Host-Pathogen Interaction (Immunology)
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Lecture – 15
Cells of Immune System and its role in Host Defense-Neutrophils

So, in previous session we have learned about the transgenic mice we have discussed in a great length. What is the transgenic mice? What are the kinds of transgenic mice? And how it is useful in our immunology research or even in any field of biological sciences? So now, we will begin with the cells of immune system and we will look at how these cells are playing important role in immune defense.

And in this session, we will take up one very important immune cell known as neutrophils. So, first we let us see what is the composition of blood? Because blood contain various immune cells.

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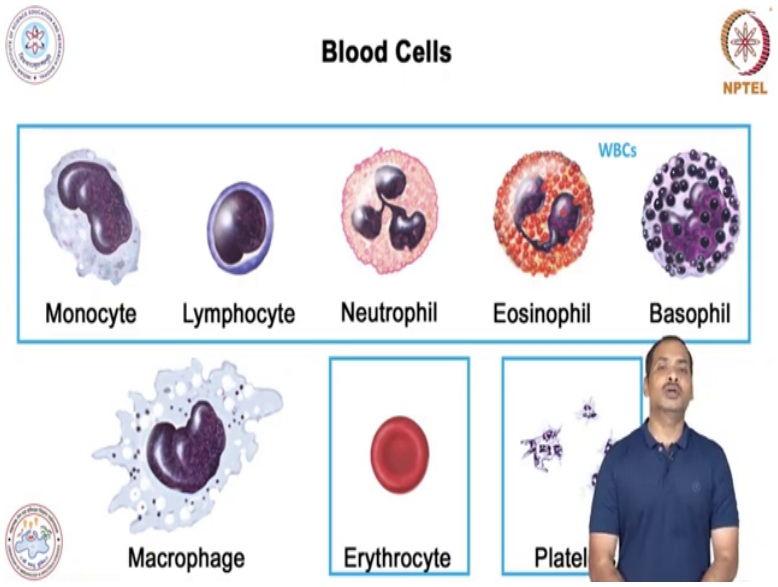

And as you can see in this slide that blood has variety of immune cells such as monocyte, lymphocyte, basophil, eosinophil, neutrophils and all these cells basically constitute which you have studied quite early in during your education we call it as a white blood cells. So, these white blood cells are playing very important role in defense and we will take up all these cells one by one.

And we will discuss in great detail there how they look like? What it contain? And how they are playing important role in defense? And if something goes wrong in these cells then what kind of problem will arise in the individual or we basically call it as some kind of immunodeficiency if there is some mutation in some gene, as in some very critical genes then that results to the development of some kind of disease we will discuss all those things.

And this is white blood cells and another is a red blood cells or we also call it as a erythrocytes. So, erythrocytes you are very well aware that what is the role? So basically, they are playing very important role in transportation of gases. So, these RBCs take the oxygen from lung and deliver it to the all tissues. And they also take carbon-dioxide from tissues. And then it is this carbon-dioxide is released from the lungs.

So, you know this thing very well from beginning you might have studied. So, RBC has a few unique properties or characteristic which you should know one is that you know the mature RBC they do not have a nucleus. So, since they do not have a nucleus, it is very easy to transfer from one individual to another individual. Of course, you need to take care of one of the surface antigen which constituted the blood group.

Another very unique property of red blood cells or erythrocytes are they are biconcave in shape. So, this is very interesting that most of cells are basically biconvex, but this RBCs biconcave in nature. I was quite curious that why it is like that? So, of course, I did not perform any kind of research or experiment why it is biconcave? But I looked at a huge amount of literature before coming to take this session.

And I found out that this biconcave structure has a few very important ~~property~~properties one is that this provides a much more stability in terms of basically, if you see the blood, so blood is containing most of cells are erythrocytes and they are quite tiny in size and they are huge in number. So, when they flow in the blood then there is a lot of stresses over the cell. So, this structure seems quite stable.

It does not rupture so easily. So, probably this is one of the reason, another reason is that these biconcave structure can very well fit in the capillaries. So, you know that there is a artery, bigger artery, and then this eventually there will be arterioles and then it will form a

capillaries and capillaries are has a very less space. So, RBCs can transport in those capillaries and it from literature.

It seems that this biconcave structure is also playing an important role in movement of RBCs to that fine capillaries. So, this is I am not very much all are kind of prediction by physicist or biophysicist and some structural physics and biology people. So, next very important cell and this is not the scope of our this discussions I just shared my knowledge with you.

Another cell is which is playing a very important role in defense is thrombocytes or platelets and you are I think, very well aware about the platelet and platelet counts. Because recent in recent past there is a outbreak of Dengue viruses. Every year we have a dengue virus infection and probably you might heard that individual platelets are reduced. So, platelets basically play a very important role in blood coagulation.

And if their number will be reduced in the individuals then that will cause ~~hemorrhage~~haemorrhage. There will be internal bleeding and all those things. So, platelets are plays a very important role in blood clotting and this blood clotting is also playing very important role in defense. This will discuss later when I will take the platelets.

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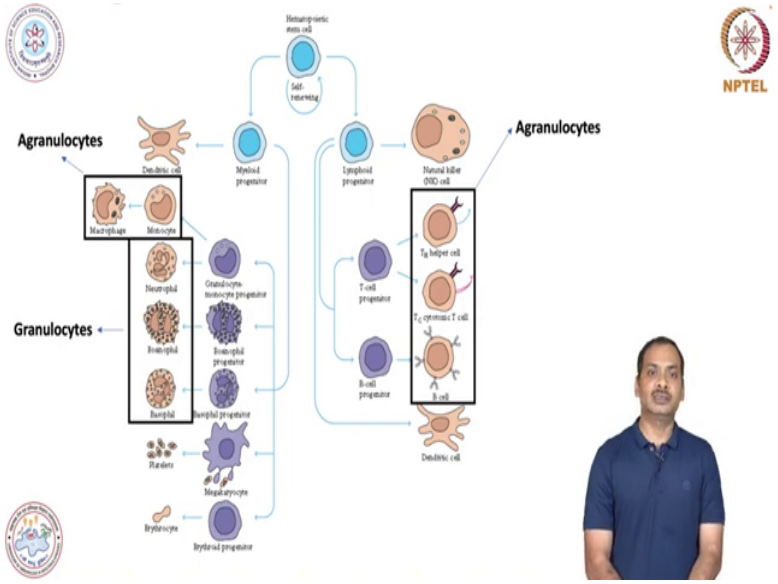

So, how these blood cells are coming? So, there is a common cells which makes this blood cells that is hematopoietic stem cells and these hematopoietic stem cells, basically differentiate into two major lineages. One is myeloid and another is lymphoid. And this

myeloid and lymphoid lineage basically give rise to the set of cells, so myeloid cells basically give rise to dendritic cells as you can see in this slide.

This can also differentiate into the monocyte and basically monocytes are floating macro floating phagocytic cells in the blood. And this can also give rise to the neutrophil, eosinophil, basophil and platelets. So, in addition, this also makes a erythrocytes, so this myeloid lineage results to the these kinds of cells. Another lineage is a lymphoid lineage, which basically which is a precursor for the lymphocytes.

And this lymphoid lineage basically result to the differentiation of or like T cells, B cells and this can also result to the formation of natural killer cell which plays a very important role in virus infection. So, in this way this all these blood cells are formed. In addition, there are few more cells like a mastuscle cells so muastcle cells is generally they are not present, it is present in the tissues.

They play also very important role in defense or they are over activation can result to the immunopathogenesis. So, most probably I will take the masst cell in next session. So, let us focus on what are the cells? So, here you can see that there is a set of cells which consists of a neutrophil, basophil and eosinophil. We call them in a very simpler way. We call them granulocyte. Why we call them granulocyte?

Because these cells, if you observe this cells after staining under the microscope then you will find out that there is a granule kind of things in the cytoplasm. So that is why we call it as a granulocyte and there are another cells this is a quite broad and old classification but still we use it. Another set of cells we call it as agranulocyte means the cells which is not showing this granular cytoplasm.

We categorized in as agranulocyte. So, they are basically monocyte, macrophages, lymphocyte, both B and T cells and even natural killer cells but they do have some granular things. They have a packets of toxins which is released during virus infection to the target cells. So, natural killer cells are not very clear but anyway we can study the natural killer cell as a one category and there is a one more cells which is present in this slide if you see carefully which we call it as a dendritic cells.

So, dendritic cells are originated from both myeloid as well as lymphoid lineage and they play a very important role in defense. And these cells we also call it as an antigen presenting cells. So, we will discuss when we will take up the dendritic cells.

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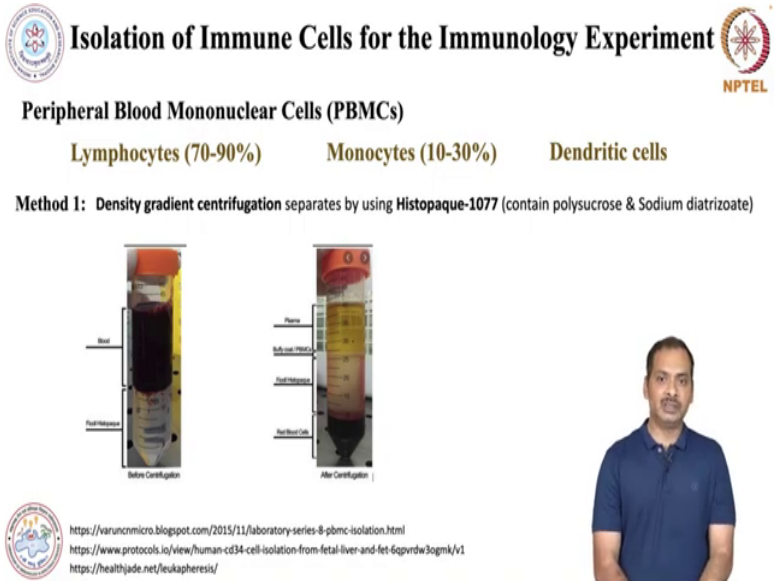

Now, the next question is how we can isolate the these cells in order to perform any experiments? So, here I will just give you the glimpse of how we can isolate these cells from the blood? For performing various kind of experiment, or even it is used for the diagnostic. So, the method which is commonly used in the laboratory is a very simple method if you look at the principle.

What we do? We use a one reagent which we call it as a histopaque, and histopaque or histopaque ficoll this has some unique density. So, this density is 1.077 gram this density, this histopaque we basically use it for the separation of these immune cells. And the method which we are using which is very commonly you can commonly used in the laboratory or it is a well-known thing.

We use basically density gradient centrifugation. It is a very, very simple. So, what you do you when? For example, you have a volunteer you have collected the blood from that individual and after collection of that blood so please note that you have to take this blood along with anticoagulant. If it will coagulate then it is a meaningless. You cannot isolate the cells.

So, you have to collect the blood along with anticoagulant so that the blood will not clot. And after that you will dilute this blood with say one is to one dilution you can use any appropriate buffer such as which is commonly used is PBS or you can use some media. Generally, we use DMEM or RPMI so you mix the blood with the same volume of these media or buffer and then you take the tube.

And first you have to put this reagent histopaque or ficoll or both are generally, we use the histopaque which is commonly available from the companies. And then you overlay that the diluted blood over this reagent and then subjected to the centrifugation and so when initially it will be like that here you can see in this image that there is a at the bottom there is a ficoll histopaque or histopaque and over there is a diluted blood.

And then you spin and after spinning you will see something like that. And what is this? This will be you can see there are three distinct phases. The topmost phrase is basically a plasma, plasma you understand plasma. And there will be a one interface between plasma and histopaque and over there you will see a very thin layer of white colour ring and this white colour ring is basically we call it as a PBMC

peripheral blood mononuclear cells. And these peripheral blood mononuclear cells or PBMC is basically rich in immune cells. Mainly this contain lymphocyte which is about 70 to 90 percent and we can use it for various experiments and this also contain about 10 to 30 percent monocytes. And there are some dendritic cells also present. So, the dendritic cells are very less in general in blood, the dendritic cells are in very less amount.

So, there are some dendritic cells. And below that you will see there is a layer of this histopaque. And at the bottom you will see the RBC and just above RBC there will be a thin coat of cells which is white in appearance basically, those are like a granulocytes. So, all these cells are there. And in that way, you can just aspirate this intermediate section and then you can use it for various experiments.

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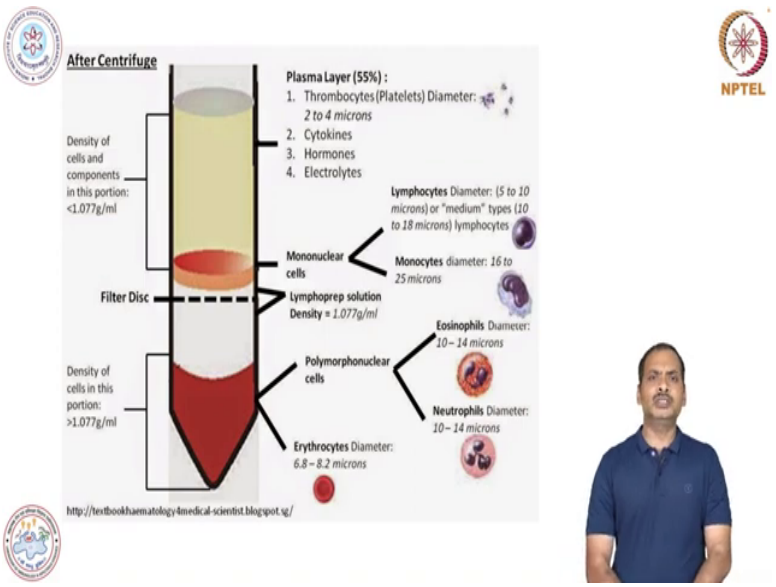

So, here I am giving you just a little more detail how this it is a schematic? And I think it will be very convenient for you to understand. So, here you can see there is a plasma layer which basically contain all soluble factor or very small size, some very small size cells. So, here you can see, there is a some platelets and cytokine, hormones, or some electrolytes are also there.

And in this mono-layer which I was saying the interface between the plasma and histopaque this mono layer which is rich in lymphocytes and this also contains the monocytes which as I have explained you earlier. And you can see there is a histopaque and there is a RBCs which over there will be a thin layer of these granulocytes. So, why it is separated like that I think you can understand very simple it is not so difficult to understand.

So, the density of this histopaque is 1.077 gram per ml. And the density of these PBMC is matching with that. So that is why they are separated and why RBCs went down? Because the density of RBCs are more that is why they went down. And the density of plasma is less that is why you are seeing the plasma layer is on top and in between plasma and histopaque there is a ring of this PBMCs.

So, in that way we purify or we isolate the PBMC and we conduct variety of experiments and these experiments are very useful because in general, most of people perform the experiment with some kind of cell line. And cell line is basically derived from the cancerous cell and they were grown ~~for~~ from ages. So, generally, these cells are transformed and whatever phenotype which you are seeing in these cell lines, they may be not appropriate.

So, therefore, this in order to prove your whatever discovery you have done it is better to use the primary cells. So, primary cells are directly derived from the animal or human and this is a very easy primary cells, especially for performing all immunological studies.
So, I think this is very use full reagent and it is a very easy to isolate these cells and perform the experiment use after isolation, you simply wash it, count it.

And then stimulate and perform whatever experiment you want to do it. So, this is basically more this method is basically used more commonly in research setup.
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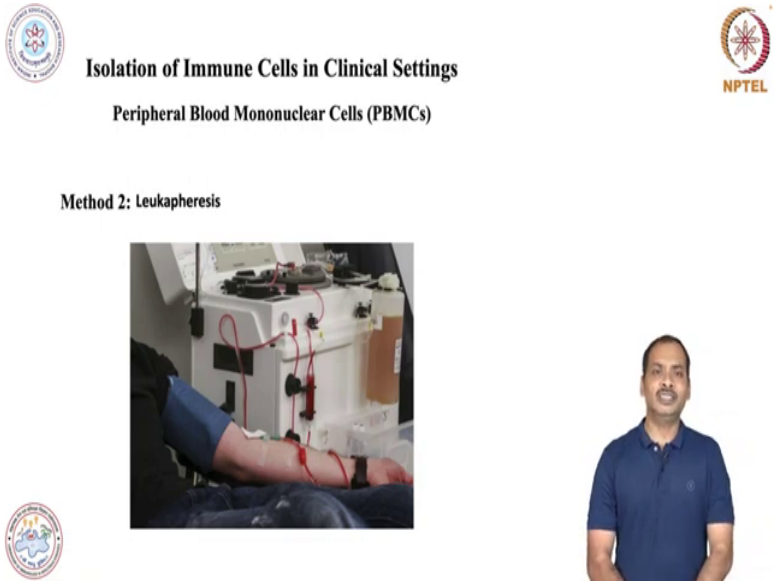

But the PBMCs are also separated from the red blood cells and this is basically done in clinical setup as well. For example, an individual who met an accident and that in individual needs there is a severe loss of blood. So, in that case, what we are doing? Or during long surgeries, we basically give the during long surgery there is a severe loss of blood. So, in that scenario, what we do? We give the only RBCs, not immune cells.

So, and if you need this RBCs is immediately then what we do we perform one method which we call it as a leukapheresis. So, this is in real time, so blood will go to someone one equipment here this is the image you can see, so the blood will go to this instrument and that will separate the RBC and then RBC will be collected and rest of immune cells and plasma will be again transfused in the individual.

So, in that way, this isolation or separation is much more in the clinical setup we do not use in our experiment? So, I think we will stop here in this session and in next session we will

discuss about the neutrophils and we will in subsequent session we will take up another immune cell, such as basophil, eosinophil and masst cell. Thank you. Thank you very much.