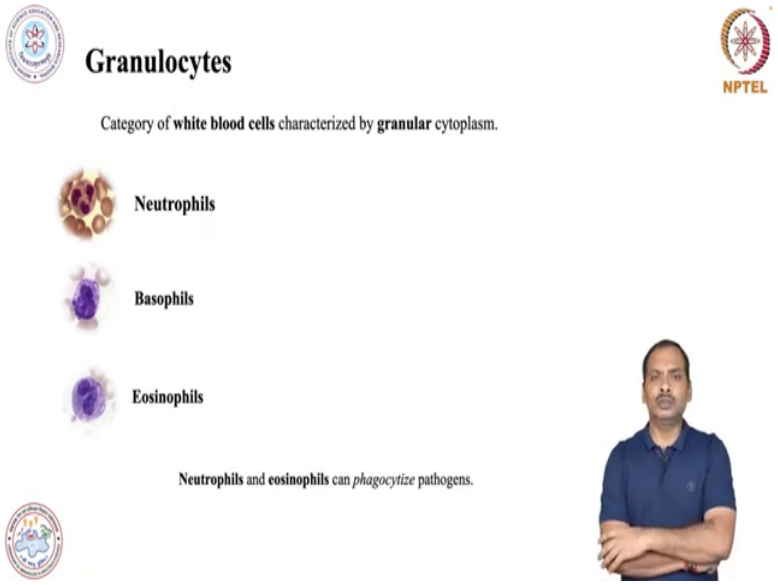
Host-Pathogen Interaction (Immunology)
Prof. Himanshu Kumar
Laboratory of Immunology and Infectious Disease Biology
Department of Biological Sciences
Indian Institute of Science Education and Research (IISER) – Bhopal

Lecture – 16
Cells of Immune System and its role in Host Defense-Neutrophils

Hi in previous session we have learned about the various components of blood and how these blood cells are arising and how these blood cells are or from blood cells, how we can isolate the immune cells and these immune cells, how we can use it for various experiments? So, in this session now we will take up the neutrophil and this neutrophil is a one of the very most important cells and this plays a very important role against the defense against various viruses.

And in fact this is a first responder during infection this is a first responder from the immune side. So, what I mean to say that when there is infection, neutrophil will come first and they will try to clear the infection. These cells are very aggressive as well.

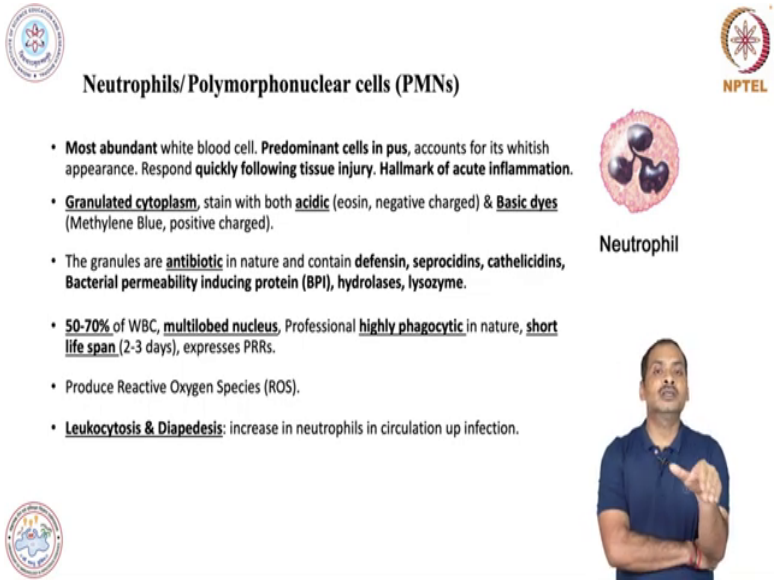
(Refer Slide Time: 01:25)



So, basically neutrophils is ~~is~~ a granulocyte and as you know that granulocytes are basically consists of various cells and they have a granular cytoplasm and it is consists of neutrophils, basophils, eosinophils. And one very important thing about the neutrophil is that they are phagocytic in nature, please remember this thing. It is not only macrophages who phagocytose, it is a neutrophil.

Neutrophil is a far more aggressive phagocytic cells. And besides neutrophil, the another cell which has a some capability of phagocytosis, is eosinophils. So, eosinophil can also phagocytose the pathogen or other things and or another microbial pathogen in more precisely and they can clear to some extent but they are their primary function is not the phagocytosis. Eosinophil plays another role which we will discuss in subsequent session.

(Refer Slide Time: 02:50)



Now, let us talk about the neutrophils, so neutrophils are also called as polymorphonuclear cells. Why polymorphornuclear cells? Because of they have a multi-lobe of nucleus. Here, you can see that there are multi lobes of nucleus. That is why we call it as a polymorphonuclear cell or in short form PMNs. They are most abundant in blood, and they play a very important role in a defense against any microbial.

As I said, they are the first responder. So, they are the predominant cells. So, during battle there will be a lot of damage from both sides the pathogen side as well as the host side. And that will result to the accumulation of pus. Pus is basically dead cells and there are some live cells. And in pus basically, the predominant thing is these neutrophils and that is why it is white in appearance because neutrophils are colourless.

And they play a very important role in acute inflammation. So, here there is a word known as "acute". Let me elaborate a little bit about the acute and there is a one contrasting term which we use as a "chronic". So, chronic, when we use chronic and acute they have some specific

meaning. And these words are quite frequently used in various disease or various condition, for various pathological condition.

So, acute is basically anything or any response which is extremely high for short duration. So, we call it as a acute and the chronic is there it will be not so high but it will be for long duration. So, we use the term chronic like chronic disease. Which is not so creating too much problem but it is a persistent and for a very long time. So, this neutrophils are playing a very important role in acute inflammation.

They have a granulated cytoplasm and they these cells these granules can be stained by both acidic like eosin, dye or it can be also stained by basic dyes like methylene blue which is quite commonly used in the laboratory. And that is why these cells are called as a neutrophil? There are some cells which is stained by the acidic dye and there are some cells which can be stained by basic dye. So, on that basis the names were given.

Neutrophils so, when we observe under the microscope, they have a granules. So, what is this granules? So, these granules are antibiotic in nature. Antibiotic in nature means they contain the substance which inhibit the growth of microbial pathogen and basically it is consists of a variety of antimicrobial protein and peptide, such as defensin and defensin which is playing very important role in innate immunity.

Seprocidin and cathelicidins, so these are the protein which is present in the granule. Besides this, there is a bacterial permeability inducing protein. So you can understand so in bacteria if this protein act it will disrupt the permeability and then there will be a loss of iron and other molecules and eventually it will cause the death of that particular microbe.

This contains some enzymes such as hydrolases, variety of hydrolases like proteases. There is a enzyme which can break down the complex sugar and there is a also enzyme lysozyme. You know that lysozyme is acting against peptidoglycans, it is also present in tear. So, these are the composition of these granules and basically, they constitute about 50 to 70 percent of white blood cells.

You can see this is a quite big number having multiple nucleus. They are phagocytic as I told you and one very important thing is that they have a very short life span that is 2 to 3 days.

But they are very aggressive, so very aggressive means if the neutrophil will see the pathogen then they will kill the pathogen only way to escape from the neutrophil which can be adopted by the pathogen is to kill the neutrophils?

Then these microbial pathogen can escape otherwise microbial pathogen cannot escape. They are more it is like a it is a very aggressive killer, aggressive killer means they have a lot of weapons in order to kill which I will explain you in subsequent slide. So, once the pathogen will come in contact with neutrophil, the pathogen will die that is a for sure. If pathogen need to survive then the pathogen need to take some way by which this neutrophil can be killed.

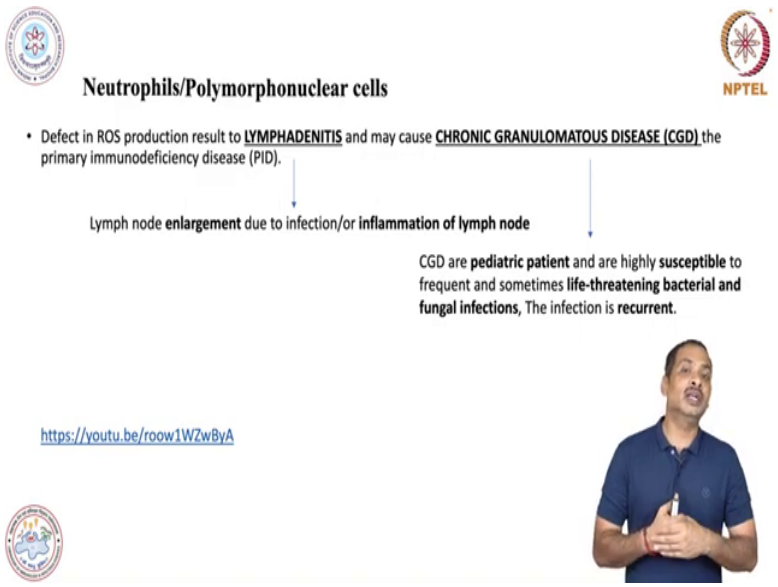
If it is surviving they cannot escape and that is why their life span is very short and this property is unlike macrophages. Macrophages are not so aggressive. We will discuss the difference between neutrophil and macrophages in subsequent session. They produce most fatal the most fatal weapon is that reactive oxygen species. Reactive oxygen species you can understand and that this reactive oxygen species can react with anything.

And you can imagine that this reactive oxygen species is produced inside the neutrophil and if the pathogen will come in contact with this reactive oxygen species then that will definitely die. And basically, this reactive oxygen species is produced by the action of one very important enzyme known as NADPH oxidase. And if there is some problem in NADPH oxidase then that individual will develop some kind of disease which I will discuss in subsequent slide.

Neutrophils, show two unique properties that is leukocytosis and diapedesis. Basically, leukocytosis is the increase in number of neutrophils in the circulation upon infection this is the meaning of leukocytosis. The diapedesis, it is a quite interesting phenomena and it is not true for only neutrophils. It is true for various immune cells. Diapedesis means there is a movement of this cells which is immune cells across the blood vessels without rupturing.

So from blood vessel to the immune cell can migrate from blood vessel to the tissues without rupturing the blood vessel wall, that we call it as a diapedesis. So, neutrophils show these properties.

(Refer Slide Time: 11:52)



So neutrophil as I told you in previous slide that there is a mutation or if there is a some problem in NADPH oxidase, basically it is consists of two subunits, one is catalytic unit and another is a; which is another subunit is essential for maintaining the conformation and structure. So, if there is some problem in NADPH oxidase then that will result to very severe disease which we call it as a lymphadenitis.

It means there is a inflammation of lymph node. And why this inflammation of lymph node is there? Because in lymph node, since the pathogen is not cleared so these pathogen as you remember in previous session, I have told you that pathogen or antigen will be transported to the lymph node. And over there, there should be activation of appropriate immune response and then there should be a clearance of that pathogen.

But since the pathogen is not clear, they will go in the lymph node and then that causerves the problem in lymph node. Wherever you see this word or suffix ITIS. It means it is like pancreatitis, gastritis. It means inflammation of that particular organ. So, thisese discuss cause the inflammation of lymph node and that result to the accumulation of pathogen or microbial pathogen and that will cause variety of severe problem.

For example that will result to the enlargement of lymph node. So, lymph node will keep on increasing in size. And there will be inflammation in the lymph node means it is a kind of frustrating situation for the immune system. The pathogen is there but they do not have a weapon to clear because NADPH oxidase is not functional. So, pathogen will be keep on accumulating immune cell will keep on frustrating.

They will release the factor and that will cause the increase in size of that particular lymph node. Another which another disease which is caused due to the lack of this NADP~~energy p~~H oxidase, is chronic granulomatous disease and this is a very kind of dangerous disease. And, basically, all these disease we call it as a primary immunodeficiency disease and these primary immunodeficiency disease is quite life threatening and this is a in the in the ~~pediatric~~paediatric patient.

All these diseases are common in ~~pediatric~~paediatric patient. And why it is common in ~~pediatric~~paediatric patient? Because this due to some congenital defect, these proteins are not expressed properly the subunit of NADPH oxidase. It is not expressed properly. That is why they are not in correct confirmation and they cannot make the reactive oxygen species, and that result to the accumulation of pathogen.

So, all these things cause a very severe disease and this is a primary immunodeficiency disease and in this disease the kids will they suffer a lot. They will have a recurrent infection of common bacteria or some fungi. In general, these we keep on having these infection but we efficiently clear but these kids cannot clear those infection and then, of course we do not have a appropriate cure for the disease.

But there is a some way by which you can stop one could be which you can understand very well, the individual or the kid we need to give the some antibiotics, keep on giving antibiotic. But you can imagine when you take the antibiotic for short duration, you will have a variety of another problem, such as your gastric system that severely affect because there is a some friendly bacteria in your gut which is needed for the proper function.

So those are also dying and if the individual is taking for very long time you can understand this is a very complicated situation. There are some more ways by which people just manage this disease, so this disease cannot be cured, this can be just managed. Another way is that they infuse some immune factor, for example, interferon gamma which may activate the macrophages or another cells.

In order to clear this bacterial thing so, all these things are there. In finally, I would like to say that CGD is a quite severe problem and now, or previously people tried to do the bone

marrow transplantation in order to overcome the disease. But I am not sure because I am not clinician how much it is successful. Now, we are hoping for gene therapy, I do not know if we will have a gene therapy by which we can replace this defective gene.

Then probably that will cure this disease and the kids will become healthy. So, in this connection I have a one very nice video and this video will be helpful in understanding more about the CGD. **(Video Starts: 18:15)** We humans, control, inflammation and infection by using white blood cells and those white blood cells have to kill infections and they have to control inflammation or swelling.

Those cells are damaged in chronic granulomatous disease and they fail to make bleach. And bleach turns out to be really important for how you kill things and for how you control inflammation. When the white blood cell encounters a bacteria it takes it inside and it puts it in a little container. Now, you got a kill it. How do you do that? You assemble a bunch of proteins on this container they take a chemical in the cell called NADPH.

They turn it into NADP+ because they have taken an electron from there and they give that electron to oxygen. That makes super oxide and then that gets turned into hydrogen peroxide and then that gets turned into bleach. And that bleach is involved in killing the bacteria that is inside the cell. In chronic granulomatous disease people are missing one of these proteins, so they cannot make the bleach they have trouble killing the bacteria inside the cell.

And chronic granulomatous disease is just like it says it is chronic, goes on for a long time and it is granulomatous that is, they form little balls of inflammation in different parts of the body that can get people in trouble and make them have fevers or be very uncomfortable. Chronic granulomatous disease often shows up as recurrent infections and those can start out as swellings in the neck or the armpit or the groin, as fevers, as diarrhea, as pneumonia, as abscesses in the liver or other parts of the body.

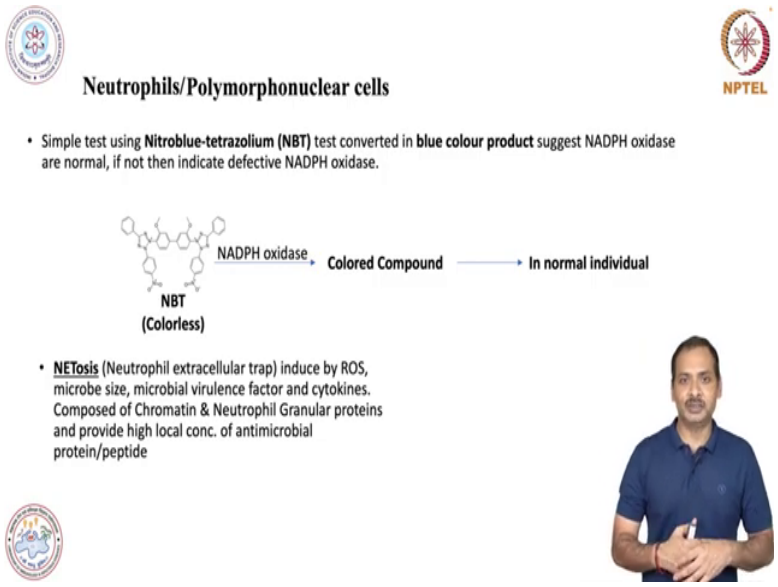
There are about 40 people with chronic granulomatous disease born each year in the United States. When I started as a young doctor, this disease was called fatal granulomatous disease of childhood because there was no treatment and over the last 40 years, the treatment has grown from nothing to the use of immune stimulants, to the use of prophylactic antibiotics, to bone marrow transplantation, to looking into the future gene therapy.

People sometimes ask why do you study patients with rare diseases when you are the National Institutes of Health. And the reasons are first that we give patients hope but two they serve as windows to understand some common diseases. Although patients with chronic granulomatous disease have problems with infection, these patients also have some reduced inflammation because the products that are missing, such as hydrogen, peroxide and bleach are very pro-inflammatory.

We began to think. Maybe the enzyme that is abnormal in CGD could actually become a new target for a new drug to prevent inflammation in certain clinical settings in people who do not have CGD. And if we can do that we will demonstrate that you can take the journey from understanding a very rare disease to understanding certain diseases that are common and maybe coming up with a new drug to treat things that you never imagined you would be able to do. **(Video Ends: 22:01)**

I hope you have seen this video and it is very much informative to you and this video basically is provided by National Institute of Health, USA and I hope you can understand the importance of neutrophil and the above all you understood the importance of NADPH oxidase in our defense system.

(Refer Slide Time: 22:32)



So, one very important thing how we accsess the function of NADPH oxidase. So, NADPH oxidase is a very pivotal and very key for the function of neutrophil or any phagocytic activity. So, there is a one very simple experiment by which we can show that whether

NADPH oxidase is functional or not. So we use a one very simple, reagent it is a colourless reagent which we call it as a nitroblue-tetrazolium and this test is we call it as a NB test or NBT?

So, in this test, what we do we take the cells and you break the cells and then mix it with this reagent. If the reagent will turn to deep blue colour then that indicates that this your enzyme NADPH oxidase is functional and the individual is normal. If the intensity is less, then it is less functional. If it is almost colourless then the one can consider that this individual is lacking the functional NADPH oxidase.

So, this is a all about the NADPH oxidase and neutrophil and recently there is a discovery of one very important phenomena known as a NETosis. NETosis is nothing it is a neutrophil extracellular trap. And this neutrophil extracellular trap is a induced by reactive oxygen species. And microbes also play a very important role in in making this NET or NETosis.

And basically, this is a kind of environment where this pathogen is entrapped or after entrapment. There will be a lot of in addition there will be a broken neutrophils which will release this chromatin and then basically this will kind of quarantine in a body not in body, it will make a separate kind of a thing, so that other things will be not interfered and then that will be a very active area lot of ROS, cytokine and all those things will be there.

And then that pathogen can be easily cleared from that infection. Please note this is a quite recently discovered thing NETosis this phenomena and to best of my knowledge the evidence in under physiological condition is not very well understood. So, in in vitro experiment we can see this kind of phenomena. So this is all about the neutrophils and in next session we will talk about the basophil, eosinophil and Mast Cells. Thank you. Thank you very much.