Essentials in Immunology

Prof. Dipankar Nandi

Department of Biochemistry

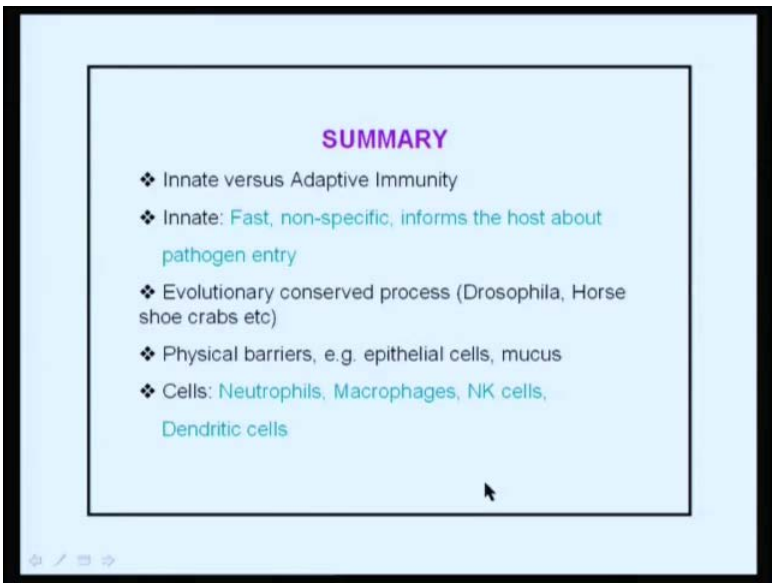
Indian Institute of Science, Bangalore

Lecture No. # 06

Innate Immunity – Part 2

Today, we will be starting off with the second part of innate immunity.

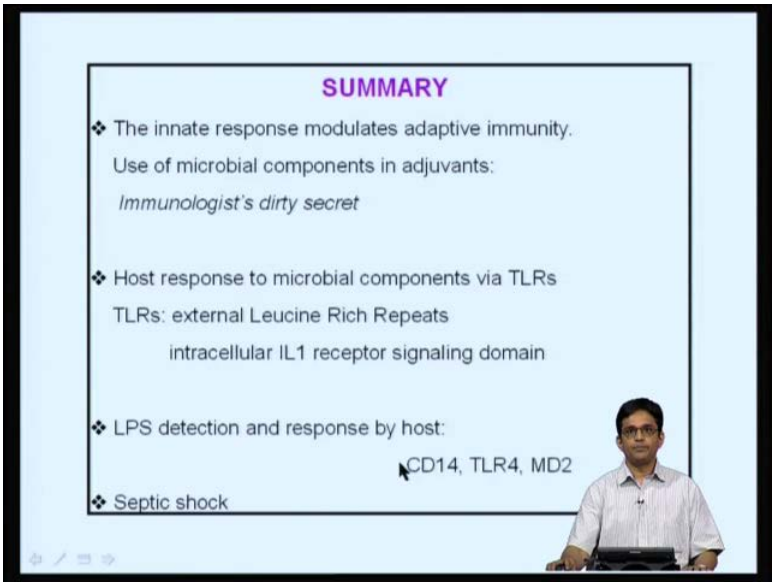
(Refer Slide Time: 00:25)



Before we go into that, it might be a good idea to summarize some of the main aspects of the last lecture. The first is the importance of innate versus adaptive immunity. As mentioned previously, it is a quick response; it is non-specific; it does not differentiate between different types of bacteria, but it tells the host that there is some sort of invasion that has taken place. And, that is the important thing. It is an evolutionary conserved process and it is present in the lower organism, such as drosophila, horse shoe crabs, etcetera. So, it would be good idea for you to sort of think about where the drosophila and horse shoe crab were useful in the previous lecture. For example, the identification of a toll receptors were shown in drosophila and the measurement of lipopolysaccharides, which is a potent endotoxin is used using a lysate from the horse shoe crab.

We also talked about physical barriers that are important in innate immunity, for example, epithelial cells, mucus. So, mucus that is produced, traps these microbes and that is useful in sort of containing their spread. There was also discussion on different types of cells, for example, neutrophils, macrophages, NK cells and dendritic cells. So, we will briefly go a little bit over them. Neutrophils are one of the first cells that the host response to; and, they are the first ones to arrive at the site of pathogen entry. Subsequently, they produce chemotactic factors and macrophages are recruited. Macrophages are important in processing and presenting antigens to T cells; then, you have natural killer cells, which are important for what is known as antibody dependents or toxicity. Once antibodies are produced, the eyes target cells; they are also important for tumors. And, dendritic cells are perhaps the physiologically most important antigen (( )) cells, which enter different antigens to T cells, and so, you can turn on the adaptive immune response.

(Refer Slide Time: 02:50)

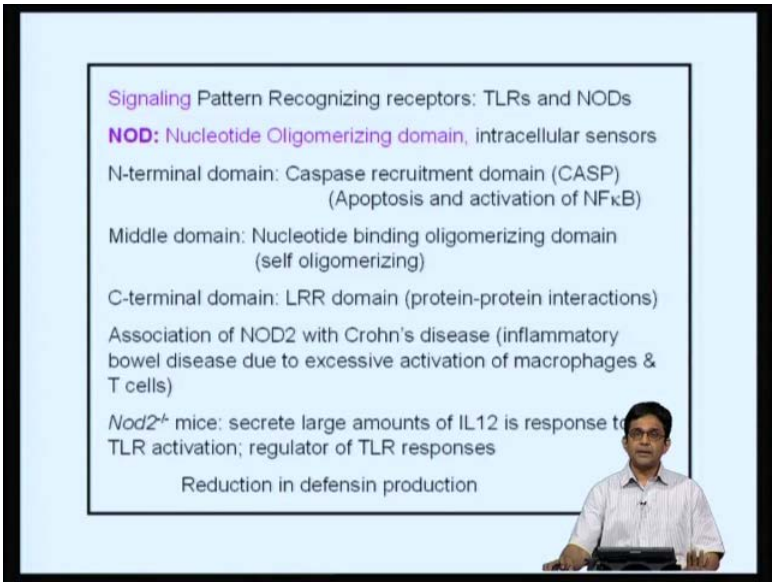


We had trust about the fact that the innate immunity modulates adaptive responses, and this is mainly seen in the use of microbial components in adjuvants. So, for example, complete (( )) adjuvants contain killed mycobacteria. And, this was known as the immunologist's dark secret by Charlie Janeway, who first propounded that the innate components needed to be activated to get an optimal immune adaptive response. And, subsequent studies resulted in identification of toll-like receptors that are present in the host, which recognizes specific microbial components. So, TLRs, for example; they

contain this external leucine rich repeats and this LLRs; or, leucine rich repeats are important for protein-protein interactions and it contains an internal IL1 receptor signaling domain by which the signal transduction can be done.

We also discussed the ways by which LPS is detected and the response by host. So, LPS binds to LPS binding protein in the serum, and this complex is transported and it is recognized by CD14. And, TLR4 is important for recognition of LPS. And, this complex alone cannot signal; it needs a signaling molecule known as MD2, which is important in signal transduction. So, you turn on cytokine response and you turn on an acute innate response, which is often manifested with respect to cytokine release, increase phagocytosis, killing of target cells or pathogens, etcetera. We also discussed an important part, which is septic shock. And, septic shock is quite prevalent especially in hospitals, are post infections. And, this is important, because you have an acute inflammatory reaction, because of the presence of microbes, and the host responses is so strong that it often leads to multiorgan failure, low blood pressure and sometimes even death. So, it is important that these aspects are revised by you before we move on to the next part.

(Refer Slide Time: 05:19)



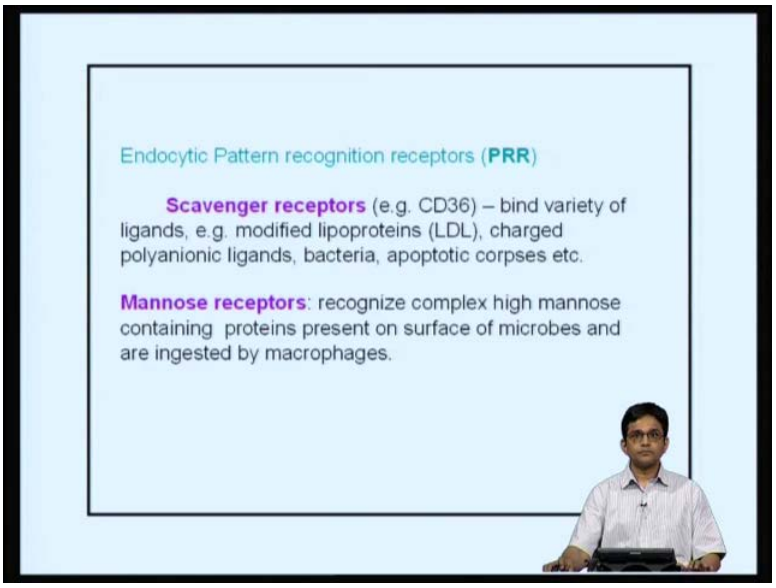
We had mentioned the role about signaling pattern recognition receptors. And, the ones that we mainly discussed in the last lecture were toll-like receptors. Now, there are two main types; there are others too, but the two main signaling pattern recognizing receptors

are the TLRs and the NODs. So, what are the NODs? The NODs are the nucleotide oligomerizing domain. These are intracellular sensors. If you remember, most of the TLRs – TLR2, TLR4, etcetera – are present on the surface. Some TLRs are present in endosomes, for example, TLR9. But, in case of NOD, they are primarily intracellular sensors. So, they sense microbial entry pathogen within the cells. And, these are characterized by particular domain. So, NOD proteins contain an N-terminal domain, which contains the CASP domain or the caspase recruitment domain, which is important apoptosis and activation of NF kappa-B. We will discuss this important transcription factor, NF kappa-B subsequently.

The middle domain contains the nucleotide binding oligomerizing domain, and hence, the name NOD, which is important in self oligomerization. The C-terminal domain contains the leucine rich repeat, which is important for protein-protein interactions. You will remember that TLRs contain LRR domain, but it is present on the external surface; whereas, in NOD, it is present in the C-terminal end. The importance of NOD has been shown with its association with Crohn's disease, which is an inflammatory bowel disease. Now, mutations in NOD2 have been associated with Crohn's disease, which is an excessive activation of macrophages and T cells in the bowel.

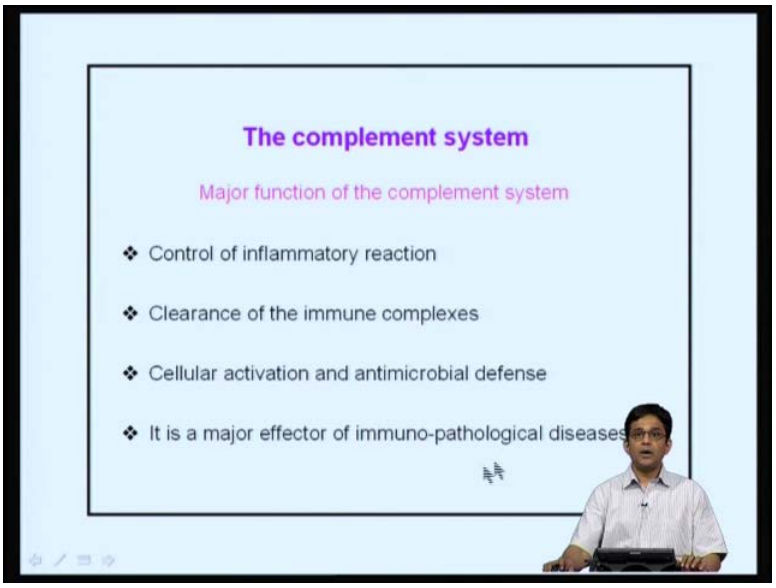
One of the possible reasons for this has been shown that NOD regulates the TLR2 signaling. So, for example, in TLR2 knockout mice or mice that lack NOD secrete large amount of IL12 in response to TLR activation. So, it is possible that NOD displaying the regulator of the TLR activation. And, since the mutations in NOD are unable to control this, it results in greatly exaggerated activation possibly resulting in inflammatory bowel disease. There is also a reduction in defensin production, which are important anti-microbial peptides in NOD2 knockout mice and that may also be important, because as previously mentioned, that the production of anti-microbial peptides is important in reducing the number of bacteria that is present in the intestine.

(Refer Slide Time: 08:05)



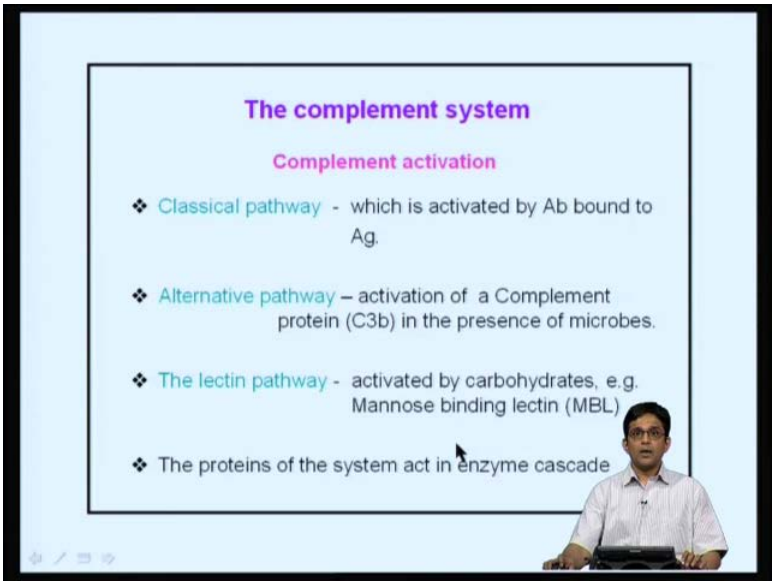
There are other types of pattern recognition receptors and two of the important ones are endocytic. Now, these do not signal by themselves; they are endocytic, which means they will bind to complexes and these receptors internalize and clear of these bound ligands. So, one of the important ones are scavenger receptors. Scavenger receptors are particularly important, because they bind to modify lipoproteins, for example, LDL, which is important in transport of cholesterol. So, they also bind to charge polyanionic ligands, bacteria, apoptotic corpses, etcetera. And, this part is important, because for example, when there is increased cell death, you want to remove off the dead cells and scavenger receptors may be playing an important role in these sorts of processes. There are also mannose receptors that are present on macrophages. And, these recognize high mannose containing proteins, which are present on surface of microbes and which are then ingested by the macrophages.

(Refer Slide Time: 09:09)



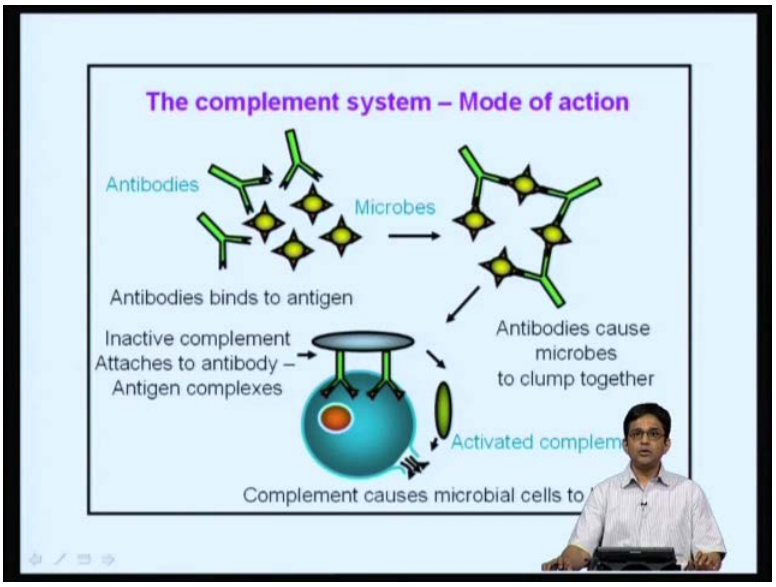
We will next be moving on to the complement system. And, the complement system has several functions. It is important in the control of information. It is most important in the clearance of immune complexes. So, especially when you have antigen-antibody complexes, they need to be cleared off and the complement system comes in place over here. They are important in activation of the antimicrobial defense and we will see parts of that subsequently. And, it is a major effector of immuno-pathological diseases.

(Refer Slide Time: 09:40)



So, there are different ways by which complement can be activated. The most classical and well-studied is the antigen bound to antibody. So, you have antigen bound to antibody and this activates complement. And, this is useful in the body and it is also useful for doing in vitro experiments, where you want to isolate certain population of cells. You have an antibody to a particular cell type and you can use complement to deplete that particular cell type. So, complement has a variety of uses. But, in terms of innate immunity, one of the ways that it plays an important role is to the alternative pathway, where you have the activation of complement protein (C3b), which binds to certain microbial surfaces, and then, gets activated and remains activated; and, as a result of which the cascade initiates. The other way by which complement can be activated is through the mannose binding lectin, which binds to two pathogen surfaces. And, we will discuss these in slightly greater detail. The important part of the complement pathway is that the proteins in the system act as an enzyme cascade. So, one protein gets activated, in turn, activates the other one, and so on until the microbe is lysed. And, we will see that somewhat later.

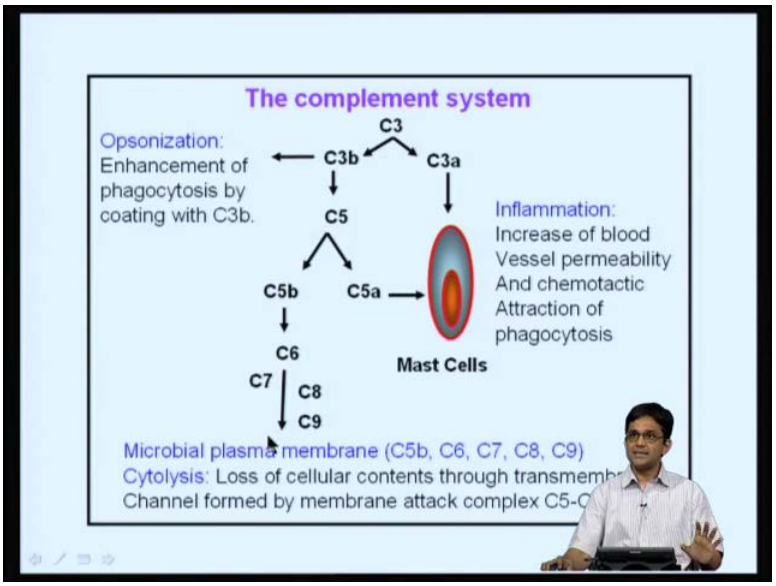
(Refer Slide Time: 11:00)



What is shown over here is the classical pathway. So, here you have antibodies and these antibodies have been produced against the microbes. And, these results in what are shown over here as antigen-antibody complexes. And, these antigen antibody complexes are clumped together and then complement binds to these, and what it does is, it results

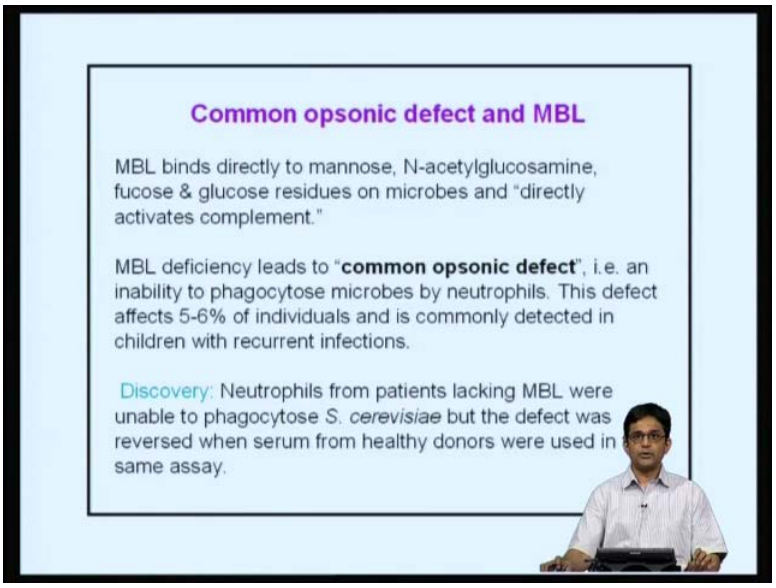
in lyses of the microbes. And, as a result of which, these antigen-antibody complexes and the microbes are lysed.

(Refer Slide Time: 11:30)



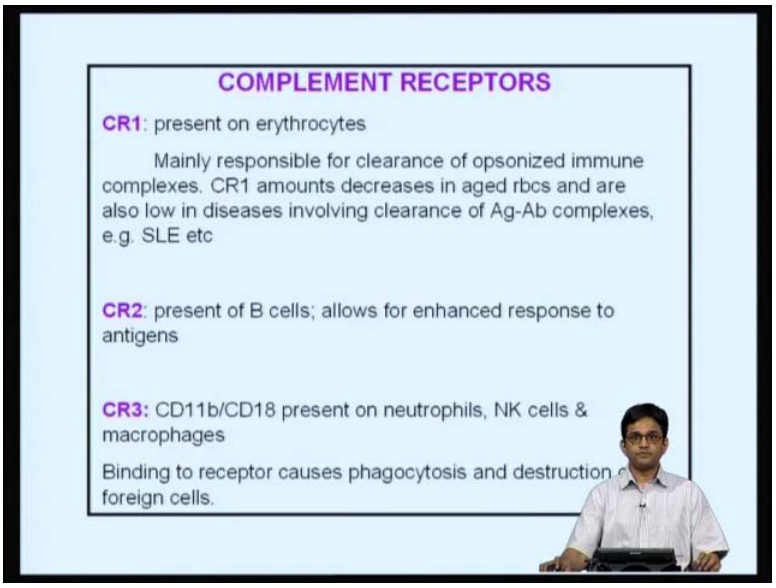
And, what is shown over here is the cascade as I was talking about. C3b is an opsonin, which means it enhances the phagocytosis by coating. So, once C3b is coated on microbes, it enhances phagocytosis. That is the process of opsonization. It also results in activation of the complement and you can see the cascade leading to the microbial plasma membrane loss or lyses of microbe. There are other processes involved in here. Complement plays an important role in inflammation. So, it increases the blood vessel permeability and the chemotactic attraction during phagocytosis.

(Refer Slide Time: 12:13)



And, important disease that is related to this particular pathway is the common opsonic defect and its relationship with mannose binding lectin, something that we have just discussed in the previous slide. The mannose binding lectin is the host protein; it binds directly to mannose, N-acetylglucosamine plus fucose residues, etcetera that are present on microbes, and directly activates complements. So, this is the third part pathway by which complement can be activated. What is important is that deficiency in MBP leads to common opsonic defect, that is, an inability to phagocytose microbes by neutrophils. And, this defect affects 5 to 6 percent of individuals, which is fairly high and it is commonly detected in children with recurrent infections. So, how was it discovered? What was found is that neutrophils from patients, who lack MBL or have mutations in MBL, were unable to phagocytose yeast, which is saccharomyces cerevisiae, but the defect was reversed when serum from healthy donors was used in the same assay. So, there was something in the serum that was missing and subsequently it was identified to be mannose binding elected.

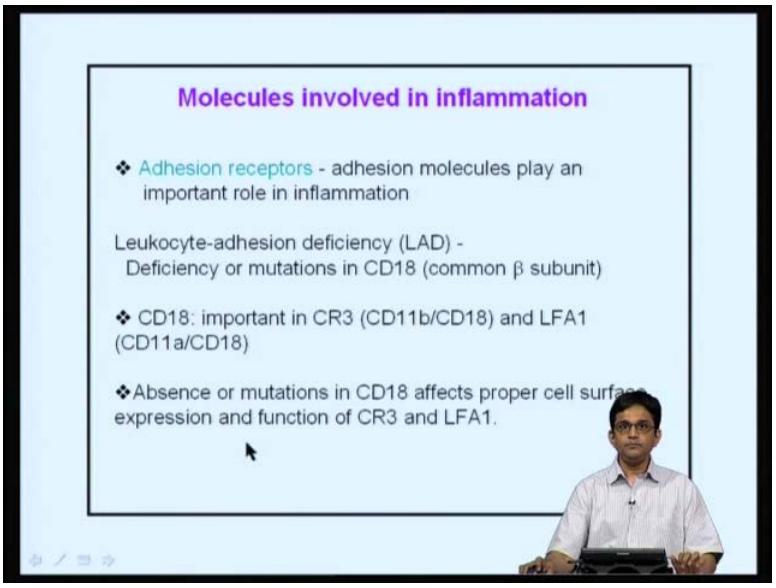
(Refer Slide Time: 13:22)



Complement receptors are important over here. Perhaps, the most important one is CR1, which is present on erythrocytes of CR, stands for complementary receptor. And, it is mainly responsible for clearance of opsonized immune complexes. CR1 amounts decrease in aged rbcs. So, as rbcs get old, these amounts decrease. And, they are also low in diseases involving clearance of antigen-antibody complexes. For example, systemic lupus erythematosus, and that is again something that will be discussed in the lecture on auto immunity.

CR2 – the complement receptor 2 is present on B cells; and, it allows for enhanced response to antigens. So, you can imagine a situation with B cells. And, if you have the antigen with the CR2 complement and bound to antigen-antibody, and is binding to B cells, it is internalized efficiently. And, this allows for better presentation and activation of B cells. So, CR2 is important for enhanced B cell responses. CR3, which is shown over here as CD11b and CD18, is present on neutrophils, NK cells and macrophages. And, it is important for phagocytosis and destruction of foreign cells. We will see the importance of this particular subunit, CD18 subsequently.

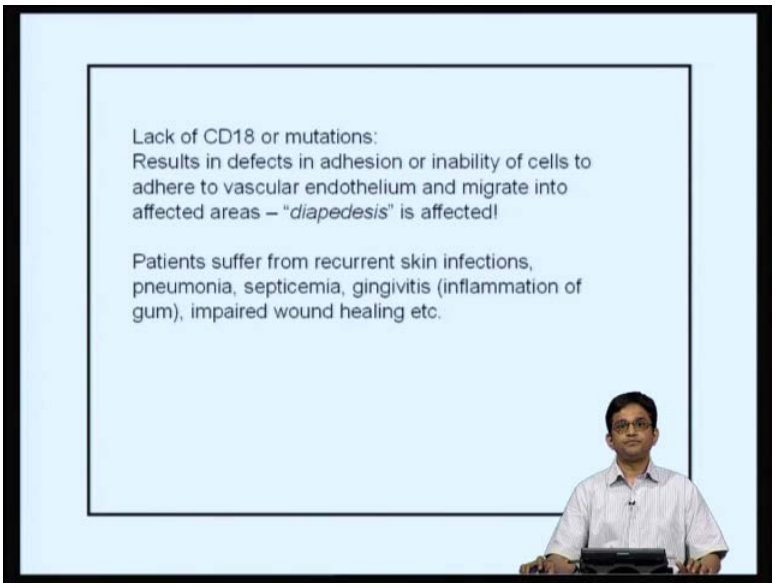
(Refer Slide Time: 14:51)



The other molecules that are important in inflammation are adhesion. During the process of information, adhesion receptors increase. So, both receptors as well as ligands increase. And, this adhesion is important especially because neutrophils, macrophages have to leave the bloods circulatory system and travel into tissues, where the damage has taken place. So, in order to do that, adhesion plays an important role by which they can go to particular areas within the part, where tissues are affected. And, adhesion receptors and ligands play a very important role in this process.

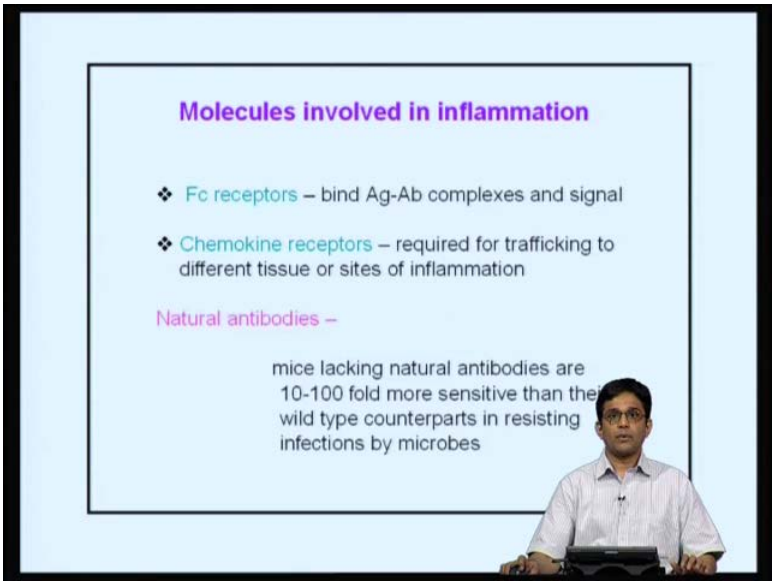
One important disease is known as leukocyte-adhesion deficiency. And, this results due to mutations in CD18, and CD18 is the common beta subunit. Now, this beta subunit is associated with different types of receptors. For example, CD18 is important in CR3, which is the complement receptor 3. Here CD18 is associating with CD11b. And, alternatively, CD18 is also important for LFA, which is an important adhesion receptor. Here the alpha subunit is different. It is CD11a and which associates with CD18. So, you can see here that CD18 is common in both these two different types of receptors, but the alpha subunit is different. So, if you have patients that have mutations in CD18, what it does is, it affects proper cell surface expression and function of both complement receptor 3 and LFA molecules.

(Refer Slide Time: 16:46)



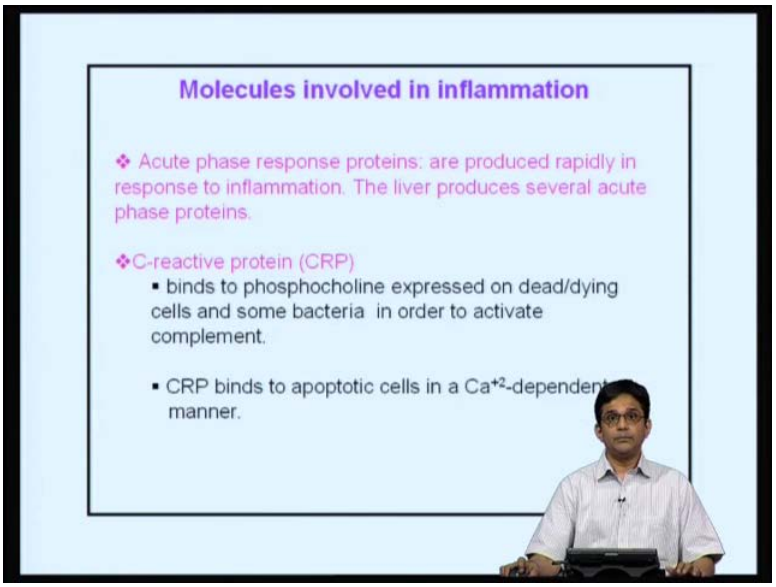
What happens as a result of this is, it results in defects in adhesion. As a result of which the cells will not be able to migrate to the affected area. As a result of which diapedesis, which has this ability to migrate, is affected. And, consequently, patients suffer from recurrent skin infections, pneumonia, septicemia, gingivitis, which is inflammation of gum, impaired wound healing, etcetera. So, it shows you clearly, these examples are there to show you the importance of particular subunits in the innate immune response.

(Refer Slide Time: 17:22)



There are other molecules that are important. FC receptors – FC receptors will bind to antigen-antibody complexes; and, these signal. FC receptors are particularly important, for example, in signaling during allergies. Then, you have chemokine receptors, which are required for trafficking to different tissues or sites of inflammation. An important chemokine receptor is CCR5, which is important for entry of HIV. In the last class, we had talked about CD5 positive B cells present in the peritoneum or live1 B cells in the mouse as the unknown. And, these are often responsible for production of what is known as natural antibodies. So, these antibodies are produced in response to different types of microbial pathogens. So, it is naturally present. And, mice lacking natural antibodies bodies are 10 to 100 fold more sensitive than the wild type compartments in resisting infections by microbes. So, it clearly shows you that natural antibodies are also playing an important role in innate immunity.

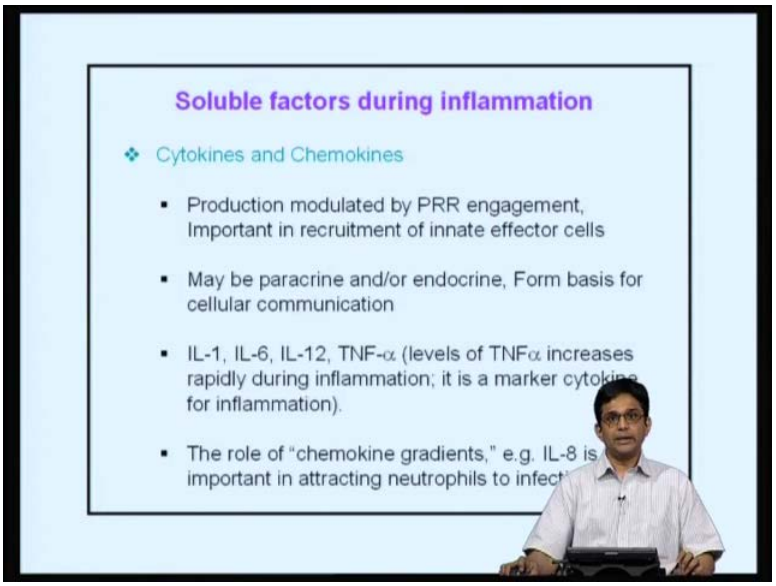
(Refer Slide Time: 18:30)



An important class of proteins that plays a response in innate immunity is acute phase response protein. These proteins are produced rapidly in response to information. And, the liver is responsible for production of several acute phase proteins. An important acute phase protein is the C-reactive protein. It binds to phosphocholine present on dead or dying cells and some bacteria in order to activate complement. So, as a consequence of that, it binds to apoptotic cells in a calcium-dependent manner. So, CRP is important for (( )) of dead cells and it is a way above which you know, once inflammatory reaction is

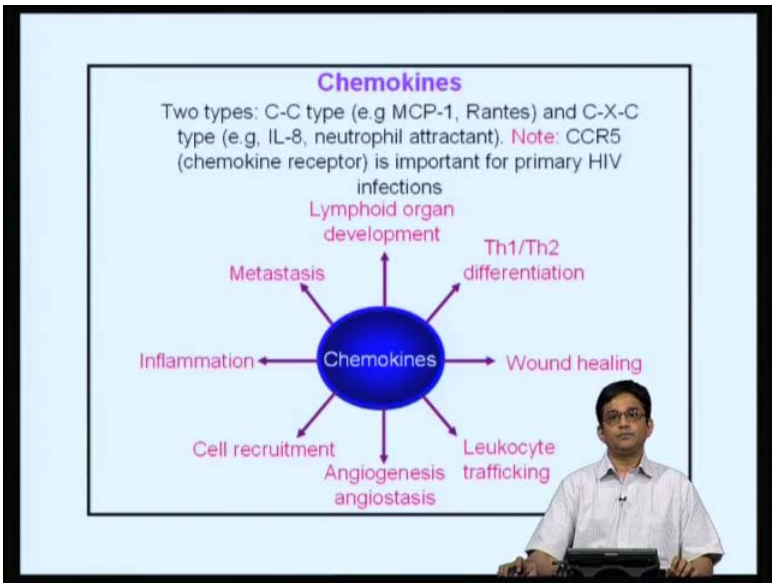
over, you are sort of down modulating and getting rid of all the debris that is around. It is the way the body has developed by which dead/dying tissue can be removed efficiently.

(Refer Slide Time: 19:27)



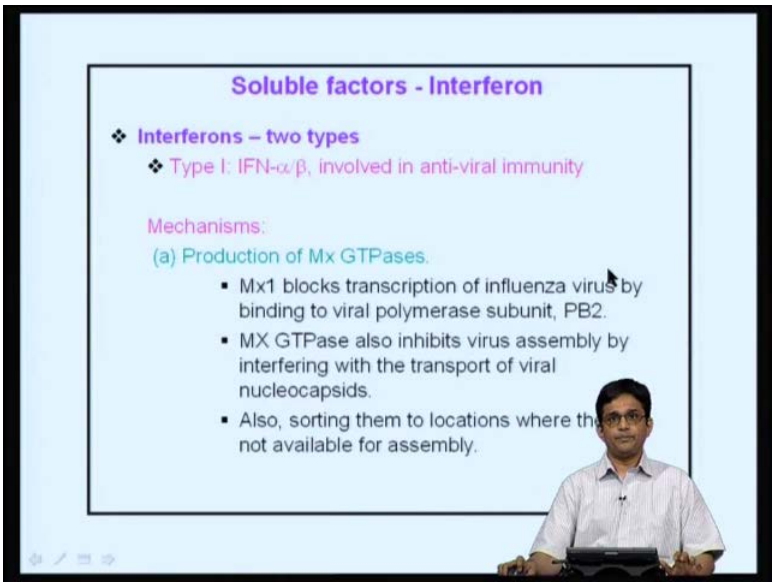
Among the soluble factors that are produced during inflammation are cytokines and chemokines. Some of the important cytokines are IL-1, IL-6, IL-12, TNF. And, in fact, TNF is a marker, because so rapidly, it is one of the quickest or the fastest produced cytokines during inflammation. So, it is often thought to be a marker for inflammation. Now, what happens often during signaling, we are the pattern recognition receptors. One of the downstream consequences is our production of cytokines, which have a variety of effects. Also important is the production of chemokines. And, chemokines are especially the example shown, is that of IL8, which is important in attracting neutrophils during infection. So, the production of IL8 attracts neutrophils to the site of infection.

(Refer Slide Time: 20:26)



This slide depicts the main types of chemokines. You have the C-C types shown by MCP, Rantes; and, the C-X-C shown by IL8, which is a neutrophil attractant. And, as I mentioned to you that CCR5 is important in playing an important role in HIV infections. So, chemokines have several different roles. And, at this point, we will not dwell on this further then to show you that they play a variety of roles.

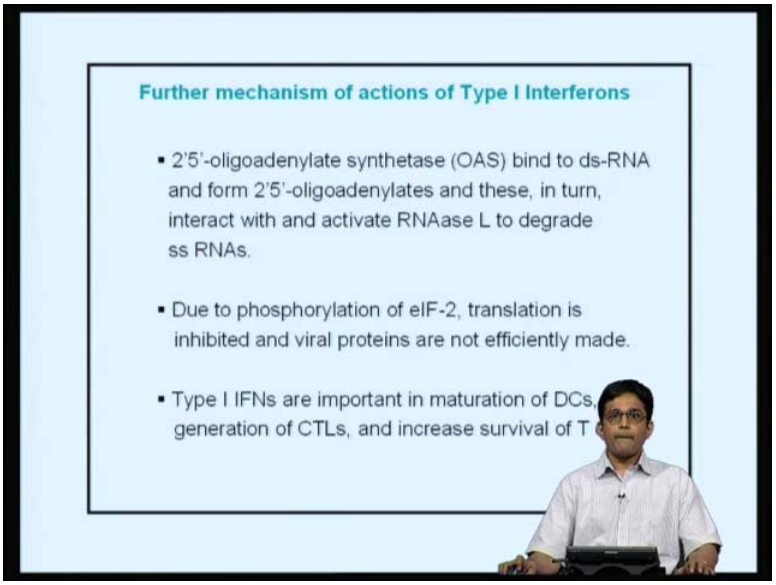
(Refer Slide Time: 20:54)



In cytokines, important cytokines are known as interferons. Interferons originates from the word interfere; and, interfere, because interferons will discover to interfere with viral

replication. So, in terms of anti-viral immunity, the interferons are known to play an important role. There are two main types of interferons: type I which is IFN-alpha beta, IFN-alpha, IFN-beta; or type II, which is interferon gamma. And, the type I interferon, which is interferon alpha beta is involved primarily in anti-viral immunity. It has other function, but its main role is very well-known to play an anti-viral role. So, how does the type I interferon function? There are several mechanisms that are known. One of the important mechanisms is we are the production of MX GTPases. What these GTPases do is, they inhibit transcription in one case, but more importantly, they inhibit viral assembly. So, they interfere with the transport of viral capsids; they also sort them to locations, where they are not available for assembly. So, the GTPases prevent or slow down a viral assembly.

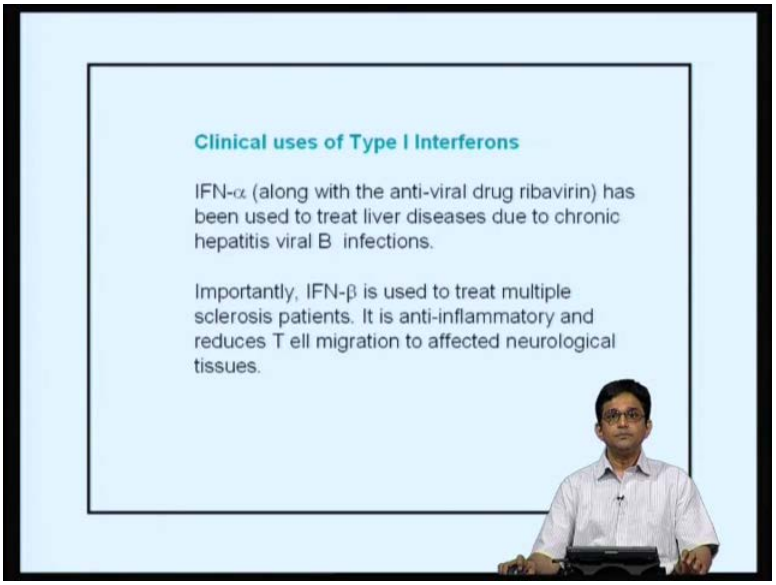
(Refer Slide Time: 22:23)



The other main way is through the production of this enzyme known as 2 5-oligoadenylate synthetase. What this does, it binds to double-stranded RNA and form this 2 5-oligoadenylates. So, they adenylate these adenylation and as a result of which, this in turn, activates RNAase L, which degrades single-stranded RNA. As you will remember, that these are important during production of viruses and often also for replication of viruses and transcription viruses. So, this is one way by which it acts in the anti-viral manner.

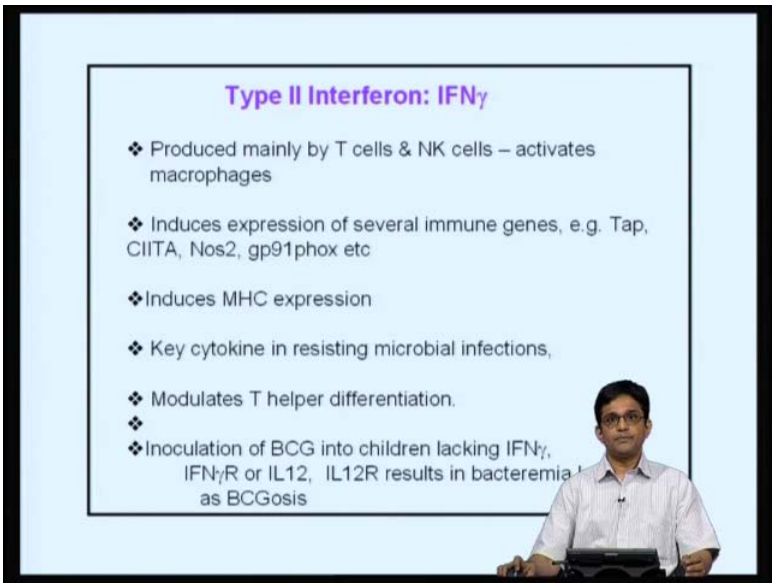
The other way is, there is phosphorylation of eukaryotic initiation factor-2. As a result of which, translation is inhibited and viral proteins are not efficiently made. Apart from their strict anti-viral roles, type I interferons are now shown to be important in other processes in modulating host immunity. One of which is the in maturation of dendritic cells, generation of cytotoxic T lymphocytes. And, in some cases, they have been shown to increase the survival of T cells.

(Refer Slide Time: 23:41)



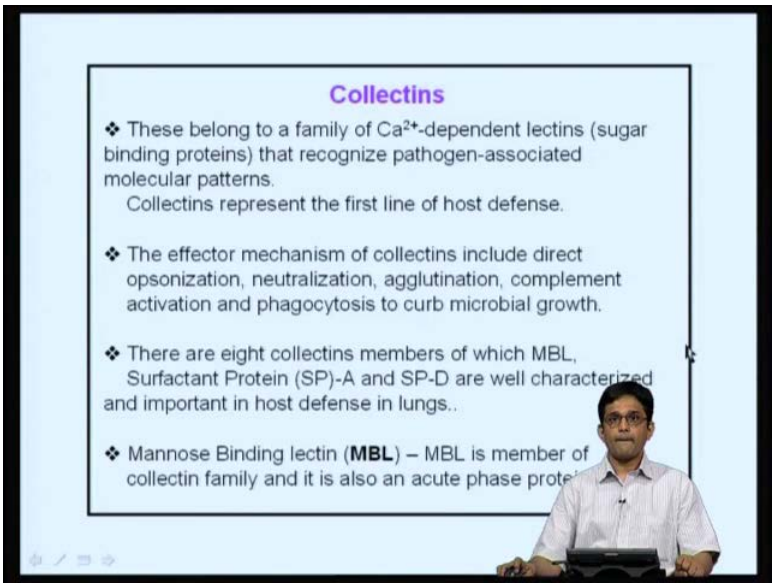
Type I interferons are of use clinically, for example, type I or interferon-alpha along with the anti-viral drug ribavirin is used to treat liver diseases with chronic hepatitis B infections. The second case is interferon-beta is used to treat multiple sclerosis. Now, how does it do? In this particular case, interferon-beta is anti-inflammatory and reduces T cell migration to affected neurological tissues. Also, it increases the production of anti-inflammatory cytokines.

(Refer Slide Time: 24:22)



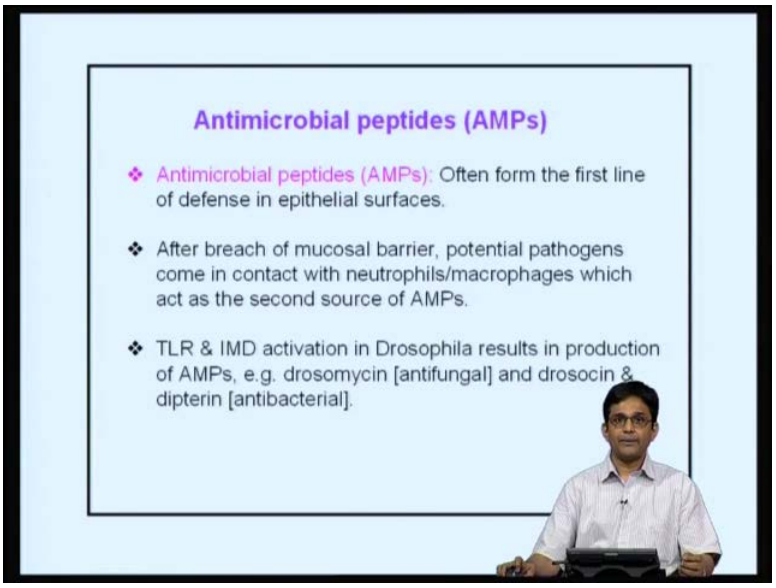
The other interferon is the type II interferon: interferon gamma. Now, interferon alpha beta is produced by all different types of cells; whereas, interferon gamma is produced mainly by T cells and natural killer cells. And, what interferon gamma does is, it activates macrophages. And, the way interferon gamma functions is primarily through induction of expression of several immune genes, for example, transport associated with (( )) processing, C II TA, which is an important transcription factor for MHC class II, Nos2, gp91phox; Nos2 is important in the production of nitric oxide; gp91phox is important in production of superoxide radicals. And, these two are one set; we will see a little bit later. Interferon gamma is a potent inducer of MHC class I and MHC class II expressions. Once the MHC molecules are increased, the chances of peptides that derived from pathogens are also increased, because overall, the production of MHC molecules increases. This is especially important during inflammatory conditions during infections. It is a key cytokine in resisting microbial infections and it modulates a T helper differentiation. Perhaps, the most important role of interferon gamma is seen in patients, where inoculation of BCG, which is a live vaccine given to prevent tuberculosis. And, if this live vaccine is given to children that lack interferon gamma interferon or its receptor IL12 or IL12 receptor, it results in bacteremia known as BCGosis. So, one of the primary roles of interferon gamma is in boosting up of immunity against intercellular pathogens.

(Refer Slide Time: 26:24)



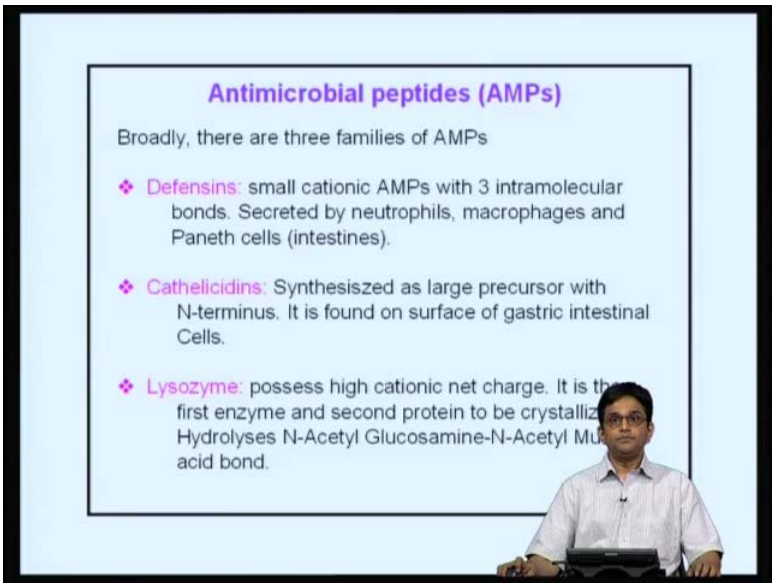
There are other types of molecules and we will briefly mention or go over the different other types of molecules. One of which are the collectins. And, these are calcium-dependent lectins. What do you mean by lectins? These are sugar binding proteins that recognize pathogen-associated molecular patterns. And, these are important, because they are involved in direct opsonization. Opsonization – if you remember, opsinization is the process by which there is enhanced phagocytosis of opsonized bacteria or microbes; neutralization, agglutination, complement activation and phagocytosis to curb microbial growth. There are different collectin members. And, if you remember, the mannose binding lectin is an acute phase protein; that means it is produced rapidly during the inflammation; and, it is also important for complement activation, is a member of the collecting family. Two of the member of collectin family, namely surfactant protein-A and surfactant protein-D are well characterized to play an important host response in the lungs.

(Refer Slide Time: 27:40)



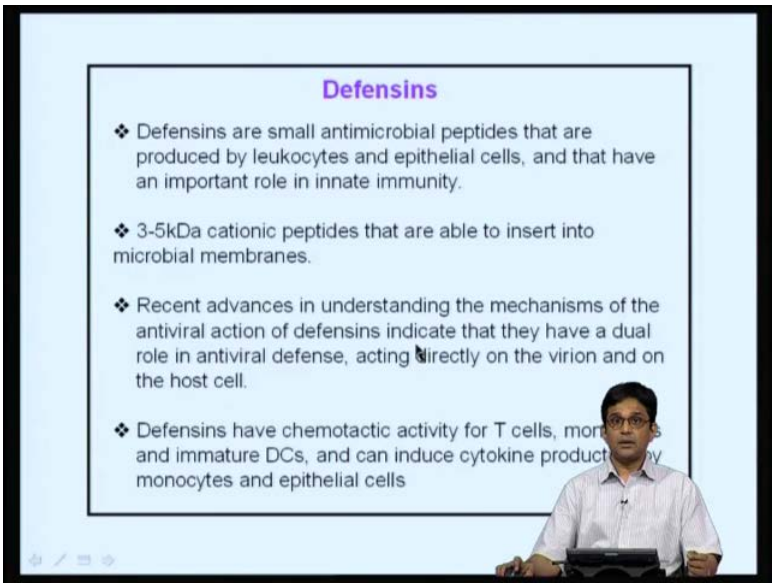
So, these are ways by which these are different proteins that are produced by the host in order to be able to tackle different types of microbes, because we are constantly under attack. We have discussed antimicrobial peptides in the previous class and we will again (( )) were some important aspects of it. These represent one of the first line of defense in epithelial surfaces and this is especially true in the intestine or the gut (( )). And, you will also remember that TLR and the IMD pathway activation in drosophila results in production of antimicrobial peptides, for example, drosomycin, which is antifungal, and drosocin and dipterin, which is antibacterial. TLR activation is thought to increase cytokine production in others, in mammals, but in case of drosophila, the main role has been shown to be in production of anti-microbial peptides.

(Refer Slide Time: 28:28)



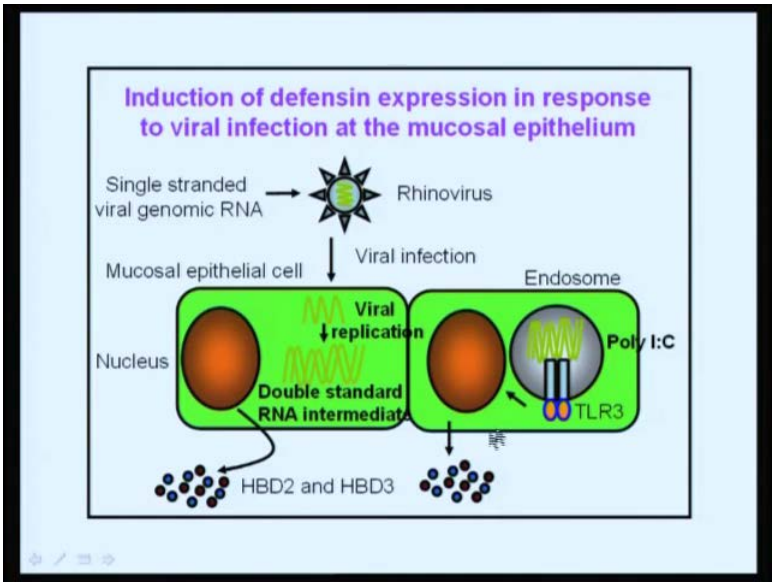
There are different families of antimicrobial peptides. You have defensins, which are small cationic antimicrobial peptides and defensins are produced by neutrophils, macrophages and Paneth cells. Paneth cells are present in the intestine and they are potent sources of defensins. Cathelicidins – (( )) they are produced as large precursors, and then, they are trimmed to produce these antimicrobial peptides; and, they are found in the surface of gastric intestinal cells. The other one is lysozyme. And, lysozyme as you should know, it is the first enzyme and the second protein to be crystallized. It hydrolyses N-acetyl glucosamine and N-acetyl muramic acid bond, which is present in several bacteria. Lysozyme is present in our tear secretions and other fluids. And, it helps in cleavage of bacteria or lysing of bacteria in a nonspecific manner.

(Refer Slide Time: 29:25)



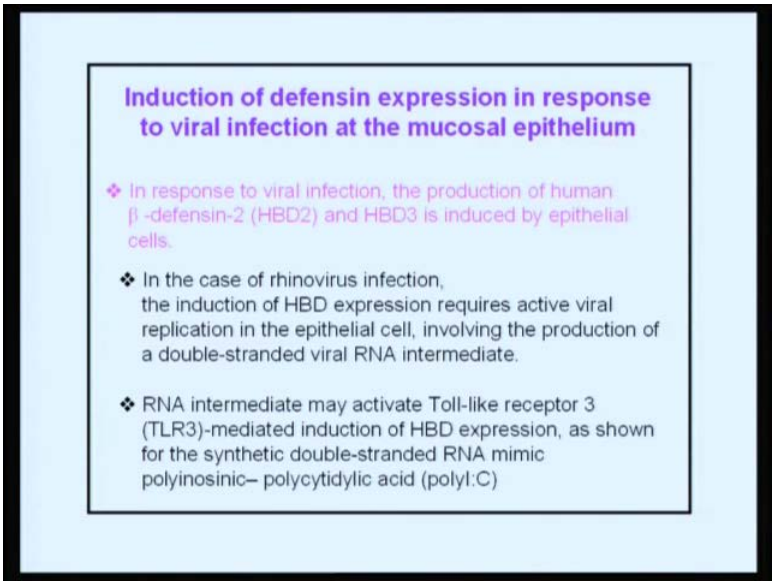
Once people started working on defensins, what was shown is that these are small peptides and they are able to insert themselves into microbial membranes and cause their lyses. What has been shown is that their direct antimicrobial action was well-known. What people are beginning to appreciate now is that defensins also play a role in host immunity. So, for example, in anti-viral defensins, they act both on virus as well as on the host cells. They have been shown to have chemotactic activity for T cells, monocytes, immature dendritic cells, and they induce cytokine production by monocytes and epithelial cells.

(Refer Slide Time: 30:24)



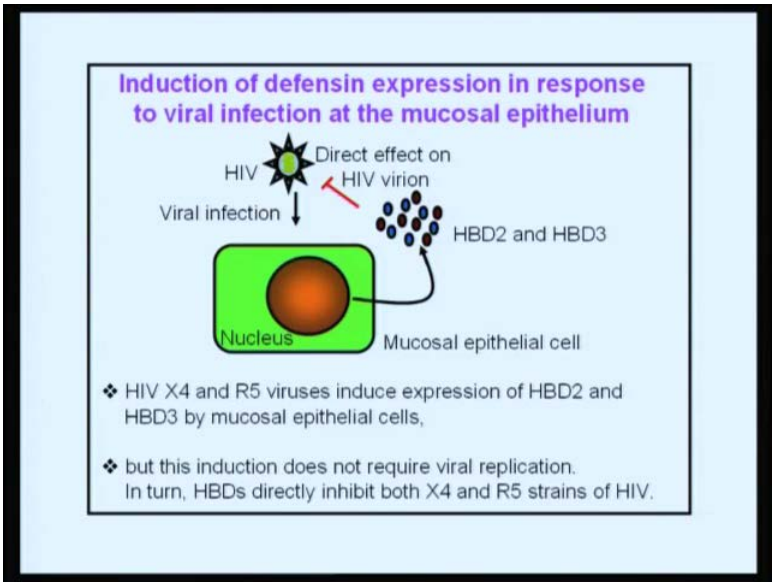
So, this is an example to show you the role of human beta defensins during rhinovirus infection. So, over here, you have rhinovirus infection. It is affecting this mucosal epithelial cells and this is produced in response to double-stranded RNA intermediate that is present. Instead of the double-stranded intermediate, you use poly I:C and activation TLR3. That also results in the production of human beta defensins 2 and 3. So, essentially that was shown.

(Refer Slide Time: 31:01)



Whatever I said over there is written over here.

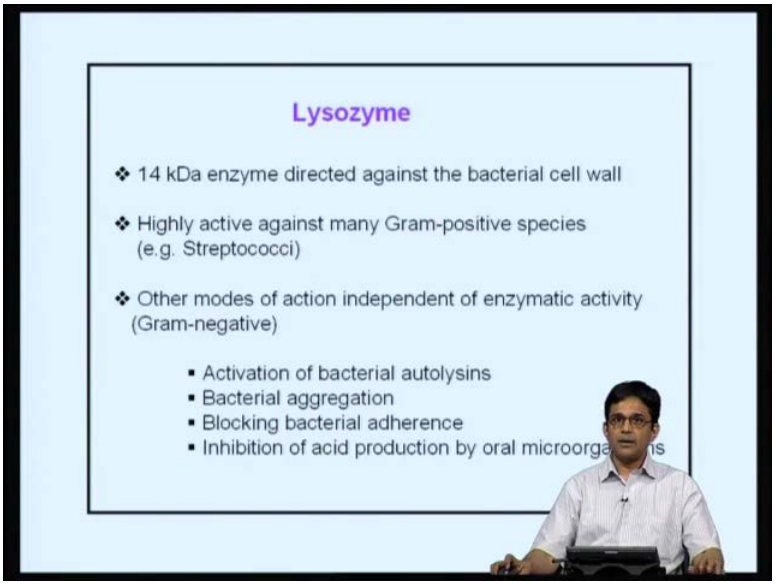
(Refer Slide Time: 31:08)



But, in case of another viral infection (( )) In this case, HIV, the production of human beta defensins 2 and 3 does not require viral replication. So, you know situations, in which, in some virus, you need viral replication for production of these defensins; in other virus, you do not need these. Nevertheless, they play an important role in anti-viral immunity.

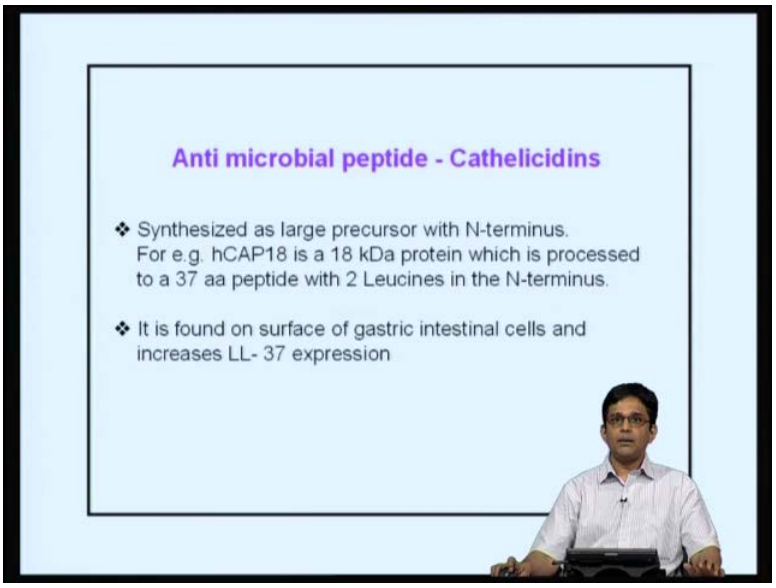
As I was saying so, the production of human beta defensin in 2 and 3 in some cases are (( )) the production of an RNA intermediate and in some cases, (( )) viral replication; and, whereas, in other cases, it does not require. Nevertheless, the defensins play an important role in antimicrobial immunity and this was an example to show you a role of these.

(Refer Slide Time: 32:52)



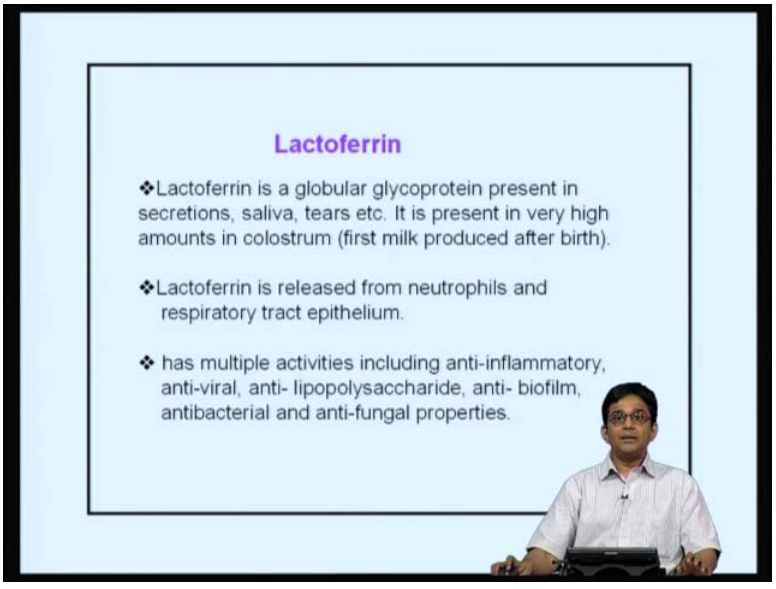
This is a lysozyme. Again, it is highly active against gram-positive species mainly because of the part that it cleaves the muramil peptide bond, is present primarily in gram-positive. And, there are other means also; they activate bacterial autolysins. They result in bacterial aggregation and so on. So, they play important antimicrobial roles.

(Refer Slide Time: 33:19)



The other ones are the cathelicidins. Remember, the cathelicidins are produced as larger precursors and they get trimmed down over here. And, cathelicidins play an important role in the intestinal lumen and they are present on the surface of gastric intestinal cells.

(Refer Slide Time: 33:38)

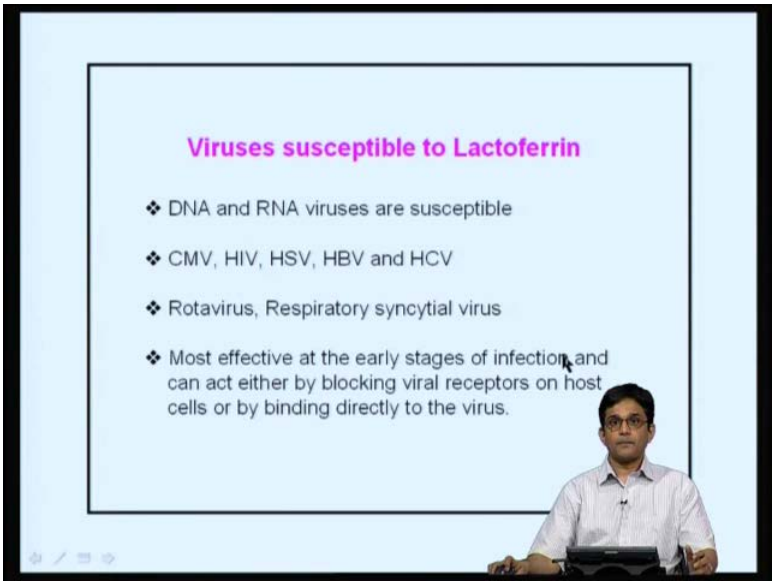


Lactoferrin is a globular glycoprotein and it is present in secretions of the saliva, tears, etcetera. It is present in highest amounts in colostrums. So, why is colostrum so important? Colostrum is important because it is the first milk that babies drink after birth. And, it is possible that lactoferrin is playing an important antimicrobial role because it is

helping the baby in fighting immunity; because once the baby is born, they are most susceptible; they do not have immune system of their and they rely a lot in the mother; their immune response from their mother. So, perhaps, for the initial few months, lactoferrin may be playing an important role, because it is present in very high amount in colostrums, which is in the first milk that is produced after birth.

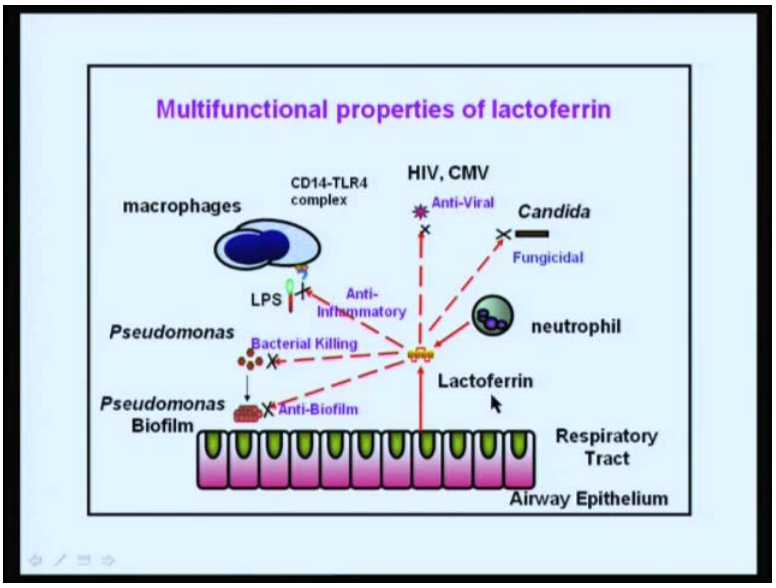
Lactoferrin is also released from neutrophils and respiratory tract epithelium. It has multiple roles; it is anti-inflammatory, anti-viral, anti-LPS, anti-biofilm. And, biofilm is important because what happens in some cases, bacteria form from these film-like structures. So, they form a sort of a colony of their own in different tissues. And, these biofilms are highly resistant to antimicrobial drugs. So, lactoferrin has this anti-biofilm property, which is very useful.

(Refer Slide Time: 35:04)



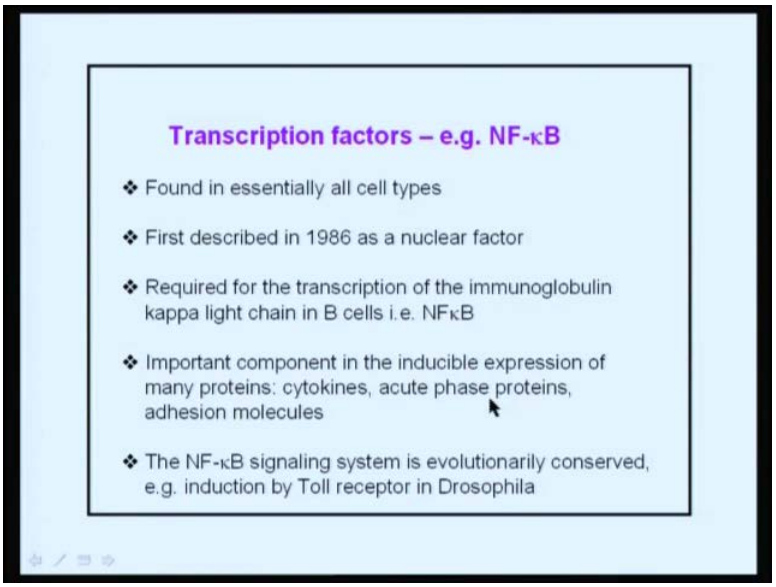
This is the list of different viruses that are susceptible to lactoferrin.

(Refer Slide Time: 35:12)



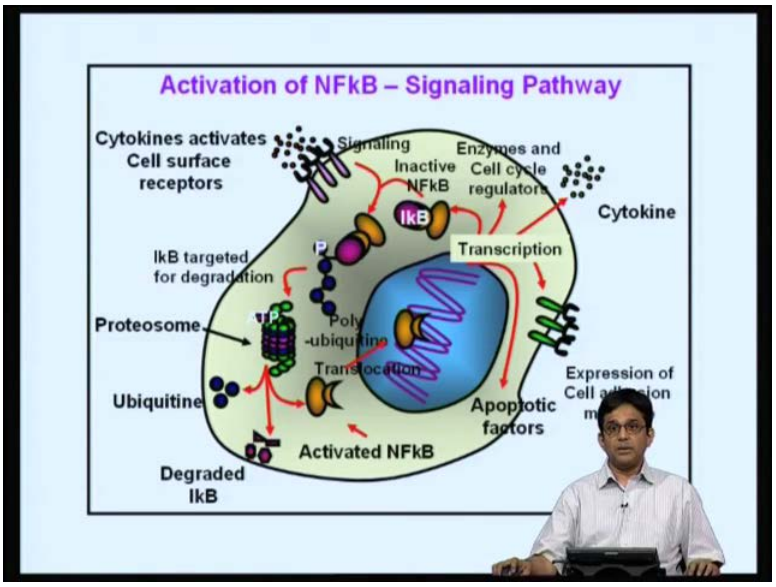
And, this slide summarizes the different roles of lactoferrin. So, basically, it has its fungicidal, which means like for example, shown against candida; it has anti-viral roles; HIV, CMV shown over. It has anti-inflammatory, because it is anti-LPS. It is also important for bacterial killing. And, most importantly, it has anti-biofilm properties, which make it important, because you can see that this is a film-like structure that the microbes are sort of developing, which makes them very resistant to treatment with antibiotics, and so on. So, the anti-biofilm property of lactoferrin is very useful.

(Refer Slide Time: 35:56)



During the studies on the signal transduction of TLRs, we had shown importantly, the activation of NF kappa-B. This NF kappa-B and NF kappa-B equivalent in drosophila is the signal transduction cascade, is almost similar. So, for toll activation and toll-like receptor activation in mammals, NF kappa-B is playing an important role. So, you can see the conservation of both the receptor, the signal transaction and especially an important transcription factor like NF kappa-B. And, it tells you about the conserved signaling and processes during innate immunity in lower organisms as well as higher organisms, such as mammals. So, NF kappa-B was first described as a nuclear factor a long time back. At that time, it was shown to be important in transcription of the immunoglobulin kappa chain in B cells, and hence, the name NF kappa-B. Subsequently, it has been shown to be important in production of wide variety of molecules, especially those related to a inflammation. So, for example, in production of cytokines, acute phase proteins, adhesion proteins, NF kappa-B plays an important role.

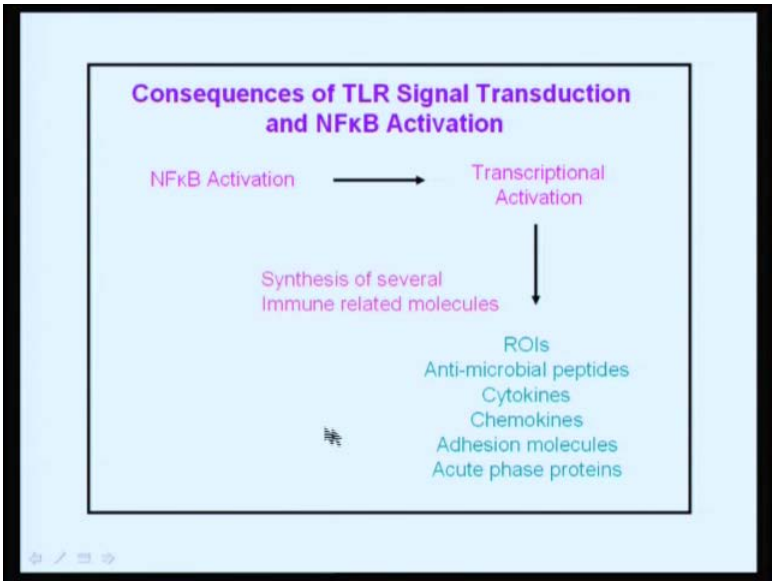
(Refer Slide Time: 37:30)



What is shown over here is the signal transduction pathway. So, NF kappa-B is usually present in the cytosol and it is associated with another protein known as inhabited kappa-B, which is shown over here. So, you have NF kappa B and I kappa B in this complex. Upon signaling, what happens is that there is ubiquitinization of inhabited kappa-B and degradation of the inhibitor. So, as a result of which, this degradation is (( ))26 as proteosomal pathway. And, you have over here, what is shown as the activated NF kappa-B. Once NF kappa-B is activated, it can now go from the cytosol into the nucleus,

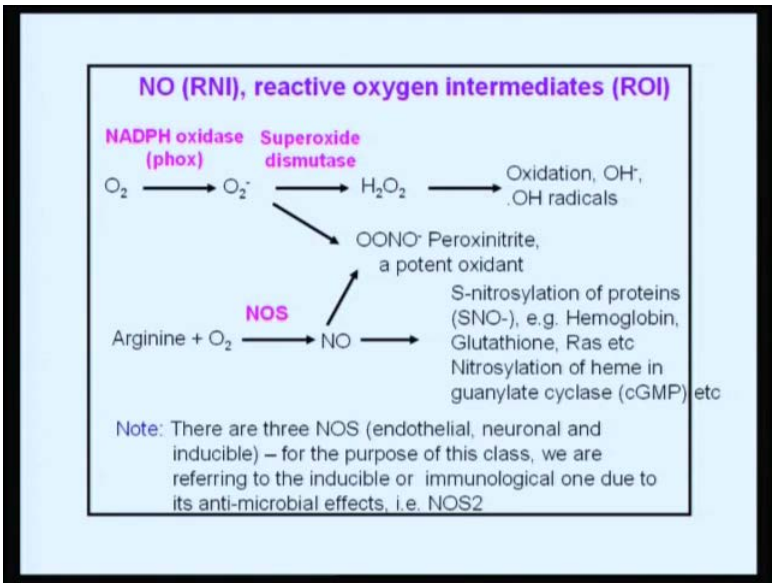
where you can bind to its particular cognate binding sites in front of promoters and turn off transcription of several genes. And, what is shown over here is apoptotic factors, cytokine, cell cycle regulators, so on and so forth. So, this pathway of activation of NF kappa-B is important. And, it is very important for students to understand this particular pathway. Remember, the NF kappa-B activation pathway is again conserved between drosophila and mammals. And, the signals are also conserved for this activation, which is the toll and the IMD pathway resulting in activation of drosophila NF kappa-B; whereas, in mammals, it is the TLR (( )) of the NF kappa-B pathway. And, it results in a wide variety of responses; a very important concept for students.

(Refer Slide Time: 39:06)



So, this is shown over here; NF kappa-B results in transcriptional activation. And, you have synthesis, are now of several immune related molecules. You have reactive oxygen intermediates, antimicrobial peptides, cytokines, chemokines, adhesion molecules, acute phase protein and so on. This is just a partial list, but NF kappa-B is a super transcription factor. It turns on several different molecules.

(Refer Slide Time: 39:30)



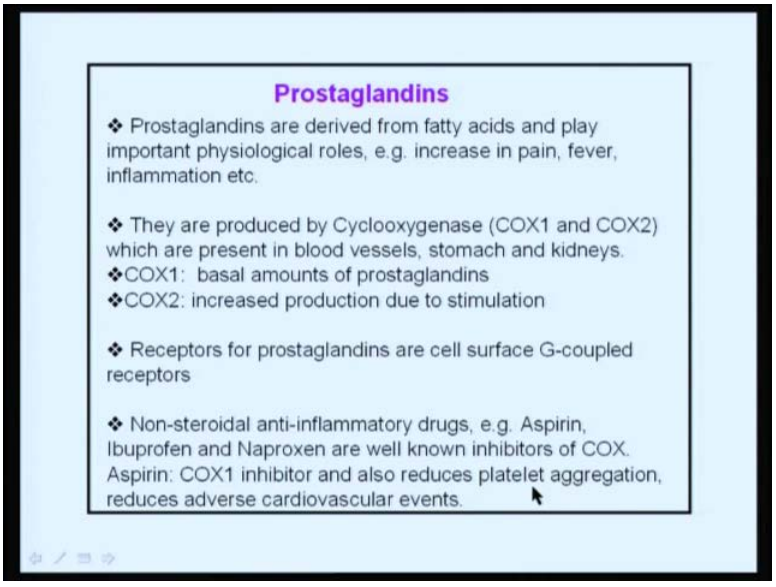
One of the ones that was shown over there was the induction of reactivate oxygen species. And, what I will do here is to discuss the importance of reactive oxygen and nitrogen species in innate immunity. Over here, ROI and RNI play a very important role in innate immunity. And, the cells that are mainly responsible for these are the neutrophils, which rely mainly on the reactive oxygen intermediates and macrophages, which rely mainly on the reactive nitrogen intermediates.

However, the two pathways can converge together to form a potent oxidant, which is peroxinitrite; and, I will go over this a little bit slowly. So, you know, you have oxygen, which is converted by NADPH oxidase. Now, NADPH oxidase needs to be assembled in the membrane upon activation. Once it is assembled, it can form superoxide. And, the superoxide is highly potent; it can be converted into hydrogen peroxide or it can combine together with nitric oxide to form peroxinitrite, which is potent oxidant. And then, the hydrogen peroxides can go on to form hydroxyl radicals and these will be part of the reactive oxygen intermediates. The other ones are the reactive nitrogen intermediates. And here, for the production of nitric oxide, arginine is the nitrogen donor; it combines over here. And, you have nitric oxide synthase, which is responsible for the production of nitric oxide. Now, in the body, there are three main types of nitric oxide synthase. You have the e NOS, which is the endothelial NOS; then neuronal NOS (n NOS); and then, you have the inducible NOS.

For the purpose of this class and especially with respect to the immune system, what we are referring to, is the inducible or immunological one or the i NOS or commonly known as NOS2, which is shown over here. Now, what happens with nitric oxide is, it can combine as shown over here with superoxide to form a peroxinitrite, which is a extreme potent oxidant. But, nitric oxide on its own has a lot of roles too. The main one that was initially shown was activation of guanylate cyclase resulting in the production of cyclic GMP; and, this cyclic GMP will have its own roles. And, the activation of guanylate cyclase is because of nitrosylation of heme, which is present in the active side of this particular enzyme. The other way by which the nitric oxide function is by S-nitrosylation of proteins; so, SNO, which is cysteine and then SNO. So, that is how it nitrosylates different proteins, for example, hemoglobin, glutathione, Ras signaling proteins, so on and so forth. So, this is an important way by which the reactive nitrogen intermediates and the reactive oxygen intermediates play an important role.

And, remember, (Refer Slide Time: 42:45) these are potent molecules and they would kill different microbes that are ingested. And often, the cells also themselves get killed, and which are then subsequently cleared off by the process of phagocytosis. So, the cells sort of not only kill microbes, but they might get themselves killed, because of production of these radicals. And then, ultimately, the body is saved, because of the fact that the microbes are killed. And then, the debris and all are cleared off later.

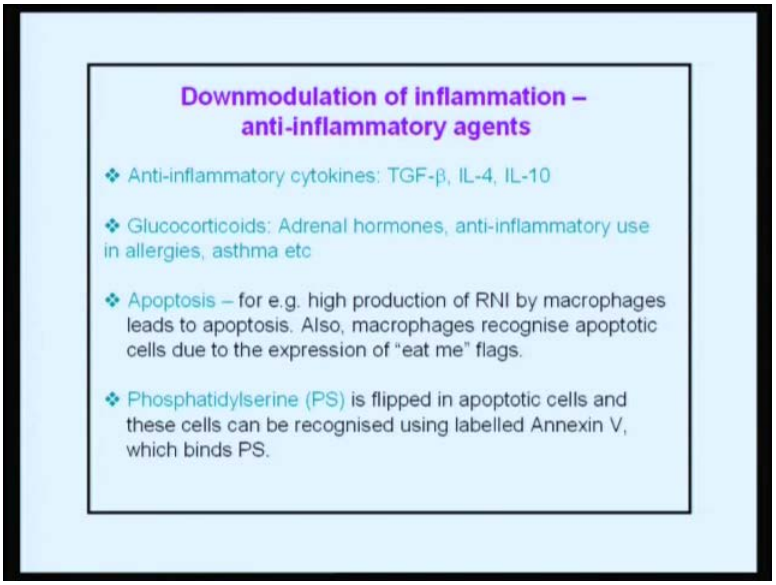
(Refer Slide Time: 43:17)



And, important molecules in the inflammatory process are prostaglandins. And, they are derived from fatty acids; and, arachidonic acid is an important one and they play important physiological roles, for example, pain, fever, inflammation, so on. The enzyme that is responsible for production of prostaglandins is cyclooxygenase – you have COX1 and COX2; and, which are present in blood vessels, stomach, kidneys. COX1 is responsible for the basal production of prostaglandins. And, the increased production due to stimulation is done by COX2. The receptors for prostaglandins are cell surface G-coupled receptors.

Now, the importance of the prostaglandin comes into focus by the use of non-steroidal anti-inflammatory drugs, which several of us take whenever we have head ache, pains; we take Aspirin, Ibuprofen, Naproxen, so on. These are belonged to the category of NSAIDs. And, the way it functions is that they are inhibitors of COX. Now, Aspirin is particularly important, because not only it is an inhibitor of COX1, it also reduces platelet aggregation as shown. And, this reduces adverse cardiovascular events. And, for heart patients, often the doctors prescribe Aspirin. And, now, you know, what the mechanism of action is, because it reduces platelet aggregation and reduces the risk of adverse cardiovascular events. So, Aspirin has two roles: one is COX1 inhibitor and also reduction of platelet aggregation.

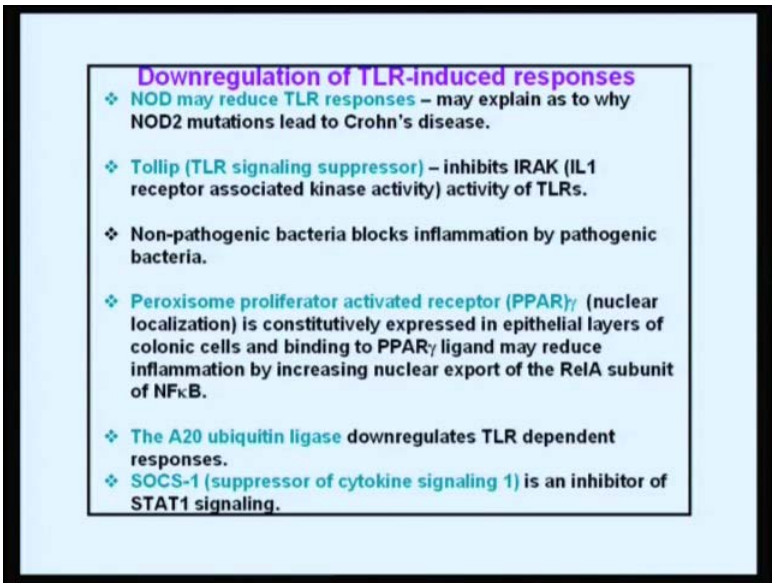
(Refer Slide Time: 44:58)



Now, once you have the inflammatory process, anything that goes up needs to come down, because remember, if the activation keeps on in sustained manner, it results in problems to the cells and to the host. Sepsis is a good example of that, where you have so much activation of immune cells that it becomes very difficult to control the process, and ultimately, it leads to multiorgan failure and death. So, in the immune response, whatever goes up, if the immune response is induced, it needs to have some regulatory mechanisms by which it can be reduced. And, this brings to us an important aspect, which is downmodulation of inflammation or downmodulation of the inflammatory response. So, with inflammation, anti-inflammatory processes are also induced. For example, anti-inflammatory cytokines have TGF-beta, IL-4, IL-10. These are well-known anti-inflammatory cytokines.

Some hormones also play an important role, for example, glucocorticoids. And, these are produced by the adrenal glands and they are anti-inflammatory; they are useful in case of allergies, asthma and so on. In case of heightened immune reaction as observed in case of autoimmune disease, sometimes, glucocorticoids are also prescribed. The other way – once you have debris, they need to be cleared off, is apoptosis. So, macrophages are often responsible for recognizing apoptotic cells, because of the expression of eat me flags. So, these cells that are undergoing apoptosis, express certain cells of those receptors. These are recognized and then they are phagocytose by macrophages. One of the eat me flags is phosphatidylserine, which is usually present inside; but, in the apoptotic cells, it is present on the cell surface. And, this can be recognized using Annexin V, which binds to phosphatidylserine. Now, this particular assay is useful in detection of apoptotic cells. So, if you wish to look at cells undergoing apoptosis, often you can look at the surface expression of Annexin V, which is a marker for apoptotic cells.

(Refer Slide Time: 47:29)

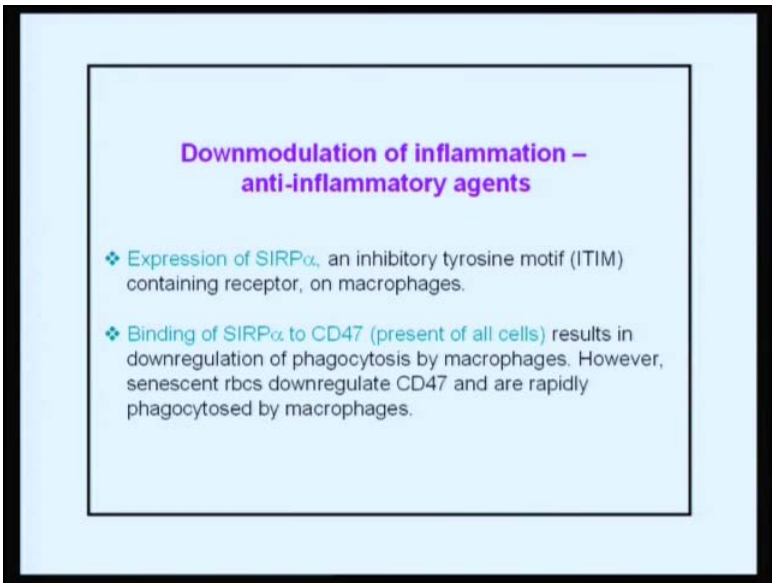


Now, in terms of TLR induced responses, again there are different mechanisms in place to reduce the TLR activation, because microbial infection is often associated with TLR responses. So, initially I had shown you the role of NOD2 in regulation of TLR responses. And, it is possible that NOD2 mutations and may explain as to how NOD2 mutations lead to Crohn's disease. Now, another molecule shown, tollip; now, tollip was shown up in my previous classes lecture slides on TLR, but I did not explain it, because there was not sufficient time to explain each and every molecule. But, tollip inhibits IRAK and this IRAK activity is associated with TLRs. So, you have IRAK activities associated with TLRs; you have a regulator of this particular activity. So, you can reduce TLR activation. Non-pathogenic bacteria also reduce inflammation, and in fact, the block inflammation induced by pathogenic bacteria. You will remember that in our gut, we have lots of non-pathogenic bacteria. And, there are different mechanisms in the gut to take care of these huge load of bacteria that are living in the gut.

The peroxisome proliferator activated receptor PPAR gamma – now, peroxisome proliferator activated receptors are nuclear transcription factors, which get activated and they lead to wide variety of responses. In this particular case, peroxisome proliferator activated receptor, which results in increased production of peroxisome; and, that is why, the name peroxisome proliferator activated receptor. And, these might reduce inflammation; these have some role in inflammation. And, by increasing nuclear export of the RelA subunits of NF kappa-B. So, NF kappa-B goes into the nucleus once it is

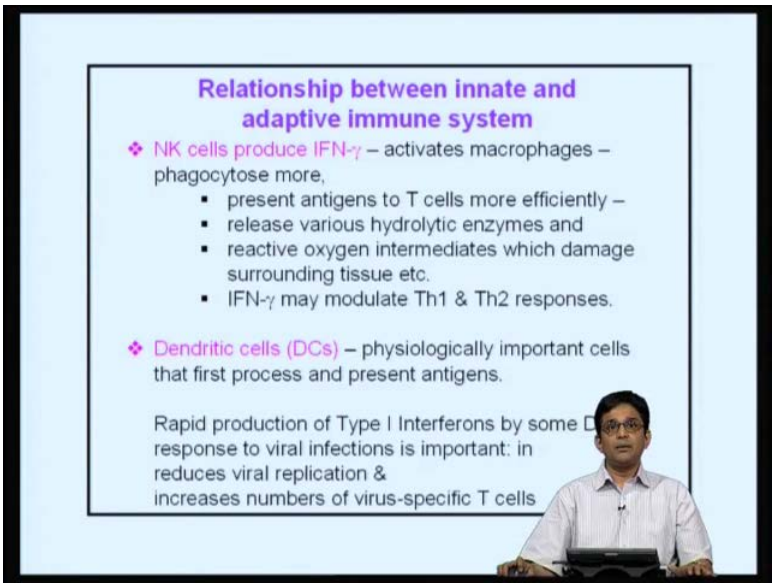
activated. And, what this could be going is that it is exporting this NF kappa-B back into the cytosol. And, by increasing the export of this, you are reducing activation. So, there are different mechanisms in place by which downmodulation of responses can occur. The other example is shown by the A20 ubiquitin ligase, which downmodulates TLR dependent responses. The other molecule, which is well-studied, is a SOCS-1, which is a suppressor of cytokine signaling. It is an inhibitor of the JAK STAT pathway, which is important in signaling we are the interferons.

(Refer Slide Time: 50:18)



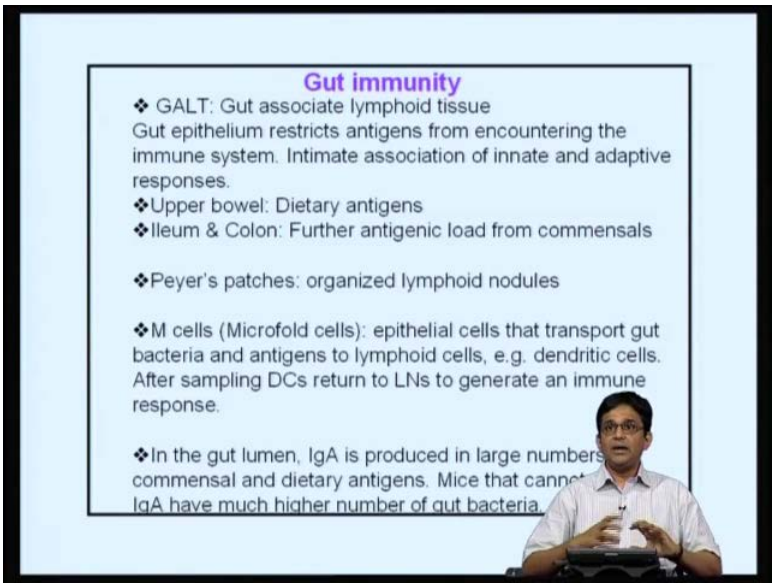
One of the examples of downmodulation of inflammation is the example that is shown over here, is the expression of SIRP alpha, which is an inhibitory tyrosine motif containing receptor, on macrophages. Now, usually, binding of this to CD47 results in downmodulation of phagocytosis. However, as rbcs age, they downregulate CD45 and these aged rbcs are rapidly phagocytosed by macrophages. So, this is an example again of phagocytosis; about molecules that play an important role in phagocytosis; and, how you can downmodulate the responses.

(Refer Slide Time: 51:02)



I will briefly now talk about the relationship between the innate and the adaptive immune system. The NK cells produce interferon gamma. These activate macrophages. And, once macrophages get activated, they will present and process more efficiently to T cells, and consequently, they will modify your T cell responses, which may be of Th1, Th2. And so, you can see a role, where NK cells, which play a role in innate immunity, are also modifying the adaptive immune response. The second one is dendritic cells. Some dendritic cells produce large amounts of type I interferons in response to viral infections. And, these have been shown to play not only an important role in reducing viral replication, but they also result in increased number of viral-specific T cells. So, again you it is a case by which a classical innate cell is modulating the adaptive response.

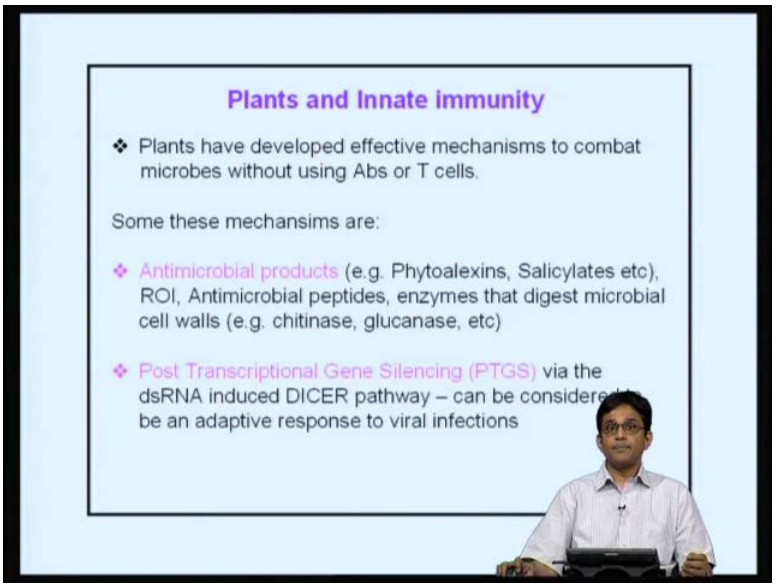
(Refer Slide Time: 52:00)



A fine example of immunity or this interrelationship between adaptive and innate is seen in the gut, because in the gut, you have huge numbers of bacteria in there. And, the gut plays an important role, because you have this gut-associated lymphoid tissue. And, the upper bowel has lesser number of bacteria, but it reacts to dietary antigens; whereas, the lower ones, in addition to the dietary antigens, you have huge commensal organism living there.

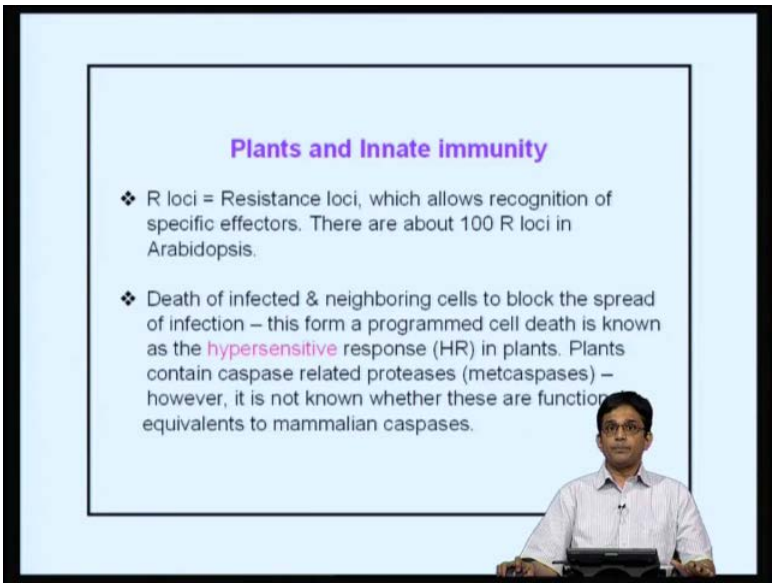
Over here in the intestine, you have specialized patches of organized lymphoid tissue known as Peyer's patches. And, among the important cells, which are responsible for uptake of antigens and then giving it to the dendritic cells and the other cells in the lymphoid tissue, are known as M cells. And, these are epithelial cells, but which play an important role in this process. The IgA that is present in large numbers in the gut lumen is also an important role. In fact, mice that cannot produce IgA have much higher numbers of gut bacteria. So, the gut immunity or the gut process is very important because of the fine interaction between innate and adaptive arms.

(Refer Slide Time: 53:25)



I will also briefly discuss how plants and innate immunity functions. So, what about plants? We have been discussing animals so far. So, before ending, I will just briefly mention the different mechanisms by which the plants also have it. So, plants are also susceptible to viruses, bacterial pathogens, so on. So, how do they deal with this? First, is by the production of antimicrobial products, for example, phytoalexins, salicylates, antimicrobial peptides, enzymes, chitinases. So, they also can shutdown transcriptions and they do so by the dicer pathway. So, it will turn transcription down.

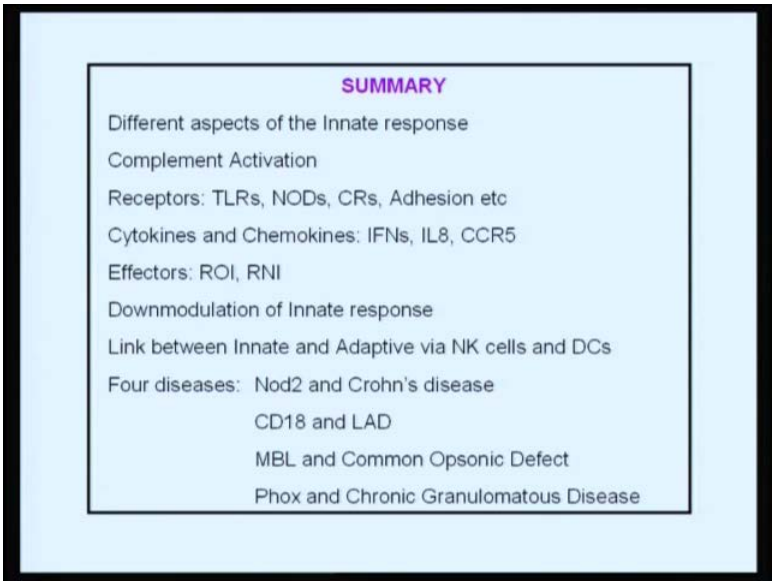
(Refer Slide Time: 54:06)



Then, you have what is known as resistance loci or the R loci, which allows recognition of specific effectors. Several of the R loci proteins contain LRRs, which are the leucine-rich repeat domains in their proteins, and which is an important mechanism. So, you can see that the R factors and the LRRs and the relationships, there is some commonality that one can see over here.

An important response by which plants take care is through the production of what is known as the hypersensitive response – which is, upon infection, the cells around that particular infected tissue die off. And, how they die off is not clear, but it is possible that plants are known to contain caspase related proteases and whether these are playing an important role in our needs for the studies. But, the hypersensitive response is clearly an important way by which plants handle the infection by pathogens.

(Refer Slide Time: 55:15)



I will now briefly summaries and just go over some of the main aspects that we have covered today. First is that what we have tried to do is, study different aspects of the innate response. You have the complement activation. We have looked at complement activation. There are different pathways. There are three different pathways: the classical, the alternate and the lectin pathway by which they can be activated. And, you have receptors that are involved; you have the TLRs; you have the NODs; you have the complement receptors; you have the adhesion receptor.

We also studied the important roles of interferons especially the type I, type II interferons. IL8, which is an important chemokine, which is an important neutrophil attractant; CCR5, which is important for HIV infections. Effectors – the reactive oxygen intermediates, the reactive nitrogen intermediates; here phox NADPH oxides is important for it. Reactive nitrogen intermediate – NOS2 is the key enzyme that is responsible for the production of nitric oxide.

We also looked at downmodulation of innate responses. The production of anti-inflammatory cytokines like TGF beta, IL-10, IL-4; and, you have further ways by which the production of a glucocorticoids, so that you can suppress immunity. You have different proteins for example, that will suppress immunity, for example, A20 ubiquitin ligase; the toll up, which inhibitor; or, SOCS-1, which is an inhibitor of the JAK STAT signaling pathway, which is important by which interferons signal. We also studied the link between the innate and the adaptive immunity; especially the role of NK cells in producing interferon gamma and modulating T cell responses. Some dendritic cells produce high amounts of type I interferons and which in turn affects the anti-viral activity and the production of anti-viral T cells.

Now, most importantly I would like to conclude with a study of four diseases; sort of review of these. NOD2 is an intercellular censor. It has been shown to play a role in… or it is associated with the Crohn's disease, which is an inflammatory bowel disease. So, mutations are not to result in increased inflammation of the bowel. It is possible that NOD2 is playing an important regulator of the T cell responses. CD18 is the common beta subunit. It is important for adhesion, leukocyte adhesion deficiency. Then, you have mannose binding lectin; common opsonic defect, which is quiet common and it is important in opsonization and complement activation; phox, which is NADPH oxidase and the production and its association with chronic granulomatous disease. So, if you do not have a NADPH oxidase or you have mutations in NADPH oxidase, which are not functional, you have chronic granulomatous disease, which results in recurrent bacterial infections.

Overall, what these two lectures – innate immunity, have shed light on the cells, the mechanisms by which our innate cells play an important role. So, they not only act as the border security force, but they are important in modulating the adaptive immune response. Thank you.