Drug Delivery Principles and Engineering
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Lecture - 44
Intravenous Administration Approved Nanocarriers and Immune System

Hello everyone. Welcome to another lecture of Drug Delivery Engineering and Principles. Just a quick recap of what we learned in the last class.

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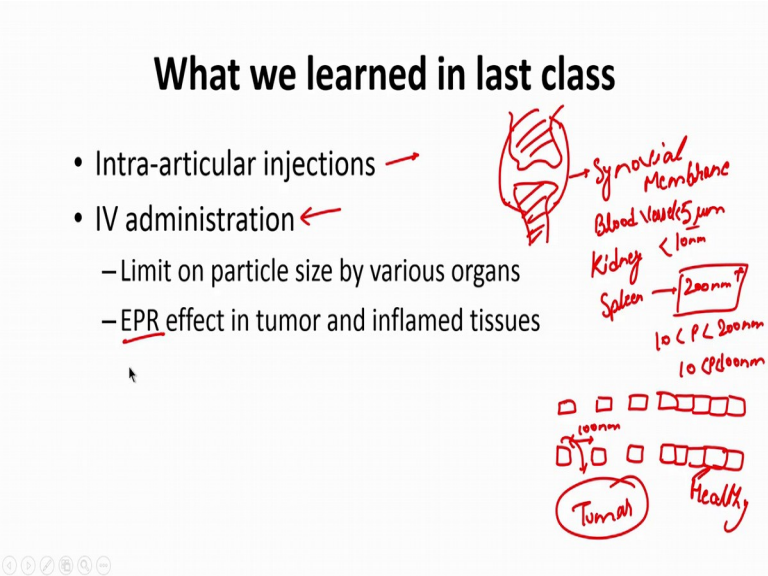

First we talked about intra articular injections. So, these are injections which are given into the joint space. So, if this is my femur, this is my tibia. You can directly inject things in here and that will result in much higher residence time. What we also learned is this synovial membrane contains lots of blood vessel and lymphatics and they can clear out the things fairly rapidly, if they are smaller size. So, if you want to inject something to reside very for long durations, you want to inject bigger particles that can be drugs or the drugs itself may be big. So, somewhere anywhere between above 1 micron would start to give a much higher residence time and then anything below that.

Then, we started IV administration. One of the most widely used methods in clinics specially for things that you want to have instant action on the body. We then discussed various organs that put the limit on a particle size. So, if you want to do a control through some bio engineering approaches, we discussed that first of all kidney will clear things

below 6 nanometers so, or below 10 nanometers. So, you want the particle sizes to be above 10 nanometers, then we said that anything with the size above greater than 200 nanometers, the spleen will clear it away; 200 nanometers plus particles. So, you want the sizes to be below 200. Of course, the biggest blood vessel is 5 microns, the smallest blood vessel sorry.

So, you should definitely have less than 5 microns and then we also discussed that liver is another major organ that is going to clear quite lot of your big particles. So, basically you want something in the size range of 10 to 200 nanometers okay! Then we talked about EPR effect in both tumor and inflamed tissues. And what it is? The blood vessels that are feeding your tumor tissue are fairly dilated whereas, the blood vessels in your healthy tissue fairly open.

So, this is healthy tissue, this is tumor tissue and these pores are about 100 nanometers in tumors. And they become larger as a tumor grows as well as there are also pores in inflamed tissue when the vessel dilates to allow more cells and more nutrients to go in. And because of that if you have size ranges in between 10 to 100 nanometers, you will have a much higher efficiency of delivery and this is called the enhanced permeation because they will go here, but not here. And then this tumor micro environment also has very poor lymphatic system. So, it cannot really clear things away once they go in and that is the enhance retention so, which is nothing, but EPR effect. So, that is something you can utilize as well; utilize quite a bit in the literature ok.

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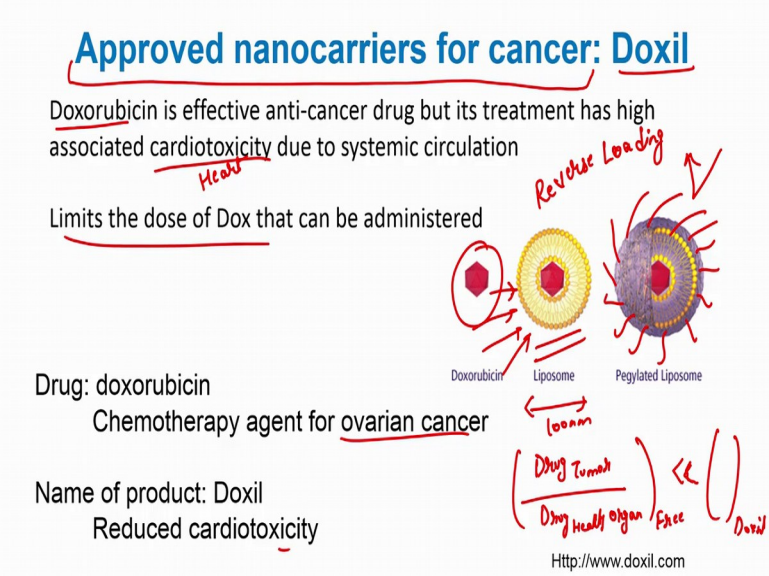

So, let us continue our discussion on the IV route that we were talking about and we are going to talk about drug called Doxil. And this is similar to the EPR effect. It utilizes that effect and is an approved nano carrier for cancer drug delivery and doxil contains doxorubicin as the major effective drug. The doxorubicin is a very effective anti cancer drug. In fact, most initial cancer studies are done with doxorubicin and but the problem is it has a very high cardio toxicity. So, cardiotoxicity means that something little to the heart. So, it starts to kill heart cells and then; obviously, when you inject something IV, it is definitely going to go to the heart as well.

So, that is a problem and that limits the amount of dose or dox solution you can give to your patient. Because eventually at a certain dose all the demon cells will die, but before that dose is reached the patient may die because of all this cardiotoxicity and even other types of toxicity. So, that puts a limitation on the dose of doxorubicin that can be administered. So, to counter that what was done is the doxorubicin was taken, it was encapsulated into a liposome; this was done through reverse loading. I hope you guys remember what reverse loading is. So, reverse loading is nothing, but using the property of molecules to diffuse into the lipid membrane if they are non ionic at a certain pH.

And once they go into the membrane, you make the liposome such that and the inner environment has a different pH and they will become ionic. So, that will cause the movement of the drug to go into the liposomes and effectively build up a very high

concentration in the liposome. So, that is what was done and then these were actually further PEGylated. So, again, I hope you remember PEGylation.

PEGylation prevents the interaction of various cells in protein with your biomaterial surface and because of that it will not lead this particular liposome get adsorbed with several proteins or get cleared away by the immune system. So, it increases the residence time and this resulted in quite a bit effectiveness of this. It was used very heavily for ovarian cancer, still used and it also reduced the cardiotoxicity and because it was using that EPR effect.

So, what happened is this was made of size of about 100 nanometers and because of that the vessels in the heart, are fairly healthy in tumor tissues. So, this liposome was not going in releasing doxorubicin in the to the heart cells. But then in the tumor areas where the vessels were fairly dilated and these particles can go and permeate and these were accumulating in the tumor areas. And actually, effectively what you did is you let us say, if you had a ratio of drug in tumor versus the drug in healthy tissue that for your free drug was much less than for your doxil. And because of that this therapy took off and was used in the clinics as well and it and is still used in the clinics.

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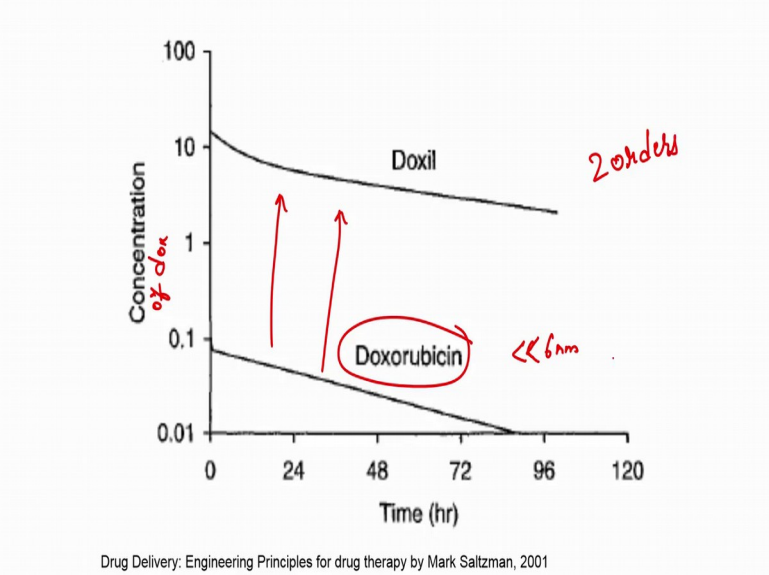

Drug Delivery: Engineering Principles for drug therapy by Mark Saltzman, 2001

So, here is also showing that not only that you are actually improved the pharmacokinetics also because doxorubicin is a very small molecule much less than 6 nanometers.

So, doxorubicin gets cleared away from the system very quickly as well. So, you have to continuously infuse doxorubicin in the patient if you want any therapy whereas, doxil since its now about hundred nanometers, it has residence time that is very high and it slowly releases the doxorubicin into the system. So, as you can see the concentration of dox was more than 2 orders of magnitude higher than what you had in case of free drug.

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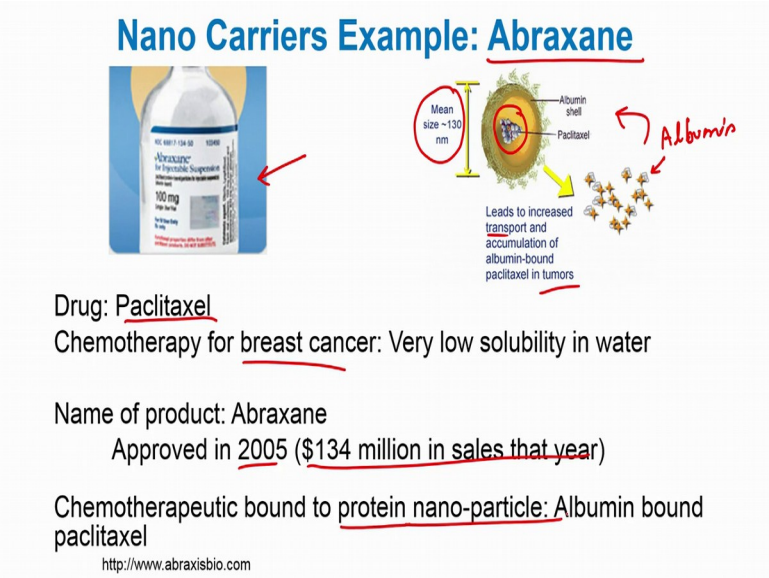

So, here is another example. So, in this example, the product name is Abraxane and this is basically one of the vials you can find in the market. And what it is? It is carrying a drug called paclitaxel again very effective against lots of cancer cells especially breast cancer, but it actually has a very low solubility in water. So, what that means, it is fairly small molecule. So, what that means, is you cannot really inject too much because it will precipitate out and again may clog your heart vessels or your brain vessels causing heart attack or stroke respectively.

So, what was done is this paclitaxel molecule was conjugated to albumin protein which is one of the most abundant protein in our blood. And it then self-assembled into a structure, because paclitaxel is fairly hydrophobic and it did not really want to interact. So, it self-assembles into a structure like this where paclitaxel ended up being in the core, and albumin changes conformation to reflect on the outside and interact with water with a mean size about one 130 nanometers. Again, close to that EPR effect range that we

wanted and that resulted in very enhanced and increased transport and accumulation in tumors.

So, this drug was approved in 2005, had bumper sale that year and still is being used quite a lot. So, as I said its nothing, but a protein bound drug that you are using and forming a nano particle out of that and using the EPR effect that we talked about.

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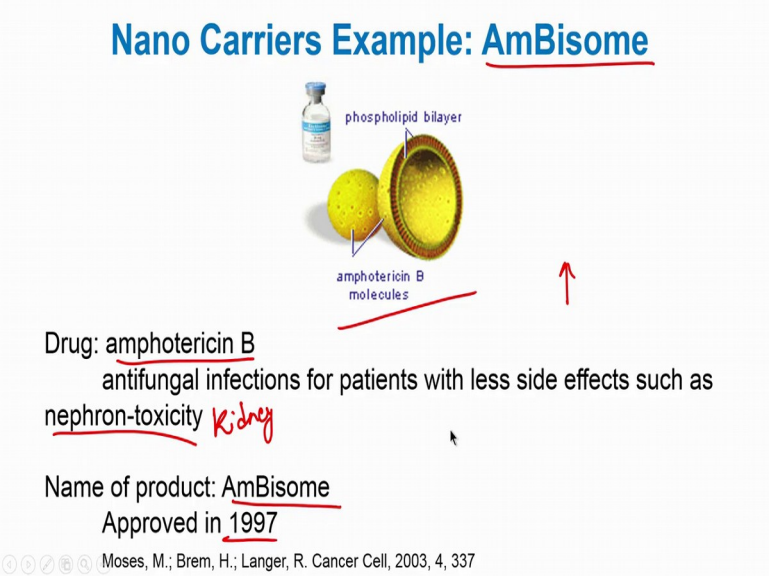

Yes, another example. This in this case, we are looking not a tumor, but at inflamed sites or infectious site. So, the product is called AmBisome and what it is? Again, a liposomal formulation in which the drug being used is amphotericin which is an antifungal drug. And, but then this drug causes lots of side effects in nephron basically, kidney.

So, that was causing limitation of how much drug you can use, but what these folks did is they encapsulated this or actually attached it to the surface of the liposome into the lipid membrane layer that caused first of all increase in the size. So, it did not really accumulate in the kidney and the secondly, controlled release as the liposomes release this drug out. So, this was the product and there was approved in 1999 and again was used quite often.

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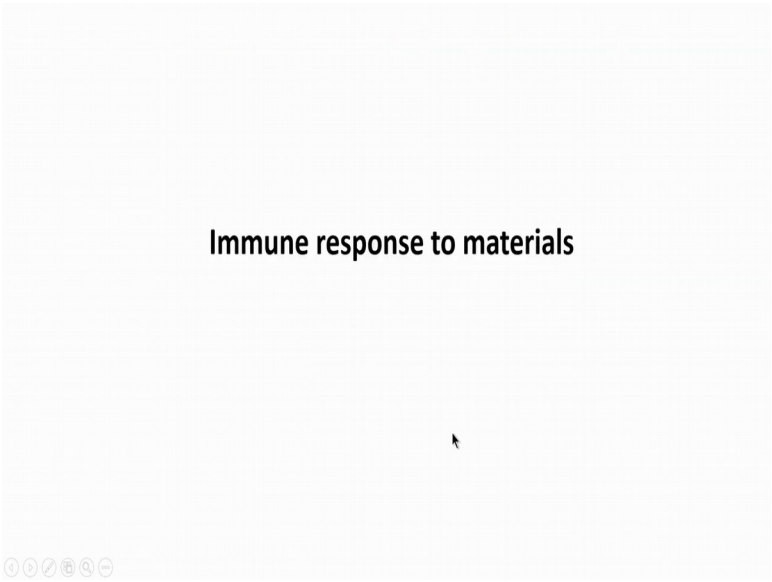

So, this concludes our session on the route specific delivery. We are now going to change and start a different module which is immune response to materials and so let us get started on this. So, if you have any material that you are putting in and we have talked about this briefly as we went down through this course but if you are introducing something new, the body is going to identify this as foreign materials and there will be some immune response against it.

So, what we will do is, we will try to understand what kind of immune response builds up in these circumstances and how do we tackle this, how do we make sure that this immune response is not rejecting a material they will be putting in, and it is not affecting the performance or whatever the function the material was supposed to do. So, this is what we are going to learn. So, before we learn that, we have to learn a little bit immunology that will be required to understand this module.

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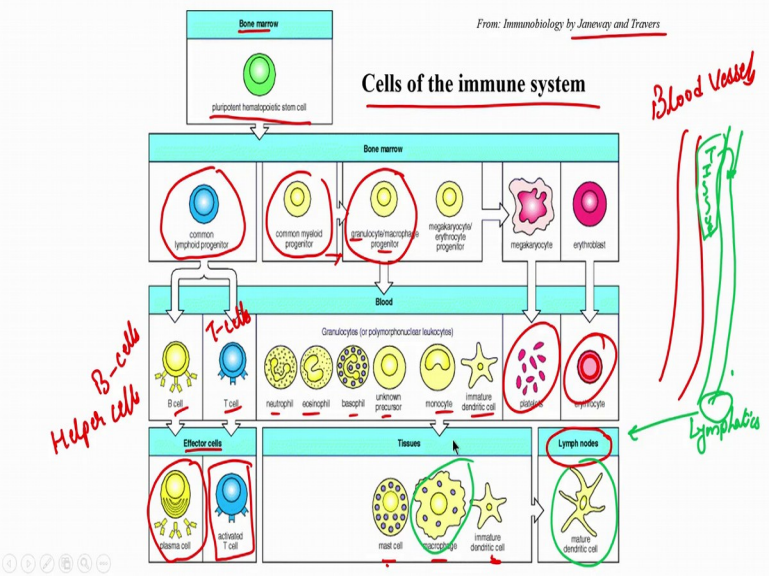

So, let us start with this. So, this is a very classic figure. This is taken from Janeway and this is showing different cells of the immune system. So, you have a bone marrow which is located in the long bones. It is the cavity within the long bones that you can find and that is a site which is extremely rich in pluripotent, hematopoietic stem cells. And what does this pluripotent mean? That means, that these stem cells can give rise to several different cell types.

And so, these give rise to several different cell types as I mentioned. Some are called common lymphoid progenitor; some are called common myeloid progenitor. The myeloid progenitor goes in and further differentiates into granulocytes and macrophage progenitors including some of the blood cells as well and then they further go in and differentiate into blood cells and platelets.

Whereas the common lymphoid progenitors have a much more complex route they go and differentiate into B cells and T cells. Again, there will be lot of biology, but bear with me and we will describe these as we go along. So, you have B cells and T cells and then they also give rise to some of these granulocytes which was from the myeloid progenitor will also give rise to neutrophils, eosinophils, basophils and there are some unknown precursors as well monocytes, dendritic cells. These are all different types of immune cells which are responsible for different functions and we will describe some of them as we go along through this module.

The B cells and T cells can then have different types as well; they can have effector cells or they can have helper cells. So, both B and T type of cells can have both effector and helper cells. So, some of these B cells are responsible for secreting antibodies something that I am sure you must of all of heard by now and then there are T cells which are directly responsible for killing any rogue cells and any cell that is not functioning well and immune system is not liking it.

And some of these monocytes and granulocytes derived things can then go in and become mast cell macrophages and dendritic cells. And these are cells that are more responsible for presentation of anything to the immune system which the thing is foreign. And then there are lymph nodes which are nothing, but they are these so, I said that there are blood vessels. Then along the blood vessel, these are another parallel network that is called lymphatics.

So, let me take another color for that. So, along with the blood vessels there is another parallel circulatory system that is called lymphatics at a much lower flow rate, but nonetheless flowing throughout our body. And this lymphatics from time to time and from different places actually harbors secondary lymphoid tissue which is called lymph nodes. And what these tissues are? They are depot of these immune cells which has a sampling around. So, obviously, this is tissue. So, what it is? They are sampling whatever is coming out from this tissue going into the lymph node where immune cells are present in very high numbers.

All these B cells, T cells and other types of macrophages and dendritic cells are present in a very high number and they are continuously signaling as to if there is something foreign. So, let us say there is a bacterial protein that is going to come in here. It is going to be picked up by one of these immune cells particularly dendritic cells and macrophage. And it is going to be then presented to one of these effector cells or memory cells or the helper cells which will then mount an effective immune response against it.

And again, if you are unaware of this, do not be worried; we will describe it in a little more detail as we go along. So, these are some of these common terms cells of the immune system. Most of them are actually fairly important for this module. So, it will be good if you can start to remember some of these cells.

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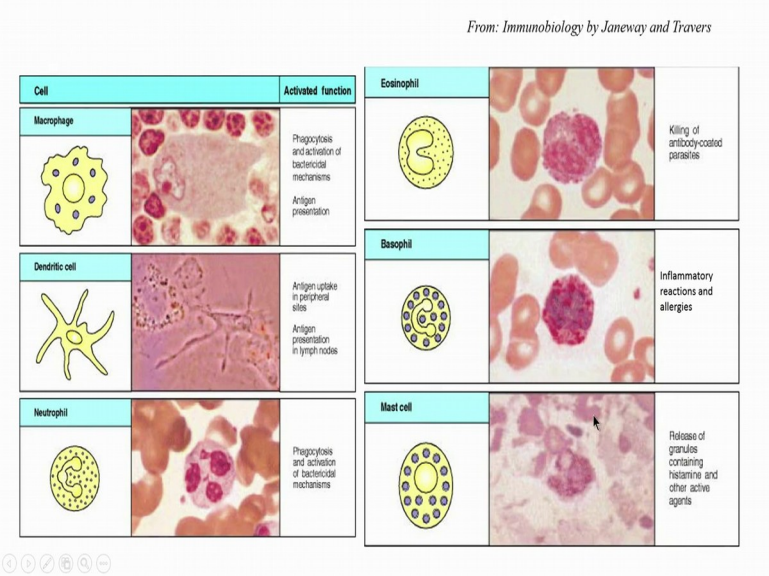

And so, let us talk about function of these cells. So, macrophages which, again we have discussed before already; these are cells that take up foreign objects, foreign bacteria foreign viruses, their major job is to first of all do phagocytosis which is a process through which large particles and large foreign cells are being taken up. And then they get activated and start secreting lot of bactericidal enzymes and bactericidal proteins that kill them. And then they are also responsible for antigen presentation which means that they are the ones who even when they kill them or even when they harbor them, they will present it to the rest of the immune system, saying that hey look! there is something foreign here.

So, that is the major rule for these. Dendritic cells like macrophages are again very similar and they are also responsible for phagocytosis and antigen presentation. Neutrophils are slightly different in that regards, they do a lot of phagocytosis too; however, they are not as involved in antigen presentation as the macrophages and dendritic cells, but they are mainly responsible for killing the bacteria. So, they even act as a kind of a suicide bomber where they carry lots and lots of harmful bactericidal molecules. And when they find that there are bacteria in the surrounding, they will just burst and release all that near the bacteria vicinity; killing the bacteria in the process.

Then we have Eosinophils and one of the function responsible for them is killing of the antibody coated parasites. So, they are more responsible for big worms like pathogens

and they can even be effective against some of the smaller pathogens too. And these basophil, mast cells are not as widely studied and understood probably their function in the immune system is either not very understood very well or they do not really play a very major role. But their major function is to cause inflammatory reactions and they are actually also responsible for allergies. The same with the mast cells, they also release granules containing histamine and other active agents to kill the surrounding environment bacteria and pathogens.

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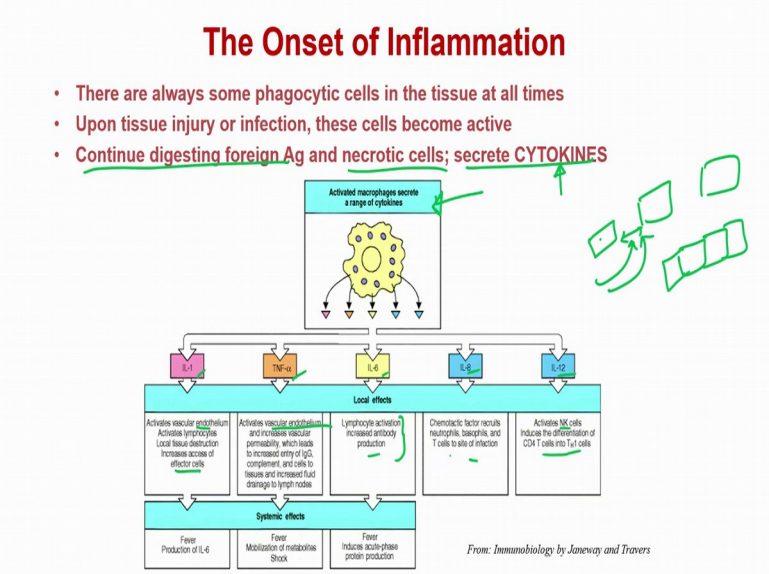

So, how does the inflammation start? So, this is important because we need to understand how does it starts that and we put our material, we must make sure that this does not happen. So, let us learn first of all how it does it start. So, again the immune system is very vast and contains lots and lots of cells and they are surveying pretty much every part of the body that we have.

So, all tissues that we have contains some one or other type of the immune cell. So, what happens if there is some injury ok? These cells become very active. So, they will then go to that injury site, they will sample that to see if there is anything that is foreign that caused this injury, why did the injury happen. And if they do fine, they will continue to digest this foreign antigen and end the cells that are dying there because; obviously, if there is some injury then you must have some dead cells, your skin may have ruptured. So, skin cells are dead, some of the blood vessels may have a ruptured. So, those

endothelial cells may be dead and if they do find something that is causing this, they will secrete cytokines.

Sometime, I mean most of times they will even do that without any the initial injury is enough for them to start secreting some cytokine. So, let us say this was macrophage that was sampling that area which is one of the major cells responsible for also sampling tissues. So, then they will start secreting several classes of these cytokines which are and we will talk about in the next slide, but which are nothing, but protein-based molecules involved in signaling and amplifying the immune system.

So, some of them being IL-1, TNF-alpha, IL-6, IL-8, IL-12; these are some inflammatory cytokines and they all have their own functions, which some of them are overlapping. Some of them are systemic so, they will actually involve the whole immune system of the body, some of them are local which is more at a localized level.

But what will basically happen is like an IL-1, if it is released, it is going to cause activation of the vascular endothelium. It is going to activate any kind of lymphocyte, which is a type of immune cell in the surrounding, causing the local destruction of the tissue and will even cause they increase in the effector cells which are the cells which are going to kill anything foreign or going to help in an aggregation of that to come into the side. So, another class of molecule is TNF-alpha similar to IL-1. It is fairly inflammatory; it activates again the vascular endothelium and increases the permeability.

So, as I if you remember in the tumors part of the talk, I was talking about, that if you have inflamed tissue, you have again vessels that are more permeable. They are not as tight as they are in healthy homeostatic tissue and that is because these factors are being released, which are signaling these cells to increase a junction diameter. And what that does is it causes more cells to come in, more growth factors, more immune cells, more cytokines to come in and that is how the inflammation is leakier, then inflamed sites are leakier.

And so, that causes entry of antibodies complement different cells into the site. Then we have IL-6 which are again responsible; all of these IL-6, IL-8, IL-12. These are responsible for again activating different immune cells; some of them NK cells, some of them causing antibody production and Th1 which is the T helper cells. They are all essentially accumulating at the site.

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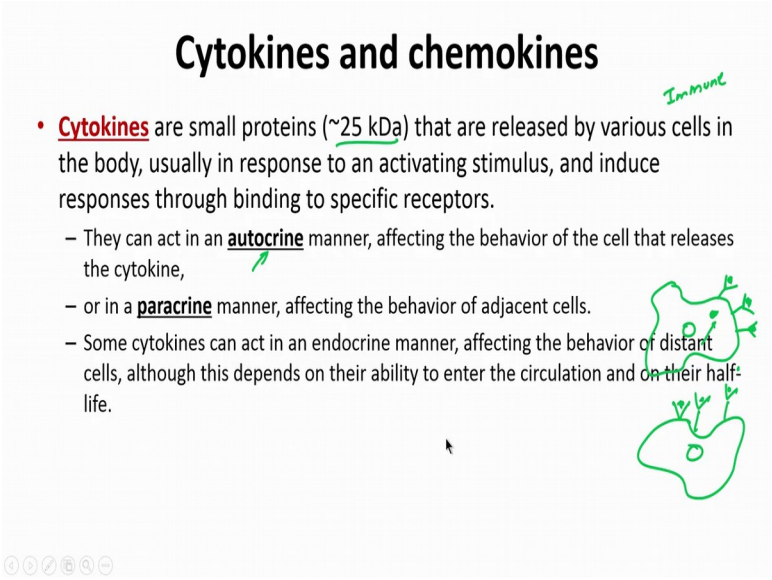

So, I mention cytokines and chemokines. Let us quickly define what these are. So, cytokines are small proteins of typically about anything between 25 to 30 kDa released by various immune cells. So, typically this is immune production although other cells can also release them and they say they are usually released in response to a certain stimulus that might be given. And then they induce responsible news responses by binding to a certain receptor.

So, lots of cells have receptors for these cytokines and they go and bind to that receptor which then signals the cell to either get activated, to start migration, to start causing the vessel dilation. So, all of this is controlled by these cytokines in the several classes of them. So, one thing as we are going along, I will I like to point out is immune system is fairly dynamic research topic every 3-4 years, the whole immune system understanding changes by quite a bit amount.

So, what I am giving you now is what is currently well accepted and well hypothesized and, in the books, but all of these are fairly lucid definitions and they may change as the time goes on. So, these cytokines connect in an autocrine manner which basically means that they can act on the cells that are actually releasing them. So, let us say if this is an immune cell and it releases this cytokine out into the media.

These cells themselves have receptors for these cytokines and when these cytokines bind to the same cell, they will activate the cell further. So, this is kind of a positive feedback

loop where cell is getting activated, it is losing this cytokine and these cytokines are binding to the cell itself and activating them further. Or they can act in a paracrine manner where once let us say these cells have released it. There is another cell in the surrounding, that goes and binds to it and this cell now gets activated. So, now the signal is amplifying because the neighboring cell is also becoming inflamed.

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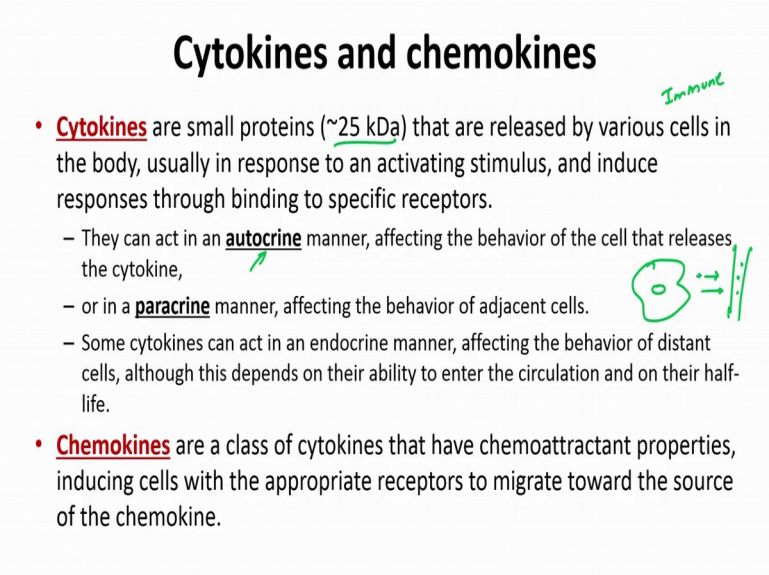

So, and then some of these cytokines connect in an endocrine manner where; that means, that let us say a cell has released in its certain organ, that cytokine, is now going into the blood vessel and that blood vessel is taking it everywhere in the body where to even distant parts of the body; it is causing this thing to activate the cells. However; obviously, since now this is going to involve some time as well as exposure to lots of different proteins. This will depend on the ability of the cytokine to first of all enter the circulation and then finally, once the end of the circulation then how stable their what is there half life in the circulation because if the half life is very low, then they will not be able to go ahead and do activation in a distant organ.

And then there are chemokines. Chemokines like cytokines are also secretary by immune cells. They are actually a type of cytokines. So, chemokines are part of a cytokine and then they act as a chemo attractant property. What that means, is let us say if there is in problem at this site or this cell and its secreting some of these cytokines which are then building up in the surrounding. So; obviously, what will happen is near the cell, you will

have quite a bit away from the cell it is going to become less and less. So, let us say there is another cell bit far away from this cell, but as it secretes that more and more cytokines are coming from this direction.

This cell will then move and get closer to this. So, it is acting as a signal to call more immune cells to come to the site. So, that is what chemokines do.

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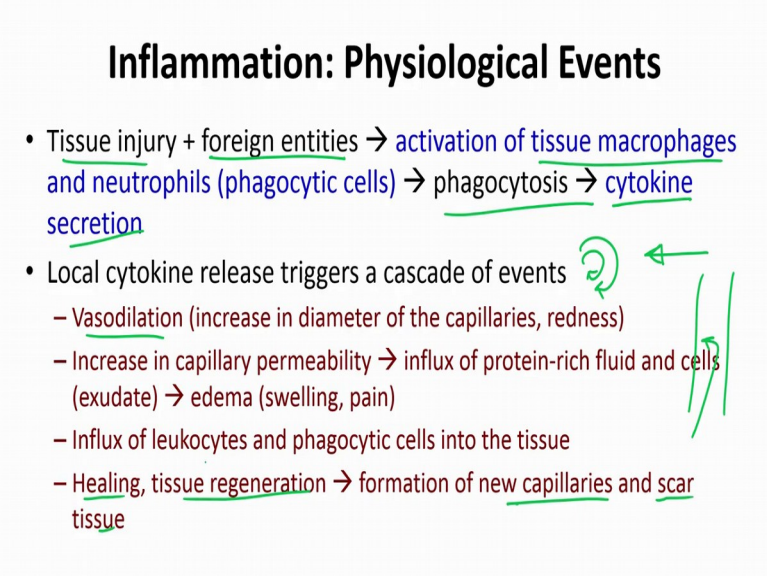

So, let us talk about some physiological events. So, let us say if there is a tissue injury and some foreign entity is also present. So, what is the first thing that is going to happen? The tissue macrophages and the neutrophils will get activated; so, neutrophils are not early resident whole lot, but they are in quite high numbers in the blood. And if there is a tissue injury, then you have also ruptured the blood vessels and the neutrophils are present at the site. So, they start doing phagocytosis to whatever was damaged and out in the surrounding. If they get activated, they will start to secrete cytokines.

Now, that they are secreting cytokine this local cytokine release is going to trigger a cascade of events right because as we discussed in the previous example, first of all these cytokines are going to act in feedback loop mechanism either on in the same cell or in the neighboring cells and amplify their production. They will start calling immune cells from the surrounding. So, more immune cells are now coming in so, more immune response is being developed and some of them will actually go far away and start activating the immune cells there as well.

And some of the other cascade of event is first of all vasodilation. So, now, they will also dilate your blood vessel which means that more immune cells can come into the site and that is why you see actually that site becomes fairly red, that is because the blood circulation is increased there.

Then you have influx of protein rich fluid and the cells are coming in. So, you now you see swelling as well now you have lot more fluid into the environment where the injury has happened. So, that is why you see swelling and that causes pain as well because nerve cells also get involved.

And then you have influx of leukocytes and phagocytic cells into the tissue. So, because of this vessel dilation, all these phagocytic cells are also again which was circulating in the blood are also coming into the tissue. And then if the next step is if you are able to clear whatever was a foreign entity and the body thinks if this is fairly compatible, then the next step is healing and tissue regeneration; which in cases of bio-material is what we want. So, more new vessels will form and maybe the scar tissue will form if it is a deep wound and all and the healing process will start.

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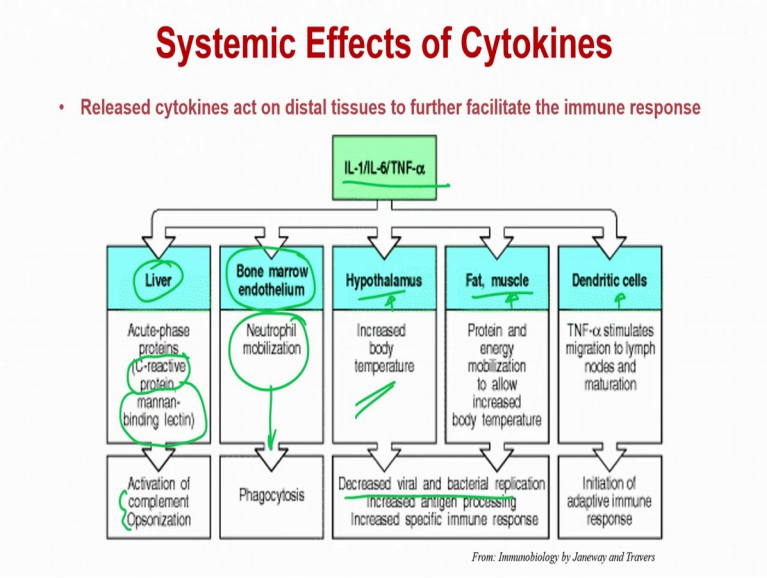

So, what are the systemic effects of the cytokines? So, the released cytokines can act on distant tissues to further facilitate immune response. So, this is on the systemic effects of it. So, some of these proteins like IL-1, IL-6 and TNF-alpha can potentially do that. So, if

they go to the liver, they will activate some other proteins like C-reactive protein and mannan binding lectin. This will lead to an activation of a complement system.

We have not talked about complement system yet I think, we will talk about it in the next few classes. So, this just hold on to this, but it activates another class of an immune response that we have not talking about yet. The other thing that they will do is in the bone marrow, they can activate the bone marrow endothelium and they will start mobilizing more neutrophils. So, as I said bone marrow is a site where all these immune cells are being produced.

So, they will start the production of neutrophils and start to cause them to come to the side. Then they can also go and act on hypothalamus which is a brain organ and that is responsible for increasing the body temperature. And the reason it is done is because our immune cells are actually in our own cells are actually much more capable of handling higher temperature than let us say pathogenic cell. So, if you increase the body temperature. So, from 37 let us say if you go to 39, the body is at a more advantage at that point, than these pathogenic cells.

Then similarly the fat and muscle tissue also get these signals and they start, because now you will need some energy to maintain that much heat to have that much influx of cell to have more cells come in. So, that will start happening and I mean both of this will cause decreased in viral and bacterial replication and then more dendritic cells are being called in again; these are also cells that are going to do phagocytosis and make sure that your bacteria are being killed. Okay! We will stop here and we will continue in the next class.