Essentials in Immunology

Prof Dipankar Nandi

Department of Biochemistry

Indian Institute of Science Bangalore

Lecture No # 05

Innate Immunity – Part 1

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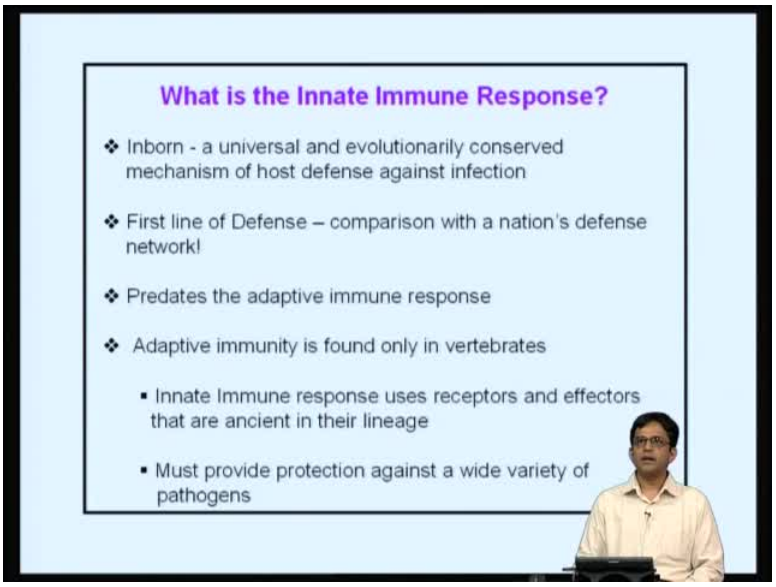

Today's lecture is on innate immunity and you are all familiar with the immune response, which can be broadly categorized into the innate immune response and the adaptive immune response. The innate immune response is the first line of defense and innate stands for inborn. It is a universal and evolutionary conserve mechanism of host defense against infection. The adaptive, on the other hand, takes some time, it is a little bit delayed and, but it is a far most specific response than the innate response.

What I would like to do is to compare the immune response to a nation's defense, defense network and nation's defense network consists of the army, the air force, the navy, etcetera, and these all come together to form the nation's defense network. Why is it, that we have all these different agencies? That is because the enemy can come in either through the sea, through the air or through the land. Similarly, for the immune

system, the, it has got several different groups of cells and molecules and network that take care of this host defense.

So, the first line of defense is the innate immune response for, in case of a nation's defense network, often our borders are patrolled by the border security force and they are, they are responsible for its security. Subsequently, the army is called, in case it is required. So, the border security force can be compared to the innate immune response and the army can be compared to the, to the part of the adaptive immune response. The innate immune response, it predates the adaptive immune response, which means, that is, it is evolutionary conserved and it is present in lower organisms, something that the adaptive immune response is not. The adaptive immune response is present only in, in, in, vertebrates; the innate immune response uses receptors and effectors, that are ancient in their lineage and they must provide protection against a wide variety of, of pathogens.

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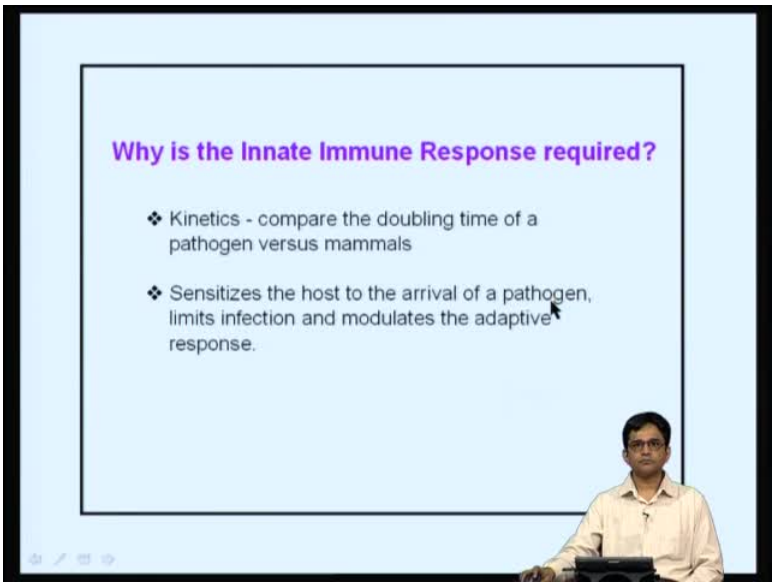

Next, we should try and understand why is the innate immune response required? And to, in order to do this, we can compare it with respect to the doubling time of pathogens versus mammals. In case of a pathogen, the doubling time is very fast. So, for example, if you, if you think in terms of microbes, such as equal or salmonella, the doubling time is about 20 minutes, whereas that for vertebrates and mammals doubling time is lot longer. Therefore, the pathogen is an, is at an advantage with respect to doubling time because they can double much faster and they can overwhelm the host. In order to take

care of that, what the host has done is to take care, of the, of, of, of having different sorts of networks, in case, in case of the immune system, that can deal with this fast doubling point. So, they have, they have to, so, that is why, you have different kinds of cells and different mechanisms to deal with it.

In essence, the innate immune response sensitizes the host to the arrival of the pathogen; it limits infection and modulates the adaptive immune response. And what we will try and do in this class is to understand the molecules and the processes, by which the innate immune response sensitizes the host to pathogens. How does it limit infections? So, it is present only, it does not disseminate to all different parts of the body and more importantly, how does it send a signal to the adaptive immune response, so that you can generate a much better and a vigorous specific response.

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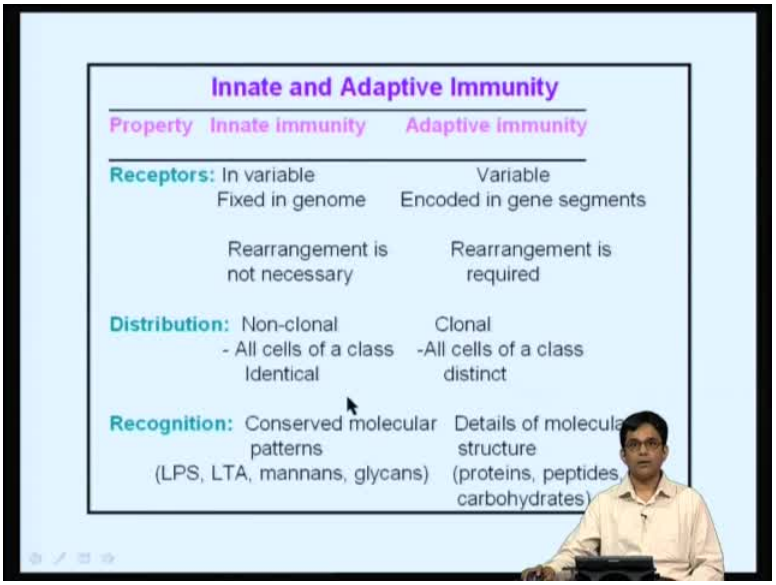

What we will do here is to compare the differences between the innate and the adaptive immune response. In case of receptors, the, in case of the adaptive immune response, you have specific, you have a specific response and specificity is conferred by B cell receptors and T cell receptors. These are variable; these are variable because they need to be specific for a particular pathogen. So, you have different combinations of genes coming together in case of B cell receptors or in case of T cell receptors, that come together to form a specific receptor, that will be for the pathogen. In case of innate immune response, the receptors are invariable, they are fixed in genome and

rearrangement is not, not required. The adaptive response is clonal; by clonal, we mean, that a particular receptor gets amplified. That means, it is clonal because it divides and that is the basis of the adaptive immune response. Such type of clonal receptors are not required in case of the adaptive, in case of, in case of the innate immune response and all the cells, all cells of a class are identical.

What the innate immune response does? It, it, it recognizes conserved molecular patterns, for example molecules, that are present on microbial surfaces, such as lipopolysaccharide, lipoteichoic acids, mannans and so on. In case of the adaptive immune response, the B cell receptor and the T cell receptor is able to find, find minute differences between molecular structures and including small changes, that are present between different kinds of bacteria or different kinds of pathogens and these can be, especially in case of, in case of proteins and peptides.

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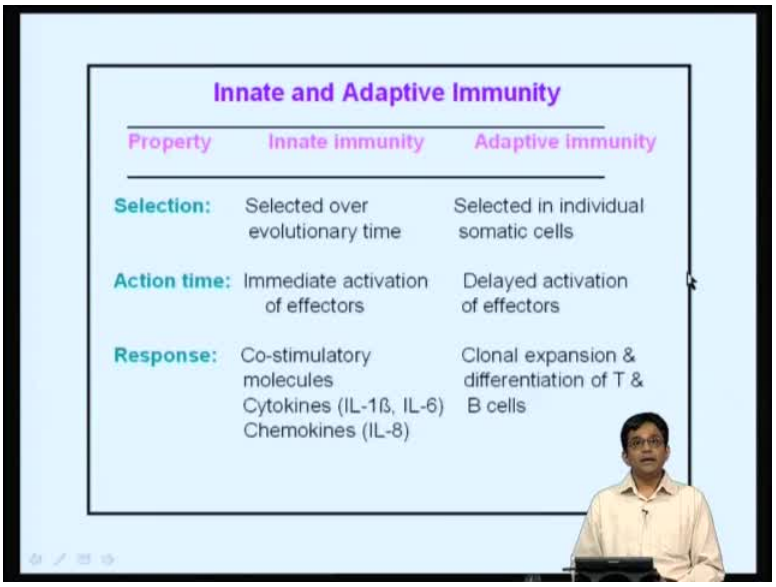

The innate immune response has been selected over evolutionary time. So, we have the innate immune response, which has been selected over time and whereas, the adaptive immune response selection is done on basis of individual somatic cell. That means, these are cells, that, that, that express this particular receptor and that is what is selected for an amplified and those are the, and the one specific for the antigens are the ones, that get amplified and proliferate.

As mentioned previously, the innate immune response is very quick, so once, once a pathogen comes in, the innate immune response quickly tries to meet this pathogen and tries to limit the or restrict the entry, and the, and the distribution of this pathogen over the body. So, therefore, the response has to be very quick and very fast. The adaptive immune response, on the other hand, is somewhat more delayed because you need selection of particular receptors, that recognize this particular pathogen and can, then, it is only after selection, that these cells will be able to target and generate a specific immune response to the, to this pathogen.

In case of a adaptive immune response, it results in clonal expansion and differentiation of T cells and B cells, whereas in case of innate immune response, what happens is, you have cytokines being produced, co-stimulatory molecules being produced, chemokines being produced. These are important in limiting the pathogen, in telling the body, that the, that the body is under attack and as mentioned previously, to modulate the adaptive immune response. And how this occurs is something that we will try and understand during this lecture.

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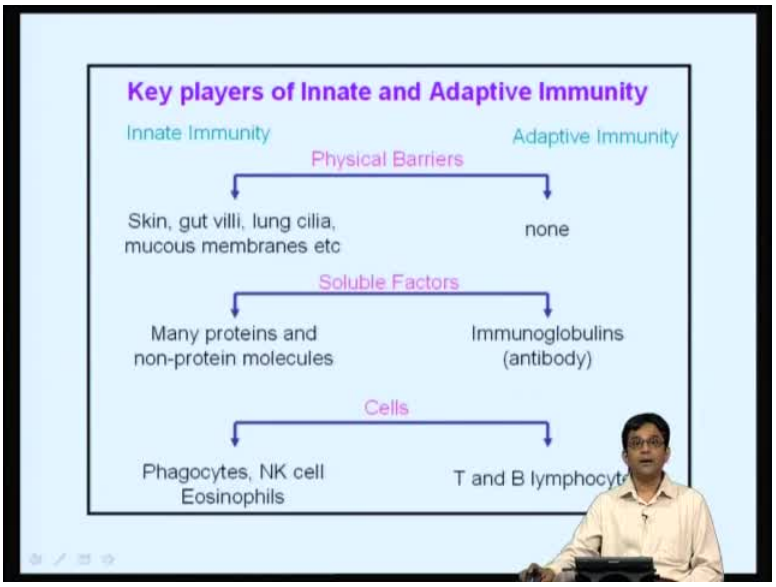

The key players in innate immune in, in, in the innate and adaptive response are briefly shown over here. In case of the innate immune response, the physical barriers are the key. So, for example, we have the skin, the gut, the lung and so on, so pathogens cannot indiscriminately enter. There are physical barriers, which are part of the innate immune

response and there are cells and mechanisms by which these, that, that prevent the entry or indiscriminate entry of, of pathogens. In case of adaptive immune, response is done mainly by the T cells and B cells and there are no physical barriers that play an important part over here. In case of soluble factors, in case of innate immune response, many proteins and non-protein molecules are involved in this response. In case of the adaptive immune response, the secreted immunoglobulins are really, the key effectors of soluble factors, in case of adaptive immunity.

In case of cells, you have phagocytes - the natural killer cells, which are shown as NK cells; eosinophils, which play an important role in innate immunity. And as mentioned previously, again in case of adaptive immune response, you have the T and the B lymphocytes.

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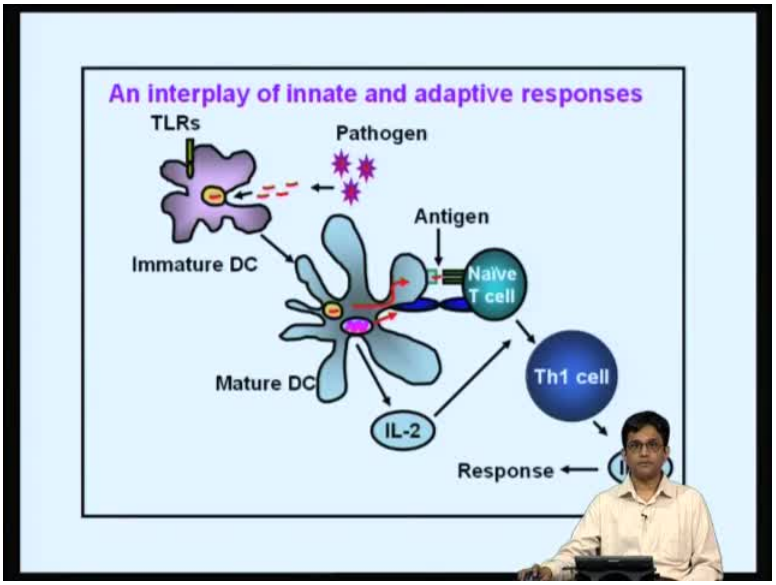

What is shown in this particular slide, is to show, is to depict interplay of innate and adaptive responses. So, what is shown over here is that there is an immature dendritic cell and this is a pathogen that is invading. These pathogens are recognized by or by these receptors that are known as TLRs of toll like receptors. Once these pathogens come in contact with immature dendritic cells, these cells then differentiate into, what is known as, mature dendritic cells. These immature dendritic cells are in different parts of the body and upon contact with pathogen, they migrate into the lymph node cells and this is where, where maturation takes, an activation of the adaptive immune response takes

place. And what is shown over here is that the mature dendritic cell then, presents the antigen to what is shown over here as a naïve T cell and this naïve T cell then, gets activated. It differentiates into a T helper cell in here again, it produces into luken2, it differentiates and it produces interferon gamma, which is a key cytokine, and so as to start off, what would be a typical Th1 response or a pro-inflammatory T cell response.

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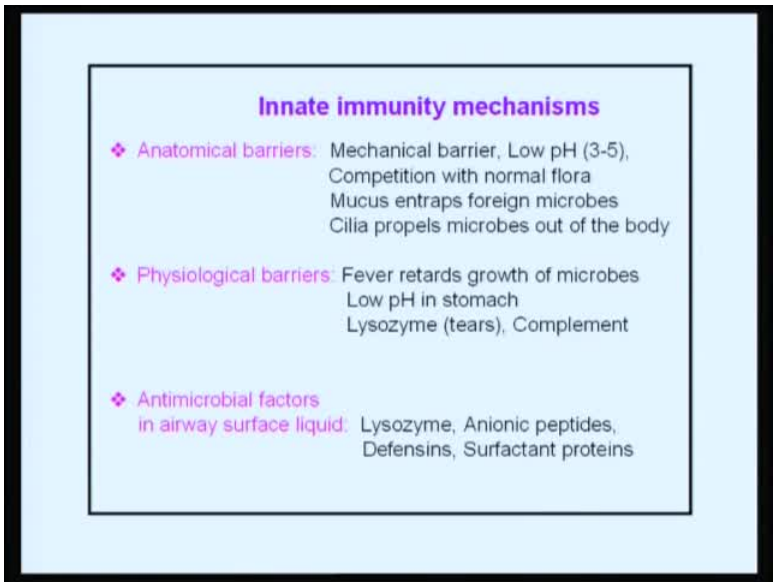

There are other mechanisms that are part of the innate immunity and which are listed over here. So, anatomical barriers, as mentioned, you have mechanical barriers, you have low pH and there is a lot of normal flora. So, there is competition; so, the pathogen has to compete with a normal flora in the body. There are ways by which you can limit the spread of pathogens and mucus entraps foreign microbes and that, that is shown over here. So, again, so again, these are all ways by which it can be limited. You have cilia that are present that propels microbes out of the body; you have other physiological barriers too. So, for example, fever retards growth of microbes; there is low pH in the stomach, which is not suitable for the growth of, of, of microorganisms; in the tears, in our tears, lysozyme is present and lysozyme will make holes in bacteria and kill them; you have complement, which is present in blood, which recognizes antigen-antibody complexes, which and also other microbial proteins, as a result of which, you have, you have killing of different pathogens.

More importantly, several antimicrobial factors are also present in, in, in the intestinal and in the airway fluids. So, for example, you have lysozymes, you have defenses, which are small peptides surfactant proteins, etcetera, that are produced, which help to, sort of, limit the number of bacteria or pathogens or microbes, that can enter into the body and cause damage.

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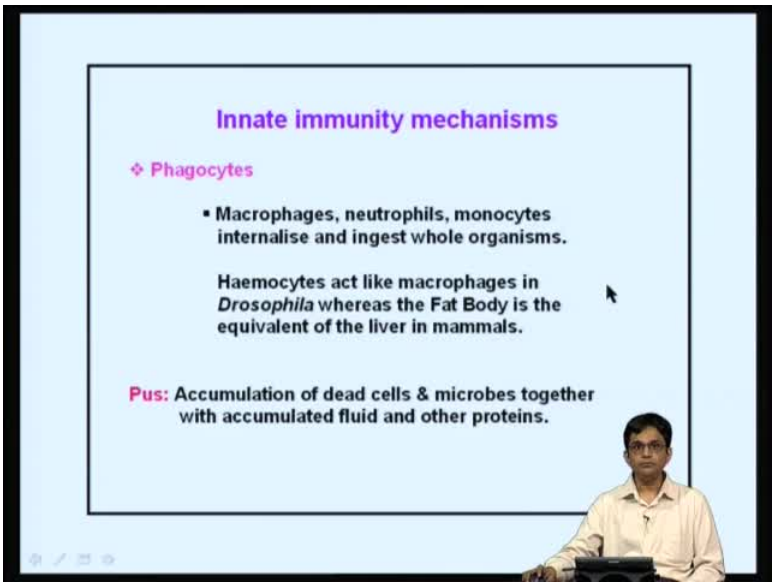

There are cellular mechanisms, that are in place over here and some of the cellular cells, that are involved, are shown. So, the main cells are, are, are macrophages, neutrophils, monocytes and they, these are internalised microbes and they ingest whole and ingest these organisms. So, as I mentioned, the innate immune response is present in lower organisms also and what is shown over here is an example of that. So, haemocytes act like macrophages in drosophila, whereas the fat body acts as the equivalent of the liver in mammals. So, drosophila would have haemocytes, which act like macrophages and fat body, which are the equivalents of the nature of the important parts of the innate immune response in, in mammals.

So, pus, which is also present, which often occurs, is actually an accumulation of dead cells and microbes together. So, again, you can see a way, by which the response is being limited.

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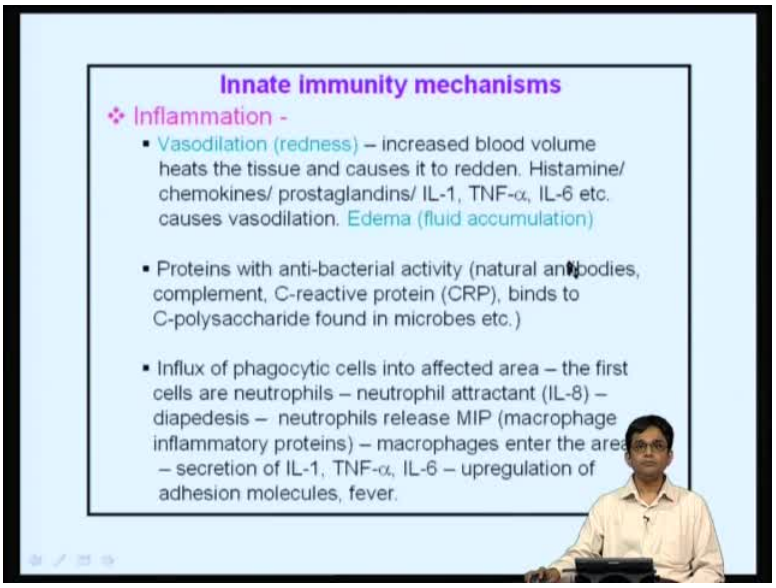

One of the important hallmarks of the innate immune response is inflammation and by inflammation we mean, that, you know, there is an inflamed surface, which often happens during a cut or invasion, and this result, and this is due to vasodilation and edema or fluid accumulation. So, what happens over here? There is increase in blood volume and the tissue heats up and it causes to redden. What happens is, certain molecules, such as histamines, prostaglandins, etcetera, are in cytokines, are produced, which cause this vasodilation. So, this leads to accumulation of fluid and these are all signs and signals that are sent by the body, saying that it is under, under attack and you have different mechanisms then coming in place. So, you have certain proteins that are present in the serum, which contain antibacterial activity.

So, for example, you have natural antibodies. These are antibodies that are, these are a part of the innate immune, immune system because they, they recognize some, conserve the carbohydrate determinants, that are present on several microbes. You have again a C reactive protein, which is part of the complement pathway, which binds to the C polysaccharide, again found in several microbes. What happens often is you have an influx of phagocytes that come into this affected area, which is now red and which is filled with fluid. The first cells to arrive are the neutrophils and these come in because they respond to a particular neutrophil attractant, which is lukin 8 and they enter tissues by a process of diapedesis. And diapedesis is the way by which cells that are in blood or in these things, they can enter into tissues and subsequently, macrophages also enter, and

they result in cytokines, and they up-regulate adhesion molecules. There is also fever, which increases the body temperature and which is not suitable for the growth of microbes.

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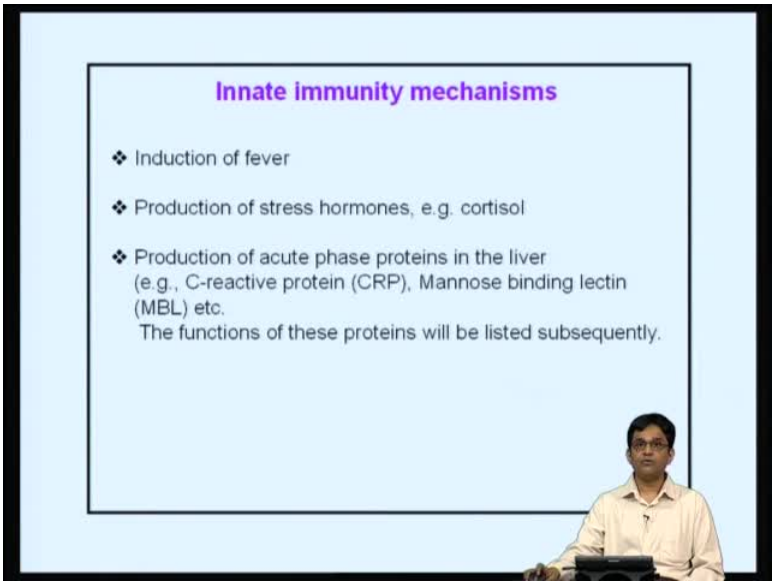

So, some of the mechanisms, that are shown are, have been listed, I am going to stress again, on a few. First is the induction of fever. Cytokines are produced, especially something like, IL-1, which is, whichever results in the induction of fever, you have production of stress hormones, for example cortisol. Cortisol is actually, thought to suppress immune responses and these could be part of a network to regulate the innate immune response, your production of acute phase proteins in the liver. So, the liver plays an important role and some of these acute phase proteins, that are well known, are CED active protein, mannose binding lectin. These are all, these all have antimicrobial functions and the detailed mechanisms of these will be discussed subsequently.

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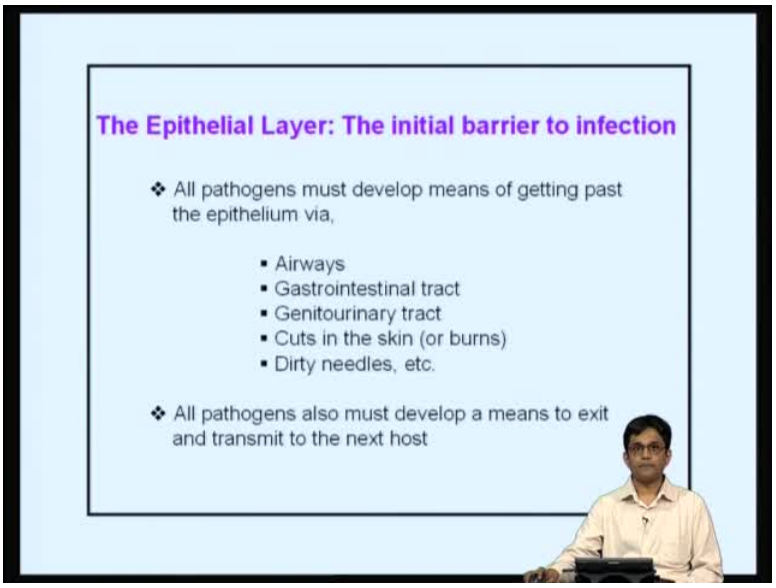

One of the initial barriers to infection is the epithelial layer and the pathogens must develop a way, by which they can get pass the epithelial, and the epithelium is present in several surfaces. So, for example, the airways, the gastrointestinal tract, the genitourinary tract cuts in the skin or burns and so on. So, pathogens must develop some means by which they can enter and go on to the next host too. So, they have, to, to, to, to conquer 1 host and then be able to transmit so that they can, they have other host, so that they can spread their numbers.

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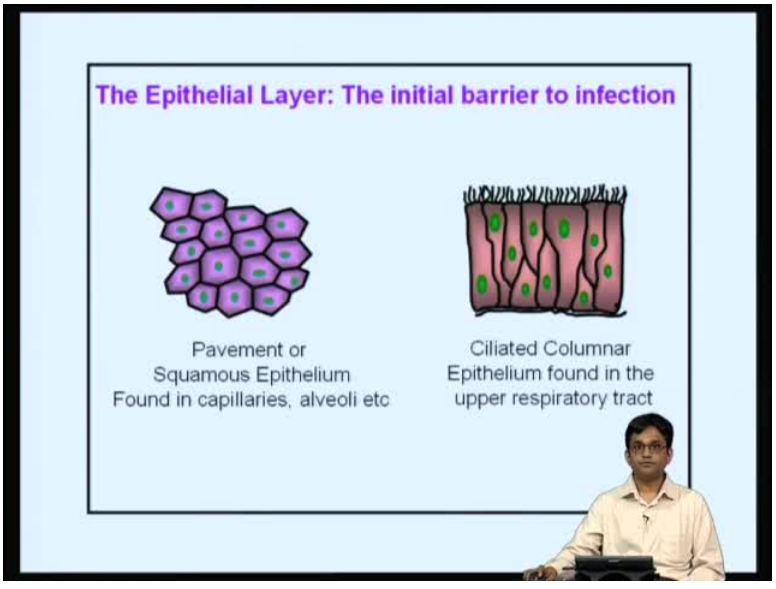

So, this is an example of a squamous epithelium, which is present in capillaries, alveoli and so on, and then you have the ciliated columnar epithelium, which is present in upper respiratory tract. You will note the cilia present there and the cilia helps to get the pathogens out, and so they help in movement so that they can, sought of, move and may be, come in contact with some macrophages or some others that are present and hopefully be ingested, rather than entering the body.

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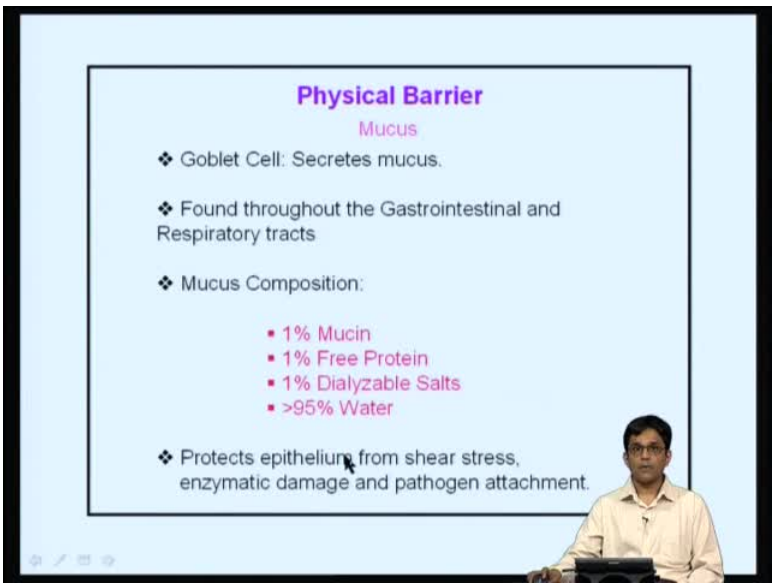

We will discuss a little bit about some of the features of the, immune, innate immune response - one is the physical barrier as mentioned, mucus. Mucus is produced by the goblet cells and these again are, are there, which help in, sort of, limiting the spread of microbes and these are present in throughout the gastrointestinal and respiratory tracts. The composition of mucus is shown, but the basic idea of mucus is that, it protects the epithelium from shear stress enzymatic damage and pathogen attachment.

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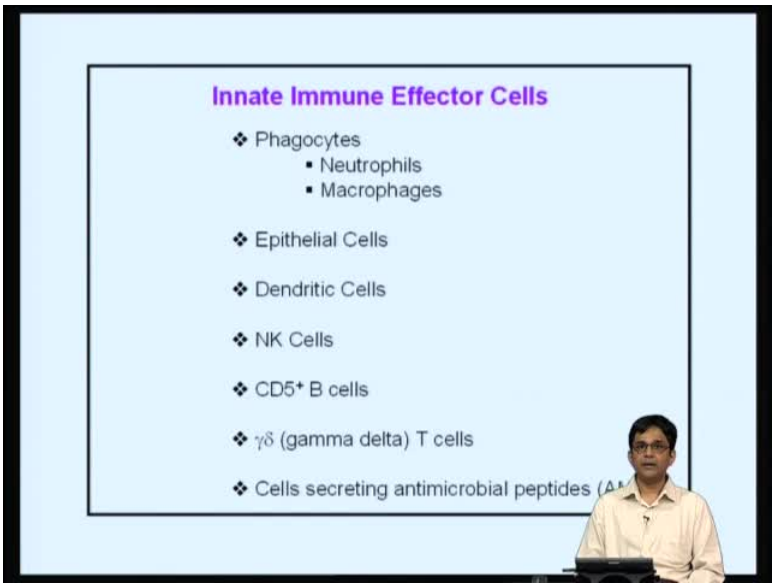

There are several types of cells, that are, play an important role in the innate immune response - your phagocytes, your epithelial cells, dendritic cells, natural killer cells, the CD5 positive B cells, gamma delta T cells, cells secreting antimicrobial peptides, and I will be discussing some of this in greater detail, in the lecture.

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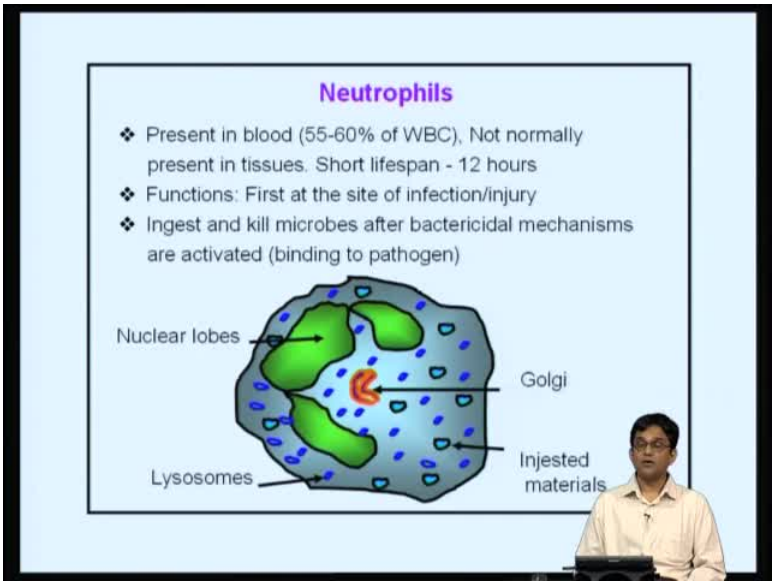

So, the first important cell, that pathogens usually come in contact are, are neutrophils and these are present in large numbers. For those of you who have done blood counts, you would know, that neutrophil count is very important because usually, if the

neutrophil count is increased, it often suggests that there is an active infection and that is what doctors often ask, when they ask for blood counts. They are looking for, to see if there is increase in neutrophil numbers and if neutrophil numbers increase, it often tells, that there is an active infection, that is, that is going on.

So, neutrophils are like a nation's BSF. They are the first ones to guard and first ones to respond to an injury and their job is to, they ingest and they kill microbes and there are several bactericidal mechanisms present in, in, in these neutrophils. Neutrophils are characterized by multi-lobed nuclei, which is shown over here in (( )), so these actively ingest and they kill microbes.

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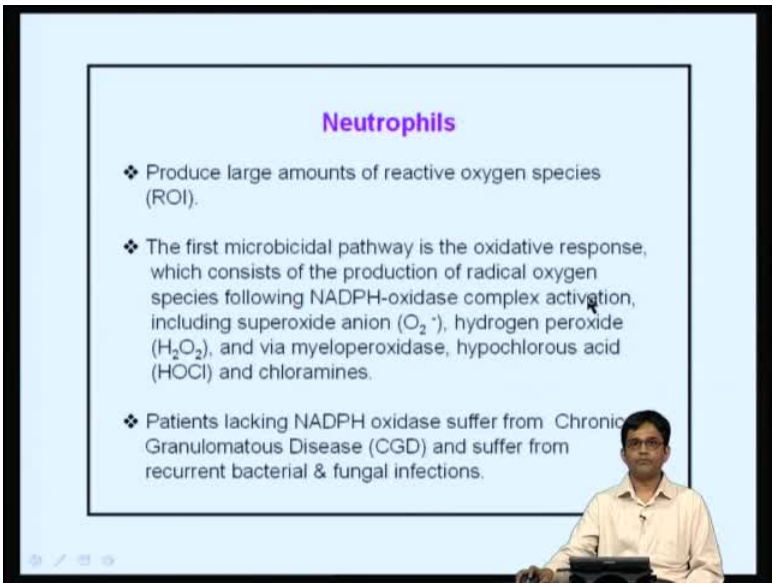

One of the main mechanisms by which neutrophils kill is, where kill microbes is, we are the production, we are the production of reactive oxygen species or also known as ROI. And in fact, the main enzyme, that is responsible for this is the NADPH oxidase complex. So, the NADPH oxidase complex gets activated and it produces superoxides and this superoxide can react with other molecules to form hydrogen peroxide and via myeloperoxidase, it forms hypochlorous acid, so on, and all these are antimicrobial in nature.

One of the most important roles of neutrophils has been shown in patients who suffer from chronic granulomatous disease or CGD and these patients suffer from recurrent bacterial and fungal infections. That is because the NADPH oxidase is deficient or

absent in their neutrophils and macrophages, which allows the pathogens to have an advantage and so they are able to cause these infections in these patients. So, CGD is an important, important disease and one should be somewhat aware of it. CGD also shows the importance of the role of neutrophils and especially the enzyme NADPH oxidase.

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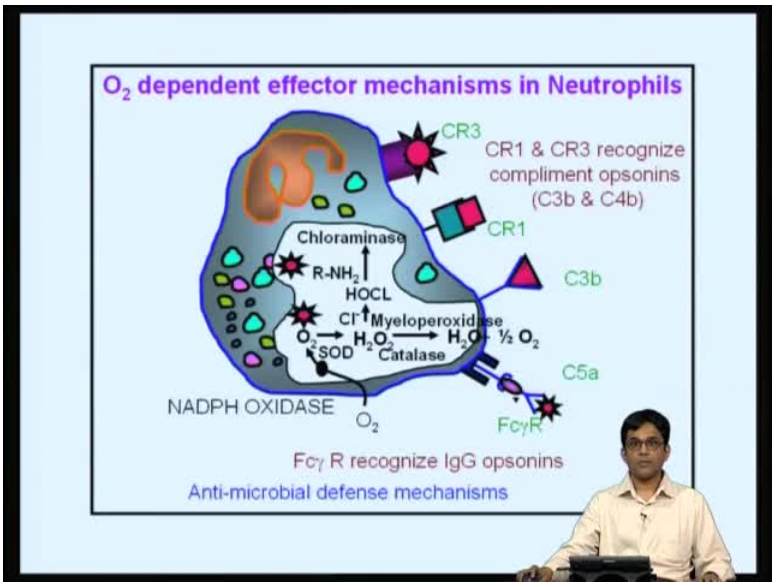

There are, apart from the oxygen killing mechanisms, neutrophils are also involved in other pathways of limiting the spread of pathogens. One is, they express these complement receptors so they can recognize the complement, that is, our complement proteins that are bound or that bind to these pathogen surfaces, so the, so these complexes can be ingested, rather quickly. You also have FC receptor of gamma, so these, so antigen-antibody complexes can also be taken in or phagocytized by, into these neutrophils, where these complexes are, sort of, degraded and as consequently, the immune response kicks in, in a very strong manner and these are part of the antimicrobial defense mechanisms.

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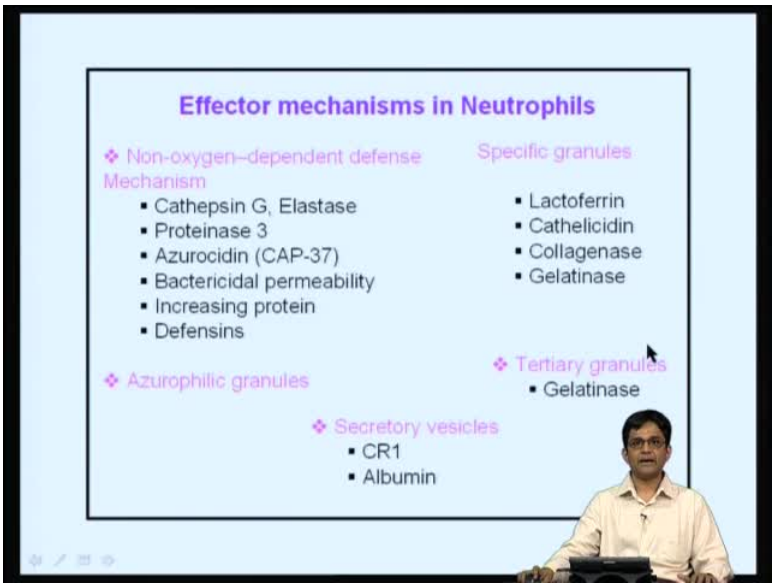

Some of the effective mechanisms in neutrophils are listed over here. You can see, there are several, you have, there are specific granules in them for, with, that contain for example, that is shown over here as a Cathelicidin, which is an antimicrobial peptide. You have other enzymes now, which are elastases, Cathepsin G, so on. These are all part of the antimicrobial defense network.

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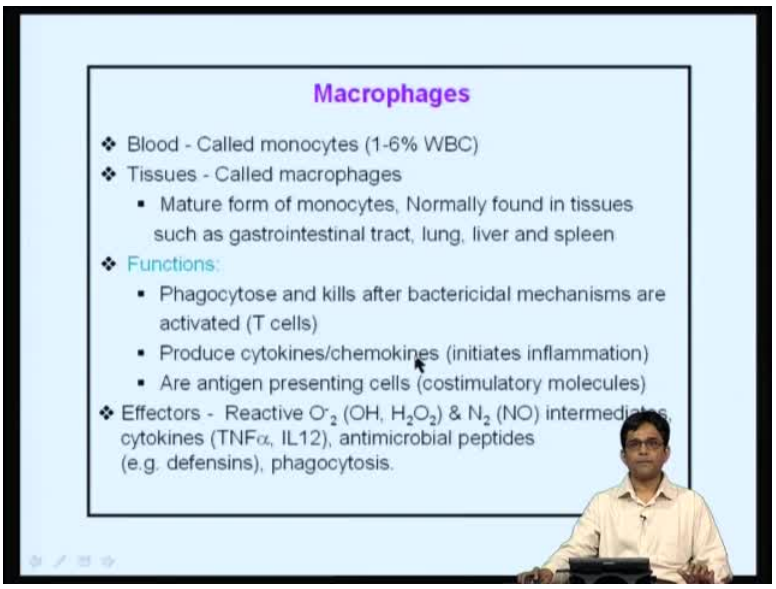

The other important cells are the macrophages. So, usually, when pathogens attack, the first response is by neutrophils and then subsequently, followed by macrophages. The

macrophages, the number of macrophages are lot less in the blood and they are usually known as monocytes in the blood, and in tissues known as macrophages and their main job is to phagocytoze and kill pathogens. The second important role, that they have, is that they process antigens and they present it to T cells and this is where I mentioned, that modulating the adaptive immune response is an important aspect of the innate immunity.

Macrophages also produce cytokines and chemokines, so which are important in initiating inflammation and they act as antigen presenting cells, and this is very important because not only do they express MHC 1 and MHC 2, the roles of which we will be discussing subsequently, which are, and these MHC 1 MHC 2, they present peptides to T cell receptors. They also, macrophages also, have the presence of co-stimulatory ligands. So, upon pathogen entry, macrophages up-regulate the expression of co-stimulatory ligands and these are important, these play an important role in T cell activation and we will study the roles of these, the, in the appropriate class.

Macrophages contain several effectors, so they are, they produce high amounts of reactive oxygen species, ROI, they also produce high amounts of reactive nitrogen species and microbial peptides, antimicrobial peptides, they phagocytose.

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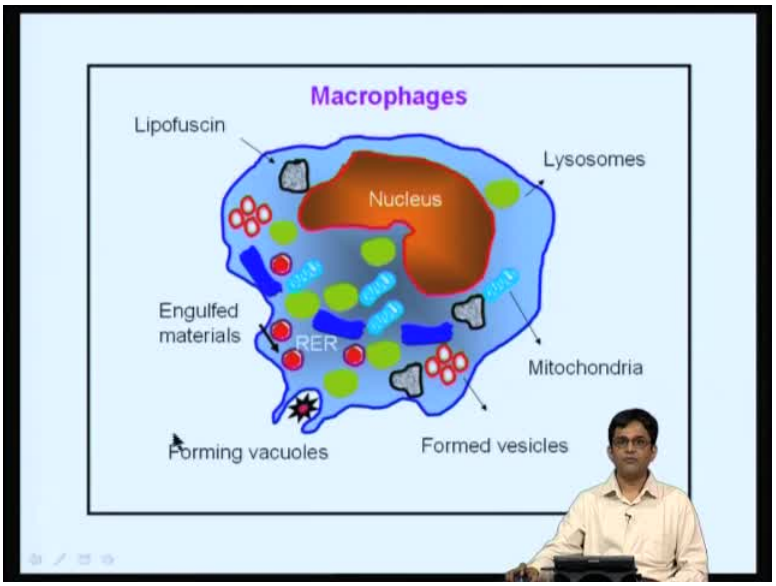

So, overall, macrophages play an important role in innate immunity and this is what is shown of a typical macrophage. You can see, they engulfed particles, they form vacuoles

and these vacuoles, this is how pathogens get digested and parts of the pathogen are shown up on the cell surface, along with MHC molecules.

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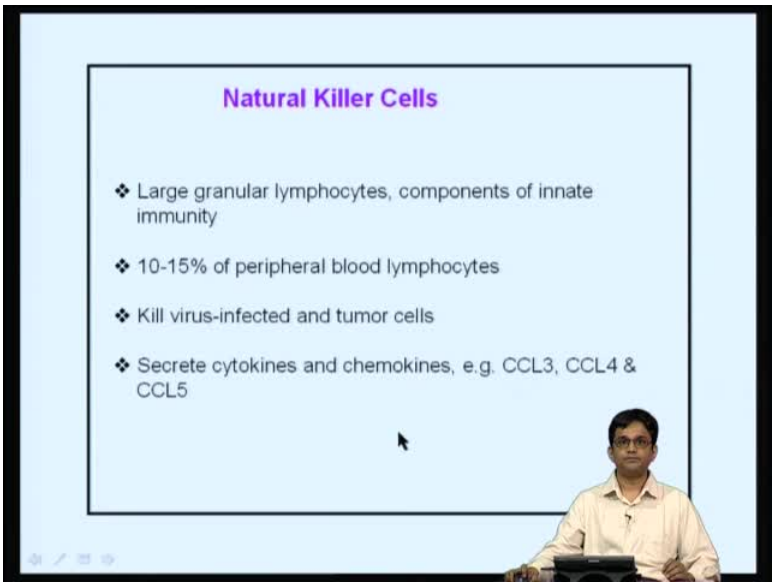

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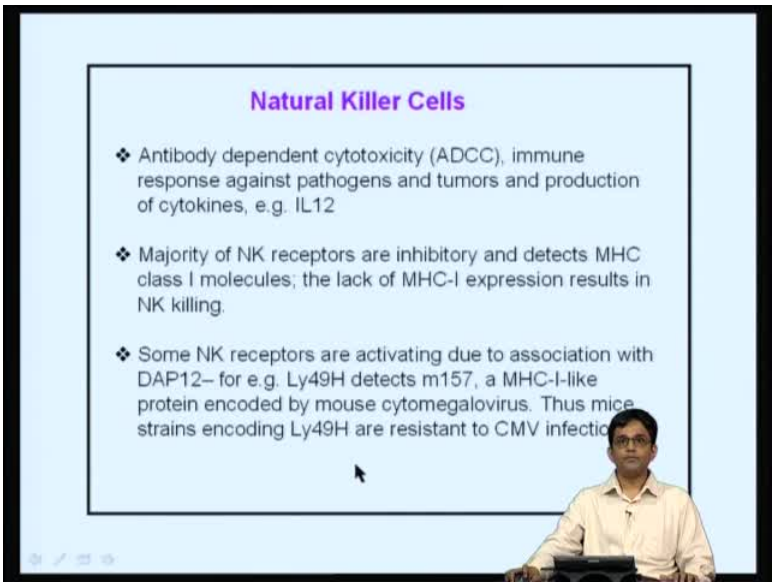

The other important cells that play an important role are the natural killer cells and these are the large granular lymphocytes. And they are important because they kill virally infected and tumor cells, they are also important in secretion of cytokines and chemokines. One of the main roles of the natural killer cells is antibody dependent cytotoxicity or known as ADCC. They are extremely good at ADCC, which means, they

can capture antigen-antibody complexes; antigen-antibody complex, that are bound to pathogens and can clear the body of antigen-antibody complexes. So, main role of NK cells is in ADCC and as mentioned, they are also important in production of tumors, sorry, in production of cytokines, example IL-12, which play an important role in anti-tumor mechanisms. And as mentioned, NK cells play are important in terms of, play a major role in the anti-tumor host response and one of which is through the production of IL-12, which is again an important cytokine, for, not only for initiation of T cell responses or modulation of T cell responses, especially in terms of anti-tumor immunity.

Now, NK receptors, there are 2 types of NK receptors – one, that are inhibitory in nature and the other, that are activating. So, you have the, activating, activating receptors and the inhibitory NK receptors. So, the majority of the NK receptors is inhibitory and detects MHC class 1 molecules, and so the lack of a MHC class 1 expression results in NK killing. What often happens is during tumors, there is lowered MHC expression because what the tumors are trying to do? They are trying to bypass the sedate response and as a result of one, one of which, they do this is to lower MHC class 1 expression and so, that is why, the host has the NK cells and were lowered MHC class 1 expression, these 2 killing, of these, of these tumor cells. So, NK cells take art sort of survey, the body for lowered MHC class 1 expression, which is a tumor strategy.

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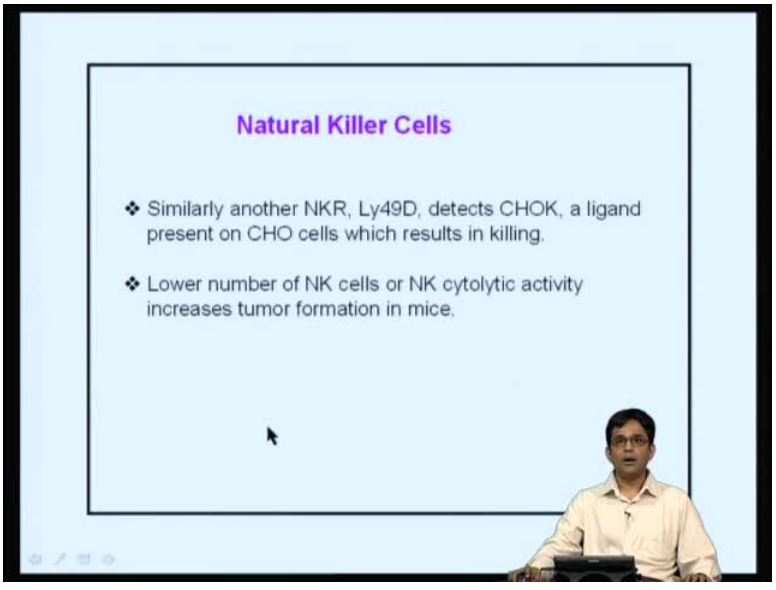

In some cases, NK receptors are activating and especially, they are activating because they associate with the molecule known as a DAP12. So, one activating NK receptor is Ly49H, which detects MHC like molecule, and which is encoded by mouse cytomegalovirus and therefore, mice strains encoding this particular NK receptor are resistant to CMV infection or cytomegalovirus infection. So, what, this, this is an example, to show how NK cells play an important role in antiviral as well as in antitumor immunity. So, one example of that is NK receptor Ly 49D, which detects CHOK, which is a ligand present on Chinese hamster ovary cells or CHO cells, which results in killing.

And as mentioned previously, low numbers of NK cells or NK cytotoxicity, a lower, lower numbers of NK cells or, or lower amount of NK cytotoxicity increases tumor formation in mice. So, overall, NK cells play an important role in antiviral and in, in surveying the body for, for tumors. They also play an important role in the antibody dependent cytotoxicity, especially in clearing of antigen-antibody complexes.

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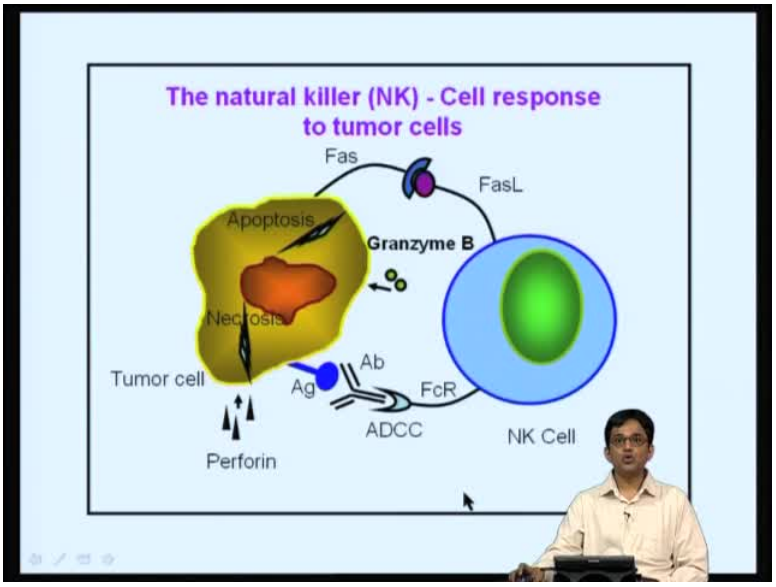

This is an example, to show, how NK cells respond to tumors. So, what is shown over here is an NK cell, it has these receptors, known as the FasL. It expresses to Fas, which results in death of the tumor cells and over here, through a process of antibody dependent cytotoxicity. It, this is a particular antibody that is secreted by the host, which is recognizing this particular tumor antigen that is present over here. So, the NK cells recognizes this and is getting, has got activated because with the FZ gamma it produces

perforins, it produces Granzymes. Perforins are ones that, that make holes in these tumor cells, so that, so that molecules such as Granzymes, which are like molecules, can enter and initiate the death cascade, which will result in death of these tumor cells. So, this is just an example or a model, to show the inter relationship of how NK cells play an important response against tumor cells.

At this point, students must be aware that the immune response is important not only for the anti-pathogen response, but it is also important for surveying the body for tumors. So, they may, the robust immune response reduces tumor formation, as well as, it reduces infection by pathogens.

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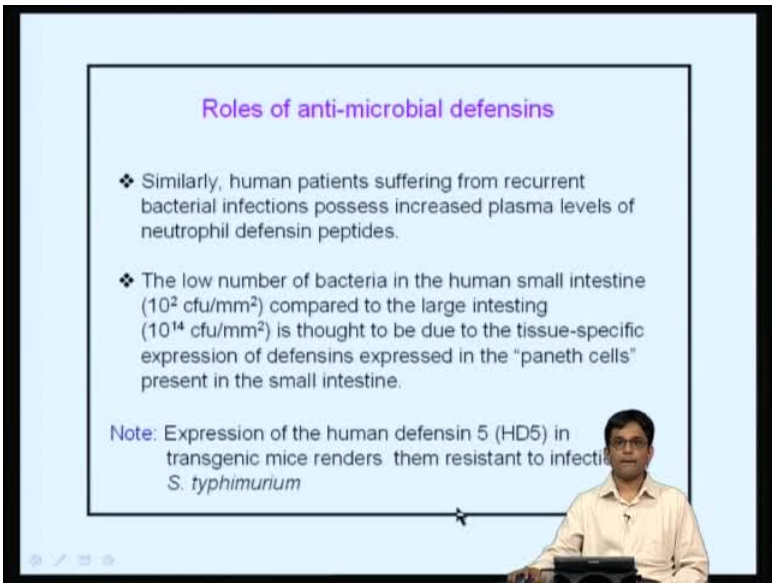

One of the effectors of innate immunity is antimicrobial defenses and these are antimicrobial peptides that are produced by innate immune cells of the body. So, an example of this is shown by the fact, that human patient suffering from recurrent bacterial infection produces increased plasma levels of neutrophil defensin peptides.

Now, one of the reasons, that the number of bacteria in the human small intestine is less compared to that in the large intestine, where there is, you know, a tremendous increase, we see this number, these numbers are greatly increased in the large intestine and it is thought, because of the tissue specific expression of defensins in the paneth cells in the small intestines. So, you have the cells, known as the paneth cells, in the small intestine, which produce these defensins and which reduce the numbers of bacteria present in

there, whereas the large intestine does not have paneth cells and therefore, there is, there are, there are cells, that do not produce high levels of antimicrobial peptides, as a result of which, it, this may be the case.

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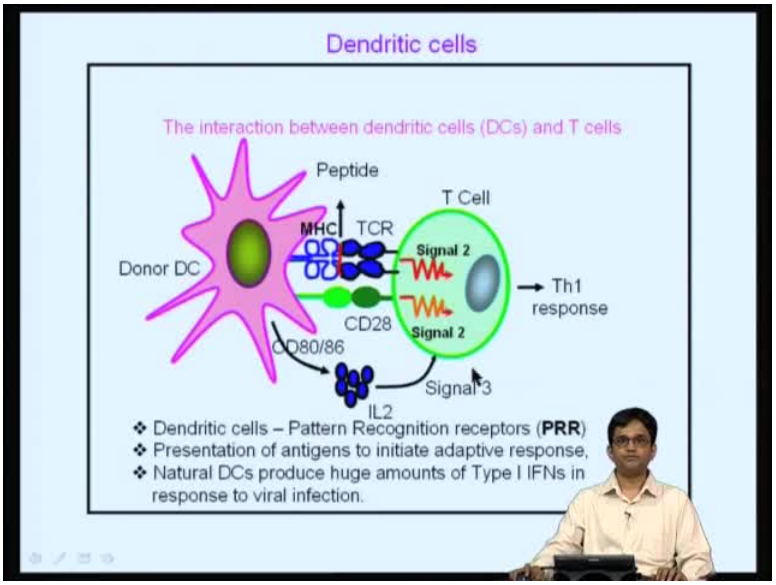

An example of this was shown by the fact, that expression of defensin-5 in transgenic mice renders them resistant to infection by salmonella. So, what this clearly shows? The relationship or the importance of antimicrobial peptides in defense against pathogen and in this case, it is salmonella. Now, in terms of modulation of the immune response, important cells are dendritic cells. I had shown a cartoon, where upon pathogen entry, you have these immature dendritic cells, that are patrolling, that pick up antigen and then, travels to the lymph node, where they become, where they activate T cells and that is what is shown over here. This aspect is important because they are important in modulating the adaptive immune response and that is what is shown over here. You have dendritic cells over here and dendritic because they are, because of the presence of dendrites over here, and this dendritic cell is presenting MHC class 1 and with peptides to a T cell. The important aspect about dendritic cells is that physiologically, they are probably the more important or the most important antigen presenting cells because they are very efficient at presenting MHC peptide complexes, and they also express these co-stimulatory ligands, known as CD80/86, which are important activation of T cells. So, for activation of T cells, you need the T cell receptor, you also need co-stimulatory

receptors, which bind these ligands on dendritic cells together. This signal is important for initiating the Th 1, in initiating Th responses that are shown.

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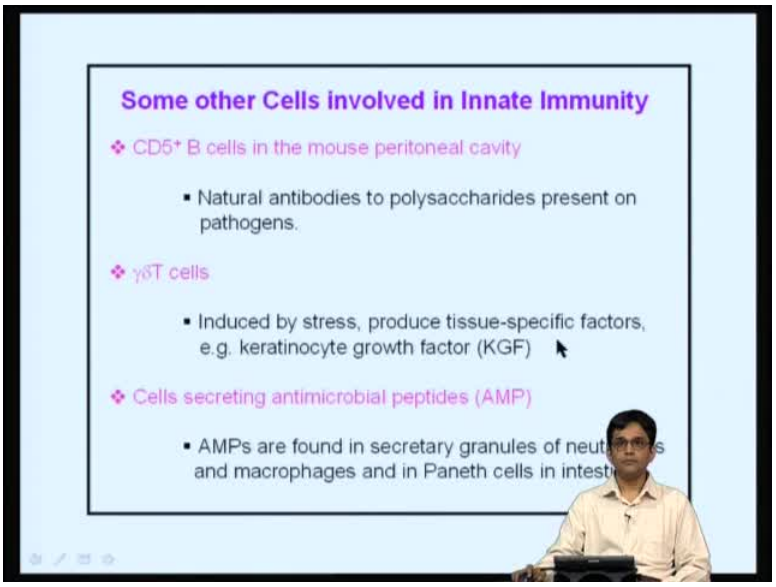

There are some other cells that are also involved in innate immunity. You have the CD 5 B cells in the mouse peritoneal cavity, which are also known as the Ly1B cells and what these B cells do is that, the, they produce natural antibodies to polysaccharides present on microbes and as a consequence of that, they sort of, it is, again it is a part of innate response. So, it is inborn and so they, sort of, again limit the spread of pathogens.

The other example are gamma delta T cells and in this case, gamma delta T cells are present, in the, in the, gamma delta T cells are, some of them are present in, on different epithelial surfaces and they are, some are also present in blood, but in this case, we are talking about gamma delta T cells, which are induced upon stress and they produce tissue specific factors, in this case, keratinocyte growth factor, which is important in healing of the skin. So, in different ways, innate immune cells may play an important role in the defense network. I have also, I have already mentioned the role of antimicrobial peptides and you have paneth cells in the intestines, which are important for production of antimicrobial peptides, which lower CFU numbers in the small intestine.

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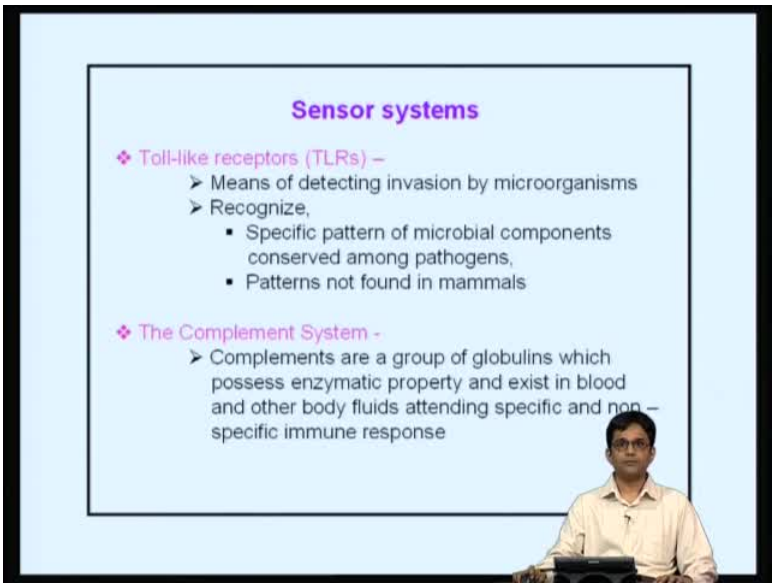

Now, what are the sensor systems that are in place to take care of these different pathogens? The first one is toll like receptors; these tolls like receptors are molecules, that detect different parts of microbial or molecules present on microbes. So, they are different, different molecules, these are detected by toll like receptors and we will discuss a little bit in greater detail. Subsequently, what they do is to recognize specific pattern on microbial components and whereas, these patterns are not present in mammals, so you can generate a specific response to microbes.

The other is the complement system and the complements are a group of globulins, which possess enzymatic activity, they are present in blood and they are important for specific as well as non-specific immune responses. So, how do, how do, how does one explain, that the specific response is because, it, they are important in recognizing antigen-antibody complexes and antibody, as mentioned, is part of the adaptive immune response. It is a specific immune response and so, when antigen-antibody complexes are, are present, complements results, it results in activation of the complement system and which, the pathogen is killed because usually what happens is, the pathogen is covered by antibodies, different antibodies and these are recognized. What about the role of the complement system in non-specific immune responses? As mentioned, sometimes complement gets activated by certain components present on microbial surfaces, this and this activation of the complement results in lyses of, of pathogens or microbes, as the case may be.

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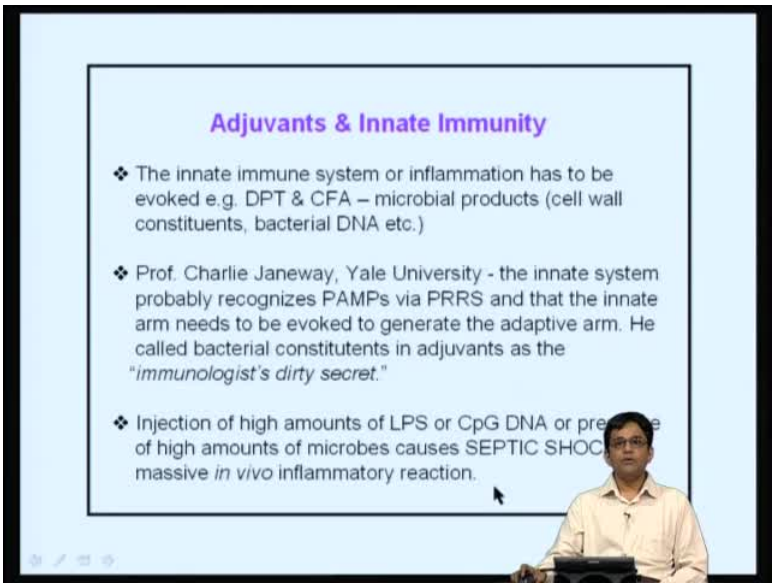

We will talk a little bit about adjuvants and innate immunity. The innate immune system or inflammation, in order for a good antibody response, the innate immune system are good antibody, as or as cellular response, good innate immune system needs to be evoked and what was found is that if you just give the antigen alone, the response was rather poor. However, if the antigen was mixed along with an adjuvant and this adjuvant can be from bacteria or which is in, in, what is shown over here, is an example of complete funds, adjuvant or microbial products, then you are able to generate a very robust and a very good adaptive immune response. And so, this is where innate immune, the innate immune system modulates adaptive immunity, was clearly shown.

And Professor Charlie Janeway from Yale University was the one who recognized the importance of this and he realized that the innate immune had to be evoked to generate a robust adaptive response. And he called the use of adjuvants by immunologists as the immunologist's dirty secrets because what the immunologist's view is to say, that it is a specific response. However, to generate the specific response, you had to mix the antigen along with dirty components or in this case, components of microbes, so that you know, you could generate a better adaptive response, and that is why, it was thought to be immunologist's dirty secret and which is, as part of the, it has become very famous. What is known is that parts of the pathogens, so for example, lipopolysaccharide or a CpG hypo-methylated DNA, that is present in bacteria, causes SEPTIC SHOCK and this is something again, or a massive in vivo response and this is something again, which we

will discuss a little bit later. So, so parts of, or molecules that are present on microbes cause a host reaction and it is a non-specific reaction, but it is a massive reaction and sometimes SEPTIC SHOCK is, can cause even death and that is an, and therefore, it is an important aspect that we need to understand.

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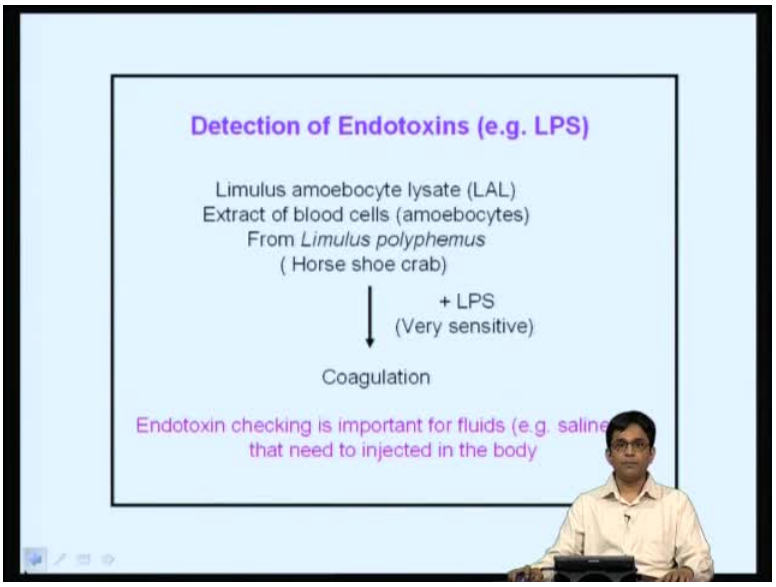

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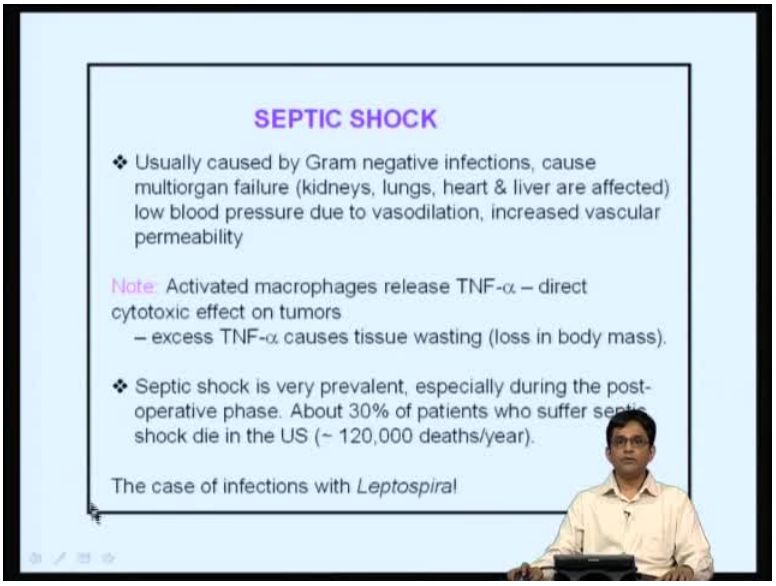

Now, one of the parts that were shown is, so we just go over here, so we will just, by, by, by discuss a little bit about SEPTIC SHOCK. So, usually SEPTIC SHOCK is caused by infections, usually gram negative and what happens over here is because the infection,

the infection is causing and it causes a massive host response. So, SEPTIC SHOCK is actually a massive response by host cells and, and it is because the response is so massive, that it leads to multi-organ failure of, you know, different organs are effected, there is low blood pressure due to vasodilation, increased vascular permeability and often, it contributes to death.

Now, as mentioned, you know, macrophages release upon activation, release TNF alpha, and this is an example of that, macrophages release TNF alpha and this, which TNF alpha is cytokine and it was discovered because it had a cytotoxic effect on tumors and that is why, tumor necrosis factor; that is how it came up. However, now, what is also known is that excess TNF alpha causes tissue wasting or loss in body mass. So, you can see that cytokines are produced in, as part of the host defense. However, if too much cytokines are produced then, there is a problem in, in, in, our body is unable to respond to this huge burst of cytokines and which are, all contribute to septic to this condition, known as SEPTIC SHOCK. It is important to realize about septic shock because septic shock is prevalent and especially, it comes up in the post-operative phase.

And to give some statistics about 30 percent of patients of, who suffer septic shock die in the, in the US. So, often, these cases are post-operative and, and due to post-operative complication, there are sometimes infections and these infections, if they are not controlled, they can lead to sepsis or septic shock. I will now talk a little bit about, about an infection that is prevalent in India, this occurs especially during the monsoons and it is caused by a Spiro key, known as a Leptospira.

Leptospira is, is, is, is, humidity plays a very important role for leptospira. Therefore, it shows up in monsoon seasons, but otherwise it is usually prevalent in, in rat kidneys and therefore, the name rat fever. So, the urine is secreted in, in, in, in the streets and all, but during monsoons, and all the water carries these and if there are cuts in the skin or something, leptospira can enter into the body and cause rat fever. Now, this rat, the leptospira again, enters and then proliferates in the kidneys, releasing several millions of these organisms. Now, what happens with leptospira is leptospira has an extremely potent lipopolysaccharide, we had mentioned this, and this lipopolysaccharide, which is a part of the component of the cell wall of bacteria, it evokes an extremely strong host response and therefore, leptospira can be tackled very easily with penicillin.

So, therefore, in endemic cases where leptospira is often present, if a patient shows up with, with symptoms, that suggest, that it could be a case of leptospira, the patient is often put on to penicillin because penicillin kills leptospira very easily and one of the problems that doctors face is that if there is delay in treatment of, of penicillin, often you may, what may happen is, if you are, if there is delay, you can kill the, kill the bacteria, but you can still lose the patient. That is because even dead bacteria will evoke a very strong host response and it is this host response, which is so tremendous, that when it results in high production of cytokines and ultimate multi-organ failure, which is a major problem. So, therefore, a sepsis or septic shock is an important aspect and we should try and understand, that it is reaction by the body against these, these, these different pathogens, but it is a host response, is the uncontrolled host response, which results in, in multi organ failure and in some cases death of patients and we need to understand the importance of this in, in, the importance of this cannot be emphasized any further.

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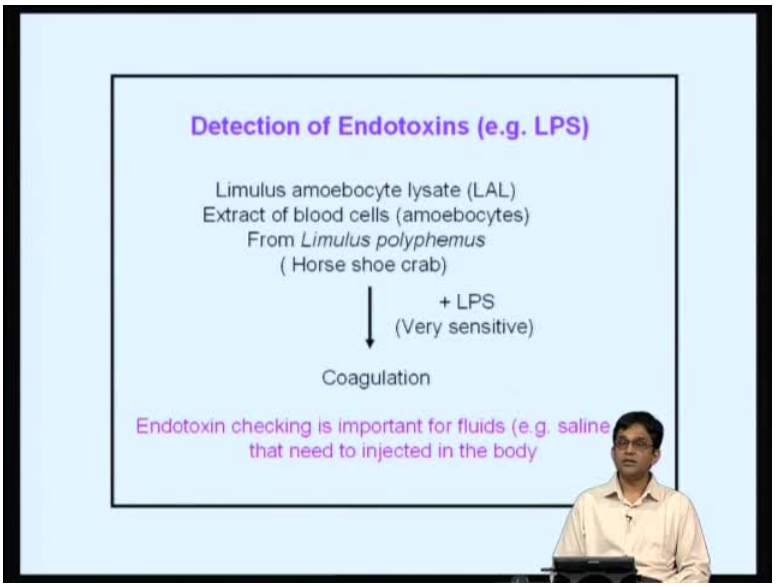

So, given the importance of LPS, also known as endotoxin, it is important to know, how it is measured? So, any fluid for example, that needs to be injected in body needs to be checked for the presence of endotoxin or LPS, and one of the ways by which LPS is measured is to use the limulus amoebocyte lysate. Now, what is the limulus amoebocyte lysate? It is nothing but an extraction of blood cells or the amoebocytes from, or the horse shoe crab. And what was found several years back is that this lysate is extremely

sensitive to small amounts of LPS or endotoxin, so what happens is, it results in coagulation.

So, you can see, even in the crab, the system in the crab has been used as the system to, for the detection of endotoxin or LPS. And endotoxin checking is extremely important because as mentioned, that if these components of microbes enter our body, our body will generate a host response and this is an example of that. And I, I, I have shown this because it is an excellent example of the use of the study of innate immunology for, which has, which has some value in terms of patient, has some translational value because it is, the lysate is from horse shoe crabs, they are extremely sensitive to lipopolysaccharide, so they coagulate. So, the coagulation is to limit the disease or limit the spread and so this has been brought up in an assay for the detection of endotoxin. So, all major fluids, that need to be injected into the body, need to be checked and it is done usually using often the LAL kit.

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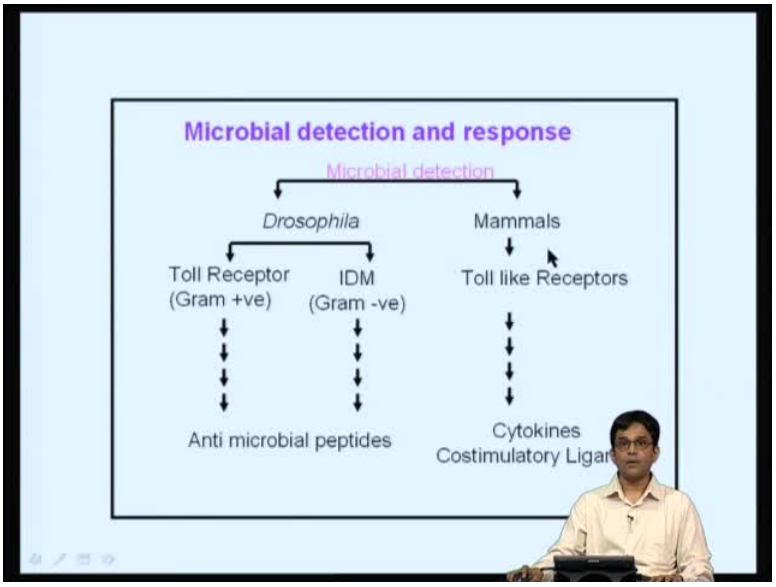

I will now briefly mention a little bit about the microbial detection and responses, and 2 important parts will be emphasized - one is the detection in drosophila and importance of drosophila is there because the toll receptor, which is important for detection of gram positive bacteria and fungal pathogens, was discovered in, in drosophila. So, that toll receptor is important for this and through a series of pathways, it is important for the production of antimicrobial peptides. Now, for you have another pathway in drosophila,

known as the immuno-deficiency pathway or the idiom pathway, which recognizes gram negative bacteria. Now, we have ortho, we have orthologs of the toll receptor in mammals, which are known as the toll like receptors and what happens over here is again, through a series of pathways, you have the production of cytokines and co-stimulatory ligands. So, what is shown over here is this pathway of response or of detection of microbes and response is more or less conserved, right from drosophila to mammals. And as mentioned over here, the toll receptor was initially shown in drosophila and in mammals you have toll like receptors, but the basic pathway is conserved.

However, in drosophila, you have the production of antimicrobial peptides, whereas in mammals you have the production of cytokines co-stimulatory ligands, so on, which will, which are important for the adaptive immunity. But this is the good example of innate immune response because you can see that the major players are actually conserved in both drosophila and mammals.

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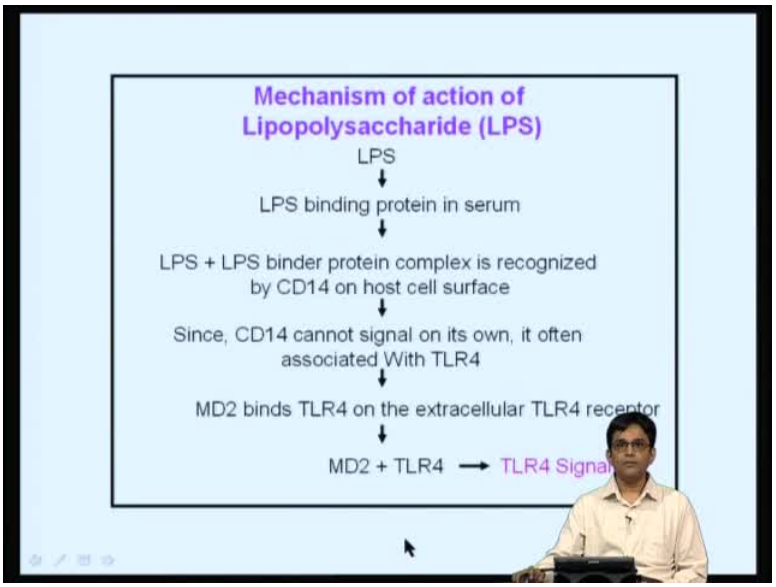

Now, again, we need to understand, we said, that lipopolysaccharide plays an important role; how is lipopolysaccharide detected in mammals and how is it recognized? So, lipopolysaccharide is, once it comes into the blood, it binds to lipopolysaccharide binding protein (LBP) and it is this LPS and lipopolysaccharide binding protein is recognized by molecule, known as CD14 on the host cells. Now, CD14 can bind to this

complex of lipopolysaccharide and lipopolysaccharide binding protein, but it cannot signal on its own. In order for it to signal, it needs to be associated with a toll like receptor 4 in this case. And now for optimal signaling TLR4, which is present on the extracellular domain is present on MD2. Now, there are 2 important parts over here for TLR4, optimal action of TLR4, you, it needs CD14 for the, CD14 for, to bind to LPS and LPS binding protein; for optimal signaling, it needs MD 2. So, you can see this complex that is an important part that plays an important part in TLR4 signaling.

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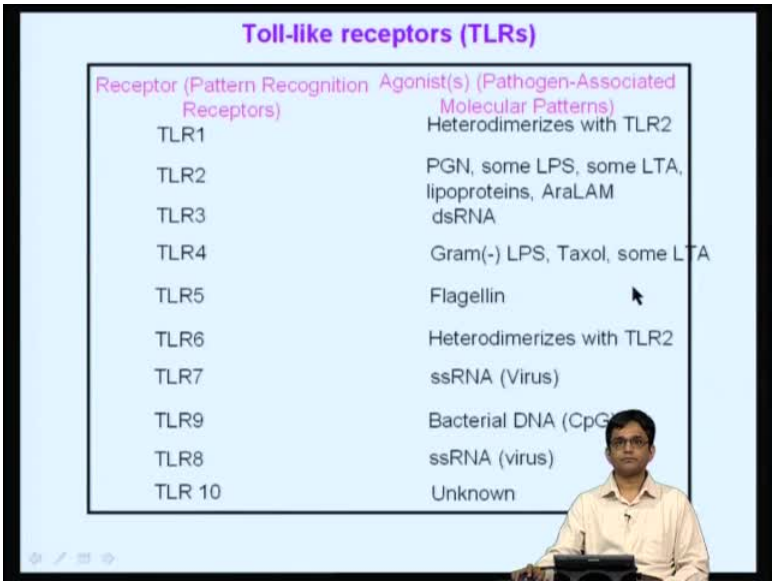

Subsequently, in mammals, several orthologs of and paralogs of the TLR receptors have been, have been shown, so I will highlight a few of them. You can see in humans, there are, there are 10 of them and TLR2 for example, it recognizes peptidoglycans lipoteichoic acids; TLR4 as mentioned, recognizes LPS; TLR5 recognizes flagellin, which is present on, which is important for flagellated bacteria; TLR7 recognizes single stranded RNA, which is present in viruses; TLR9, very important, which is present, it recognizes hypo-methylated DNA, which is present in bacteria. Note, our DNA is often methylated and therefore TLR, TLR9 is unable to recognize. However, microbial DNA is often hypo-methylated and that acts as a good way for differentiating this response. Note, the innate immune response recognizes bacterial or microbial DNA because it is primarily hypo-methylated.

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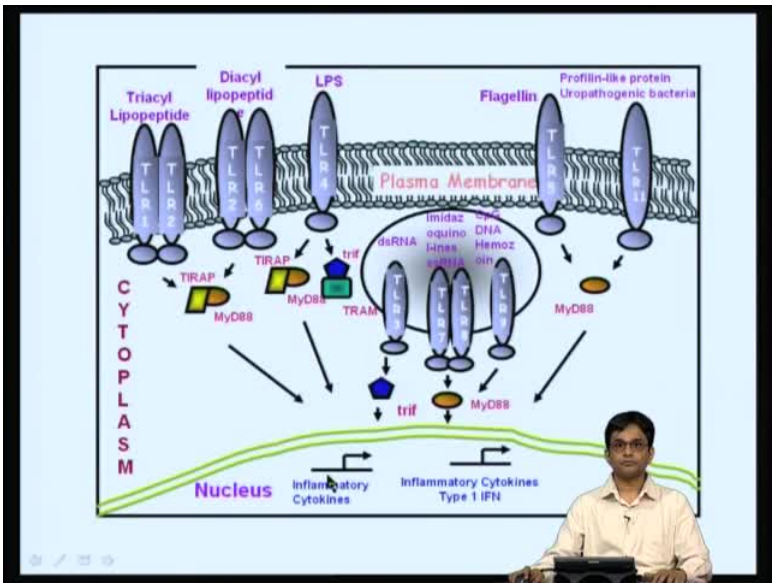

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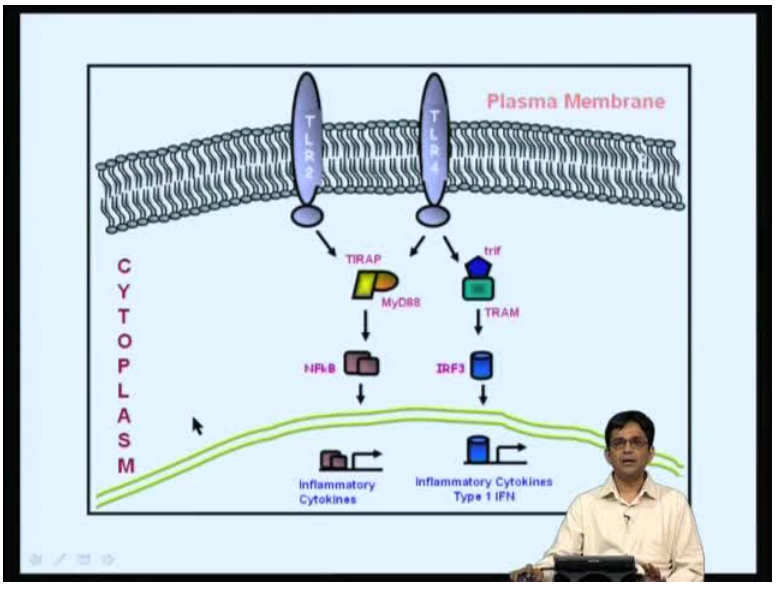

So, this is the rather complex slide and I will just briefly summarize it for you. You have different types of TLRs and that is shown over here, some of the TLRs are present on the cell surface and some of them are present in endosomes or intracellular, suffice to say, all of these signal and they result in production of inflammatory cytokines, and you have different pathways over here, that is shown. I will, I will move on to show, I will try and simplify it to show, how a surface TLR, which is, in this case TLR 2 or TLR4. It signals via certain molecules, known as MYD88, which is an adapter molecule in NF kappa B, which are an important transcription factors and these result in production of cytokines.

What is also shown over here is, is a pathway by which is a MYD88 independent and this is important activation of IRF 3, which is an important, in production of type 1interferon and type 1 interferon and an important player in innate immunity. So, this is to show you, that there are different pathways by which TLRs can function, some via MYD88 and some, that are independent of MYD88. But nevertheless, you have production of different cytokines and one of which is type 1 interferon, which plays a very important role in antiviral immunity.

This one summarizes the TLR9 and other TLR that are present in endosomes. Again, they go through different pathways and which produces different types of cytokines, some of which are, are, are type 1 interferon and the others, other cytokines, but nevertheless they are important in case of immunity.

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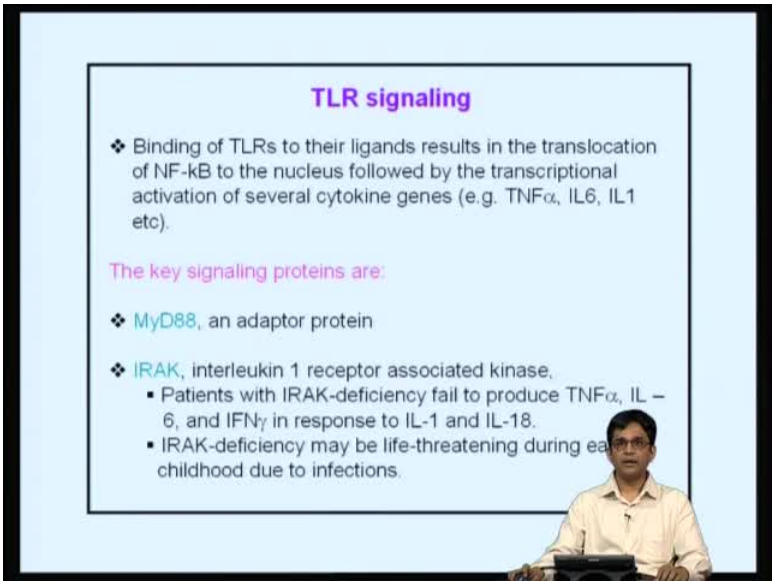

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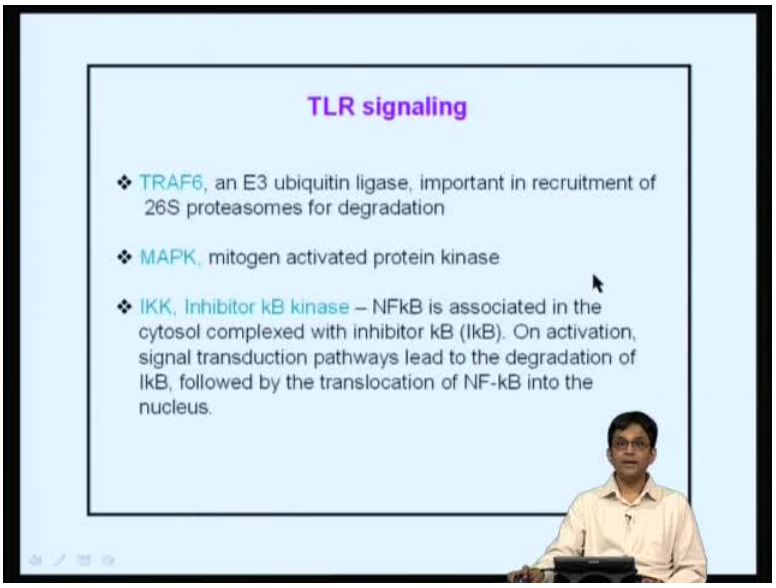

I will, I will just briefly summarize over here to, to, to mention, that the binding of TLRs are extremely important and this TLR signaling pathway is conserved between drosophila and higher organisms. The key signaling proteins are MYD88, which is an adapter molecule IRAK, which is an interleukin 1 receptor associate kinase and in fact, patients that, that lack IRAK fail to produce these cytokines and it can be, and having, and not having IR of K can be life threatening. You have TRAF6, which is E 3 ubiquitin ligase, which is important for the signaling pathway because certain proteins may need to be degraded; you have the mitogen activated protein kinase pathway; finally, you have the IKK or the inhibitor kappa B, which needs to be degraded, so that NF kappa B can translocate into the nucleus and set off the signaling pathways.

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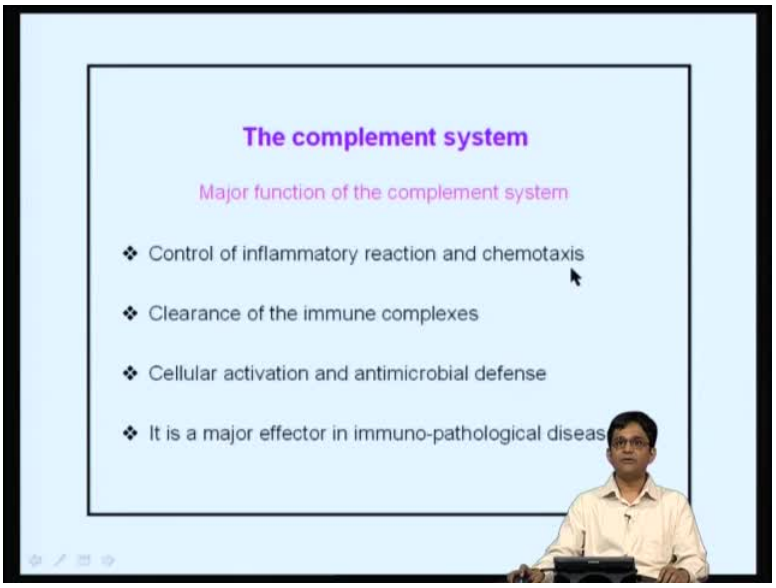

So I will, I will, before going to the complement signaling system, which will be taken up in the next class, I will briefly summarize this class to say, that what we have tried to study in this class is the innate immune response; the different cells, that are playing an important role in the innate immune response, for example neutrophils, macrophages, the NK cells; you have different molecules, that play an important role, the antidefensin peptides, the TLRs and the TLRs are especially important because of the conservation of the pathway between drosophila and higher, higher animals, and it, and it, this is really the core of innate immuno-immunity. I also would like to mention the importance of the LAL kit to detect endotoxin because here is, where you can see a response of a crab to LPS has been used as, as, say, to detect LPS. And endotoxin detection is extremely important because any fluids, that need to be injected in patients are, have to be tested for the presence of endotoxins and this is important because high amounts of microbial components will result in response by the host cells, and too much of a response can result in a condition known as sepsis.

Thank you.