**Host-Pathogen Interaction (Immunology)**
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**Lecture – 19**
**Cells of Immune System and its role in Host Defense-Macrophages**

Hai, in previous session I have discussing great length about the dendritic cells or I can say the various kind of dendritic cells. And in this session, we will take the another very important immune cell known as Macrophages and most of you are I am quite sure you are aware about the macrophages. But let us see I will give the glimpse of macrophage in as a immunologist point of view.

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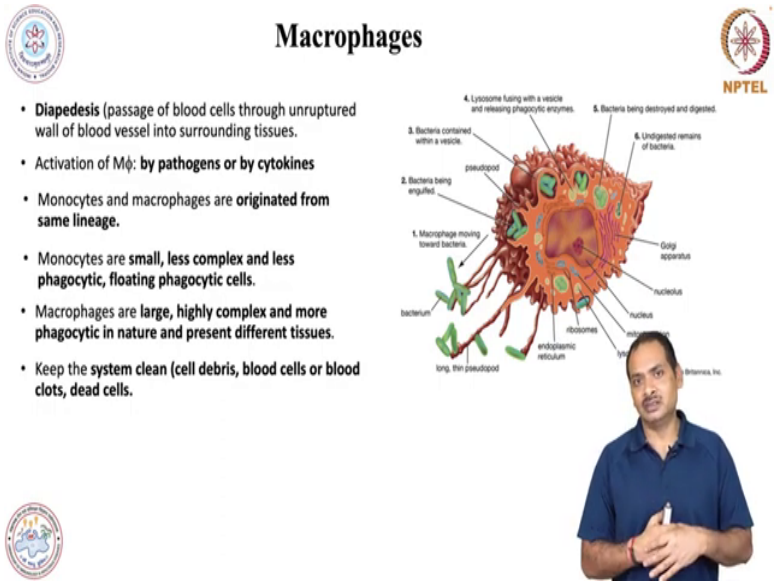


So, macrophages are probably you are aware that it was first discovered by Metchnikoff and at that time I think, you remember my previous session when I discussed the history of Immunology. So, it was first discovered in the starfish and they are basically readily taking the particles and there was at that time people were thinking that the defense against infection is mainly taken place by this kind of cells.

And those group of people they were known as cellularist there are two school of thought I have discussed those things in history of immunology. There are two school of thought, one is cellularist and another is humoralists those who are believing there is a some soluble

component in the blood which is taking up the antigen or clearing the antigen or protecting us from the infection.

So, this macrophage was discovered quite early now you can understand. So, basically the macrophage is something look like this it is a schematic here you can see that they have a projections or we call it as a pseudopodia. And this pseudopodia is basically helps in taking up the microbial pathogen and once it is taken up by the microbial pathogen then it will undergo phagocytosis.

In a simpler word there will be some receptor for this pathogen and this receptor basically attached there will be a clustering of this receptor when it will come in contact with the membrane of macrophages. And after clustering of this receptors there will be activation of downstream signalling pathway, which basically re-arrange the cytoskeleton and this re-arrange cytoskeleton is basically caused the invagination in the macrophages.

And eventually it will be taken up in the form of particle means this particle will be taken or pathogen will be taken up and it will present in a in a membrane-bound structure. And here you can see after phagocytosis there is a fusion of one cell organelle which you are also aware that and we also call it as a suicidal bag. And this suicidal bag is lysosome. And these lysosomes are loaded with a variety of hydrolysis.

So, these hydrolysis breaks down this this microbial pathogen and then this will ~~be~~ further activate various kind of immune response. It will present the antigen along with MHC class II molecule, it will also do various kind of responses that I will discuss in a short while. And beside this the macrophages are other organelles as other cells have so, I am not going to talk about those things.

Macrophages show a very unique phenotype and it is quite common in macrophages known as diapedesis. I think I have explained you in previous session if you missed it then I can again explain. It is a basically movement of this cell across the blood vessel, un-ruptured blood vessel to the surrounding tissue so, this we call it as a diapedesis. So, macrophage is a very uniquely show this property.

And macrophages are generally two major state of the macrophages are there, one is resting stage and another is activated stage. So, let me explain what is this activation of macrophages or activated what is activated macrophages? So, activated macrophages are showing some unique properties, one is that they produce a lot of inflammatory cytokine number one.

Number two they can be more aggressive phagocytic in nature, the activated macrophages another is they will present the antigen and they will also express the adhesion molecules. Adhesion molecule which is needed for the T cell activation and they are very strong stimulator of T cells. So, all these property together we call it as a activated macrophages or activation of macrophages.

And this can be achieved by two major way, when the macrophages see the pathogen, microbial pathogen then they get activated number one. Number two, these macrophages also get activated when they are treated with some immune factor and these immune factors we call it as a cytokines. So, if you treat the macrophages with some cytokine for example, the interferon gamma.

So, interferon gamma is commonly used as a stimulator of macrophages or if you can stimulate with some component of pathogen for example, LPS which is coming from the gram-negative bacteria. So, if you treat the macrophages with LPS then they get activated. So that what I mean to say that macrophages and monocytes are originated from same lineage.

And if you remember my previous session, I explained you that macrophages and monocytes are derived from myeloid lineage. So, hematopoietic stem cells makes two lineage myeloid and lymphoid and this macrophages is coming from the myeloid lineage. Monocytes are basically when we call the monocytes then they are in the blood I have explained you earlier. So, monocytes are basically present in the blood and they are very small less complex, less phagocytic.

And overall if I can say that in a simple word they are floating phagocytic cells but with compromised property but we can differentiate these monocytes to the macrophages if we treat with some factors. If we treat with some reagent such as cytokine or some pathogen derived molecules then we can differentiate these monocytes to the macrophages. In general, we use this differentiated macrophages in some experiment.

We treat we isolate the PBMC and then we treat with some factors cytokines and then differentiate to the macrophages and then we perform the various experiments. So, macrophages on another hand macrophages are highly complex if you take the tissue macrophages they are large, very complex and more phagocytic in nature and in different tissue, we call it as a different by different name.

Of course, it is not only the different name they are having some different properties as well. So, they I will discuss this thing in upcoming slide, what are the different kinds of macrophages? Besides immune function macrophages are playing a very important role in scavenging our whole body. What I mean to say that, you know that every moment there is a some death of cells, some damage of cell there is a some damage of blood cells.

So, all this thing is cleaned up by mainly by macrophages. So, macrophages eat up all those damaged cells and then keep the system clean. So, macrophage plays a very important role in maintaining the homeostasis overall I can say.

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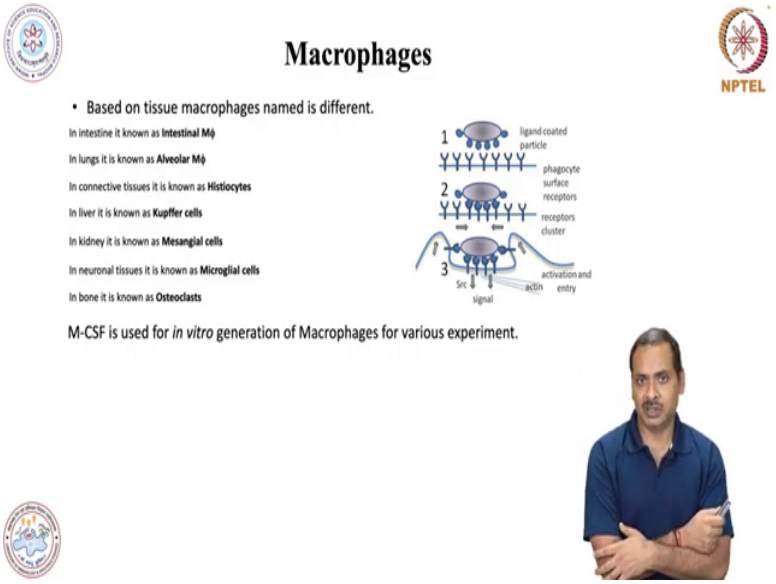


So, based on different tissues their location in different tissues. They are of a different kind and they have a different name and of course they have some variation in function as well, some has a more phagocytic activity some has less or all other properties. So, the macrophages which is present in intestine we call it as a intestinal macrophages. The macrophage is present in lungs, we call it as a alveolar macrophages and they play a very important role in all lung associated infection.

They are also present in connective tissue, we call it as a histocyte or histiocyte and in liver these macrophages we call it as a Kuppfer cells and they play a very important role in processing of antigen again. In kidneys also there are cells which can phagocytose and we call it as a Mesangial cells and in our nervous tissue or neuronal tissues they there are specialized phagocytic cells and we call it as a microglial cells.

And it is a very interesting that macrophages do have do present in the bone and they are very much important for the bone homeostasis and these cells, we call it as a osteoclasts. And these osteoclasts and there is another cell osteoblast their activity basically maintain the mineralization and other things of the bone. So, this is playing a very important role in mineralization and whole activities in the bone.

So, how these macrophages phagocytosis? As I told you that when the pathogen or some bead will come in contact here you can see that there is a bead which is a coated with some ligand. And there will be a receptor for those beads over the macrophages and once they will come in contact then there will be a clustering of these receptors. And this clustering of receptors basically result to the activation of downstream signalling.

And that downstream signalling basically result to the polymerization and de-polymerization of cytoskeleton protein and then that will result to the formation of phagosome. And eventually the particle will be taken up and then there will be a formation of phagosome and then once it is phagocytose it will fuse with lysosome and then it will make a phagolysosome and finally and the microbe will be degraded.

So, like another cells like dendritic cells, we can also prepare the macrophages in the dishes, we basically take the bone marrow cells as I have explained you previously. We prepare the bone marrow cells and stimulate with M-CSF macrophage colony stimulating factor and once you stimulate with this for about a few days to a week time then you will notice that there is a lot of cells are very strongly adhered to the bottom of your culture dishes.

And generally, we use the EDTA in order to remove this macrophages for doing further experiment. Basically, EDTA take out this calcium ion and calcium is needed for the

adhesion so, in that way we can prepare the macrophages and we can conduct a variety of experiments.

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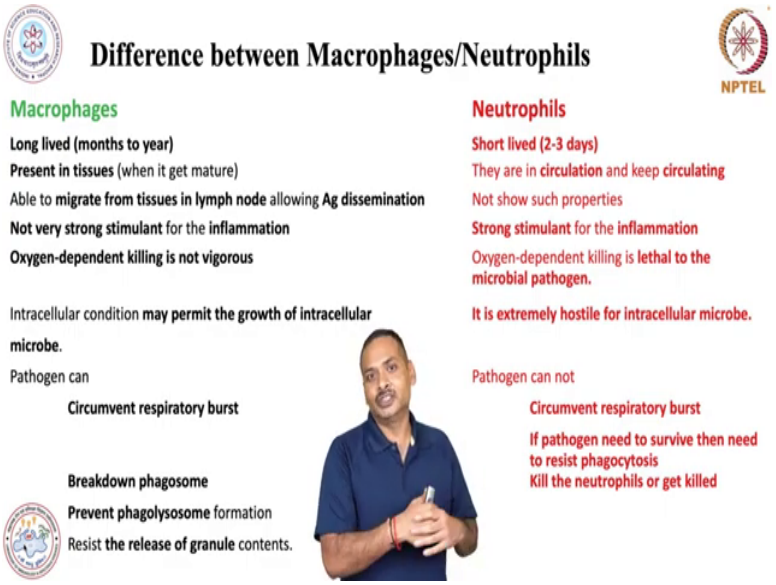


Now, I would like to talk about the difference between macrophages and neutrophils. But before going to the difference I would like to tell about the similarity between macrophages and neutrophils and if you remember that the initial slide or previous sessions where I have discussed about the origin of these immune cells. So, if you see care if you remember that thing then you may remember that this macrophages, monocytes and neutrophils are originated from the myeloid lineage.

Both are phagocytic cells that is also one of a key similarity and they both macrophages and neutrophils basically they can produce a variety of reactive oxygen species or reactive nitrogen species in order to kill the target microbe or pathogenic microbe. Now, let us look at what is the difference between macrophages and neutrophils. So, here you can see that the macrophages are very long-lived their life span is very long and that could be from month to year.

On another hand this neutrophil which is very aggressive which you have seen in previous slides they are very short-lived, they live only for two to three days. So, therefore their production is keep on going on. Another difference is the macrophages basically present in the tissues as you have seen in previous slide there are variety of tissue macrophages as you if you remember that there is a histiocyte which is present in connective tissue there are osteoclast they are present in bone.

And so, these macrophages basically present in the tissues and they get mature and they work over there they have a variety of functions such as cleaning and all those things, cleaning cell debris and other things. On another hand this neutrophils they are present in circulation and they are keep on circulating in blood and they basically encounter very frequently the blood-borne pathogens or blood borne microbial pathogen.

So, another difference is a macrophages are able to migrate from tissue to lymph node. So, they if you remember if they see the antigen then they can take in a tissue if they find the antigen, they can take this antigen and then they can transport it to the lymph node and in that way they can disseminate the antigen. So, this dissemination is very important because if the antigen will be not disseminated then it will be difficult to induce this antigen specific adaptive immune response that is B cell and T cell mediated immune response.

So, macrophages are very good in in taking antigen and disseminating to the nearest lymph node but neutrophils do not show such property. Macrophages are not very strong stimulant for the inflammation compared to the neutrophils. They do produce inflammation through the production of variety of inflammatory cytokines such as IL-6, TNF, IL-1 beta they produce they do induce inflammation.

But they are not very strong stimulant for the inflammation but on another hand if you see the neutrophils they are very strong stimulant for the inflammation they are basically induce quite aggressively the innate immune cytokines which will see when we will discuss about the cytokines. Macrophages are they produce free radicals or reactive oxygen and nitrogen species.

And oxygen dependent the skilling through this reactive oxygen species or through this reactive nitrogen species is not very vigorous compared to the neutrophils. In case of neutrophil they are very vigorous and they are quite lethal or fatal to the microbial pathogen. So, this is a one of a very big difference and that difference is makes the neutrophil more aggressive. So, in case of macrophages intracellular condition may permit the growth of intracellular microbe.

Here I would like to give an example of a mycobacterium tuberculosis so, micro mycobacterium tuberculosis can grow inside the macrophages variety of macrophages. And why because this intracellular condition is not so aggressive and that is why the microbe basically take this advantage and then they can replicate but this is not true for neutrophils. So, it is the environment inside environment in neutrophils is extremely hostile to this intracellular microbes.

And at last but not least there is a pathogen this microbial pathogen can circumvent this respiratory burst so, respiratory burst is taking a lot of oxygen and then producing this reactive oxygen and reactive nitrogen species. So, in case of macrophages the micro can evade or circumvent but it is not true for the neutrophils. In case of macrophages the microbe can break down this phagosome or phagolysosome.

And basically, they can escape from the from this phagosome and phagolysosome and they can grow. In case of macrophages they can prevent the micro can produce some molecules which will prevent the fusion of phagosome and lysosome. You will you can understand that this is very important in order to clear the intracellular pathogen So, in case of macrophages since the condition is little amicable to the micro they can prevent this fusion.

They can prevent the addition of hydrolytic enzymes and in that way they can escape they can resist the release of granule contents because that lysosome they are loaded with a variety of enzymes. So, all these micro which is residing inside the macrophages they can resist the release of content. But on another hand neutrophils are they are extremely dangerous to the microbe.

So, there are only two ways to escape from the neutrophils, one is that either they should not come in contact with the neutrophils and if they are coming in contact with neutrophils then they should kill the neutrophils. In that way they can escape from the neutrophils there is no other way if the neutrophil take up this microbe then they this micro will definitely killed by the neutrophils.

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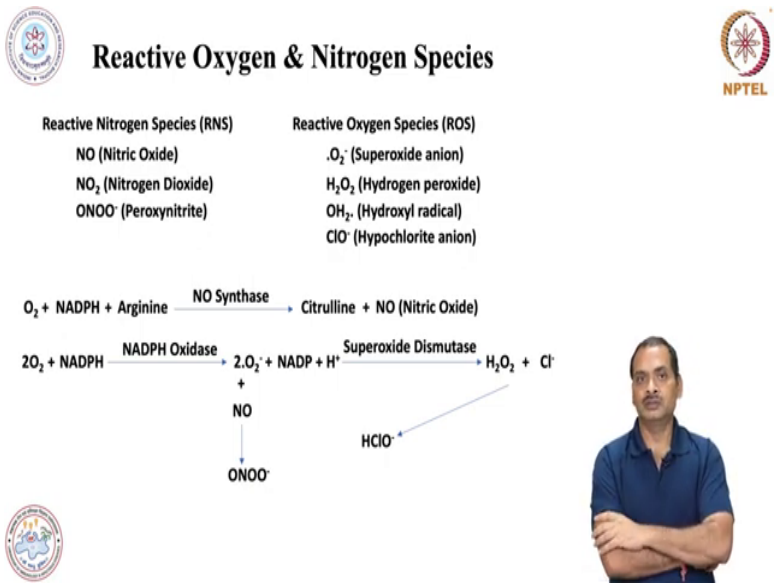


Now, I would like to tell about how this reactive oxygen species and reactive nitrogen species are generated. But let us first we know what are the reactive nitrogen species and what are reactive oxygen species? Here you can see the reactive nitrogen species are nitric oxide which is very active molecule in animal cells particularly in human, I will tell more about this nitric oxide and there is a nitrogen dioxide and there is a peroxynitrite, all these molecules basically make the intracellular condition more hostile.

And there are reactive oxygen species and you may know all these species like superoxide anion this is highly reactive this can react with a literally with anything. There is a hydrogen peroxide in inside the phagolysosome there is a hydroxyl radicals and there are hypochlorite anions. So, let me talk how these things are synthesized inside the cell so, first I will take about talk about the nitric oxide.

So, basically nitric oxide is synthesized in presence of oxygen and NADPH using one amino acid known as arginine and the enzyme which act on this reactants are nitric oxide synthase. So, by action of nitric oxide synthase there will be a formation of nitric oxide and citrulline. Citrulline is a another product it is a derivative of amino acid. And this nitric oxide is playing a very important role in defense besides this the nitric oxide is playing a very important role in variety of situation.

The most important is that the nitric oxide is a vasodilator. So, it means this any source of nitric oxide can protect the human from cardiac arrest and probably you might hurt one drug known as sorbitrate. The sorbitrate is a compound which after breakdown this releases the

nitric oxide and nitric oxide basically vasodilate, it is a vasodilator they dilate the blood vessels and then that will ease the blood flow.

So, probably you might heard that during cardiac arrest this sorbitrate and one more drug which is commonly used is a aspirin. So, aspirin and this sorbitrate basically do the same job they are vasodilator so, aspirin is basically stop the adhesion of blood cells. So, in that way the blood can flow it is a; we also call it as a blood thinner. And this basically helps in in flow of blood to the heart and then we can protect from protect the individual from the cardiac arrest.

In addition, nitric oxide is a also very important in some erectile dyis-function the individual who is having a problem in erection of a penis they use this drug which we call it as a Sildenafil ~~sildenafin~~. This drug is basically a Viagra ~~viagara~~ and this drug is basically producing an active molecule which basically inhibit the breakdown of c~~C~~GMP. And so, there will be a nitric oxide and c~~C~~GMP and these two molecules are needed for increasing the blood flow.

And that will result to the erection of penis so, this drug is also kind of inhibitor for this ~~C~~cGMP di-phosphoestrase~~rylates~~ enzyme and in that way this works. Now, let us look at come back to the immunity part so, this nitric oxide is also reacting with the various free radicals and then this generates a nitrogen dioxide, peroxynitrite and let us see how it is how other reactive oxygen species are generated inside the cell?

So, basically in it reacts with oxygen means NADPH react with oxygen in phagolysosomes. And in presence of NADPH oxidase you remember the NADPH oxidase is deficient or non-functional in case of CGD. So, this is the same NADPH oxidase and that will generate the superoxide anion and there will be a release of proton and on action of superoxide dismutase enzyme over this superoxide anion.

This result to the formation of hydrogen peroxide and there will be a there is a and this can react with chloridine ion which is available in the in the cell and that will basically makes a HClO– which is highly reactive, basically it is a hypo chloride anion. And this nitric oxide can also react with a superoxide anion and that will make the peroxynitrite. So, in that way all these reactive nitrogen species and reactive oxygen species are formed inside the cell.

Please remember all these things are very momentary they react and then they can destruct any biomolecule. It could be a protein or it could be a lipids mainly protein lipid is present in the surface of microbe. So, with this I will stop here, I will stop the macrophages and in next session I will take another immune cell, maybe NKT natural killer cell and few more and thank you, thank you very much.