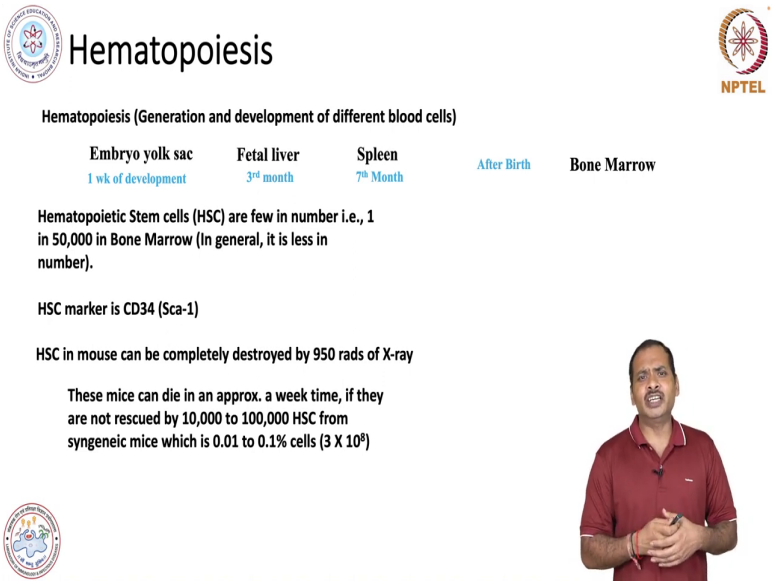
Host-Pathogen Interaction (Immunology)
Prof. Himanshu Kumar
Laboratory of Immunology and Infectious Disease Biology
Department of Biological Sciences
Indian Institute of Science Education and Research (IISER) - Bhopal

Lecture: 12
Cells of Immune System-Hematopoiesis

Hi so, in previous several sessions we have studied about the unique properties of immunity. We have learned about various immune organ and in immune organ we have studied thymus, bone marrows, spleen, lymph node, lymphatic system and mucosal associated lymphoid tissue. Now we will move on to the and the cells of immune system which we which is playing a very important role in defence.

(Refer Slide Time: 00:59)



And the generation of these cells we generation and development of these cells we call it as a hematopoiesis. So, if you remember my previous session I have shown you that this hematopoiesis is started immediately after a week and it is basically taking place in yolk sac and after thereafter it is taking place in fetal liver and subsequently it is taking place in a spleen and after the birth of the foetus it is taken over by the bone marrow.

So, this is very important to understand this hematopoiesis and basically all these hematopoiesis or development of blood cells which includes both immune and non-immune cell that is RBC as well. So, all these cells are the development of all these cells or generation

of all these cells are taking place from the one of a very unique cell which we call it as a hematopoietic stem cell.

And hematopoetic stem cell is very much essential for the reconstitution or constitution of these blood cells. And it is very interesting in adult human this hematopoietic stem cell is residing in the bone marrow. And there is a one cell in 50000 cells in 50000 bone marrow cells only one cells are there and these cells are generally in very less number and this number keep on changing depends on the need.

Need means if there is a severe loss of blood then these stem cell will divide and they will increase in number and immediately this cell will replenish the blood cells and then again they will reduce in the number. And these cells have some unique marker and these markers are playing a very important role in isolation. So, after discovery of this unique marker now we can isolate the hematopoietic stem cell.

And this hematopoietic stem cell we can use it in various kinds of therapies. And in fact we are using this marker in order to isolate the hematopoietic stem cell when we are performing various experiments. Here just I am giving a note about the mouse because whatever immunological studies we are doing we are mostly doing using the mouse model. So, here I will talk about some of this mouse experiment as well.

So, if you irradiate the mice with X-ray with 950 rads which is a unit for the radiation then you can deplete all the hematopoietic stem cell as well as you will deplete all immune cells in the mice. And if you deplete these cells or if you irradiate this mice then this mice is highly vulnerable to the infection and subsequently they will die. So, we use this thing this is a strategy for doing various kind of experiment in order to prove some of our discovery or phenomena.

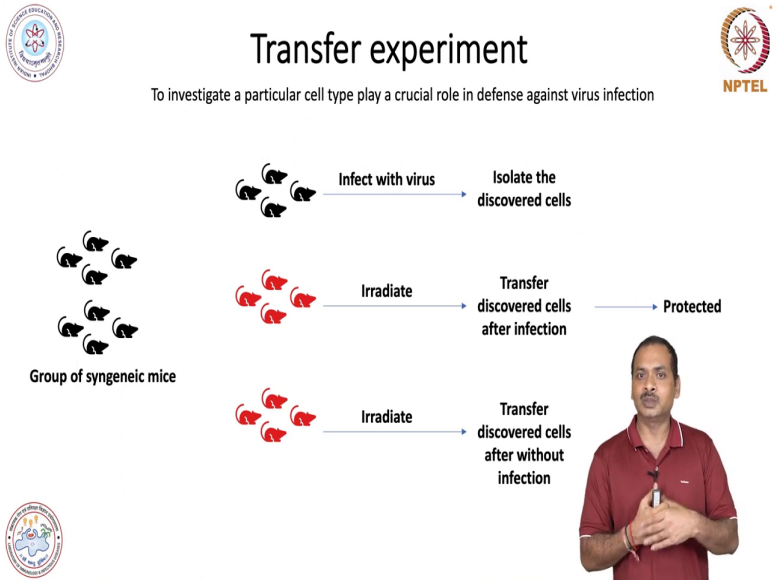
And if you irradiate these mice with this radiation X-ray and then if you can infuse these mice with say 10000 or one lakh of these hematopoietic stem cell please note this stem cells should be derived from the syngenic mice. So, there is a very big difference between the syngenic mice and other mice. Syngenic mices are those mice which are generated after breeding from brother, sister and all those things.

So, they are so, their genome is kind of consistent if you take the group of synergeneic mice their genome is kind of consistent there is no variation. So, in that scenario you if you will take some cells from one mice and then and put it in another mice then there will be no reaction these cells will be taken up by that recipient mice but this is not true for human right. So, we are outbred.

So, syngeneic mice are basically in a simple term they are inbred mice. And in case of human if you take some tissue or cell in general then and if you transfer it to the another recipient then this will develop a reaction and those cells or those tissues may be rejected probably you may know that before transplantation we do lot of analysis for tissue matching and all those things.

So, in case of synergeneic mice it is not needed and if you have a group of mice and which is irradiated with X-ray and you can rescue this is mice because after irradiation these mice will not survive but you can rescue these mice by taking this hematopoietic stem cell from syngeneic mice and transfer it in these mice. So, you can rescue and these number of cells are very less it is about ten thousand to one lakh which is approximately 0.01 percent to 0.1 percent.

(Refer Slide Time: 07:24)



So, using this strategy I will explain you one experiment for example you want to investigate a particular cell type that play a crucial role in defenceifference against viruses. By a lot of experiment by several things you have you are speculating that this particular cell type is playing a very important role in defence against sayhape some particular virus let us talk

about say keep an example of NDV Newcastle disease virus which is not pathogenic to the human.

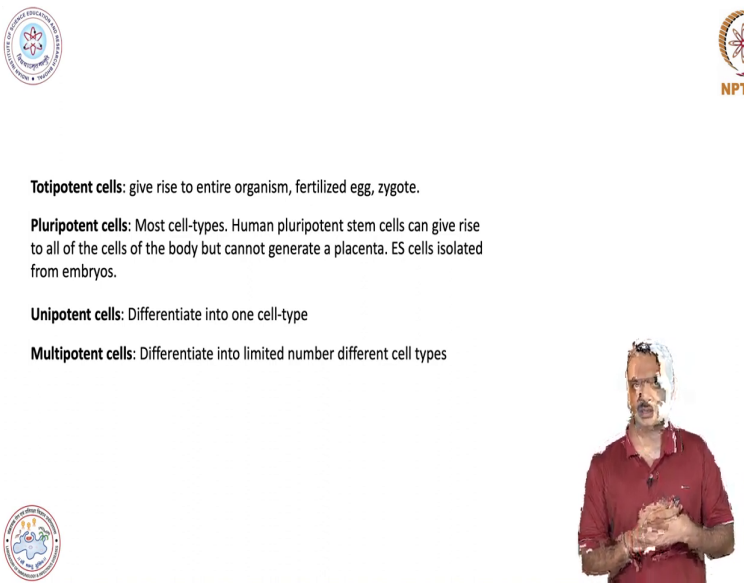
So, what we can use this strategy and for example this is a group of synergeneic mice and using this is the syngeneic mice the genome will be consistent. And you can divide this syngeneic mice in several groups for example here I have made it three groups and the then in one group you can infect with virus another group you can another two group you can irradiate with x-ray with 950 radds.

And from infected population you can isolate the cell which you have discovered you have discovered earlier which you have discovered you can isolate those cells there are variety of techniques by which you can isolate that particular cell if you know the marker. For example you can use the magnetic bead system or you can use the flow cytometer. Using these techniques you can isolate those cells.

And after isolation you can infuse these cells in one group of radiated mice and in another group you can introduce the cells or the cell type which you have discovered from uninfected mice and you will if after doing all this thing if you infect this radiated mice then you will see that this the group of mice the group of irradiated mice which received the cells from infected mice will be protected and another group will be not protected.

So, in that way you can you can show your discovery you can you can demonstrate that the discover the cell which you have discovered in by various way it is fitting well under the physiological condition that is most important. So, whatever experiment you do in vitro that is not so good. So, in that scenario you have to prove it in improve your discovery under physiological condition and in that way you can do you can prove that this cell is playing a very important role against the virus infection.

(Refer Slide Time: 10:23)



Now I will talk about some various types of stem cells. So, these are totipotent cells or totipotent cell is basically just derived from the egg fertilized egg. So, it is basically the cell at early stage immediately after fertilization. So, this single cell first it will become a zygote and then this will divide it into two cell stage, four cell stage, eight cell stage so, at that stage if you isolate the cells.

So, these cells have a potential to make a whole organism. Probably you might have seen or you have you might know that there are Mirror Image twins. So, by some during embryology during after fertilization during develop meant somehow this zygote divided into 2 parts and that generates two individual and those individuals are Mirror Image twins they are identical or almost fully identical right.

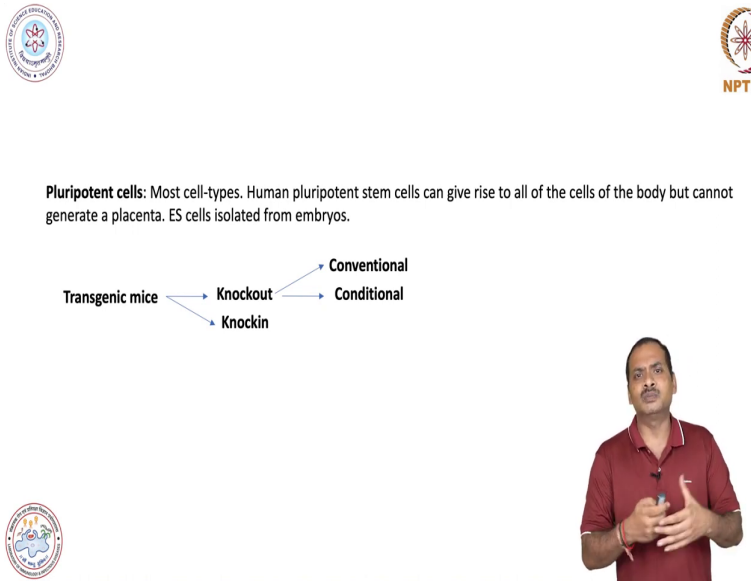
So, those cells we call it as a totipotent cell. Another is pluripotent cell so, these are the most cell type like a human ~~ch~~plurior~~i~~potent stem cells can give rise all kinds of body cells but it cannot make a placenta. So, from from this pluripotent cell we can we can make a very cell type like a bone, liver and all those things. Probably you might know that these days the doctors suggest to preserve the stem cell immediately after the birth.

But I will I would like to say that it is a currently we do not have a technology or we do not have a things which directly used in the clinical setup it is all in a discovery phase or people are standardized standardizing all over the world. So, this pluripotent cell we are in case of mouse we are using very frequently and we call it as embryonic stem cell and we can

manipulate this embryonic stem cell and we can make a variety of transgenic mice in next slide I will discuss all those things.

Another is unipotent cells and these unipotent cells are very very restricted development steps they can this unipotent stem cells can make only one kind of cells. So, that is why we call it as a unipotent stem cells. Another is multipotent stem cells. So, this multipotent stem cells can make a few cell types which is quite close to each other and biochemically or in terms of function. So, those cells we call it as a multipotent cells.

(Refer Slide Time: 13:46)



Now I will talk about the pluripotent cells because this is quite commonly used in our research and in one of the aim of this course is to educate you for research also. So, this pluripotent stem cell is basically very commonly used in mouse experiment as I have explained you and we can generate the transgenic mice using this pluripotent stem cell or in case of mice we call it as a embryonic stem cell.

So, we can create a variety of transgenic mice and these are, you can create a knockout mice. So, knockout mice is I would like to explain that this knockout noise is nothing it is just you for example you discovered some Gene and this Gene is displaying a very important role in particular phenomena. So, what you do by using molecular biology techniques you just inactivate this Gene and you can you can do it very easily.

Not so, easily it is a quite difficult but it is possible you can inactivate the gene UTR or you can inactivate the Exon and in that way this Gene will be inactivated and we call this kind of

Mouse as a knockout Mouse. Another is knocking mice. So, knocking mice is a another very interesting reagent to prove some discovery I will explain these two mice in subsequent slide by showing us very simple experiments.

So, in knocking mice for what we are doing we are introducing some gene at appropriate location in the genome. For example, you want to you might have seen in videos or in research paper there is a some mice which is giving a fluorescent green colour when you radiate this mice in blue light. So, those are basically a GFP knocking micenoise. So, what we do simply put the GFP gene under the control of that promoter for a promoter of that Gene which is expressed in the skin cells.

So, in that way we can make a knocking mice. So, that is the little fancy thing but I will tell you the importance of knocking mice in discovering a new cell type or variety of new things and all those things you can do it under the physiological condition physiological condition means in the animal. And once you show it in the animal then everything is full proof there are different kinds of knockout mice one we call it as a conventional knockout mice and this conventional knockout mice is basically you just inactivate that Gene.

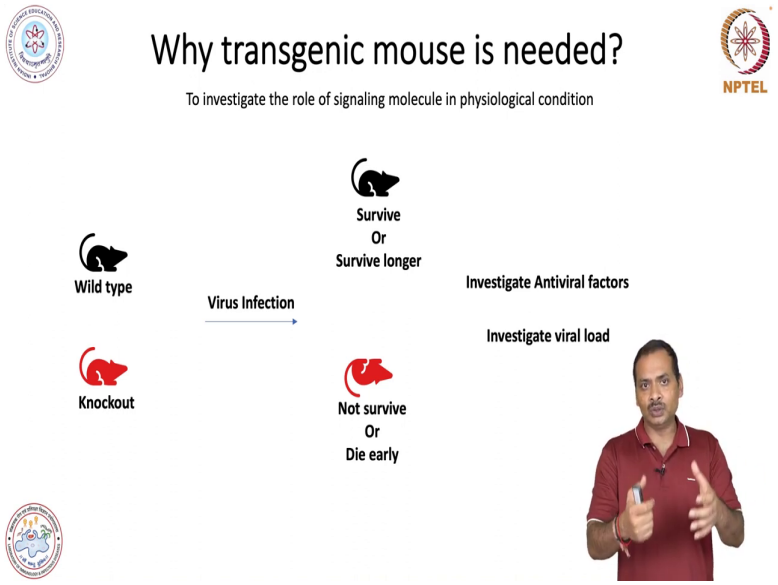
So, once you inactivate that Gene then this Gene will be inactivated in all cells of that particular animal. But sometime what happens if you inactivate that Gene and if this Gene may be involved in development some somehow in development process then this will cause the embryonic lethality means the after fertilization of this egg by a sperm and the embryo cannot develop because this Gene may be involved in development process.

So, in that scenario we make another kind of mice which we call it as a conditional knockout mice. So, conditional account mice is a it is a far more complex but here I will explain you in very simple way what you do you basically inactivate that Gene in particular cell type. For example there is a some pathogen or some antigen is there and this pathogen and antigen is basically taking playing important role in macrophages or dendritic cells.

So, when you are creating a conditional knockout micenoise you will knock out this Gene only in dendritic cells or macrophages not other cells. So, this is a very complex technique in next session I will discuss how to create this conventional or conditional knockout mice. Here

I will just explain you how these mice can be used for proving some very important discoveries.

(Refer Slide Time: 18:48)



For example why transgenic Mouse is needed? So, here one so, when I say transgenic mice it is a it is a it could be a knockout or it could be a knock-in so first I will just tell the importance of knockout mice. For example; if you have a question or if you want to investigate the role of some signalling molecule by. So, many in vitro analysis bioinformatic analysis you have discovered some molecule which is playing very important role against the virus infection.

And now you want to prove it under physiological condition whether it is valid or not because in when you perform the in vitro experiment at that time you miss so, many things. So, in order to prove it under physiological condition in animal you need to create knockout mice. So, for example this is a wild type mice, wild type mice when I say it is not wild it is not cast from the wild. It is also a syngeneic mice but they are having a gene in the intact gene.

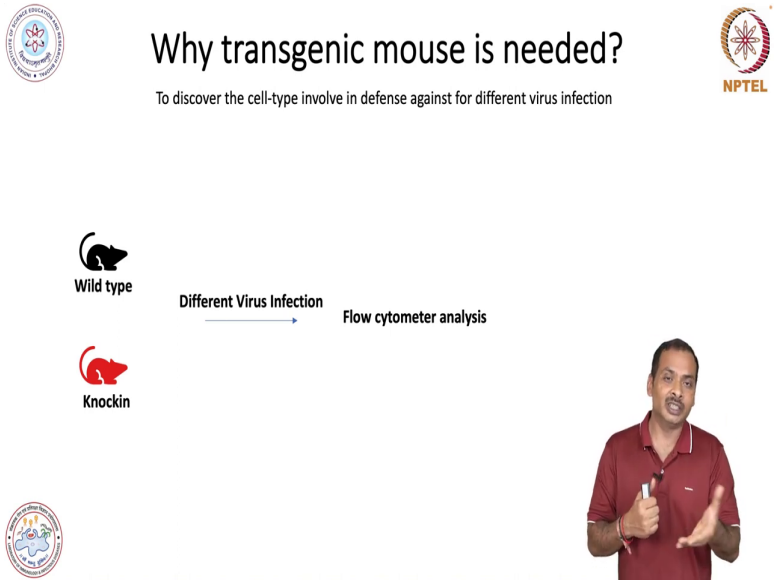
The intact gene for whatever molecule you have discovered and this is a knockout mice in this mice you just mutated the gene or you inactivated the gene the most appropriate term will be inactivated the gene. So, that the Gene which you have discovered is not functional. And then you have infected these mice with virus. So, for example the gene which you have discovered and it is playing a very important role in defence against the viruses.

So, what will happen the wild type mice will survive but on another hand the knockout mice will die and it will not survive or it will die quite early. So, in that way you can prove that this gene is playing a very very important role in defence~~ifference~~ against the viruses and subsequently you when you will do all those things you need to prove it at various level. So, for example you will investigate all antiviral factors you will estimate the antiviral factor after infection using wild type or knockout mice.

And then you will see that in wild type mice the antiviral factors are very high and in case of knockout noise it is almost abolished or it is present in very low amount. So, in that way you can prove it. Another experiment which is quite commonly people use is to check the viral load. So, since the knockout mice does not have appropriate defence system because of a mutation in that gene or non-functional of the gene.

So, you will see that the viral load will be extremely high in that mice. For example this mice is basically infecting the ~~l~~liver or lungs. So, you will take out the lungs and ~~l~~liver and you will compare the viral load in using wild type mice and then you will see that viral load is extremely high in knockout mice. So, in that way you can prove it that this molecule is playing a very important role against the virus infection.

(Refer Slide Time: 22:14)



So, this is one example another example which I would like to say about the knock in mice. For example you want to you have discovered a cell type which is playing a very important role in against a particular virus infection or bacteria infection. So, how you will prove it? So, what you will do you will discover or find out the gene which is exclusively expressed in that

particular cell type. And you will create a GSFP knocking noise you will put the GSFP molecule in that.

And then you will perform this experiment you will take this the wild type mice again it is a it is having an intact Gene and there is no transgenesis and you will use the knock and mice and then you infect with different viruses. And after that you will isolate that particular cell type and then you can show that this in case of this virus infection in this cell type there will be a there is increase in number of the cells.

So, in that way you can say that this mice is playing a very sorry these cells are playing very important role in that particular virus infection. It is a quite huge detail I am telling you I am just giving a glimpse of how you can use this transgenic mice in order to answer the questions in Immunology. So, in next session I will discuss about the various type of immune cell but before that I will take up how to create these transgenic mice.

I will just give you the Glimpse I cannot teach you in a great detail because it is a huge amount of material, thank you.