Drug Delivery Principles and Engineering
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Lecture – 45
Immune System – II

Hello everyone, welcome to another lecture of Drug Delivery Engineering and Principles. My name is Rachit and we are going continue our discussion.

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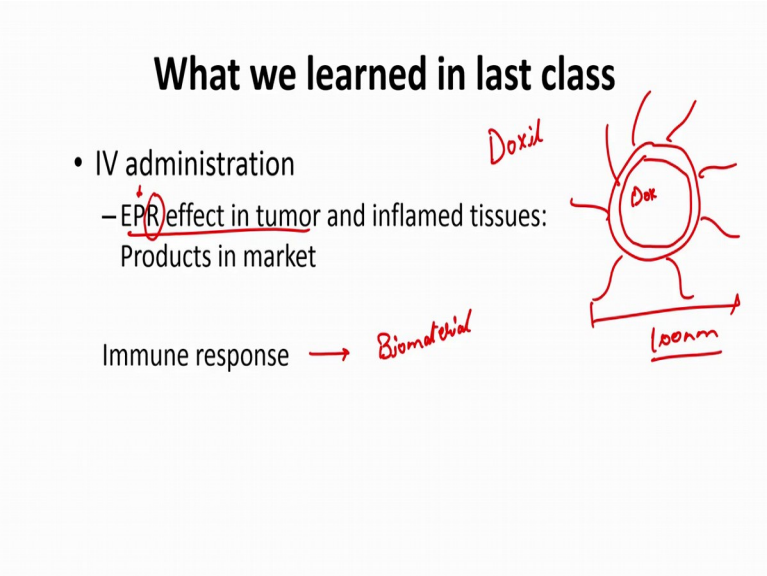

So, a quick recap of what we learned in the last class. So, we started the last class with the module on route specific delivery and we basically talked about iv administration further in lot more detail. We had discussed EPR effect which is the Enhanced Permeation Retention of a drug in the tumor regions compared to healthy tissue and that is because the tumor regions are first of all leaky.

So, it is called enhanced permeation and then the things that go in the tumor region were also retained there that is because there is quite a bit of fluid being accumulated because lack of lymphatics. So, there are products in the market on the basis of that. We discussed a few of them; one was Doxil which is a liposomal formulation. It is a lipid bi-layer carrying doxorubicin inside and this also PEGylated and this was about 100 nanometers in size, that is responsible for using the EPR effect and causing increase in the therapy.

Then we discussed this was just one example and then we started discussing the immune response. This was in context to what is going to happen when you put your biomaterial. But before we go even go to the biomaterial, we need to understand how does the immune response work, what are the different cells that are involved and so that is what we started with. So, let us continue our discussion on the immune response in this class.

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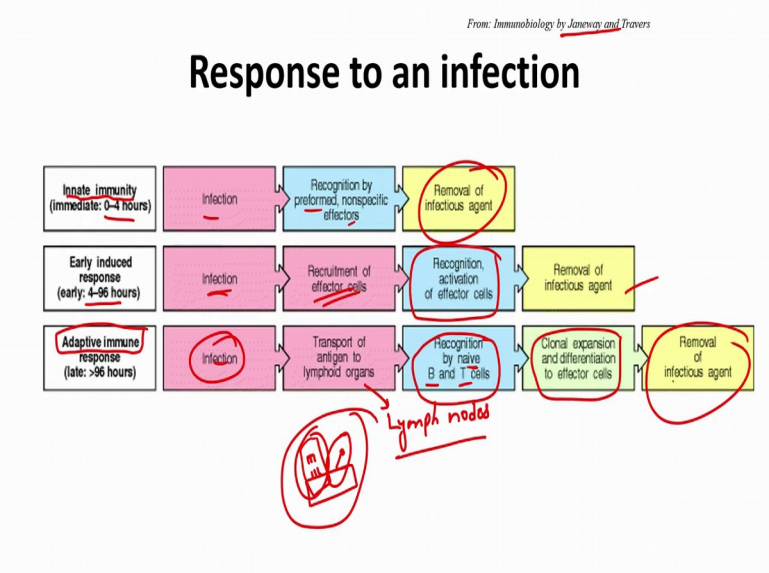

So, what is a response to an infection? So, for again this is from the Janeway book and the response to the infection involves various durations. So, the very first response is from the innate immunity. So, what is innate immunity? And we will discuss this in a bit, but innate immunity is nothing, but something that the body already knows that this is pathogenic. So, let us say there is a bacterium and it has a certain pattern to it. So, a body already has some molecules, let us say there are certain pattern to it. So, our already has some molecules that are going to bind to this pattern and immediately gets activated because, the moment they detect this pattern, the immune system then acts up and starts to eradicate this pathogen.

So, this was something that was already present. Then there is something called the adaptive immunity; an adaptive immunity is something that our body learns about this pathogen. So, it is something with the body did not really already knew; but the body can learn about it. So, let us say there is a particular protein which the body finds immunogenic.

So, the body processes it, the body learns that this is a foreign protein and how to deal with it; maybe it is using some antibodies or maybe it is using some cell mediated killing. But then, eventually the body is able to learn about it and once it does; it is much more effective in killing it then let us say by these responses.

And again, we will discuss this is in lot more in detail, but so it is obvious then that the innate immunity is going to be extremely quick. So, within the 4-hour period, the innate immunity can detect this and start to kill this. So, it is very effective for immediate response and after that if the bacteria still purses, the innate immunity, obviously, is causing amplification of the immune system. So, that we will continue for the next couple of days, next few days 4, 5 days and then within that 4, 5 day the body is also starting to learn what kind of infection is there.

And that is when the adaptive immunity will start to kick in about a weeks' time and that is going to then be able to eradicate the pathogen. So, what are the different responses? Of course, infection is common to all of these and the innate immunity is going to be recognition by preformed nonspecific effectors as I just said. So, this is nonspecific; anywhere this finds it, whether its bacteria, virus or one kind of bacteria or some other kind of bacteria; it is going to bind to it and it is going to activate the immune response; it is going to remove the infectious agent.

If that happens well and good; no further need to go forward and you do not get a disease in 4 hours because it is just too short of a time, you do not even know that you were infected with something. The other responses you do get a disease; sometimes flu is a good example to this, sometimes you do get flu, you do get sick for 1 or 2 days, but then within after 2 days you are body is actually taking over and you are well to go about it. So, in that case what is happening is; infection is happening we are recruiting more and more cells. So, maybe the threat is larger; maybe you have got larger those of the bacteria of the virus.

So, the body is developing more and more response it is causing more and more cells to come in. Some effector cells are involved and then it is able to remove the infectious agent and then finally, there could be a disease which can last for quite a long duration. So, some flu or like that, but like tuberculosis or some other form of bacterial disease

maybe jaundice! so in that one: your innate immune response is not able to take care of it.

So, there is infection now you are now transporting all these new antigens the body is sending to lymphoid organs. So, one of them being lymph nodes and at the lymph nodes are specialized cells like B cells and T cells which are initially naive which means that they are not exposed to any foreign pathogen before this exposure; they are recognizing these things and we will discuss how they do that they will then expand. And once they expand, now they are capable of handling this and then the removal of the infectious agent is taking place.

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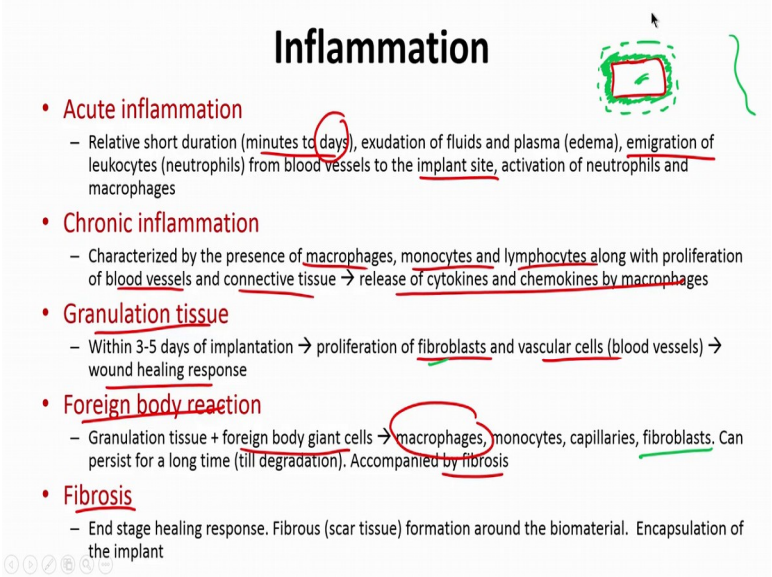

So, starting with inflammation, it could be an acute inflammation; so relatively short duration and as I said could be minutes to days. You have still the fluids if it is going to days you have swelling, you have fluids accumulating at the site because of the vaso-dilation; you have immigration of the leukocytes.

So, especially neutrophils they are coming to the sites to these dilated blood vessels to the implant site, now if you are looking at implants and this is causing activation of these cells, as well as macrophages. So, that initial part is called acute inflammation. And then you have chronic inflammation; that means, that the inflammation is lasting for quite a long duration, rather than just being a few days, it is quite a number of days.

And that is characterized by the presence of lots of macrophages, monocytes and other lymphocytes; your blood vessels are now actually starting to proliferate in that area because you have a lot more concentration of these cells. So, more blood vessels are growing in there; so, you have these blood vessels growing as well as connective tissues and you are continuously having a release of chemokines and cytokines; so, this is more a chronic inflammation lasting quite a long duration.

If that chronic inflammation is also not doing anything then after about 5 days, you have granulation tissue being formed. And so, that means, that the surrounding cells such as fibroblasts and vascular cells are forming a granulation tissue; essentially proliferating and filling up the site. It is a wound healing response, if the things are not still able to clear it then, there is a foreign body reaction. So, the body thinks this is something foreign; we cannot clear it. So, big giant cells start to form and these macrophages start to combine.

So, maybe 10 macrophages will be combined to from this big giant cell and they will persist at the site till they can degrade whatever it was in the surrounding; if they do not like it. This is accompanied by fibrosis which is disorganization on the tissue structure with lot of secretion of ECM, but in a very disorganized manner. And eventually leading to a full-fledged fibrosis which is the end of the healing response, now the body thinks it cannot really do anything with this, it had tried whatever it could. So, let us just really remove it out from the system and cannot really move it out from the system.

So, what it is doing? It is just encapsulating it and isolating it from the rest of the body. So, if this is my implant, what the body is doing now is just walling this off with lots and lots of cells and extracellular matrix which are being produced by these fibroblasts. And this is essentially depositing it around a thick layer of this. And now what the body has done? It has protected the rest of the body from anything that was here. So, it feels that now it is protected. Now this is just some different steps of inflammation as it goes along.

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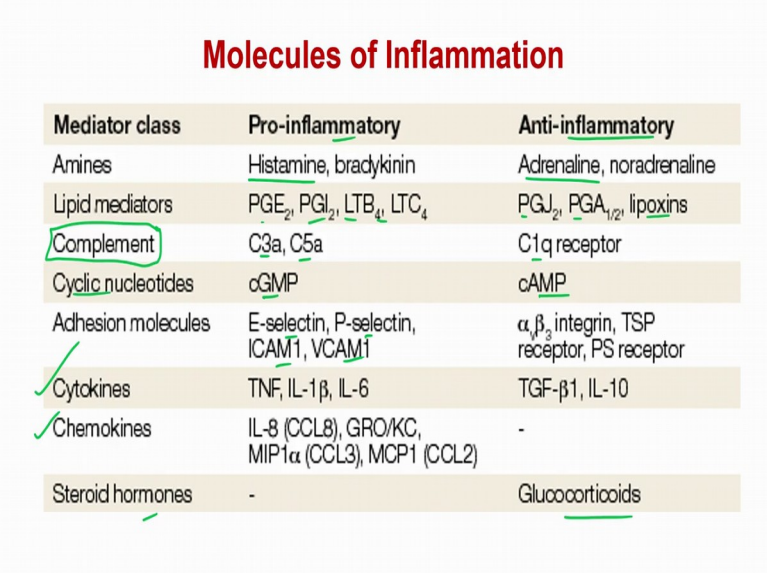

So, some more molecules of inflammation; we have lots of histamine and other things that are involved in pro-inflammatory response. You can also have adrenaline that has anti inflammatory response, you have different lipid mediators; several names are being listed here; you do not have to remember all of them, but some of them you will hear again and again. You have the complement system, that I said we will discuss later and that is still true we will not discuss today, but we will discuss it in future classes.

But there are a few proteins involved with this complement system, that is involved in both the pro-inflammation and the anti-inflammation pathway; you have cyclic nucleotides, these are nucleotides that are also making up your DNA. So, the presence of these can cause pro-inflammatory or anti-inflammatory pathways. Your different adhesion molecules: so different things to which immune cells can adhere to detect that there problem at the site and some of them are listed here and we will go into a bit of detail about some of these.

Cytokines, we have already discussed; chemokines we have already discussed. So, some of them are listed here and you can have steroid hormones such as glucocorticoid steroids. These are all anti-inflammatory, they tell the immune system to calm down and do not continue to damage their own body parts.

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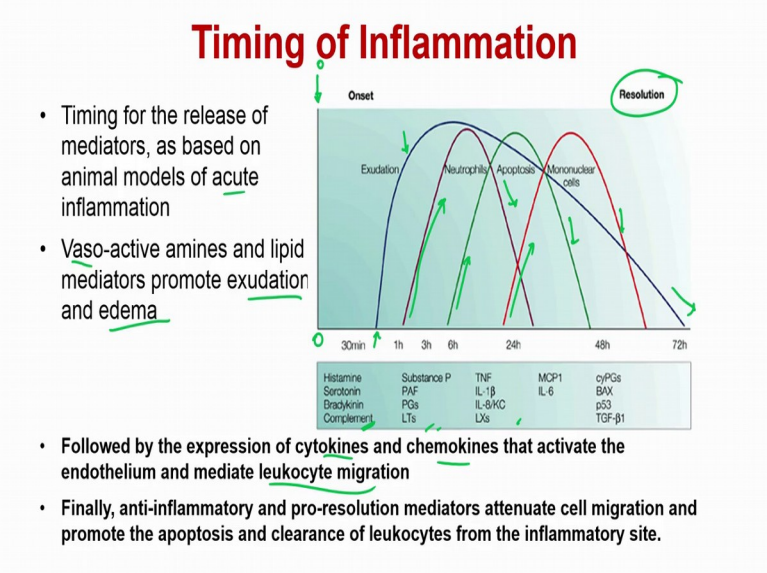

So, what about timing of the inflammation? So, we have discussed some of the timing already, but let us say some injury happens or some pathogen comes in at time T equal to 0. So, immediately the body will start responding to it. The innate immunity will kick in and you will have vessels getting dilated, once the body is detecting it.

So, once the vessels are dilating more and more cells are coming. So, you have swelling and that will start and then as more and more cells are coming; the neutrophils numbers are going up, the number of cells in the area is dying also because this is quite an extreme environment for them. And then you also have these monocytes and macrophages the more adaptive cells start to come in.

And then everything gets dissolved; these numbers will go down and eventually and the blood vessels will also go back to normal and the lymphatics will clear any extra fluid away which is called resolution of the inflammation. And so, eventually within 2, 3 days you will have this go down and again several molecules are involved at different stages and which are listed here.

Complement is again a part of the innate immune response and it acts very quickly, so that is how the timing goes. So, again in some text here listed talking about the initial acute inflammation happening; some vaso-dilation that leads to edema and swelling of the site, followed by lots of cytokines and chemokines by the endothelium and the leukocyte migration and then finally, clearance of all this if the threat is mitigated.

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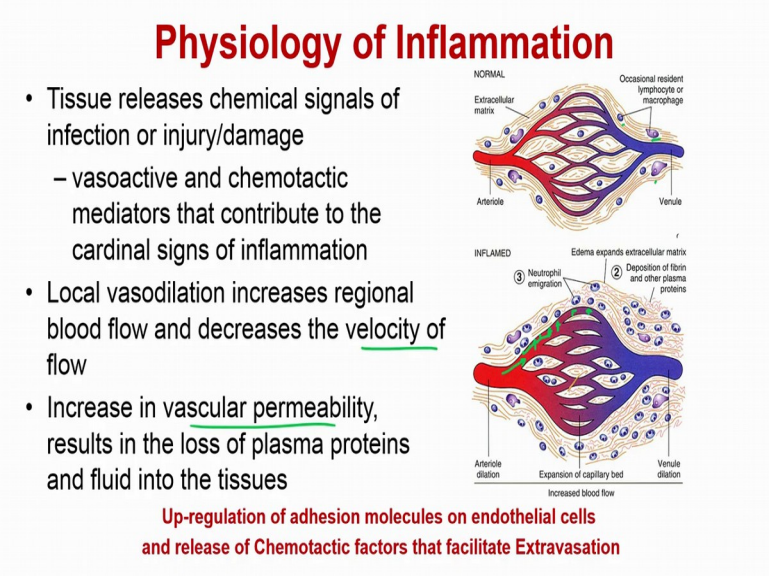

So, here is some more details on the physiology of this inflammation. So, you have tissue releasing these chemical signals of infection and injury. So, this is the normal case; you have few resident lymphocytes and macrophages as I am saying. But let us say, injury does happen to it, first of all you are seeing that these vessels are dilating, which means they are becoming expanded that causes the blood to slow down.

And secondly, the junction between these cells become more and more leakier and so, you see lots and lots of cells have come in. And that is what is essentially causing the increased blood flow and increased immune cells in the surrounding. So, with this local blood vessel dilation, the velocity is decreasing and then the vascular permeability is increasing. And then what is another step that happens is these cells that are lining here; they actually increase the expression of some adhesion molecules to which these floating immune cells can bind to and we will talk about that.

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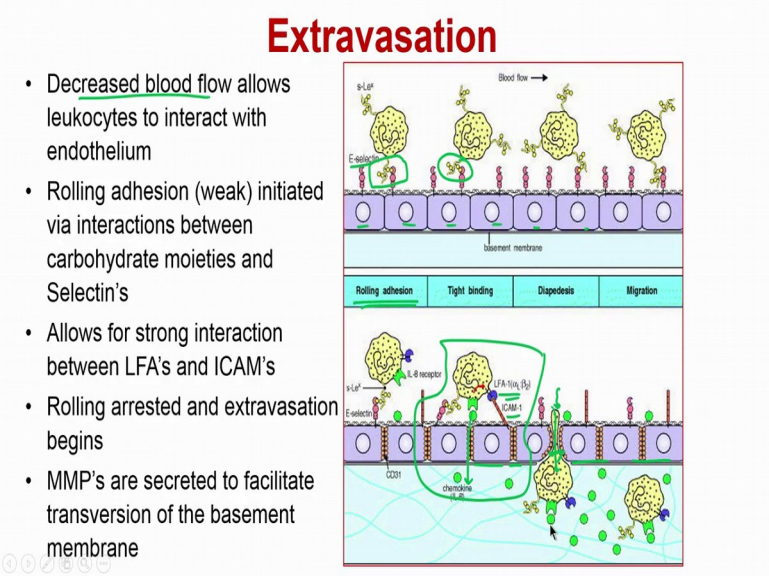

So, how does these cells actually come out from these vessels right? I mean even though the vessels are dilating, they are not dilating to an extent that they are 5, 10 microns big gaps in the vessels because then the blood cells will start coming out. So, that is not what you want; so how does this happen then? How does the immune cells come out and not the blood cells?

So, as I already said that the vessel dilation leads to decreased blood flow? This is obvious from Bernoulli's equation. If you have; if you have a certain velocity flowing through a certain cross-sectional area. So, V1.A1, and if suddenly now that cross sectional area increases because of the vessel dilation. So, this would be equal to V2.A2; So now we are saying that due to dilation A1 is small whereas, A2 is large. And because of that what you will have is the V1 will be higher and V2 will be less than V1, the magnitude of it the direction of course, remains the same. So, and because of that, what you will find is there is a decreased blood flow through the endothelium. Then I also mentioned that these endothelial cells, which are these membrane cells, then start secreting some adhesion molecules.

One of them is E-selectin and others are ICAM and all. So, what will happen is these immune cells which are now slow down a bit; they have enough time to interact with these receptors through their own receptors; so that is what you are seeing here. So, now they are starting to bind once, they start binding, they are slowed down even further.

So, now, they have more time to actually roll around on to this blood vessel because they cannot really go with the blood. Because of this interaction and they start to slow down. Once they slow down this is something called a rolling adhesion. So, they are essentially just rolling on the surface and binding more and more, once enough bonds are formed you get a conformation like this and here some more adhesion molecules that are listed here.

Once you get a conformation like this; then this cell can start to come out from this blood vessel and this is what is being shown here. So, this is actually causing the secretion of some enzymes and then squeezing through from this particular leaky vessel that is created because of inflammation. So, MMP's are secreted to make this leakier and the basement membrane; which is the membrane which is holding these endothelial cells starts to degrade and it becomes permeable.

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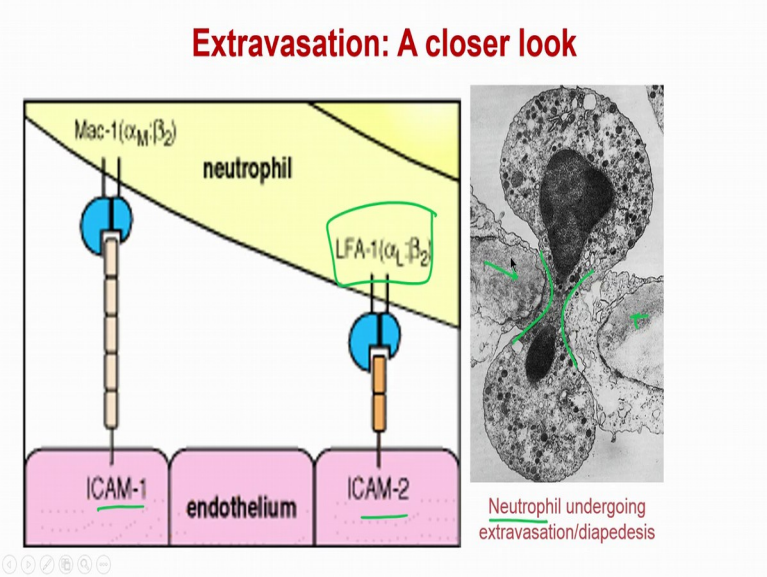

So, this is just a further closer look to it. So, again several different types of receptors you have ICAM 1 and ICAM 2; selectins we already talked about. Then you have some integrins, that are involved and what you see here is an actual SEM image of neutrophil; that is undergoing extravasation through these endothelial cells. So, you can actually see how these cells can actually squeeze through and come out on the other side.

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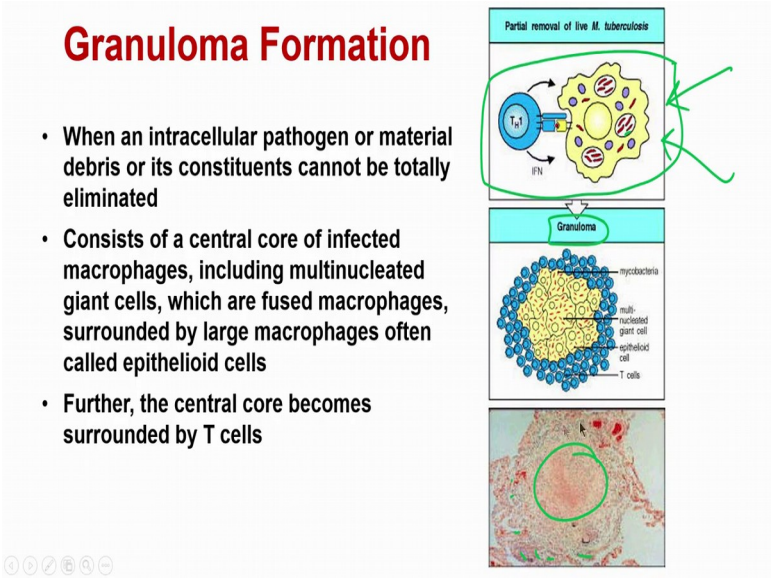

So, a little bit on the granular formation. I mean we are talked about fibrosis; there is another thing that can happen which is a granuloma formation. Again, similar to that seen quite often in cases of tuberculosis when the body is unable to clear the bacteria because sometimes the bacteria can have some strategies that the body cannot handle and that is what is shown here. So, in this case, it is showing that the immune system is actually trying to clear up this pathogen; which is also intracellular the Mycobacterium *tuberculi*.

But it is not able to kill it off causes more immune cells to come in and these bacteria actually predates on these immune cells. And because of that these bacteria are actually creating a niche for itself; which it lies and it can be replicated. And that creates this more and more cells to come in and the structure which is being shown here which is a granuloma formation.

And so, you can see that this is the rest of the lung which is well aerated, but there are certain regions here which are full of cells, causing the granuloma formation to happen. So, this is another way where is that the body is now trying to wall this off, but then in this particular case; this is also acting as a safe harbor for these bacterial cells because they like to predate on the immune cells also. So, just something that I wanted you guys to know that can also happen in the body.

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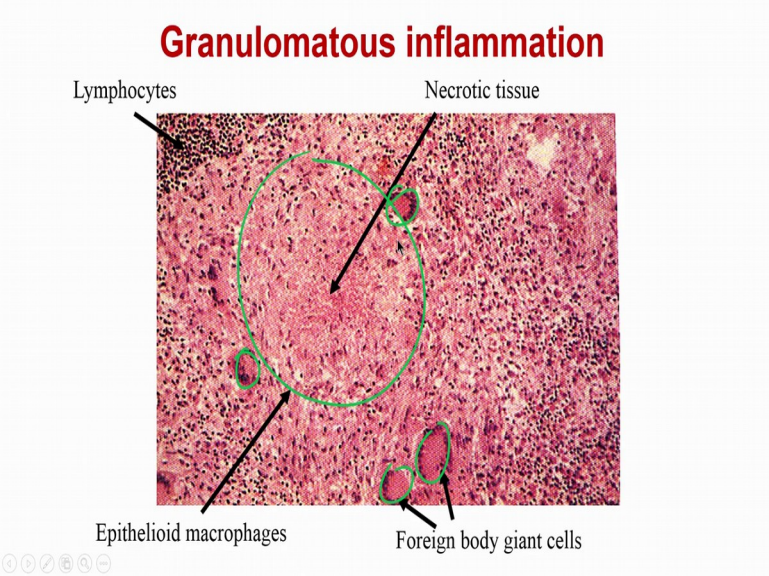

So, again a little more zoomed in image of this; so, you have this granuloma being formed, you have all kinds of macrophages and lymphocytes surrounding this area and you have even giant cells trying to clear few things up. But they are not able to clear really because these bacteria are fairly smart; sometimes they do, sometimes they do not.

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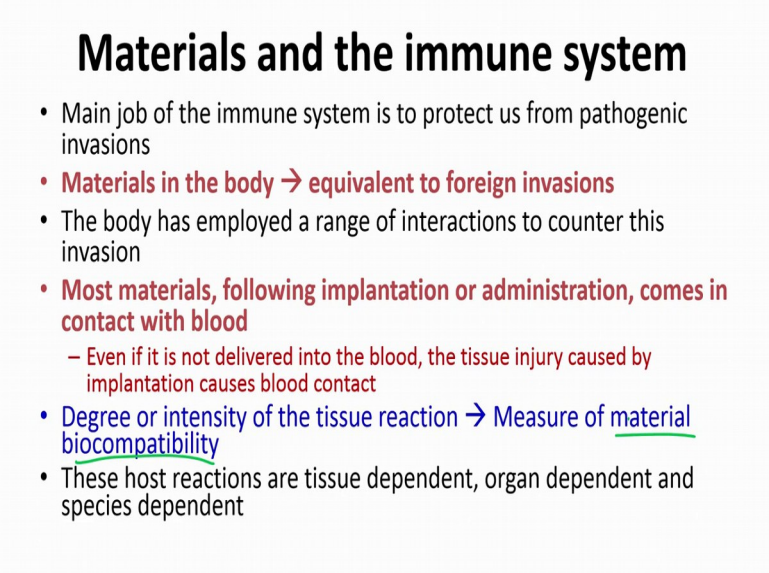

So, let us talk about how materials then interact with the immune system. The major job of the immune system is it should protect us from pathogenic invasions. So, that is fairly

clear I hope by this time, but any materials in our body, does not know that this is a foreign pathogen or a foreign material.

So, this is equivalent to any kind of invasion that is happening in the body. So, immune system does first of all its going to first survey and then if it does not like it that is going to attack it. So, the body has also employed a range of interactions to counter this invasion because of this immune system. So, when you implant something or when you inject something; these things come in contact to the blood, even if you are not delivering it to the blood right?

I mean even if its let us say a skin that carries a very dense network of blood vessel and you poke the skin with something; these blood vessels are getting damaged. So, they are coming in contact with the blood. So, because of that the immune system is going to get activated and then the degree of that activation will depend on how compatible the material is.

So, when you are trying to deliver something; which is not to in the immune system you want to make sure that this activation of the immune system is as low as possible and that would mean that your material is as biocompatible as possible. And these host reactions are again tissue dependent; so maybe the same material in a certain tissue may cause a lot more response in organ; in a certain tissue or in a certain species and so this is something to be considered. But in general, what you will find is, the materials that are immunogenic do hold true that they are more immunogenic than other materials at the same site. But the site of the implantation then also becomes important because maybe at certain site, you may get less inflammation and you get more function out of your device or out of your implant that you are putting in.

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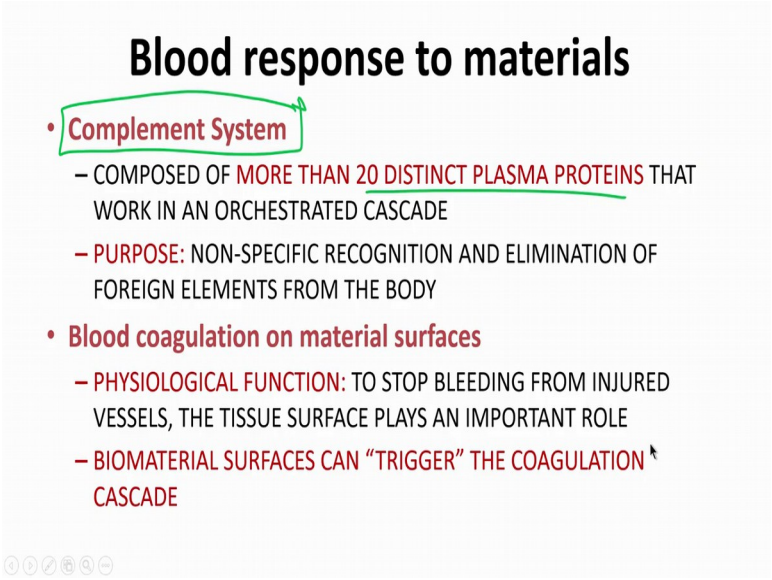

So, let us talk about blood response to materials now and you said the blood is the first thing that comes in contact. So, this composes of complement system which again we are referred to in the previous two classes.

That means, that these are innate immune responses that are composed of about various types of proteins; there are about 20 distinct plasma proteins that work in a very cascade manner; quite non specifically to eliminate some of the foreign elements in the body. And then there is of course, blood coagulation in the material surface; so, this is a physiological function to stop bleeding. So, if the blood comes in contact with anything which is not an endothelial surface; the body knows that it should not be like that, it could be because this might be bleeding out.

So, the body then triggers and a cascade of pathway again that causes clotting of the blood at the site where it's not the end he will site anymore. And so that is to prevent us from dying out right? because if let us say, I get a cut and the blood never stops, then I will eventually lose all the blood from that cut. So, that is a response that the body has evolved to perform to make sure that all the blood is not draining out and you can clot the blood on any new surface that is that the blood cells see.

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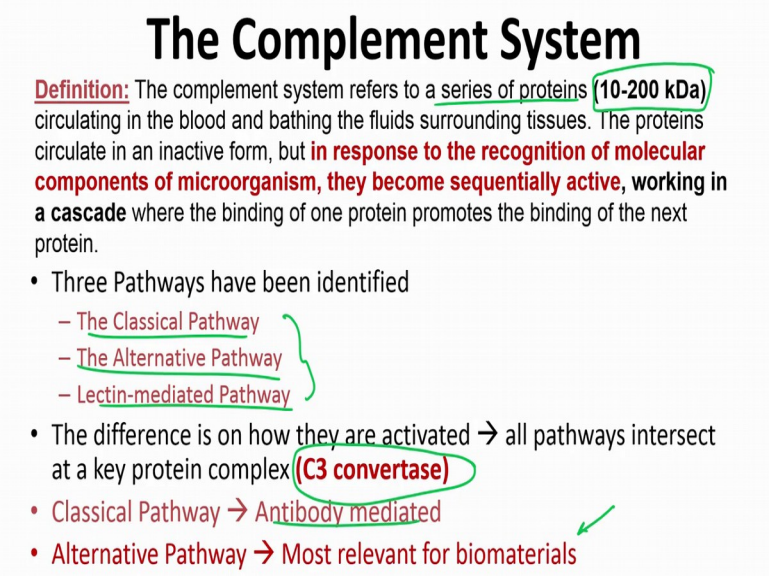

So, let us talk about complement system; I already defined it a bit, but a complement system refers to a series of proteins typically in a wide size range from 10 to 200 kilo Dalton, that are circulating in the blood they are present in different fluids and tissues.

So, they are essentially circulating and sampling everything; these proteins are initially circulating in an inactive form. But if they do recognize some molecular pattern or something foreign, they can become sequentially active and then they were in a cascade that causes the inflammation to increase. So, there are three pathways that have been identified; for this the classical pathway, the alternative pathway and lectin mediated pathway; these are again different pathways for all these series of proteins and that just is defined on the way of how they are getting activated. And the difference is basically what I said, how they are getting activated; they all intersect at a key protein which is called the C3 convertase.

And we will describe it in a bit more detail. So, classical pathway is antibody mediated, alternative pathway is most relevant to biomaterials; where this pathway is usually the one that recognizes anything foreign biomaterial, rather than a foreign pathogen.

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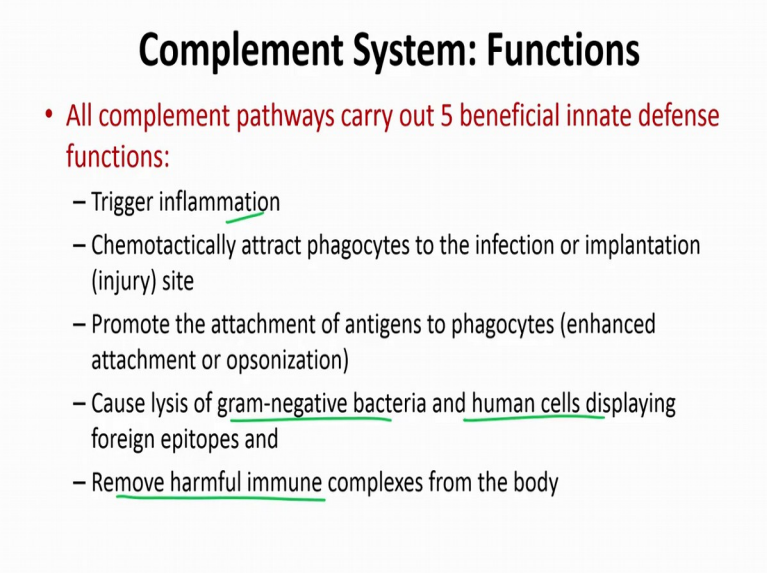

And again, the major function is to carry out the 5 beneficial innate functions. First of all, trigger inflammation, so telling the body that there is something foreign we need to remove it. Chemotactically attract phagocytes to the infection or the implantation injury.

So, get more cells to come in, basically part of this inflammation cycle itself. Promote attachment of these antigens to the phagocytes; so, you can have more enhanced immune response, more enhanced attachment and opsonization of these sites. If there is any bacteria that causes lysis of this and the human cells that are also showing signs of infections. Or maybe it is a virus that is deciding within an immune cell or within a human cell and you do not really want the virus to survive.

So, maybe it is beneficial to kill of that immune cell, so that is one of the functions they also perform. And finally, they are also involved in removing harmful immune complexes. So, if let us say the inflammation is finished or at least the threat has finished and there are few immune complexes that still remain they also help in the removal of those.

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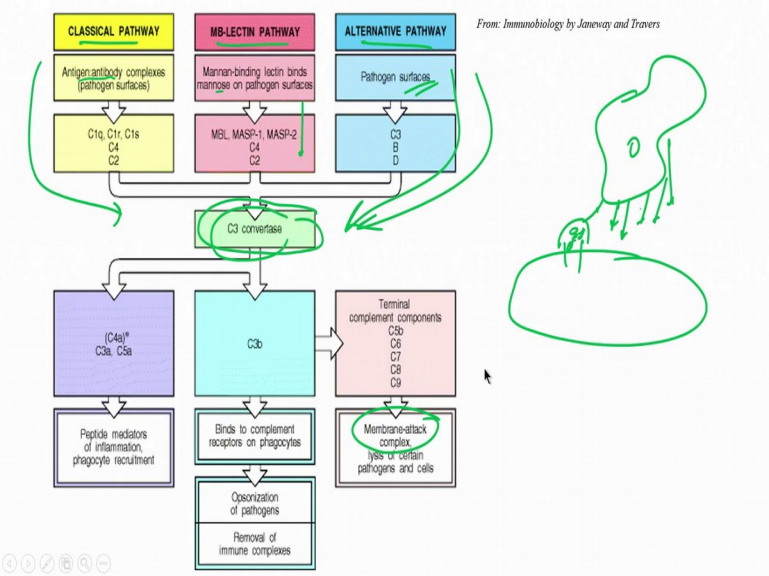

And so, this is what I was talking about there are three pathways; there are classical, lectin and alternative pathway. They all converge through various mechanism and obviously, this is a much complex chart than what I have shown here and various proteins are involved. But they all converge to a single protein called C3 convertase and from there it can then form different categories as well; so, in the classical pathway this is identifying antigen-antibody complexes, if they find any antibody getting complex to any antigen that is what activates it.

The lectin pathway this is that; it binds to carbohydrates and sugars on the pathogen surface. So, if they find anything that is not mammalian derived sugar or not a human derived sugar, they identify it and then they bind it causing C3 convertase to form. And in alternative pathway it recognizes surfaces; so, they may find surface that they have not seen before and that could cause activation of these pathways; leaving to this activation to C3 convertase. Once you get to the C3 convertase, it can then again trifurcate in two different things, you can have membrane attack complex.

So, what you can have is, let us say if this is a bacterial cell; the complex the complement pathway is identified it, it forms a series of proteins on the surface and let us say more and more proteins are coming in. And eventually what it is going to do? It is going to poke a hole into this bacterium and basically kills it. You can have these

complement systems once they bind it there are other immune cells that have receptors for these complement systems.

And once they find that there is complement, they bind to another cell, they can then directly kill this either by engulfing it or by secreting other harmful biomolecules in the surrounding. Okay! So, we will stop right here and we will continue further in the next class.

Thank you.