Host-Pathogen Interaction (Immunology)
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Lecture - 73
Virulence Factors During Bacterial Infection

Hi. So in previous session you have learned how the host is protecting themselves by bacterial infection and what are the virulence factor in the pathogen. So now you have learned about both sides, the host sidte as well as pathogen sidte. What are the virulence factor, where they are located and all those things. And for host side what are the defense mechanisms are existing. Now let us look at how these virulence factors are regulated.

Because these bacteria can exist in free living state as well and when they will get the appropriate host they can infect and multiply and cause the disease. So, if there is some changes in the bacteria that basically result to the infection and establishment of this bacteria in the host and development of disease.

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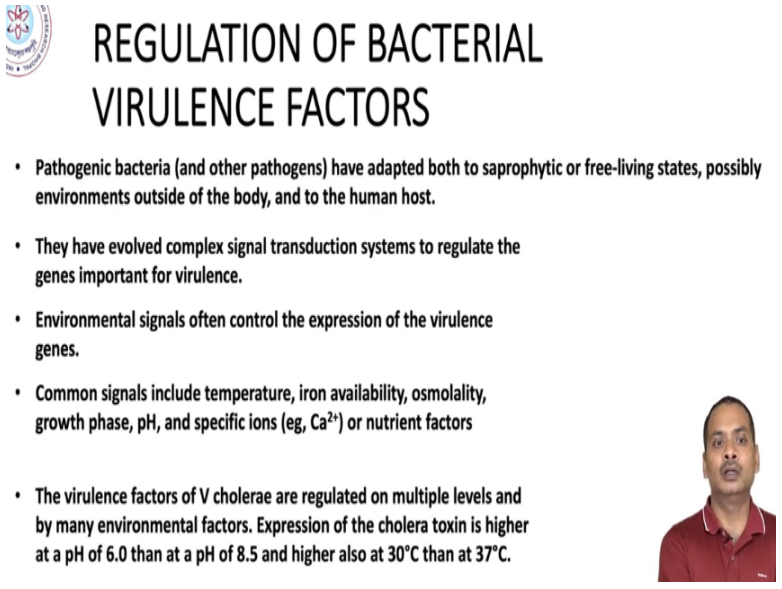

So, there are regulation of bacterial virulence factor that is very important to understand. As you know that pathogenic bacteria and other pathogen are adapted both to saprophytic or free living state possibly environment outside of the body and to the human host. So they have evolved a very complex signaling system to regulate the genes important for virulence. So

when they are present as a free living, they need not to waste their energy to make the virulence factor.

So when they come in contact with the host, it could be a human or animal, then these bacteria need to trigger the synthesis of the virulence factor. So, if you see very grossly you can understand that environmental signal often control the expression of a virulence gene. You have seen previously there are pathogenicity islands are there. You have seen the mobile elements are there which is associated with pathogenicity. So how they should express?

So here the most important thing is that these bacteria sense the environment. So, this environmental signal is most important to control the expression of this virulence factor. And what is this environmental thing? So common signal could be the temperature. As they come in contact with the host there will be stable one kind of temperature that may be a trigger point that trigger the expression of virulence factor.

It could be the availability of iron, osmolality as this is very strict in case of the host, growth phases, another is pH, need of specific ions such as calcium ion or nutrient factor. Try to understand in free living state it is not nutrient rich state, so when they will come in contact with the host it is a nutrient-rich state and probably at that time they trigger the synthesis of virulence factor. The virulence factor here I am giving one example.

Virulence factor of Vibrio cholerae are regulated on multiple levels and by many environmental factors. Expression of cholera toxin is higher at pH 6 than pH 8.5 and it is also higher at 30 degree centigrade then the 37 degree centigrade. So, these are the trigger point for expression of virulence factor here you have seen one example as well. So, for different pathogens there could be several things. It could be a more complex, it could be multiple factors, for example pH, nutrient, osmolarity, so and so.

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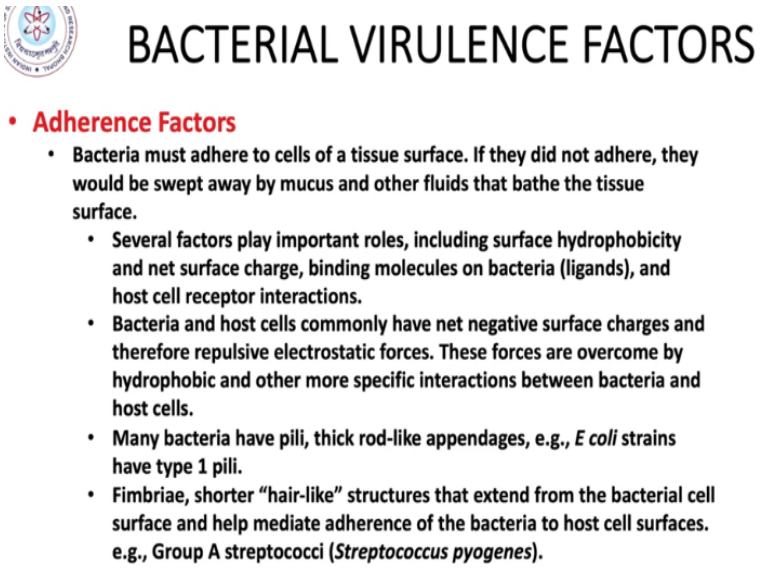

The next question is what are the bacterial virulence factor? So, it could be adherence factor. So in order to infect the host I told you there are several steps. So, one of the important steps is adherence. So bacteria must adhere to cells or of tissue surface, if they did not adhere they would be swept away by mucus. There is a continuous flow of mucus, this is an innate immune response and in some places there is cielia.

So these things does not allow to adhere on host surfaces. The bacteria cannot adhere because of these actions, there is a lot of production of mucus and other fluids also in which the tissue basically bath. Several factor plays important role including surface hydrophobicity and net surface charge. So surface hydrophobicity and there is some charge because you know that the surface of cell are having protein.

And these proteins are kind of decorated by various sugar molecules and these sugar molecules are charged also. So everything depends on the hydrophobicity as well as the surface charge. So binding molecule on a bacteria and host cell receptor interaction, so some bacteria use some, there is some expression of receptor and these bacteria can bind over there and then they may cause the fruitful infection.

Here I would like to give one very important information that bacteria and host cell commonly they have a net negative surface charge, both as net negative surface charges so in this scenario basically this will not allow the infection but on another hand bacteria has some strategy to overcome this repulsive forces and how they are doing here you can see that. They

make some more hydrophobic environment, hydrophobic-hydrophobic interaction will be there.

And then this can adhere to the host cell. So these are some strategies and another strategy could be there will be a key and lock kind of system, there will be a receptor over the cell and the these bacteria have some molecule which act as a strong ligand and in that way they can adhere. Here you can see that many bacteria have a pili, it is a thick rod-like appendage, it is a projection over the bacteria and there is one example E. coli.

Escherichia coli strain have a type 1 pilai and this pili is used in order to adhere to the surface of a host cell. There are some short hair-like structures are also there some bacteria which we call it as a fimbriae and this hair-like structure is extended from the bacterial cell surfaces and basically it mediate or facilitate the adherence of the bacteria to the host cell. And there is a one very good example there is a Streptococcus pyrogen.

This Streptococcus pyrogen express this appendage like structure fimbriae and there is a one specific strain which can cause the disease that is group A streptococci of this Streptococcus pyrogen that causes the disease. So this is the way by which pathogen adhere to the cell.

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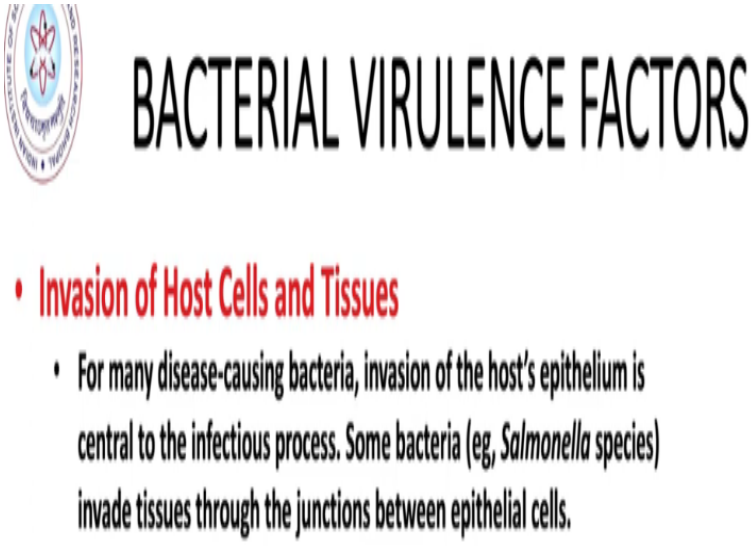

Another is invasion of host cell and tissues. For many diseases caused by the bacteria invasion of host epithelium is most important in order to establish the infection. Some bacteria such as Salmonella and some species of Salmonella like typhi evade the tissue through the junction

between epithelial cells. So these bacteria basically hook up between this junction, they find some molecule and over there they can hook up and then that cause the infection.

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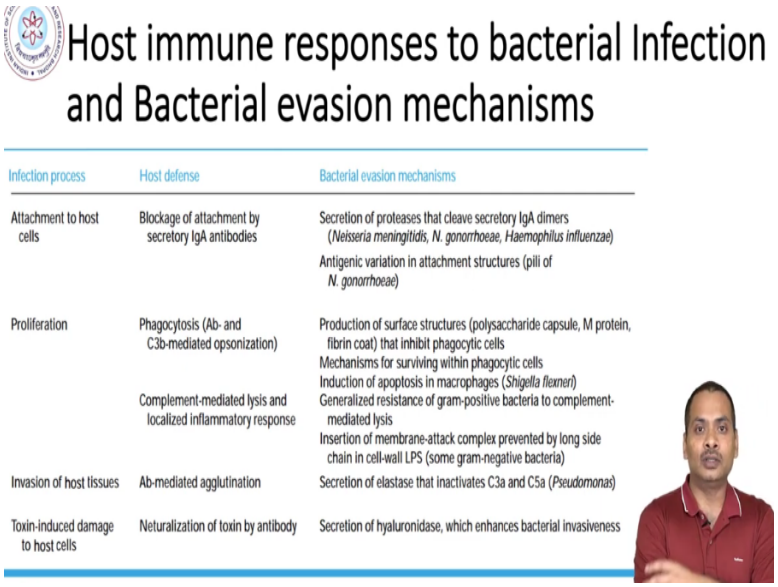

Here I am showing you a very important table, you please follow this table very carefully. So this is a host immune response to the bacterial infection and bacterial evasion mechanism, how, so here you will see the both aspect closely. So bacteria basically cause infection through attachment to the host cell, so this is the bacterial strategy in order to cause the infection that is attachment to the host cell.

So what are the defense mechanisms the host made against this strategy of bacteria? So one is that blockage of attachment by secretory IgA, you know that IgA is one of predominant antibodies which is present in our secretions. So these IgA basically avoid the attachment, but bacteria has an evasion mechanism against this IgA. So what bacteria is doing they basically secrete some protease enzyme which can break these IgA.

And there are very good examples like Neisseria meningitidis, Neisseria gonorrhea, Haemophilus influenzae. So these bacteria they throw out some enzyme in order to break this IgA and then they can establish the infection, this is one way. Another is there is antigenic variation in attachment structure that is pili for example of the Neisseria gonorrhea. They make the changes in this protein, the proteins which is involved in making this pili.

So once they make the changes they can evade the antibody mediated response and in that way they can very easily hook up to the host cell. Another way to establish the infection is

proliferation. So once they will attach then they need to proliferate very quickly in bigger number. So, what is the host defense against this proliferation thing? The most important host defense is phagocytosis.

They will eat up those proliferating bacteria and this phagocytosis is further facilitated by the antibody mediated opsonization, complement C 3b coated or mediated opsonization. So, these are the strategy by which host overcome this proliferation. So how the pathogen is evading this phagocytosis? So they make some surface structure or something which is made of a polysaccharide capsule, they make some M protein, they also make a fibrin coat and that inhibits the phagocytosis.

They escapeskip from the phagocytosis. There is a mechanism for surviving within the phagocytic cells. The one of best examples is Mycobacterium tuberculosis. These pathogens are phagocytose, but they will survive, they release some molecule which will inactivate all those arsenals which is produced in the endosome or endolysosome or phagolysosome, they inactivate like ROS, RNS, hydrolytic enzymes so and so.

Some bacteria have induction of apoptosis of macrophages like Shigella flexneri, they trigger the apoptosis of these cells. Another is this complement-mediated lysis and localized inflammatory response, this is a protective response against the proliferating bacteria. So there is a generalized resistance of a gram-positive bacteria to complement mediated lysis. There is insertion of membrane-attack complex prevented by long side chain in the cell wall LPS, this is particularly in case of gram-negative bacteria.

Another way of infection is invasion of host tissue so this is a pathogen sidte, they invade in the host tissue. And what is the protective response? There is antibody-mediated agglutination. There is a secretion of elastase that inactivate C3–a and C5–a, basically they are the anaphylatoxin, they attract the immune cells and this is mainly adopted by Pseudomonas. There is toxin-induced damage to host cell.

So some bacteria they produce some toxin and then they damage the host cell in order to establish the infection. So what is the protective response? Neutralization of these toxins by antibodies as I told you in previous session. This is one of key ways by which the host protect

against extracellular bacteria as well as the toxin produced by these bacteria. So how pathogen overcome this thing? Basically, they secrete some enzyme.

Here you can see that there is hyaluronidase enzyme which enhances the bacterial invasiveness. So, these are the ways by which here you can see the battle between the host as well as pathogen. So, pathogen also keeps on evolving and host also keeps on evolving in order to overcome. It is something like that there are hackers and there are people who basically they make some virus and there is antivirus in computer system.

So both are evolving together that is how they are enhancing and enhancing. So bacteria also produce some toxins and these toxins are basically very much helpful in order to establish infection and these toxins are mainly two types one is exotoxin and another is endotoxins.

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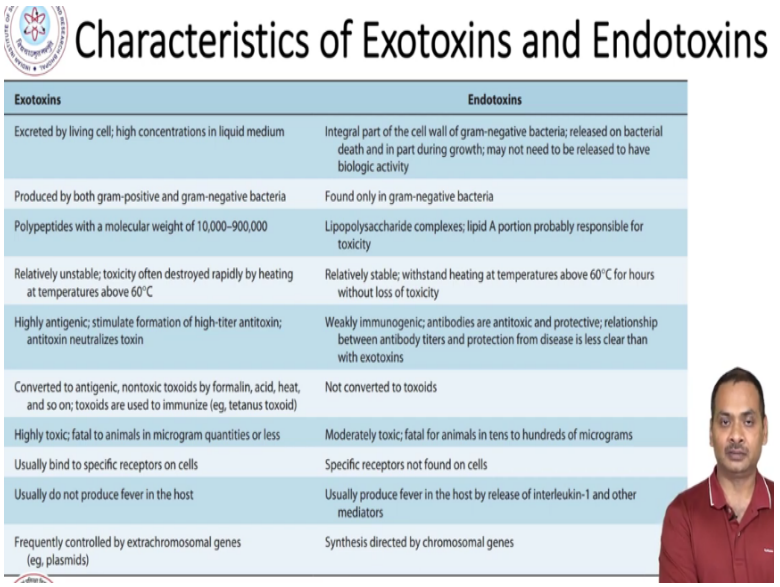

So now let us look at this, what are these toxins and what are the properties of these toxins. exotoxin as well as endotoxin? So exotoxins are basically secreted by or excreted by living cell in high concentration in liquid media. If you culture these bacteria, then they will produce in very high amount. On another hand the endotoxin which is mainly the LPS and this LPS is basically a part of the cell, integral part of cell wall.

There is an outer membrane and this outer membrane is consist of endotoxin that is LPS and it is mainly in the gram-negative bacteria released on bacterial death and in part during the growth, may not need to release to have a biologic effect. They need not to just throw this

endotoxin even if it is with bacteria, they are sufficient to cause the biological effect like basically LPS is a shock kind of condition.

This exotoxin are produced by mainly by both gram-positive and gram-negative bacteria, but endotoxin is found only in gram-negative bacteria. Exotoxins are proteinaceous in nature, they are polypeptide and their molecular weight range from here you can see that there is a 10 kilodalton to 900 kilodaltons. LPS is a very small molecule consists of lipid A and probably responsible for the toxicity.

So LPS has one component which we call it as a lipid A and that is basically associated with the toxicity. So exotoxins are relatively unstable. Toxicity is often destroyed rapidly by heating those components above 60 degree. So if you autoclave these exotoxins then this will basically denature, but on another hand this endotoxin is relatively stable with a stand heating at temperature above than 60 degree for hours without losing any toxicity.

So even if you heat the gram-negative bacteria and inject it into the room that will have almost same effect. Exotoxin can basically highly antigenic and stimulate formation of a high-tighter antitoxin, antitoxin to neutralize the toxin. So basically, these exotoxins it is a proteinaceous in nature, you can inject some appropriate host in sublethal concentration and then you can make the kind of antisera which can be useful in treatment of that disease.

So here I would like to say or describe about one term known as toxin and toxoid. So what is toxin? Toxin is as you have seen it is produced by the bacteria and then you inject it in the host then that will cause some bad effect or biological effect I will say. Toxoids are basically the toxin which is inactivated in such a way or it is treated in such a way that it will lose its toxicity but retain its antigenicity like tetanus toxoid.

So what do you understand or meaning is that the toxoid is more convenient to use in human because it loses its toxicity but retain its antigenicity means if you inject then that will elucidate appropriate immune response in order to protect against that infection. In case of endotoxin this is weakly immunogenic. Antibodies are anti-toxic and protective. Relationship between antibody titer and protection from disease is not very well understood.

Here the next point which I have explained this toxin can be converted to the antigenic nontoxic toxoid by treatment of formalin or by some acid or by heating so and so. Toxoid can be used to immunize the animal or human, the best example is tetanus toxoid, I have explained all those things. In case of an endotoxin, you cannot make the toxoid. Exotoxins are highly toxic, fatal to animal in microgram quantity or less whereas endotoxin is moderately toxic fatal for animal in ten to hundreds of micrograms.

Exotoxins are usually bind to a specific receptor, there is some specific receptor over there they will bind and then they will cause some biological effect, but endotoxin they do have a specific receptor but there are a variety of endotoxins, yeah you can say that mainly TLR 4 is involved but it is more complex. Usually, this exotoxin do not produce fever in the host, but endotoxin usually produces fever in the host by release of IL-1 family cytokine and other mediators, other inflammatory mediators.

So if you inject the animal with a lot of endotoxin or lethal dose of endotoxin then that will cause the shock condition or even if the human receive this endotoxin, we call it as a shock condition that is basically characterized by hypoglycemia, hypotension and there is weak pulses, cardiac output is also changed significantly. So, this is the effect of endotoxins. Frequently controlled by extrachromosomal gene the expression of this exotoxin such as plasmid, synthesized directly by chromosomal gene in case of endotoxin.

So this is all about the toxin produced by the bacteria that is exotoxin and endotoxin. And these endotoxins are very important to test in any important sample, why? The reason is that probably you know that previously we used the bacterial system in order to make some protein, a recombinant protein, in that scenario if you are making some protein which is used for human treatment then those protein may be contaminated with endotoxin.

So, it is very important to test the amount of endotoxin level in that sample and there is some test we call it as a LAL test and this LAL test is basically used to find out extremely low concentration of LPS in the sample. Even when we perform the experiment if you make some protein in some bacterial system and you want to test this protein in immune cells, then before that you have to test the amount of endotoxin level in that sample.

Otherwise, it is not correct because even extremely small amount of LPS contamination result to very drastic change. This LPS is a very strong stimulator of immune cell that is why you need to test in order to find out the real function of that identified protein. With this I am completing this session and in next session I will discuss about the Mycobacterium tuberculosis and the disease caused by Mycobacterium tuberculosis that is tuberculosis and my focus will be pulmonary tuberculosis. Thank you, we will see you in next session.