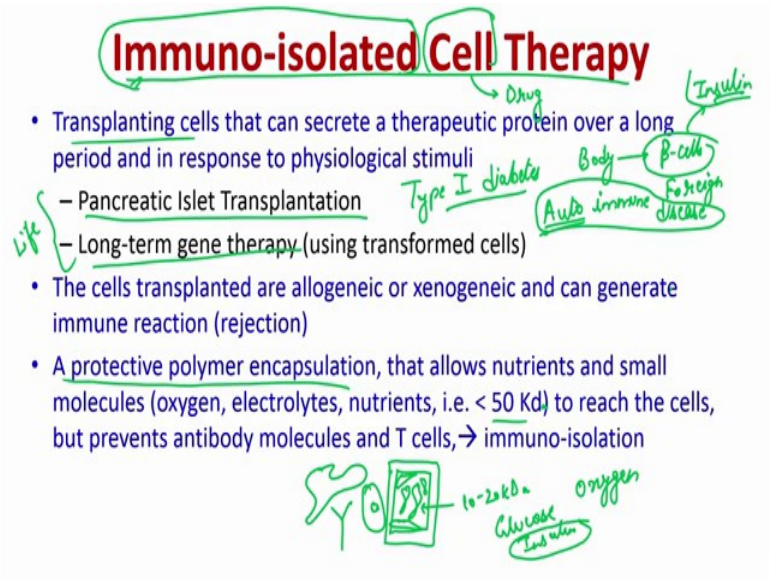

So, one way to go about it is you can put a protective polymer encapsulation that will allow small nutrients and molecules to go through. So, anything less than 50 Kd a and reach the cells, but prevents antibody molecules like T cells. So, that is they are immuno isolated.

So, what this means is we are relying on the fact that most of the immune system molecules and cells are fairly large. So, if I have a semi permeable membrane that only allows small molecules like 10 to 20 Kd to go through. So, all of these glucose insulin your oxygen these things can go through and can give it to the cells that are present inside this implant.

Whereas, the antibodies or your antigen presenting cells or your leukocytes I am not able to go through because of this barrier seigneurial barrier. So, these things can go through the effective proteins like insulin or dystrophin can come out, but your immune system is not able to attack. It is that is the whole concept of immune isolation especially, with polymer encapsulation and but there are some challenges to it and we will discuss this.

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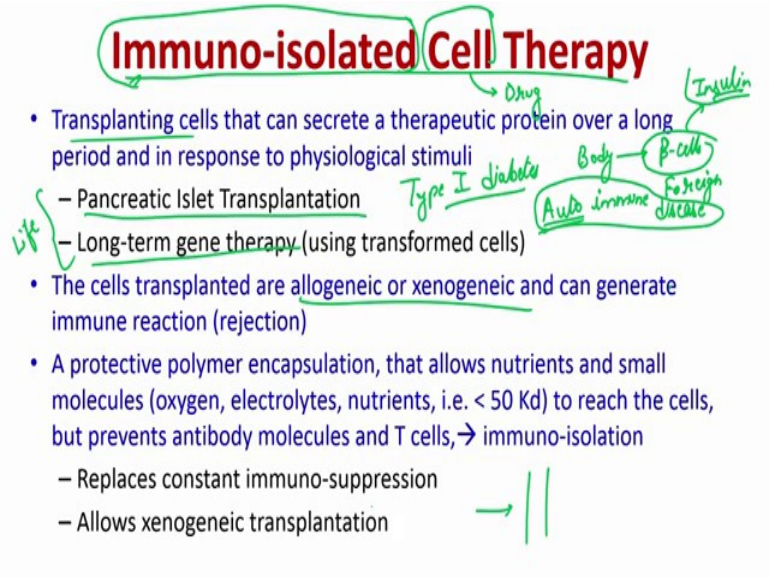

So, this replaces the constant immuno suppression everybody made it. So, in general, if you are putting anything allogenic or xenogenic you will have to then, supplement it with the immuno suppression and that would mean that your immune system is now weak. So, pathogens such as bacteria and viruses can come and attack it whereas, in this particular case you what you have done is you have because of this barrier you do not really need immuno separation anymore.

So, your immune system, you may still need it at the initial phases, but eventually the idea is to not have immuno separation anymore and because of that the patient life will be slightly better than having constantly under immuno suppression and then, suffering from several diseases. So, this can even allow xenogenic transplantation to happen. We will stop here and we will continue this in the next class.

Thank you.