Basics of Biology
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Lecture 33
Phagocytosis

Hello everyone, this is Doctor Vishal Trivedi from department of Biosciences and Bioengineering IIT Guwahati. And what we were discussing, we were discussing about the living organisms. And in this context so far what we have discussed we have discussed about declassification of the living organisms, evolution of the living organisms and then we also discuss about the different types of cells, so we have discussed about the prokaryotic cell and eukaryotic cell.

And subsequent to that, we have also discussed about the different types of biomolecules, their structure and functions. And then, we have also discussed in previous lecture, we have also discussed about the central dogma of molecular biology or the central dogma of life. And in this particular module, we are discussing about the some of the cell mediated processes.

And in the previous lecture, we have discussed about the defense responses or the immune system. So, continuing our discussion about the immune system, if you remember, when we were discussing about the antibody development, we have said that the antibodies can be developed when the antigen is going to be trapped by the surveillance systems.

So, the surveillance system is mostly been consists of the antigen presenting cells and these antigen presenting cells are either the macrophages which are going to be present at every tissue sites or it could be dendrites cells or the B-cells. So, in today's lecture, what we are going to discuss, we are going to discuss about the how we can be able to, how these antigen presenting cells are processing the antigen.

So, if you recall, when we were discussing about in the previous lecture, what we said is that, when the antigen comes in contact with these antigen presenting cells, they are being engulfed by the antigen presenting cells and then these cells then these molecules are going to be processed. So, antigen is going to be processed and then it is going to be presented along with the MHC class 2.

And then when the it is going to be presented by the MHC class 2, it is going to be recognized by the D-cells and then D-cells are actually going to give the signal to the B-cells, and then these B-cells are actually going to differentiate into the memory cells as well as the

plasma cells. And then these plasma cells are going to start secreting the antibodies, and that is how these antibodies are actually going to participate into the defense responses.

So, what you see here is that the basics of this whole process lies in the two very important processes, one is when the antigen comes in contact with the antigen presenting cells, and then the antigen presenting cells are actually going to take up these cells and they are going to be engulfed. And then the second process is that how these antigen presenting cells, when these are going to process the antigen, so that it is going to be presented along with the MHC class 2.

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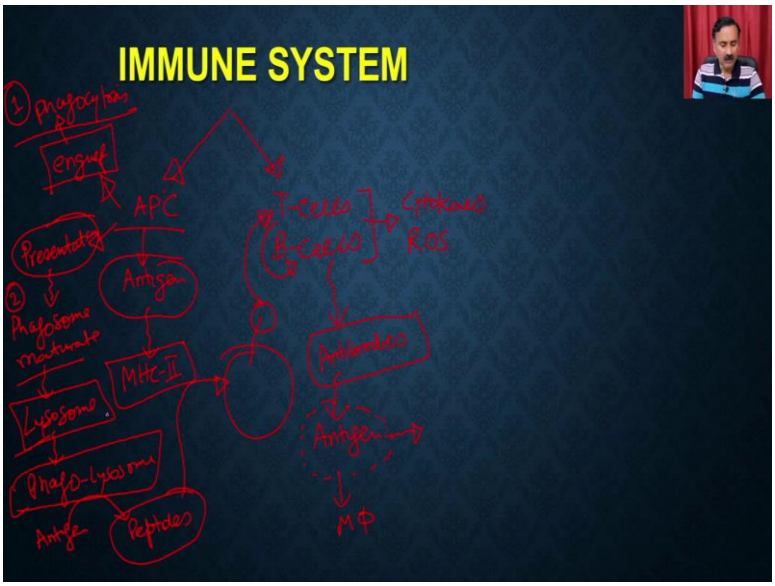

So, what we have discussed so far, what we have said is that the immune system is going to be consist of the two different types of cells, it is going to have the surveillance system or it is going to have deep excitatory cells. In the surveillance system, you are going to have the antigen presenting cells or the APCs. Whereas, in the excitatory cells, you are going to have the cells which are going to cause a different response, such as you are going to have the T-cells or the B-cells.

So, and both of these responses are actually going to secrete the different types of molecules so that it is actually going to cause the immune responses like they are going to secrete the cytokines, they are going to secrete the reactive oxygen species or the free radicals and all these molecules are actually going to be responsible for the generation of the immune responses. Compared to that the APCs are actually going to attack on to the antigens or the

organisms and then these organisms these antigen is going to be presented along with the MHC class 2.

So, as I said, what we have just now discussed at the end, when the ABCs are going to come in contact with the antigen, this antigen is going to be engulfed by the APCs and then the antigen is going to be processed and then mostly these antigens are made up of the proteins. So, these proteins are then going to be digested into the small peptides and then these small peptides are going to be presented along with MHC class 2.

So, APCs are actually going to do two functions, one is actually going to engulf the antigen. So, and this and they are also going to process the antigen so that they can be presented, so they are actually going to have the presentation of the antigen. So, they are going to have two functions, one is engulfment. So, the process through which the anti APCs are going to engulf the antigen is called as phagocytosis.

And the process through which the APCs are going to present the antigen along with the MHC class 2 is actually having a additional process which is going to be called as the phagosome maturation. And in the phagosome maturation, the phagosomes are actually going to fuse with the lysosomes and these two, then the lysosomal content is going to be delivered. So, when the phagosome is going to mature, it is going to be fused with the lysosome.

And that is why it is actually going to form the phagolysosomes. And when they will form the phagolysosome, the phagolysosome is actually going to have the content from the lysosomes. And you know that the lysosomal content is going to be degrading in nature. So, it is going to have the hydrolytic enzymes, it is going to have the different types of proteases and its pH is also going to be very low and because of that the antigen is going to be converted into the peptides, so antigen is going to be get converted into the peptides.

And then these peptides are actually going to be presented along with MHC class 2 on to the surface of these APCs. And that is actually going to give you, that it is going to give the signal to the T-cells. And then T-cell is eventually going to give a signal to the B-cell and that is how the B-cell is going to start secreting the antibodies.

So, once the antibodies are being formed, then the antibodies are actually going to attack onto the antigen. So, it is going to bind the antigen. And that is how the antigen is going to be trapped by the antibodies. And apart from that, since the antibodies are going to have the, they are called the specific receptors.

These antigen, antibody coated antigen is going to be a very good substrate or very good engulfment, so it is going to be engulfed very efficiently by the macrophages, it is going to be engulfed very efficiently by the other cells and that is how the antigen is going to be removed from the infection. So, this is just a general overview.

What we are going to discuss today is we are going to discuss about the phagocytosis, how you, what is the phagocytosis, what are the different events in the phagocytosis and how once the phagosome is going to be formed, how the phagosome will take, is phagosome is going to mature into the lysosomes.

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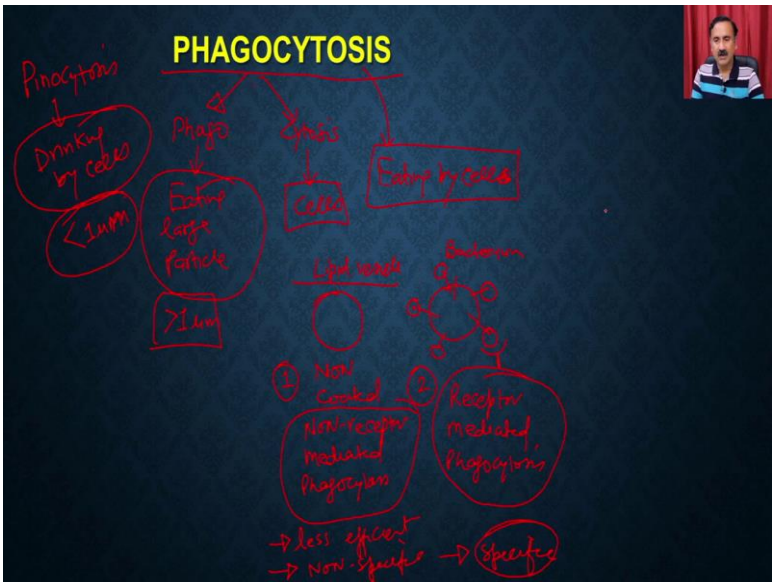

So, when we talk about the phagocytosis. Phagocytosis is a word which is made up of the two different words one is called as the Phago and the other one is called as the cytosis. So, Phago means eating and eating the large particles. So, when you do the eating, you always eat large particles. And cytosis means the cells. So, Phago, so what is literally means by the phagocytosis is that the eating by self. And apart from the phagocytosis you have another term which is called as pinocytosis.

So, pinocytosis means the drinking by self. So, if and all the pinocytosis or the phagocytosis is completely been dependent on the particle size. So, if the particle size is more than 1 micro meter, then it is going to be considered as the phagocytosis if it is less than 1 micro metre then it is going to be, a micro metre then it is going to be considered as a pinocytosis. And the pinocytosis is a process to which the cell is actually acquiring the liquids what are present into the system.

So, it is going to acquire like growth factors, different types of nutrition like amino acids and all those kinds of things, it can be achieved through pinocytosis, whereas, to the phagocytosis, it is actually going to acquire the large particles like the lipid vesicles or different types of organisms and so, on. As far as the phagocytosis is concerned, so you might see that the phagocytosis means, it is actually going to eat the large particles.

So, when you talk about the particles, the particles can be of two different types. Particles could be which are without any coating, so they can be non coated particles or they could be coated particles, which means the particle which will have some kind of ligands as for example, you can have a simple lipid vesicles.

So, you can have a simple lipid vesicle that is going to be considered as the non coated particles or you can actually have a bacterium which could be a coated particle. Because in the case of bacteria, you can have the different types of bacterial antigens which are going to be present. So, accordingly the phagocytosis also could be of, because the phagocytosis has to be take place either for this particle or this particle.

That is why the phagocytosis also could be of two different types, it could be the receptor, non receptor mediated phagocytosis, non-receptor mediated phagocytosis or the receptor mediated phagocytosis. As the name suggests, receptor mediated phagocytosis, where the receptors which are present onto the cell surface are actually going to engage the ligands what are present onto the particular particle or particular antigen, whereas, in the non receptor mediated phagocytosis, it is going to be a general process through which the particles are going to be internalized into the system.

Now, if you see the, so, this is going to be the non particle, non-receptor mediated, this is going to be called as receptor mediated phagocytosis. So, the initial engagement are actually going to be only the difference points. Apart from that, the non-receptor mediated phagocytosis is going to be less efficient compared to the receptor mediated phagocytosis, because it does not have any kind of attachments, it does not have the way to actually hold the particles.

And that is why it is actually going to be less efficient. It is going to be the nonspecific. So, it is going to be nonspecific, because it will actually going to engulf any particle which comes in the vicinity of the APCs or in the vicinity of the cell. Whereas, in the case of the receptor

mediated phagocytosis going to be specific because it is going to be mediated by the receptors. So, let us understand a general scheme.

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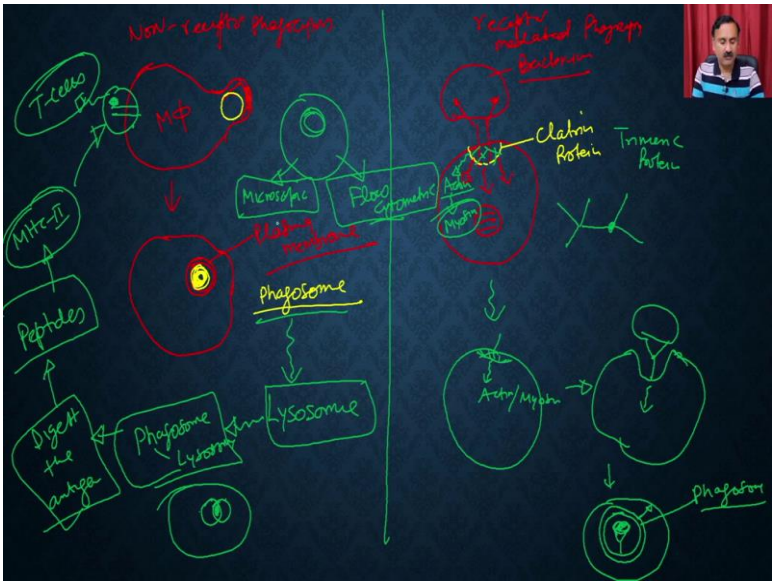

So, in general scheme what happened is that, so, in a general scheme, what will happen is that, when you have a cell like you have a macrophages. So, if you have a macrophage, and if this macrophage is going to perform, it is going to actually be able to perform the receptor mediated phagocytosis or the non-receptor mediated phagocytosis. So, once the bacteria or the particle is actually going to come in contact with the macrophages.

So, suppose this is a particle right, which is actually going to come in contact with these macrophages. So, for example, if this is a particle which comes in the vicinity of this particle. Now, you can have attachment points through which these receptor, this particle can actually attach to the macrophages so then it becomes a receptor mediated endocytosis.

But, as soon as this happens then the macrophage, what the macrophage is going to do is it is actually going to start producing its filopodia, or start increasing a filopodia. And once this particle is going to interact with the lipids, what is present onto the macrophages, they will actually going to cover the particles from the both the sides. And this will in advance.

So, as the time will pass on it is actually going to be keep advancing like this and ultimately what will happen is that these two lipid vesicles or the plasma membrane is actually going to fuse. And once that happens, then what will happen is that you are going to see that it is actually going to, is going to happen like this.

So, it is going to, so, the initial membrane which was in direct contact with the lipid, the particle is actually going to be disappear, and then it is actually going to be a part of the vesicles. So, what happened is ultimately is that in the macrophages, the particle is going to be internalized. And the particle is, so, this is suppose the, and the particle is going to be entrapped outside by the plasma membrane.

So, plasma membrane is actually going to cover the particles and this plasma membrane containing particle is going to be called as the phagosome. So, this is the particle what you have, this is the phagosome particle. And ultimately, this type of particle is going to be entrapped in a double membraned structure and this double membraned structure is going to be called as the phagosome.

In receptor mediated phagocytosis, what will happen is that you are actually going to have the macrophages. So, it is like, for example, you can have example of like the macrophages containing the receptor. So, you are going to have a receptor, and then what will happen is that it is actually going to bind to the ligand. So, it is going to be interact with the lagand, what is present onto these particular objects, so this is actually the bacteria.

And once it binds, actually, it is actually going to draw or it is going to be actually activate cellular machinery in such a way that this particular area where these, so, this is the non-receptor mediated phagocytosis where and this is going to be the receptor mediated phagocytosis. So, as soon as this receptor is actually going to engage with the ligand. So, this is actually a bacterium, and it is going to have different types of ligands.

So, when this receptor is actually going to hold the bacteria with the help of the ligand and receptor interactions, this receptor is actually going to have a downstream signaling. And because of this dominance signaling, this area which is actually going to be where the receptor is present is actually going to be coated by a proteins. So, this is going to be coated by proteins. And these proteins are called as the clathrin proteins. So, these clathrin proteins is going to coat this area where the receptor is present and this particular particle is engaged.

Now, once the clathrin is going to coat this part of the plasma membrane, then it is actually going to engage additional machinery. So, what it is going to engage? It is actually going to engage the actin, so it is going to engage the actin monomers. And clathrin is a trimetric proteins. So, these trimetric proteins are actually coating is present like this. So, these trimetric proteins are the making a mesh, so that is how they are coating.

So, these are like you can imagine like this, then it is going to have a mesh like this. And clathrin proteins are actually going to make a mesh and because of this mesh, this area is going to be completely hold by the clathrin proteins and then these clathrin proteins are actually going to be associated with actin. And then this actin is actually going to be associated in myosin. And you know that the myosin has ability to pull the whole thing.

So, then ultimately what will happen, the ultimately this whole system which is actually been coated with the clathrin proteins is actually going to be pulled by the actin and myosin system. And because of that, it will actually going to cause a depression. So, here you have receptor, which is actually having a ligand to bind and then this keep pulling, keep pulling, keep pulling like this and ultimately what will happen it is actually going to be present inside.

And it is going to be this is the vesicle, where you are going to have the receptor and that on the receptor, you are going to have the particles. So, this receptor is then going to be a lipid, double membrane, the plasma membrane, and these are called as the phygosomes. In some cases, when these are the food particles, like for example, the LDL receptors, then this receptor is also going to be in, once this is going to be it is going to be recycled back.

So, it is going to go and again to be present on the plasma membrane to take up the additional lipid molecules, but if it is a bacteria or something, then this phygosomes is actually going to fuse with the lysosomes. So, these are the two general mechanism through which the phagocytosis can happen, either it could be a generalized mechanism, where the lipid part, the particle is going to be cover by the plasma membrane, and then eventually the plasma the lipid, the particle is going to be a part of the cells.

In either of the case it is actually going to form a particle interact in a double layer, lipid vesicles. And these double layer lipid vesicle, where you have the particle is called as phagosomes. And then eventually, the fibrous form is actually going to interact with the lysosomes. So, because the phagosome is actually going to have the different types of proteins, and that is how they are actually going to be directed against the lysosomes.

And then because of this interaction is actually going to be get converted into a species which is called as the phagolysosomes. And in the phagolysosomes, you are actually going to have the phagosome, which is actually going to have the content of the phagosomes and the content of the lysosomes. And that is why within the phagosome you are actually going to

have the, so this is going to be like a phagolysosomes and eventually the phagolysosomes it is actually going to digest the antigen.

And once it is going to digest the antigen, it is going to be formed the different types of peptides. And when they will form the different types of peptides, these peptides are going to be coupled with the MHC class 2, and that is how they are actually going to be presented on to the APCs along with the MHC class 2, and that is why the MHC class 2 is actually going to have a peptide bound.

So, this is actually going to have a signal for the T-cells to give the to respond. And that is how the eventually the T-cell is actually going to give the signal to the B-cell and that is how there will be a production of antibodies. So, if you want to study the phagocytosis you have to understand that the phagocytosis means the internalization of the particle. So, it is going to be like this. So, what you have to study is you have to study the internalization of the particles.

That study you can do by two methods, one you can actually do by the microscopic method. So, you can actually use the microscopic method you can actually stain the cells and then you can observe these vesicles inside the microscope. The other is you can actually do the flow cytometry based assays and you can actually be able to understand the process by more as a quantitative pattern. So, let us understand how you can be able to study the phagocytosis in a professional Apicius like the macrophages.

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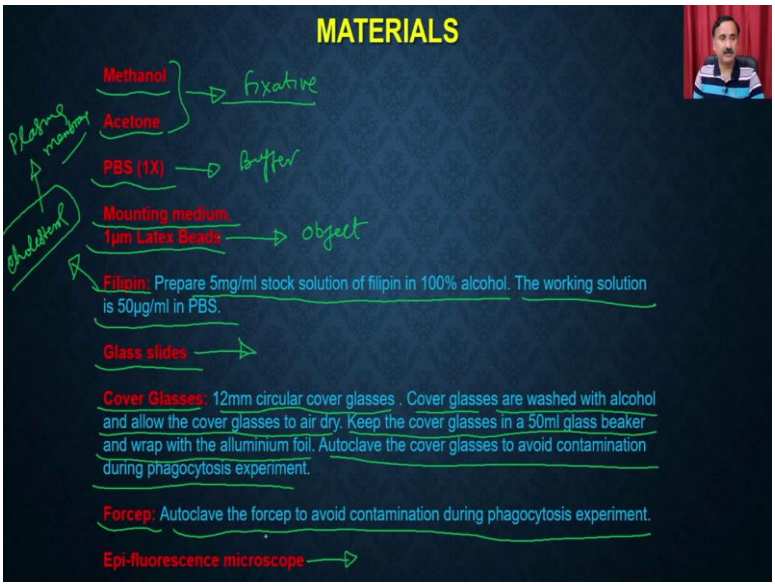

So, if you want to study the phagocytosis by the microscopic method, what are the things you require, you require the different types of reagents. So, you require the methanol, you require the acetone these are the part of the fixation system. So, it is going to be used for the preparing the fixative, then you require the PBS which is going to be a buffer. So, that is going to be used for a washing then you require the mounting media and then you require the latex beads. So, these latex beads are actually going to function as the object.

And then you require a dye which is called as the Filipin. So, the Filipin is a dye which is actually going to stain the cholesterol so it is going to be bind to the cholesterol. So, ideally it is actually going to give you the position of the plasma membrane. And Filipin is when it is interacting with the cholesterol it is going to give you the blue color fluorescence. So, you prepare the 5 milligram stock solution of Filipin in 100 percent alcohol.

The working solution is 50 micrograms per ml in the PBS. Then you require the glass slides where you are actually going to perform the experiments, then you require the cover classes. So, you can use the tall mm circular cover classes. The cover classes which you are going to use in the lab are has to be washed with the alcohol and allow the cover glasses to air dry. Keep the covered glasses in a 50 ml glass beaker and wrap it in aluminum foil.

Autoclave the classes so that you can actually be able to avoid the contamination when you are want to study the phygocytosis in your lab conditions. Then you require the forcep. The forceps are required only to handle the cover slips as well as the cover glasses. Then you saw autoclave the forces so that you can avoid the contamination during the phagocytosis experiment and then you also require the microscope, so you can also require the fluorescent microscopes.

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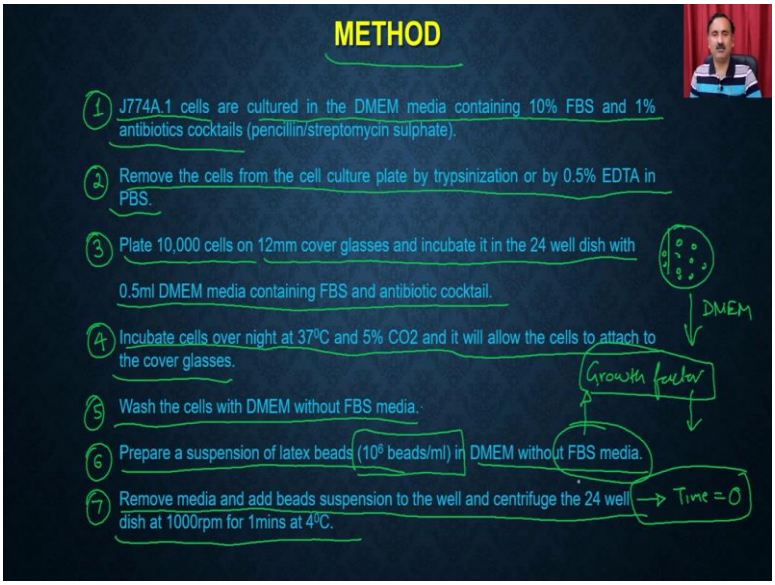

As far as the method is concerned it has a different steps. So, in the step 1, what you are going to do is you are going to take the macrophages, so these are the macrophage cell lines, which you are going to cultured in DMEM media containing 10 percent FBS and 1 percent antibiotic cocktail. So, when you are, then in step 2, you remove the cells from the cell culture plate by the trypsinization, or by the point 5 percent EDTA.

So, when you do the trypsinization, it is actually going to cleave the adapter the attachment proteins which are actually allowing themselves to attach to the dishes and therefore it is they will come out in the solutions. Then the step 3, you are going to plate the 10,000 cells on 12 mm cover glasses and incubate it into 24 well dish with 0.5 ml DMEM media containing FBS and the cocktail.

So, when you do that, the cells will go and stick to the circular cover clip. So, they will go and stick to that circular cover clips. And then you can actually be able to use them. Then the step 4, you can incubate the cells overnight at 37 degrees Celsius in 5 percent CO2 and it allow the cells to attach to the cover glasses. The step 5, you watch the cells with the DMEM without FBS, so you wash these cells with the media.

So, you can wash it with the DMEM without containing serum. So, that is how you can actually be able to remove the serum and other components because you want to do the phagocytosis under the non receptor mediated phagocytosis. And that is how you remember that we have taken the latex. Then you will prepare a suspension of the latex beads, so 10 to power 6 beads per ml what you are going to prepare in the DMEM without FBS.

So, why we are removing the FBS is because the FBS is containing different types of growth factors and these growth factors are actually going to coat the latex beads and that is how then it is going to be receptor mediated phagocytosis rather than a non receptor mediated phagocytosis. And then the 7, you remove the media and add the bead suspension to the well and centrifuge the 24 well dish at 1000 RPM for 1 minute at 4 degrees Celsius.

So, when you do that, what will happen is that the beads are actually going to come in contact with the cells. And that is how they will, you are going to start the phagocytosis. So, this is actually going to be considered as time 0 for the phagocytosis experiment. So, when you are going to spend ourselves that is going to be considered as the time 0. From this time, you can actually be allowed the cells to eat the particles for as many long time you want.

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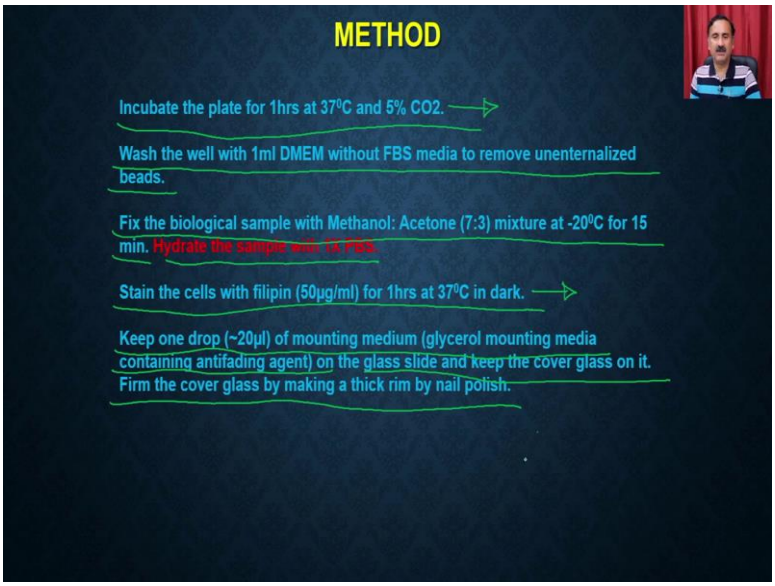

Then you incubate the plate for 1 hour at 37 degrees Celsius and 5 percent CO2. So, that is the time what you have given the cells to eat these beads. Then you wash the well with 1 ml PBS without PBS to remove the unenternalized beads. So, you are actually going to remove whatever the beads were not in phagocytosis. Then you fix the biological sample with the methanol acetone mixture at minus 20 for 15 minutes.

You hydrate the sample with the 1 percent PBS. And then you stain the cells with filipin for 1 hour at 37 degrees Celsius in dark. So, when you do that, since the filipin is going to give you the blue color fluorescence, it should be done in a dark so that it should not be the, signal should not be get quenched.

Then you keep the one drop of the mounting media, the glycerol mounting media containing the antifading agent on the glass slide and keep the coverslip onto the, coverslip onto the glass slide on and then you form the glass slide by making a thin rim by the nail polish. So, if you do so, what will happen is that it is actually going to give you the prepared slides. And now what you can do is you can take these prepared slides and absorb them under the microscope.

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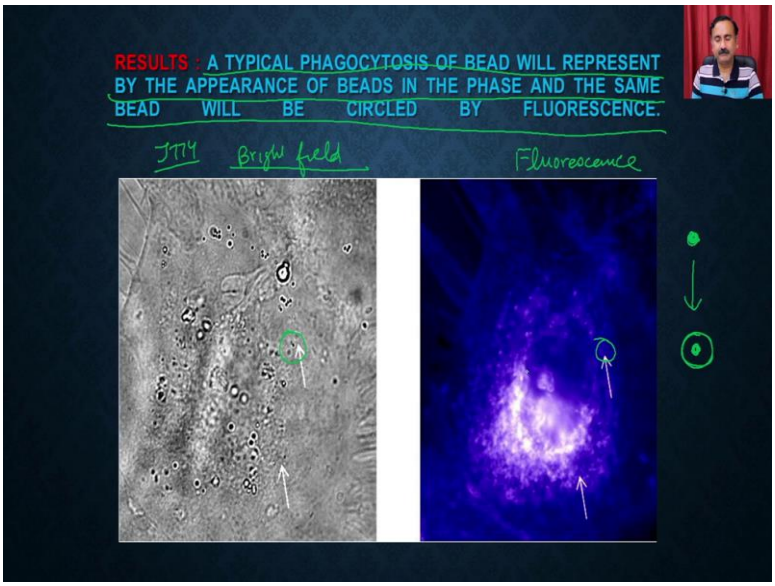

Now, what you are going to see? A typical phagocytosis of beads will represent by the appearance of the beads in the face and the same bead is going to be encircled by the blue color fluorescence. For example, you can see here. So, in this particular area, what you see here is there is a two beads which are been attached, which are look like, so these are the macrophages cells. So, these are the J774 cells. And this is the bright field image. So, how the field will look like when you see under the naked eye.

So, this is what you are going to see, these are the beads what you are going to be present on to the cell surface. And then if you convert the channel to the fluorescence, what will happen, it is going to show you the blue color fluorescence and under a blue color fluorescence this particular bead is actually having a blue color band. So, that blue color band is saying that it got internalized.

And after the internalization the it has been covered by the plasma membrane, because initially you are going to have a particle like this, but when it gets phagocytosed it is actually going to be present like this, it is going to have a plasma membrane. Because remember that I

explained you that the cell is going to engulf the particles and that is how it is actually going to be entrapped by the plasma membrane.

Now, apart from this particular assay, where you, this going to be very, very time consuming. So, this microscopic assay is going to be very, very time consuming, it is going to be labor oriented, and it actually requires an expert eye to see which bead is actually going to be internalized and which is not. So, to avoid this you can also use a flow cytometry-based phagocytosis assay. The principle of the flow cytometric assay is different from these microscopic assays.

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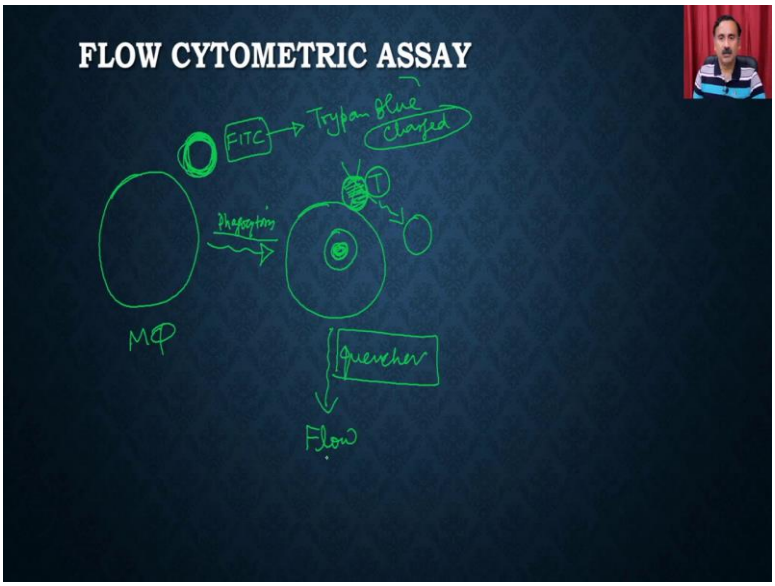

Now, what you are going to see in the flow cytometric based assay is that you are actually going to exploit the idea of that the you are going to see for example, this is the macrophages. This is the macrophages. So, when you add the particles, you are or when you give the particles to eat, for example, if I have given a bacterium. And what I can do is I can just take the bacteria and I stained them with a fluorescent dye for example, I can just sustain it with the FITC.

So, what will happen is the FITC is going to give them the green fluorescence. So, under these conditions once the phagocytosis is over, you are going to have the situation like this, you are going to have the bacteria which were in the process of the phagocytosis. And there will be a bacteria which are going to be internalized. So, there will be a bacterium which is going to be internalized. And these are the particles which are in the process of phagocytosis.

Now, the question is if you want to make a quantitation, if you can, so you have to destroy these phagocytosis beads. So, what you can do is you can just incubate these cells under quencher. So, I am sure if you do not know about the quencher means, quenches are the molecules which are actually going to kill the fluorescence of the any particles. So, what you can do is you can take the quencher. So, for example, for the FITC, I can take a quencher like that trypan blue.

So, trypan blue is a dye. So, trypan blue is a charged dye, and it does not enter into the cell because it is a charged dye. But it is actually can be good enough to quench the signal or the fluorescence of the FITC. So, if it is present outside, and if you add the trypan blue, trypan blue is going to go and bind to this particular particle, and that is how they will get converted into a non fluorescent particle.

So, because of that, these external particles are going to be non fluorescent. So, now, if you do a flow-based experiment, like if you take these cells, and if you put them under the fluorescence, these cells can be captured. And that is how you can be able to quantitate how many numbers of cells are actually having the particles which are internalized, and then you can actually be able to count and if you count, you can be able to calculate the phagocytosis.

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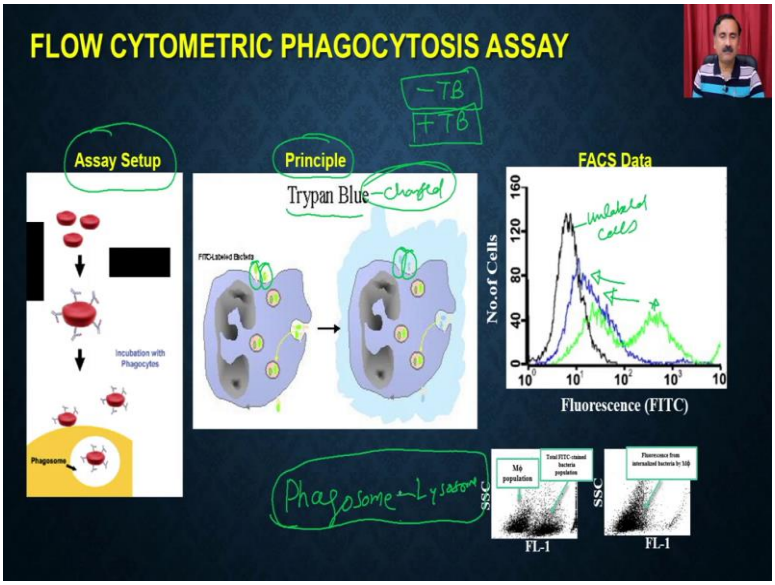

So, I have prepared this is going to be the assay setup. So, in the assay setup, what you are going to do is, you are going to take the cells, first you are going to stain it with the FITC or the fluorescein. And then this is the particle, this is the principal of the assay where you are actually going to add the trypan blue. So, trypan blue is actually a charged dye.

So, when you have a charged dye, it will not enter inside the cell, but you are going to have the two different types of particles, the particle which is outside or which is just simply attached, and the particle which is going to be internalized. So, these internalized are not sensitive to the trypan blue, because the trypan blue will not enter, but these external ones are actually going to be sensitive. So, when you add the trypan blue, this trypan blue is actually going to quench the external signal.

And that is how you see there is no signal from this. So, when you take the facts data, what you are going to see is these are the unlabeled cells. And these are the labeled cells. So, these are the labeled cells. So, when you add the cells, you can actually be able to capture the facts data under the two conditions minus trypan blue and the plus trypan blue. The minus trypan blue is actually going to give you the idea about how many number of cells are present whether it is outside or inside.

And then you add the trypan blue there should be a shift of the peak onto the left side. So, this is what is going to do. So, if you add the trypan blue this particular peak, which was present here is going to be shifted onto this side. And that actually is going to say that a large number of particles were present inside also. And that, this information, so you can actually be able to calculate the average florescence intensity for this blue color peak, and you can actually be able to calculate the average florescence intensity for this green color peaks.

And that can be used to calculate the phagocytosis. This is what it is going to show. So, this is all about the phagocytosis. So, once the phagocytosis is done, it is actually going to form the phagosome. So, these phagosomes are then actually going to fuse with the lysosomes. And that is how they are actually going to form the phagolysosome. Now, if you want to study this particular event and you want to see how the phagosome is actually fusing with the lysosomes you can use a simple assay.

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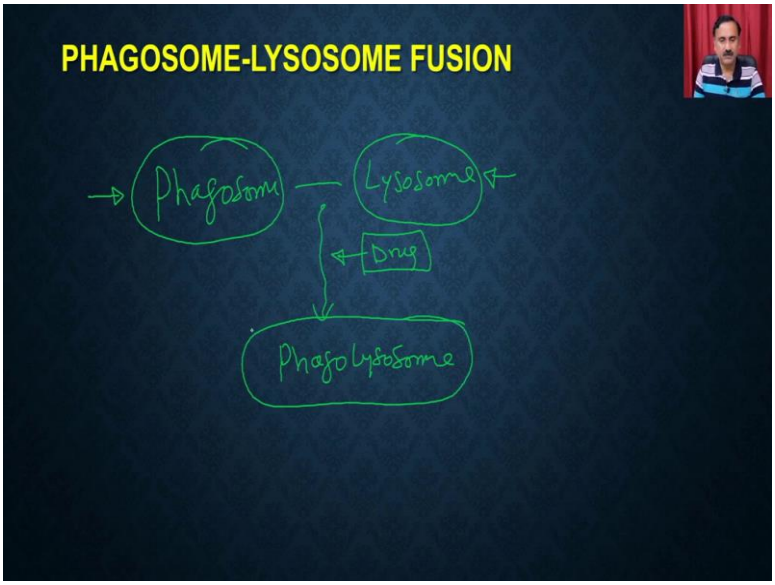

So, the phagosomes are actually going to fuse with lysosomes. So, you can actually be able to in study the interaction between the phagosome and the lysosome so that you can be able to understand how the phagosome, phagolysosome is forming and that is how it is actually going to help you to understand the process of the phagolysosome formations.

And you can actually be able to study the different types of factors and other kinds of events and even like the drug molecules, which are actually going to either accelerate this process or actually going to inhibit this process. So, in this case, when you want to do assay what you have to do is you have to first prepare the phagosomes and you also have to prepare the lysosomes and then you are actually going to mix these phagosome and lysosome together and that is how it is actually you can be able to study the phagolysosomes. So, if you want to perform this, you have to use the following materials.

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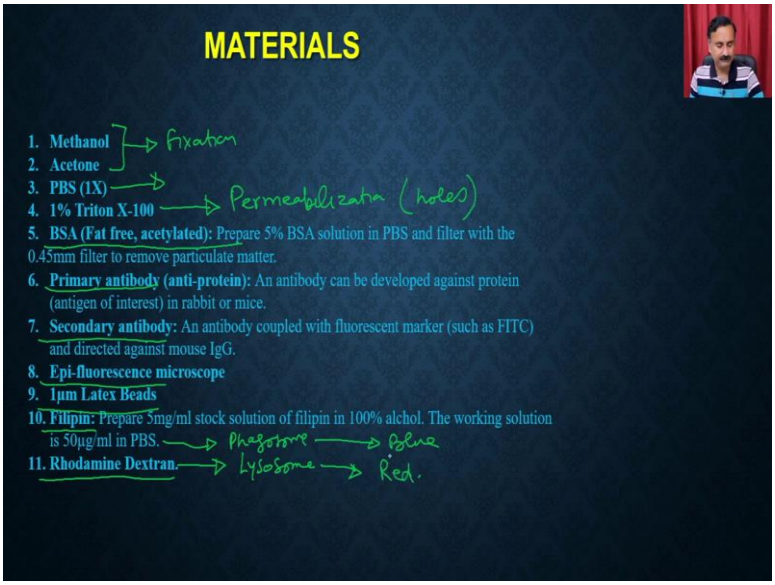

So, what you require is the methanol and acetone which is actually for a fixation. So, this is required for fixation. This is buffer. Troton X 100 is required for the permeabilization for making our holes into the cell, so that you can be able to use the different types of reagents. You can use the BSA which is for blocking agents. You require the primary antibody, secondary antibody. You require the epi florescence antibody.

You provide a one micro bowl latex beads. And then you require the filipins. And then you also require the rhodamine dextran. So, rhodamine dextran is required for labeling the lysosomes. And you require the filipins which is for staining the phagosomes. And remember that a filipin is actually going to give you the blue florescence as the rhodamine dextran is actually going to give you the red fluorescence.

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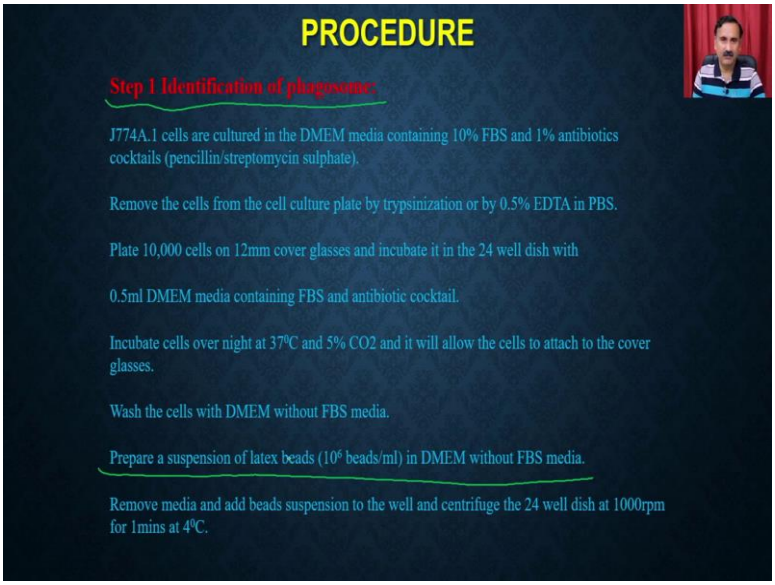

Now, first is you have to prep, step 1, you have to prepare the phagosomes. So, these are the steps I think we have already discussed. So, what you have to do is you have to take the macrophages to let it into the cells and you have to prepare a suspension of the latex beads and then you have to allow these beads to be feed by the macrophages.

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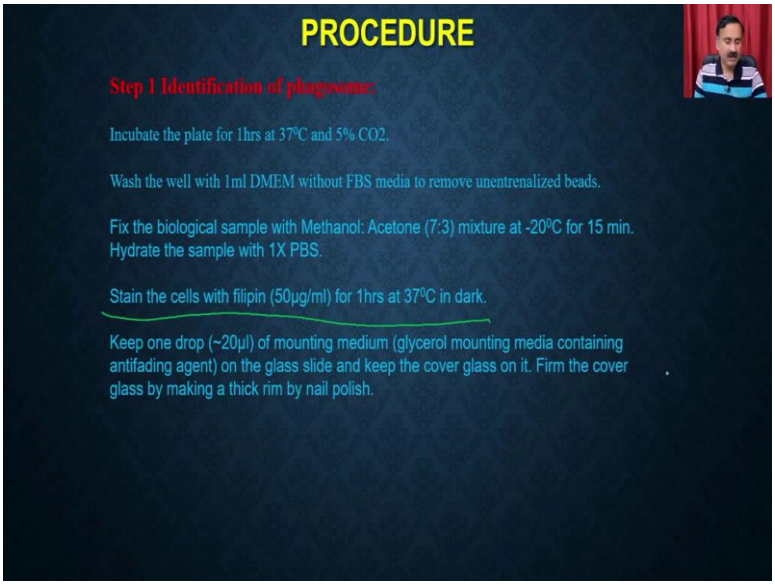

And that you are going to do and then you are going to stain the cells with the filipins. And then you are actually going to be able to monitor where the phagosomes are.

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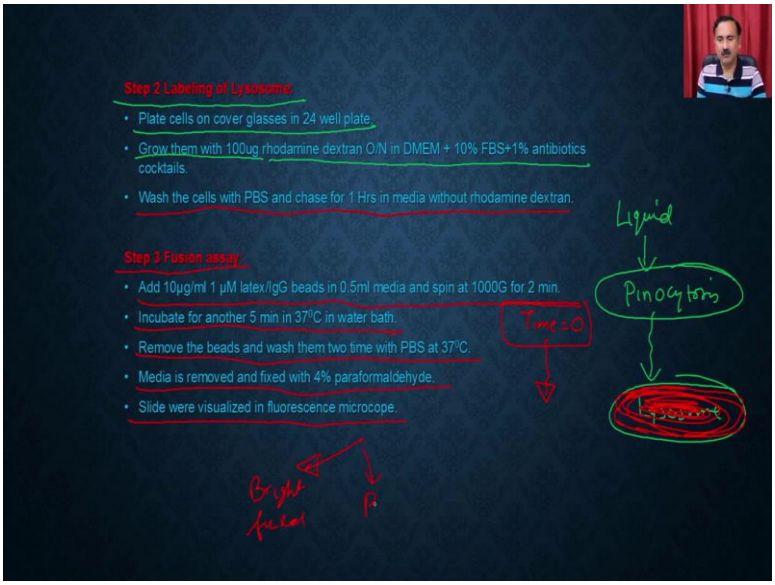

Then in the step 2, you are actually going to label the lysosome. So, what you can do is you can take the cells in a 24 well dish, grow them with 100 microgram rhodamine dextran overnight. So, when you take the rhodamine dextran, rhodamine dextran is a liquid. So, that liquid is actually going to be internalized by a process which is called as the pinocytosis.

And anything when you are going to take up by the pinocytosis will eventually going to end up into the lysosomes. So, that is how it is actually going to form a lysosomes, where internally, you are going to have the rhodamine dextran. So, rhodamine dextran means you are going to have the lysosome which is actually going to be red color. So, it is going to be filled with a liquid which is going to be red color, which means it is going to give you a red color fluorescent lysosomes.

You wash the cells with PBS and chase for 1 hour so that all the rhodamine is going to be present into the lysosomes. Then in the step 3, you are going to set up the fusion assay. So, what you can do is you can just add the 10 microgram per ml latex beads in 0.5 ml media and then you are going to spin at 1000 g for 1 minutes or 2 minutes.

That is going to be considered as the time 0, at that point the phagocytosis as well as the phagosome formation is going to start, so that from that time you can continue and you can be able to study the ribosome fusing with the lysosomes. You incubate for another 5 minutes, so that in the water bath, you incubate that in a water bath so that you can be able to initiate the phagocytosis. Remove the beads and wash them two times with PBS.

The media is removed and fixed with 4 percent paraformaldehyde. Then slides are actually going to visualize under the fluorescent microscope. And when you visualize them, you are actually going to capture the image into the three channels one is you are going to capture under the right field, you are actually going to capture under the blue fluorescence and then you are also going to capture under deep red fluorescence. And when you do that, you are going to see the slides like this.

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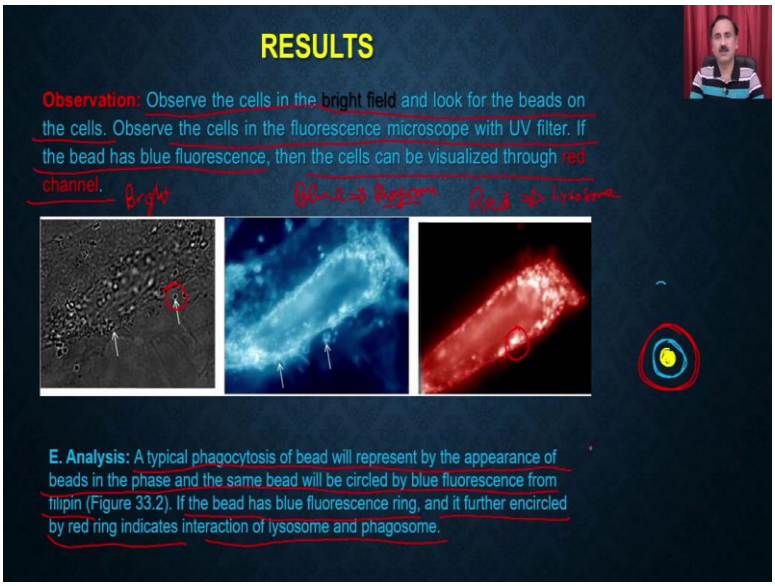

So, when you observe the cells in a bright field and look for the beads onto the cell. You observe the cell in the fluorescent microscope with UV filter. If the beads has a blue fluorescence then the cell can be visualized under the red channel. So, what will happen, what you are going to see actually, so, what you have to do is first you are going to see the cell under the bright field. So, in the bright field you are going to see whether you the beads are present on the cell or not.

So, these are the J774, this is the bright field, which means it is going to be a black and white. Then this is going to be the blue fluorescence, this is going to be the red fluorescence. So, this is going to indicate the phagosomes. Remember our previous experiment. And this is going to tell you the position of the lysosome. So, you see here there is a bead, which is actually going to be present onto the cell.

Now, if you see the same bead under the blue fluorescence, what you see here is that this bead is actually going to have a blue color fluorescence, that means this bead is actually inside the cell and it is being present inside of phagosomes. Now, if you see the same bead,

you will see that it also has a red color fluorescence which means this bead is actually having the both, it is actually forming a phagosome and that phagosome is actually being fused with the lysosomes.

So, the bead, how the bead will look like which is actually going to have the phagosomes, fuse with the lysosome. So, when you see the under the fluorescence microscope, the bead is actually going to be present as the latex bead. So, you can actually imagine that the latex beads are yellow in color. So, these are, this is going to be the beads and that is what it is going to be look like when you are actually going to be in the bright field.

Now, when you look at under the blue channels, it will look like this. So, it is going to give you a phagosome. So, it is going to have a blue color intense fluorescence. That is what you see here, this is what you see here. And now, if this beads or if this phagosome is been fused with the lysosome, then it is actually going to show you another ring and that another ring is actually going to be a red color ring.

So, it is going to have the red color ring, which is actually indication of the phagolysosomes. So, if it is having the two rings, one is the blue ring, the other one is red ring, then it is going to be said as that phagosome is actually been matured. If you see only the blue color ring, then it will say that the cell is been the objective been into life, but it does not have the any kind of maturations.

So, a typical phagocytosis of the bead will represent by the appearance of the beads in the face and the same bead will be circulated by the blue fluorescence from the filipin. If the beads has the blue fluorescence ring, and it has further been encircled by the red ring indicate that the interaction of the lysosome and phagosome is happened. So, these are the two different methods or the two different aspects what we have studied so far, what we have studied.

We have discussed about the process of phagocytosis and how that can be done by the two different methods, we can do the non receptor mediated phagocytosis and the receptor mediated phagocytosis. And we have discussed about the two different methods of the microscopic method and as well as the flow cytometric method to study the phagocytosis.

And lastly, we have also discussed about how you can be able to study the phagosome maturation. So, with this I would like to conclude my lecture here in our subsequent lecture we are going to discuss some more aspects related to cell mediated in responses. Thank you.