Host-Pathogen Interaction (Immunology)
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Lecture - 75
Bacterial Infection – Tuberculosis Tests and Pathogenesis

Hi. So in previous session we have looked at the Mycobacterium tuberculosis. We have learned that this is a very successful pathogen and the reason behind the success of this pathogen is the reasonably unique cell envelope or cell wall which is composed a variety of lipid and sugar derivatives. now in this session we will discuss about the various tests and the pathogenesis, okay how it causes the disease.

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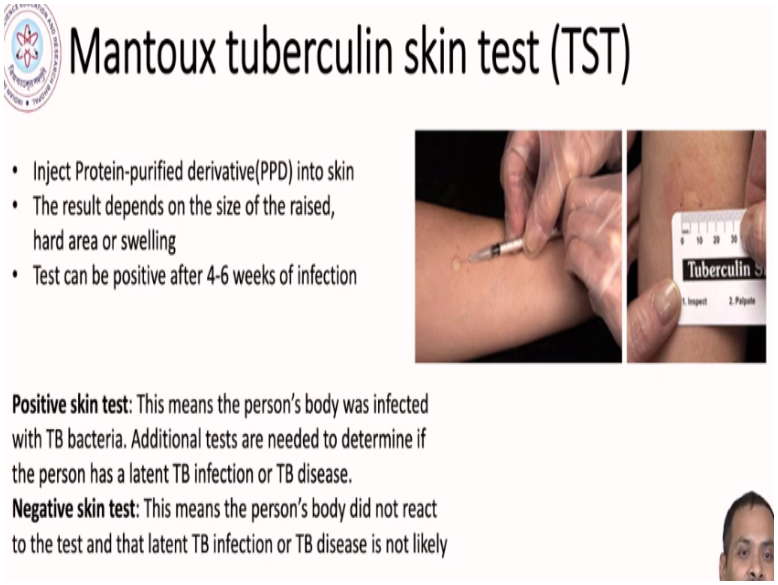

So let us look at the test. So there is a Mantoux tuberculin skin test **is** used which is now not so common but yeah still in some cases people use this thing. So basically the individual who is suspected with tuberculosis there is a very clear sign and symptom, the very clear sign and symptom probably you might have heard in radio and television that there is a chronic coughing, dry cough, there is blood in sputum which we call it as a hemoptysis, there is loss of weight.

So these are the very key features of tuberculosis and there is fever and there is night sweat, all these are the very prominent clear symptom of tuberculosis. So, if the individual is suspected with tuberculosis then they undergo various investigation. This is very preliminary,

but this is not the gold standard, the Mantoux tuberculin test. So over there what they are doing basically the doctor or the staff basically inject the PPD.

What is PPD? It is a protein-purified derivative and what is this? This is basically the protein component of Mycobacterium tuberculosis. They just inject beneath the skin and the result depend on the size and raised hard area or swelling. So after injection there will be an immune reaction and just reading that immune reaction can say that the individual may have Mycobacterium tuberculosis infection, but this is not firm.

Generally, the test can be positive after 4 to 6 weeks of infection and this is just a condition. Positive skin test what it means? This means the person's body was infected with Mycobacterium or tuberculosis. Additional tests are needed to determine if the person has a latent tuberculosis infection or TB disease. I told you this is not a firm investigation. The most firm investigation is to identify the Mycobacterium tuberculosis from the patient's sample.

If it is a pulmonary tuberculosis one has to find out the Mycobacterium tuberculosis presence in the sputum sample. Negative skin test this means the person's body did not react to the test and that latent tuberculosis infection or TB disease is not likely. If it is negative then we can say that the TB is not there, negative result is much more or relatively clear but positive result is quite not clear.

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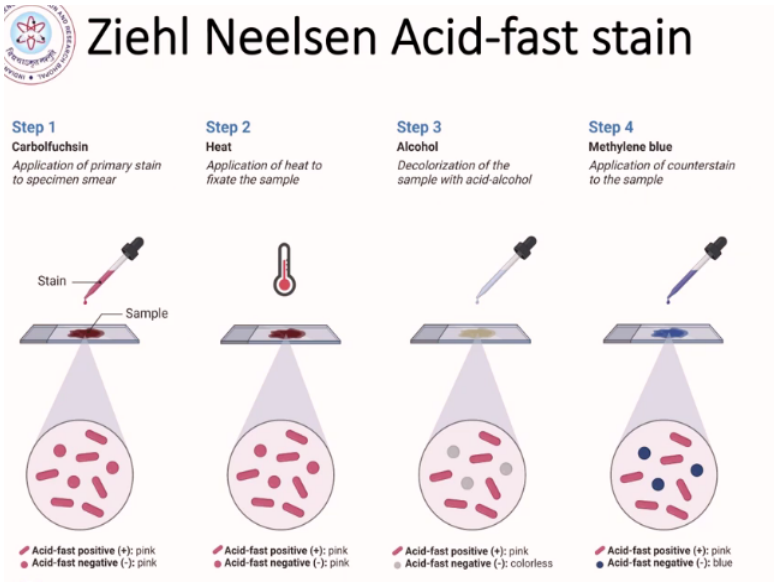

So in order to find out the Mycobacterium tuberculosis in the sample there is a test, here you can see, it is a Ziehl Neelsen acid-fast stain. This is a very simple test. Here you can see that

basically this is done with sputum smear. So first the sputum is collected from the patient and then we make the smear or the technician makes the smear and then there will be addition of this dye, here you can see that carbolfuchsin and this is application of primary stain to the specimen smear.

After that basically this sample is fixed and by heat, simple heating, you put it over the flame not too close, just apply the heat, application of heat to fix it the sample. Then this sample is a treated with acid alcohol as I explained you in previous session there is a 95 percent ethanol and 3 percent hydrochloric acid. So this sample is a treated with this acid alcohol and after the wash with acid alcohol decolourizing agent, the sample is treated with methylene blue.

So methylene blue is a kind of a counter stain. If it is negative or if there is some another bacteria, then that will take the methylene blue. And after wash you can observe and here you can see that the Mycobacterium tuberculosis will remain red. So here you can see that acid-fast positive bacteria will remain acid faster positive even after the treatment with this acid alcohol that is why we call it as acid-fast bacilli.

Generally, this Mycobacterium tuberculosis is rod shape the other bacteria other than mycobacteria will lose this initial staining solution and this will be stained with the methylene blue. So, this is the gold standard or the most appropriate method to diagnose the Mycobacterium tuberculosis.

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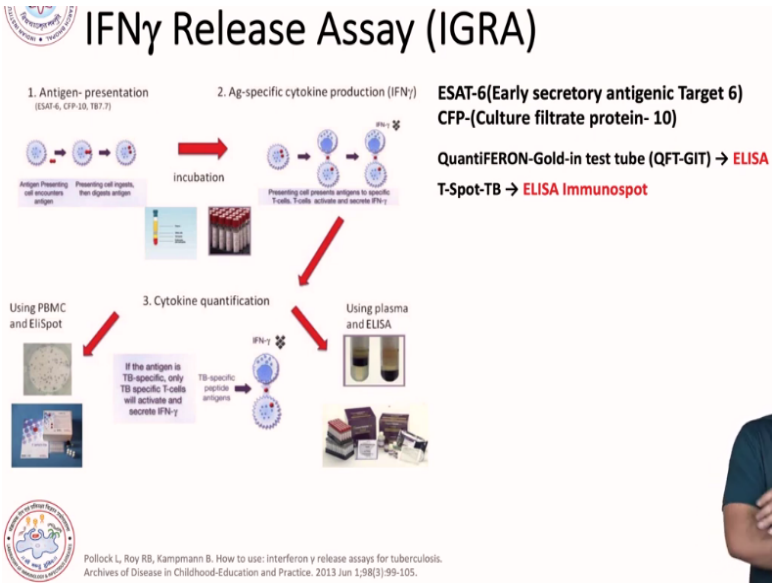

But yeah there are some another method by which one can say but the looking at mycobacteria and diagnosis is the gold standard, there are some another way, here you can see that there is an interferon gamma release assay. You know that once this Mycobacterium tuberculosis will be taken up by the macrophages then they will express their antigen and then along with MHC class 2 molecule and this is presented to the Th 1 cell.

And this antigen specific Th-1 cell will produce the interferon gamma. So if we test this interferon gamma production by using this, there are some specific antigens, here you can see that there is ESAT-6 antigen from Mycobacterium tuberculosis, this is early secretory antigen target 6. There is some protein known as CFP, culture filtrate protein 10 and this protein is also derived from Mycobacterium tuberculosis.

So if you see giving this antigen and then if you can find out the interferon gamma producing cells in presence of these antigen then you can say that the individual is having the Mycobacterium tuberculosis infection. So this is made in kit form like QuantiFERON-Gold-in test tube. There is a T-spot-TB. There is ELISA Immunospot. So here you can see the workflow. So, this is also used for the diagnosis, however it is quite expensive method compared to the finding Mycobacterium tuberculosis in the smear, but this is also used.

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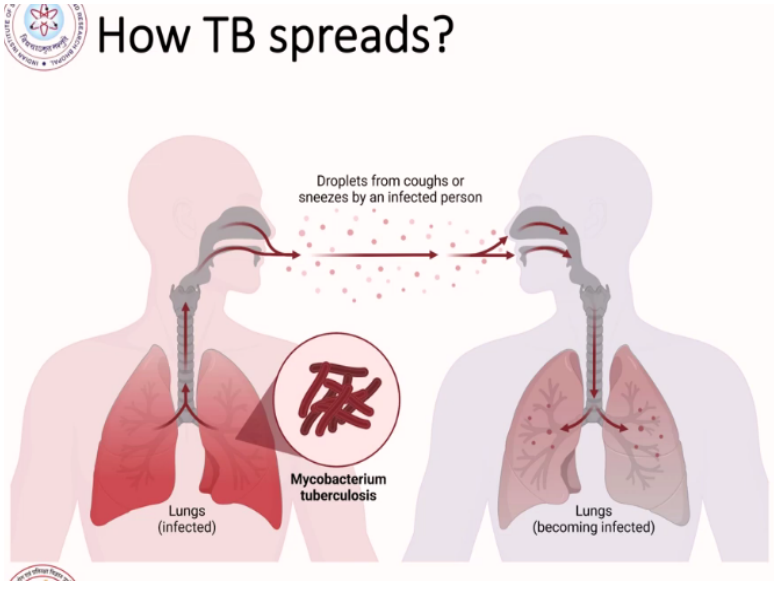

How tuberculosis spreads? I have explained you in previous session, but I can tell you again. So, this is basically the spread of Mycobacterium tuberculosis through this droplet from cough or sneeze from the infected patient. So, when this infected patient will cough and sneeze and this will release the droplet and this droplet may have the Mycobacterium tuberculosis and if

this droplet is inhaled by the healthy individual then that individual may infect with Mycobacterium tuberculosis.

But do not worry it is not going to cause disease immediately and there is a 99 percent chance if you are a healthy individual this infection will be readily cleared by the immune system. I will show you there are different categories of infection and clearance of this Mycobacterium tuberculosis infection by the host.

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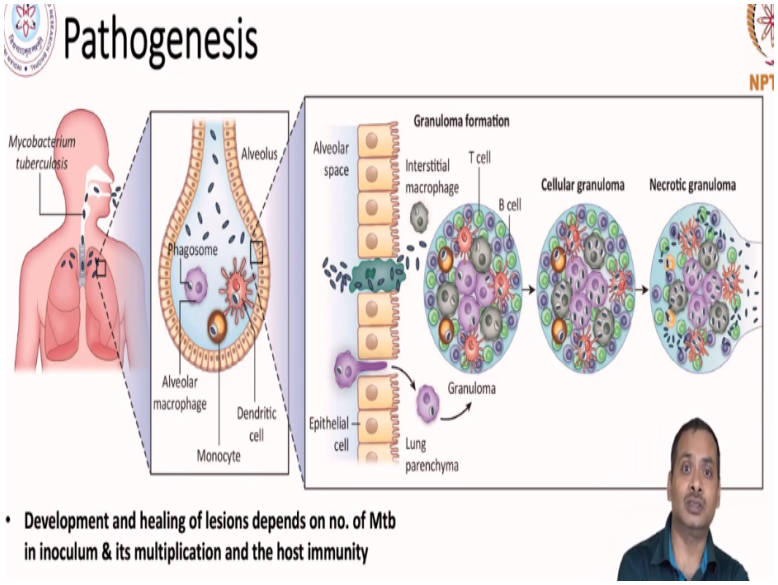

What is happening if it is going inside the lung? Then basically here you can see that this is taken up by the alveolar macrophages. Here you can see there is alveolar macrophages, alveolar dendritic cells. There are dendritic cells present in the lungs and monocytes. okay So the dendritic cells and macrophages are infected with Mycobacterium tuberculosis and sometimes monocytes are also infected. So in most of cases, this is cleared by the macrophages or dendritic cells.

However, if it is not cleared, then they will make a granuloma. So granuloma is a structure, so basically you try to understand when our immune system is not able to clear some infection so what they do, they quarantine, they keep it in one place, this pathogenic thing they will keep it at one place and this place we call it as a granuloma. So, granuloma is a kind of encapsulated structure where there is a fibrotic tissue around this granuloma.

And basically over there, there are macrophages which contain this Mycobacterium tuberculosis. There will be T cells, there is B cell. And they try to contain this infection in one

place rather than allowing to spread. So in granuloma this macrophages sometimes they fuse, several macrophages fuse together. And then they make multinucleated macrophages or multinucleated cells are formed and that contain Mycobacteria.

Initially the body try to contain the infection, but if the infection is more and bacteria is more strong than this, they will burst out and this will be released from this encapsulated structure known as granuloma. Here you can see that there is a necrotic granuloma. And then this can infect another cell or they can infect another tissues as well and that will remain as a pulmonary tuberculosis or that may result to the extrapulmonary tuberculosis.

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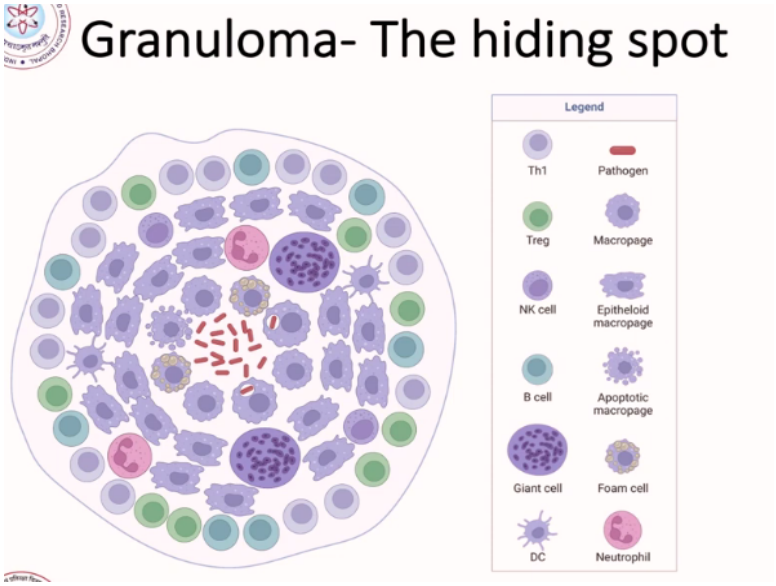

Here you can see this structure of granuloma which is a kind of hiding spot or if you look at from host side this is a kind of a containing the infection and for bacteria this is a kind of hiding spot as well. Here you can see that all these cells are there. There is Th 1 cell, so you may remember that Th 1 plays a very important role against intracellular pathogen, so there will be a lot of Th 1 cells. There will be T reg cells which regulate the immune response.

There will be pathogen, macrophages. There will be epitheloid macrophages. So basically, this Mycobacterium tuberculosis transform these macrophages and they turn to the epitheloid, sometimes they become multinucleated. There will be presence of natural killer cells. So here the strategy of the host is to encapsulate and supply all defense cell as well as defense molecules, make a more active environment in order to clear this infection, eliminate this infection.

So this is the strategy. There will be giant cell, here you can see that this giant cell has lot of mycobacteria. There is a foam cell, dendritic cells and neutrophils. So this is a kind of gross structure of a granuloma.

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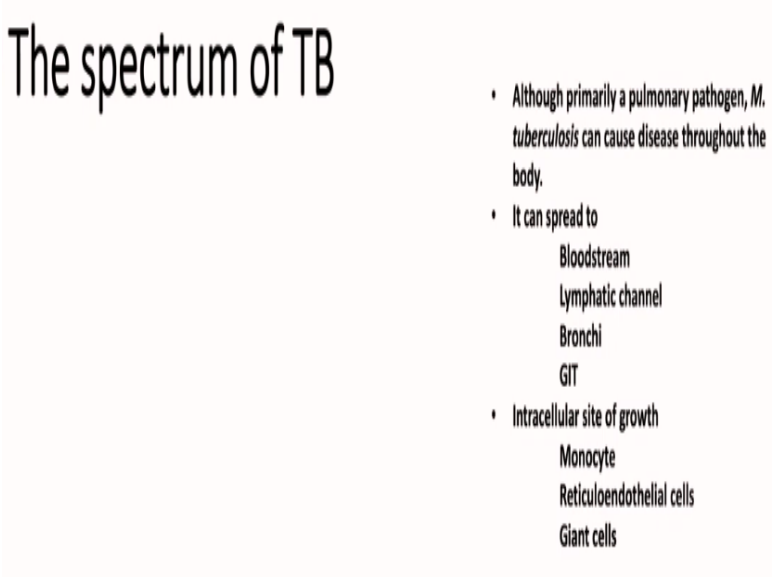

So there is a wide spectrum of tuberculosis patient. Here you can see that although primarily a pulmonary pathogen Mycobacterium tuberculosis can cause disease throughout the body, it can spread to the ~~respect~~ to blood stream, lymphatic channels, bronchi, gastrointestinal tract and intracellular sites of growth, monocytes, reticuloendothelial cells and they can make giant cells.

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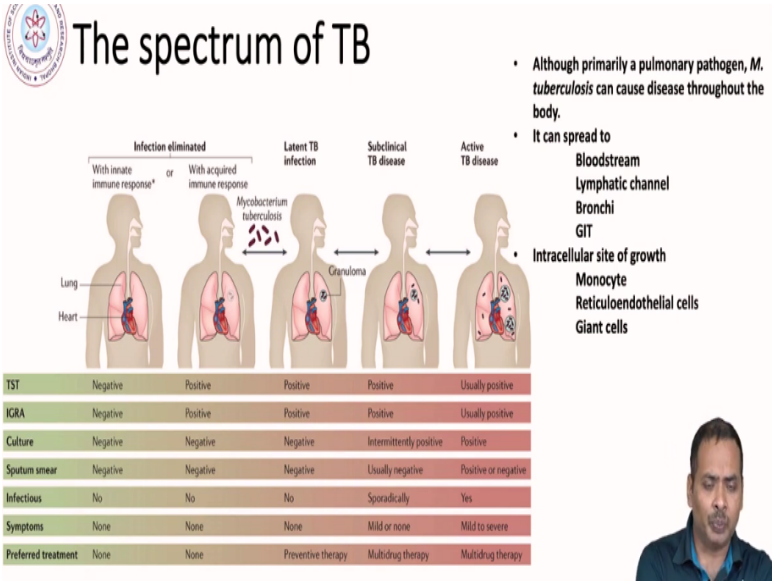

Here you can see that there is a wide spectrum of tuberculosis. After infection there are different categories of tuberculosis, I will not say all are patients, some are healthy here. Here

you can see that the individual is infected, but this infection is eliminated and the elimination could be through the innate immune response or with adaptive immune response. There are individuals who have this Mycobacterium tuberculosis, but the tuberculosis is not active, it is a latent stage.

So when there is some appropriate change in the host defense, then this Mycobacterium will proliferate and cause the full-blown disease. There is a subclinical TB patient and there are very active tuberculosis patients. So, these are the category. Here you can see, let us look at the sputum smear. So in case of infection which is eliminated in those cases this will not show the positive result, in sputum there will be a negative.

Even the latent tuberculosis will also not show the positive acid-fast bacilli, but in case of a subclinical TB disease there will be intermittently positive but in case of active tuberculosis there will be a full and clear picture of acid-fast bacilli ~~line~~ in the sputum smear. Here there are different properties given and these properties are basically the feature of infection eliminated patient, latent TB and subclinical TB disease or active TB disease patient.

With this, I will stop here and in next session I will discuss about the drugs, anti-tuberculosis drugs for this Mycobacterium tuberculosis. Thank you.