



The Sheet Team

Title of Lecture: Innate Immunity

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Refer to slide no. : None

Written by: Dalin Jihad

Today's lecture is about the immune system and its structure; how it's composed, the cells, the organs and some molecules seen in the immune system.

The first version of the immune system, the first army, is the innate, the natural immune response. Just a reminder, we said that stem cells are generated from the liver, go to the bone marrow where it develops. Then it will follow one of the two tracks, one stays in the bone marrow and develops to B-lymphocytes while the other will go to the thymus and develops to T-lymphocytes. As they mature, they'll leave the bone marrow and the thymus and go to the secondary organs of the immune system; the lymph nodes and the spleen where they'll be harbored in specific areas to produce a group of B-lymphocytes and T-lymphocytes. Their anatomical structure is very important. One in the paracortical area, the 2nd in the cortex, while the germinal center, which is respondent is in the middle. We'll see it later.

The specific immune response, the acquired response, is composed of lymph node, cells (B and T lymphocytes) and their product is antibodies and some other molecules. We'll also talk about the other part of the immune system.

Slide showing thymus: T-lymphocyte will mature there and leave to the lymph nodes and the spleen. Also, the B- lymphocytes will go there and both cells will produce the secondary immune response.

The immune response is divided into two: The Innate or the natural, again, we have certain characteristics, which are very important; we have chemical barriers, cells and physical barriers.

While the in acquired we have the humoral and the cellular immunity.

The innate immune system, we can divide the cell's immune response into 4 functions.

1. Inflammation (detailed well in pathology). The product of the inflammatory cells, like macrophages, eosinophils and neutrophils, and some of products are interferon, though not only interferon.
2. Natural killer cells, lymphocytes which are non-respondent; they have nothing to produce.
3. Complement system, which is also natural
4. Adaptive

The immune system is important; it is the main system which will try to keep the body from being invaded by organism whether they are viruses, bacteria, fungus or whatever it is.

The first thing that an organism has to do in order to disease the body is gain access to the body one way or another. The second thing is they have to attach to a cell or a membrane. Though, some grow in body fluid. There are certain receptors found on body cells which are not specific to a certain molecule, it's where the organism binds. Then, those cells must produce something to avoid this attachment to survive.

Microorganisms can enter the body by two ways, natural ways (inhalation, GI secretions [eating and drinking], urogenital organs [sexual contact] and mother to fetus) or the artificial ways (trauma, skin puncture [tattoos], injections, blood transfusion, organ transplantation, or animal/insect bites).

The natural ways to enter are all lined with mucus membranes and their secretions, which are extremely important factors; we'll talk about them later.

The natural immune system response is generally seen at three levels:

1. External levels
2. Internal levels
3. Skin, mucus membranes or secretions

How can we prevent the entrance?

1. Mechanically (tears [if something entered your eye], sneezing [smell something bad], urination [this process by itself cleans the urogenital organs], saliva and sputum [generally cleaning the upper respiratory tract because of mucus membranes], puking [if you eat something wrong], coughing, or movement of epithelial cells.

If mechanical ways are not enough to keep it out of the body, then we have a second line of defense.

2. Chemical Barriers. Skin is the largest organ exposed to the outside environment which contains a lot of characteristics. In the epidermis, which is very layer thick, we can see a few macrophages. Normally, the skin's acidic, having a pH of about 5. Organism cannot survive in acidic environment; they need neutral pH to do its job [infection]. If there is a crack, due to trauma, puncture or whatever it is that can penetrate the skins, then the macrophages gather in that area and we can clearly see them. So, in the skin there's salts, there's antimicrobial chemicals found in sweat glands under the skin and sweat gland product and fatty acids which makes the low pH of the skin.

Skin is the major part of difference between these chemicals. Chemicals are also found in the secretions [e.g. secretions in the nasopharyngeal area contains lysosomes, lysosomes are also found in tears, in mucus membranes, all over the GI tract and many other places]. These secretions will try to prevent the attachment of microorganism to membranes. If a secretion (saliva) covered the microorganism it will not be able to attach to the membrane.

In these secretions we have what we call the normal flora, which is extremely important. It will compete with pathogens to attach to membrane and the use of nutrients. So, it prevents pathogenic microorganism growth by taking away the nutrition supply. They lower the pH, help in resistance, line the immune defense and supply the body with vitamins. We coexist; they defend us and provide some chemicals that we need and we supply their nutrition. In normal conditions, the normal flora is not harmful and will not cause diseases, however; in abnormal condition this normal flora could cause very severe infection called opportunistic infection. We'll talk about it later.

So if the mechanical barrier cannot keep the microorganism outside the body and if the chemical barriers cannot keep it outside either, we'll talk about the attachment and entrance of these microorganisms into the body through the cells or through membranes, then we'll talk about the recognition and how it happens.

What kind of cells those are important in the defense against these microorganisms, non immune cells? Not T and B lymphocytes.

1. Neutrophils (macrophages)
2. Macrophages
3. Dendritic cells
4. Natural killers

Generally, these are the important cells which will react against foreign substances. Phagocytosis, which has a lot of chemicals that can destroy the foreign microorganisms as in neutrophils and macrophages. Dendritic cells also are important in producing certain chemicals. This step will destroy the foreign body by direct contact or producing an interferon (β and γ).

Once they recognize the cell, how do they recognize that these are from outside?

The microorganisms generally, have certain chemicals on their membrane and wall which we call Toll-like chemicals, and have what we call Pattern Recognition. Pattern means it is not specific, the order of the molecules on the bacterial membrane from outside. This pattern is recognized by the cells we already mentioned. All microbes have certain molecules, called Pathogen Associated Molecular Pattern, recognized by the non-immune cells. This could be:

1. Double stranded RNA
2. Lipopolysaccharide
3. Peptidoglycan

Structure on the bacterial wall could be one of those or a combination of those. This would be recognized by a receptor found on the cell, called pattern recognition receptor. This receptor drives the immune system and regulates the adaptive immune system. So, there is a connection between specific and non specific immune system through this reaction, the pattern and the receptor.

What you should know:

There is no difference between the microorganism's pattern, and this pattern will be recognized. The non-specific immune system will not recognize salmonella from streptococcus, it will only recognize the pattern they have as a foreigner. While the specific immune system can.

This is the concept of the immune recognition of the innate system, keeping what we said in mind:

1. It is non-specific
2. It activates and programs the adaptive immune system, which means it is the first line of defense.

This non-specific immune system can happen from within minutes or within one day, so a very rapid response. While the adaptive immune system needs one or two weeks to respond. The innate recognizes the pattern and the receptor; these patterns are categorized into groups.

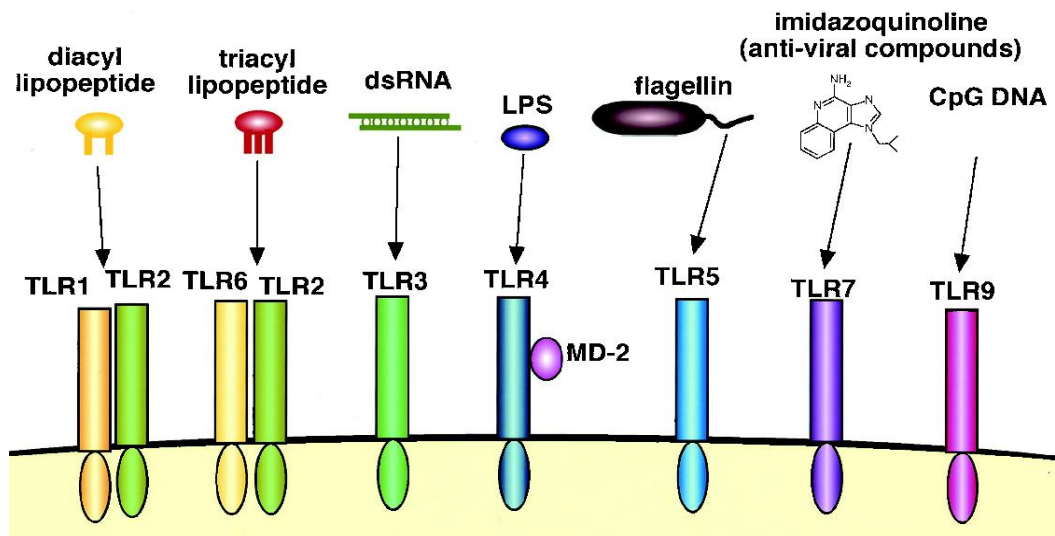
1. Mannose-Binding Lectin
2. Macrophage Mannose Receptor

3. Scavenger Receptors (found on macrophages)
4. Toll-like Receptors
5. Nod-like Receptors
6. DNA helicases

These are the receptors that recognize the pattern of the cell membrane.

If bacteria A entered the body, the innate immune system will react against it in a certain way. If the same bacteria entered again 10 days later, the system will react as if it was the first time against it. Every time it enters, the innate system will react exactly as if it was the first time it sees it. In conclusion, innate system has no recognition. During the destruction of the bacteria by the innate cell, the immune system will recognize this foreign body and develop a memory; this process needs a week to ten days.

Back to the pattern, it could be found in the soluble, cell membrane, endosome, or cytosol. So, they're not found in one compartment of the pathogen. We have about twelve different molecules for Toll-like receptors.



Refer to figure 1: all microorganisms have the same pattern, but one may have more TLR4 or one with low TLR1. In general it is the same pattern with different concentrations.

Figure 1: Toll-like receptors and recognition of pathogens

We have four different receptors which can be on the cell to recognize the pathogen. Receptors to detect:

1. Polysaccharide
2. Flagellin
3. Peptidoglycan
4. Bacterial nucleotide sequence

One each cell we have different type of receptors to detect what was mentioned above. After recognition, there will be a bind between the microorganism and the cell.

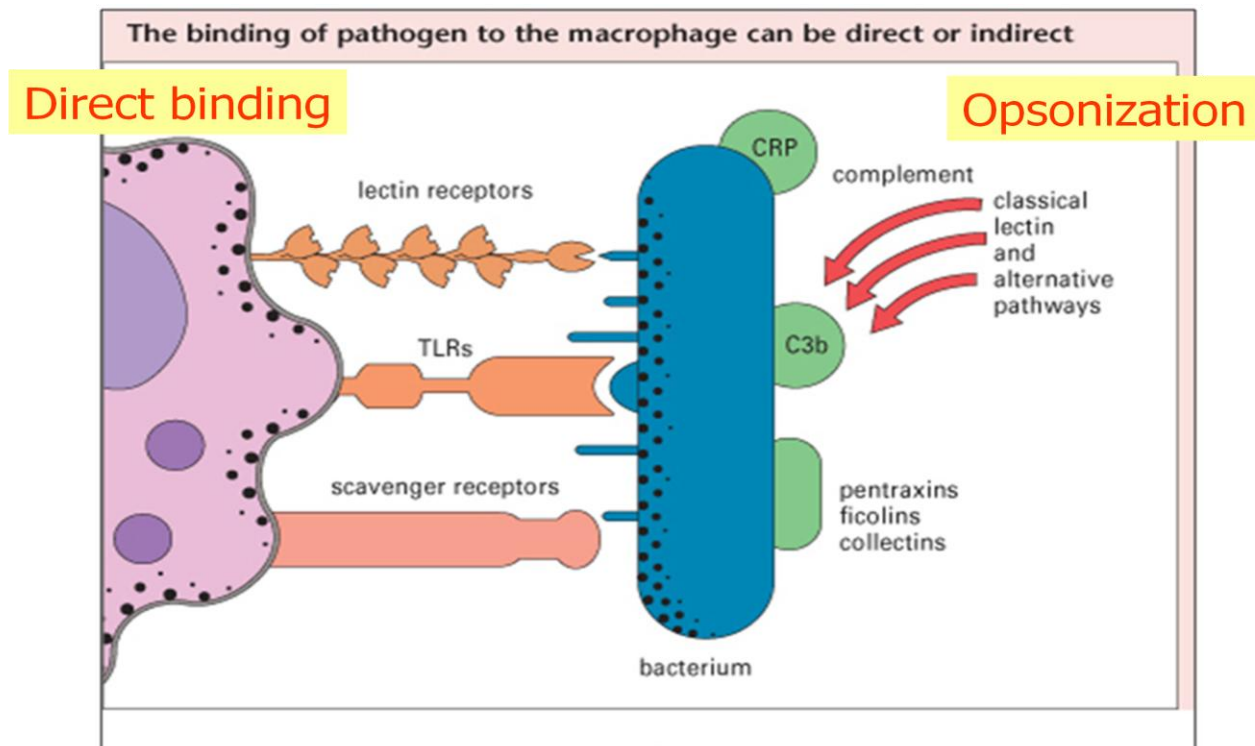


Figure 2: Recognition of bacteria by Macrophage

Scavenger means a broom; macrophages will clean the body from these things. For the reaction between the microorganism and the cells, generally, at least there has to be three receptors to bind to. Another thing we can find on the microorganism, there are certain molecules found in the secretions called opsonin; they'll cover the microorganism like another membrane. Once they're covered, cells won't recognize them and thus prevent binding between cells and microorganism. Unless the cell has receptors for the CRP or C3b (refer to figure 2) then the opsinized microorganism will bind to the cell. Opsonization can have a two way function; prevent the binding or increase the microorganism's binding.

When a reaction happens between the microorganism and the cells, there will be production of new molecules, called acute phase proteins [acute phase means immediately]. The liver will start to produce chemicals already present in the body in low concentration to reach a high concentration, like:

1. C-reactive protein
2. Ceruloplasmin
3. Haptoglobin
4. Fibrinogen
5. Alfa1 anti trypsin
6. Serum amyloid A

How will the liver produce them?

Because of the reaction between cells and the microorganism, the cells will produce new molecules called interleukins. These molecules will activate the hepatocytes.

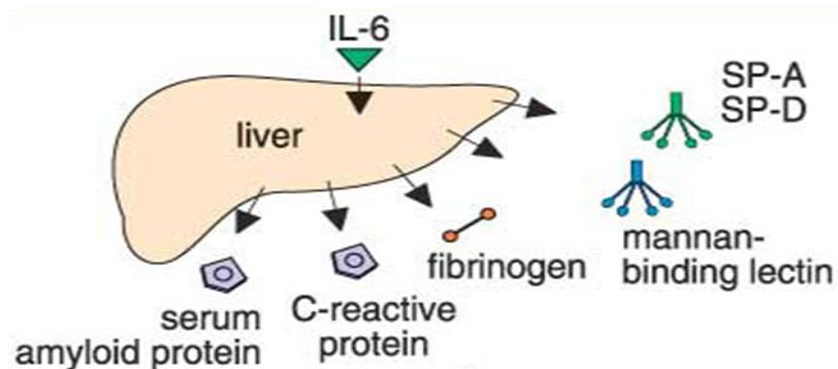


Figure 3: Acute Phase Proteins

main function mainly is activating the immune system and cells to kill the foreign body.

Let's go back to when the foreign body enters. If it entered through the normal way there is mechanical and chemical barrier.

What about the artificial way to enter? For example, through a penetrated skin. When the trauma happened, whether an infection, what will happen to the tissue and the cells?

There will be damage to the cells and the membranes in the dermis and epidermis of the skin. The normal flora present on the skin from the outside will go inside. When the normal flora changes its original position it will cause a disease. So, when microorganisms enter through the skin, they will have an influx of exudates, fluid coming there. The fluid carries chemicals like the IL-6 (interleukin 6) which will activate the inflammation at that area. Some of this exudative material could be chemotactic factors and call off the polymorphonuclear cells and later the macrophages. We will start to see some kind of granulation tissue, there'll be redness, swelling seen at the site of injury. There, the polymorphonuclear and phagocytes will start accumulate in that area.

How will they accumulate? The white blood cells are present in the circulation, but how will they leave the circulation and go to the site of injury?

Here comes the endothelial system of the blood vessels, they are who make the migration to the injury site possible. So again, an injury then production of molecules, some of those molecules can activate the endothelial system. We will start to see new molecule production in the endothelial cells which were not there. These molecules are called E- selectins. Another

molecule which is part of the membrane is CD-15, and we have an injury they will react with CD-25. Because they are in the endothelial system and have receptors to bind to, they'll trap the polymorphonuclear. When they're trapped, another signal is produced to get the chemokines to come to the injury site. These will attach to the polymorphonuclear; adherence will start to be seen. This adherence is not enough, so they'll activate more attached molecules to activate integrins which will react with the adhesion molecules.

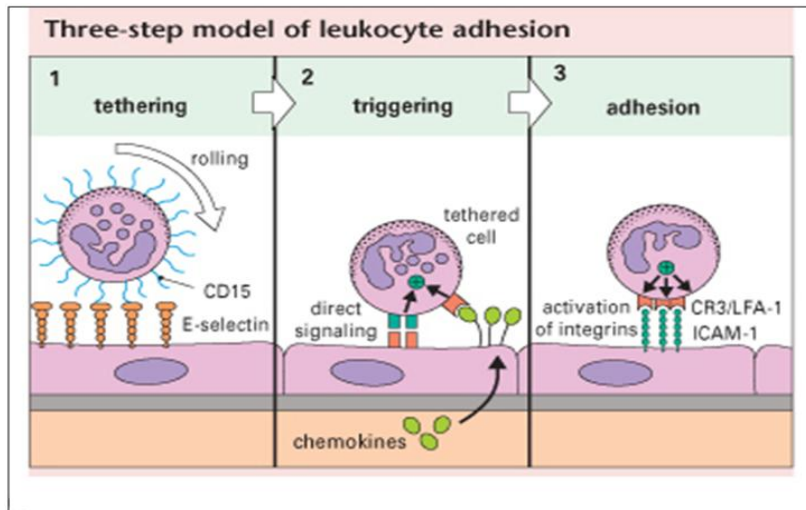


Figure 4: Adhesion molecules & chemokines control leukocyte migration

So we have tethering [means production of more molecules], triggering [trigger the reactions] and then we'll have adhesion to the endothelial membrane. Figure 4. In the end, there will be production, attachment then adhesion molecules which will increase the permeability of the endothelial giving the white blood cells access to migrate from the blood

to the injury site where they'll do their function. Their function is to invite molecules, react with receptors and

phagocytose. Along with this, we have c-reactive proteins will be produced which will activate the phagocytic receptors to react with the microorganism very strongly. Summery figure 5.

Macrophages are first discovered by Mechnikoff.

Where are the macrophages?

There are free and fixed macrophages. Free macrophages are present in the blood, synovial fluid, and in the peritoneum. Fixed are present in the kidneys, liver, lungs, brain, bone marrow and other tissues. These macrophages all of them almost do the same function, which is inducing local inflammation because of molecules they had been produced to invite them, they'll phagocytose, activate coagulation, enhance antigen presentation and initiate tissue repair.

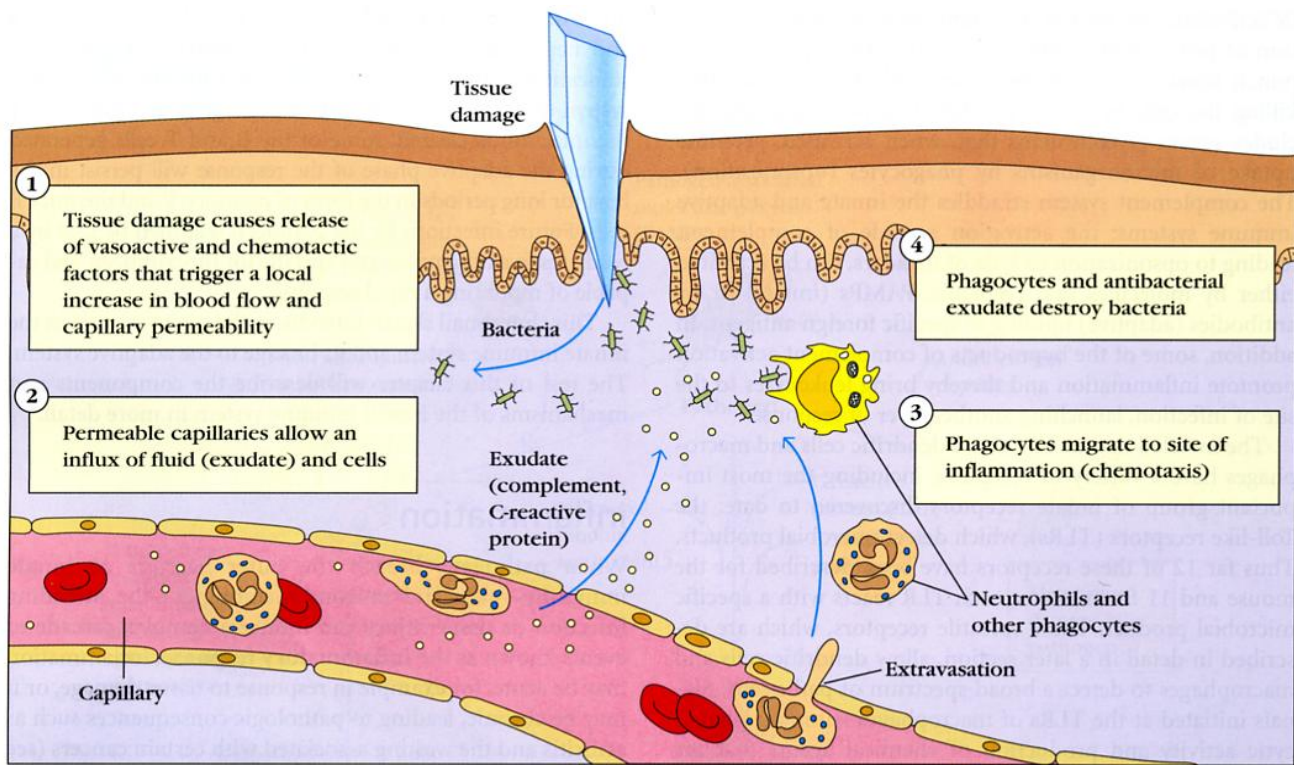


Figure 5: What happens when the physical and chemical barriers are breached?

How will phagocytosis happen?

We already said that when a microorganism enters, they'll be covered with certain molecules which have certain receptors to be recognized then they'll be taken inside the cell. Inside, there is phagosome which enters the membrane and produce invagination. This phagosome contains many molecules like lysosyme. The killing of the microorganism will happen by two ways. One is called in oxygen production which means oxidative relations. And the other anaerobic, it will destroy the organism from inside. So phagocytosis is done by producing oxygen radicals or by aneabic conditions by the enzymes. By doing this, there will be a production of reactive oxygen species; Nitric oxide to kill directly the microorganism, interleukins production at certain chemicals which will lead to inflammation, and factors that will do tissue remodeling. All of these are produced by macrophages when they react. So the microorganism covered by certain molecules, and will be taken by the macrophages. Refer to figure 6.

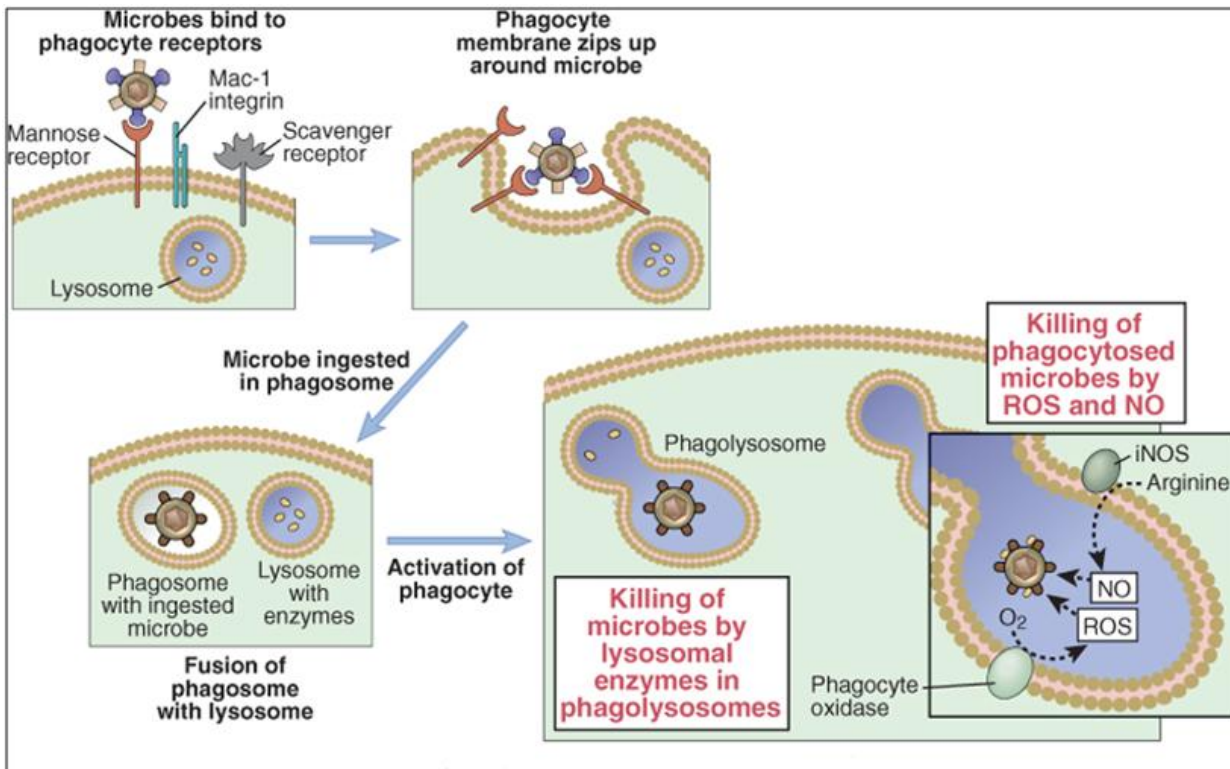


Figure 6a: Phagocytosis by innate immunity

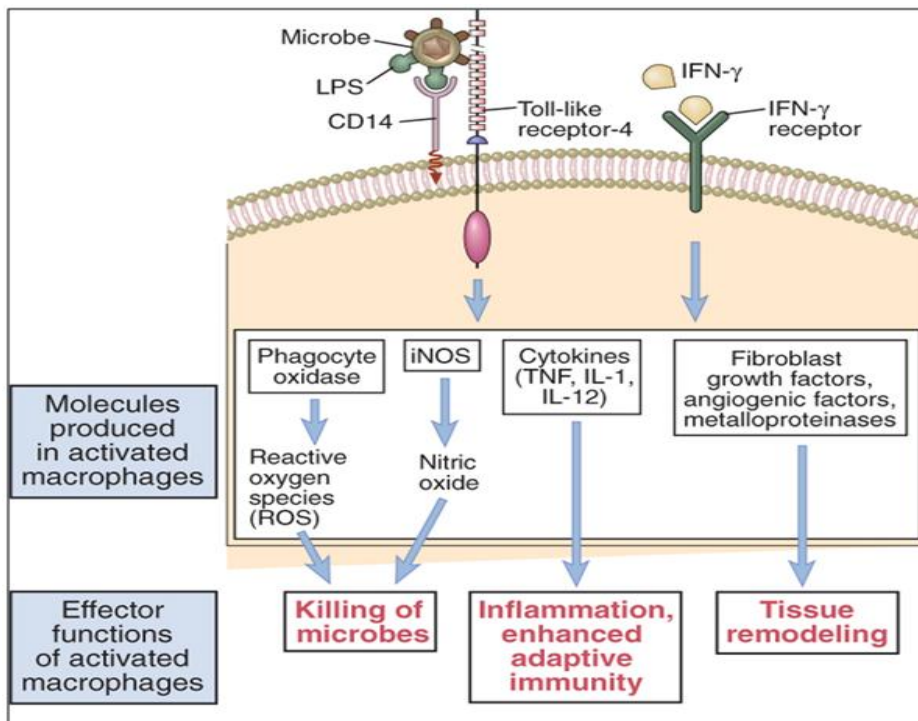


Figure 6b: Phagocytosis by innate immunity

What happens inside the granulocytes and phagosome:

1. There are primary granules already found these cells. During phagocytosis or at rest.
2. The secondary granules are produced after phagocytosis.

NEUTROPHIL GRANULOCYTE - GRANULE CONTENT

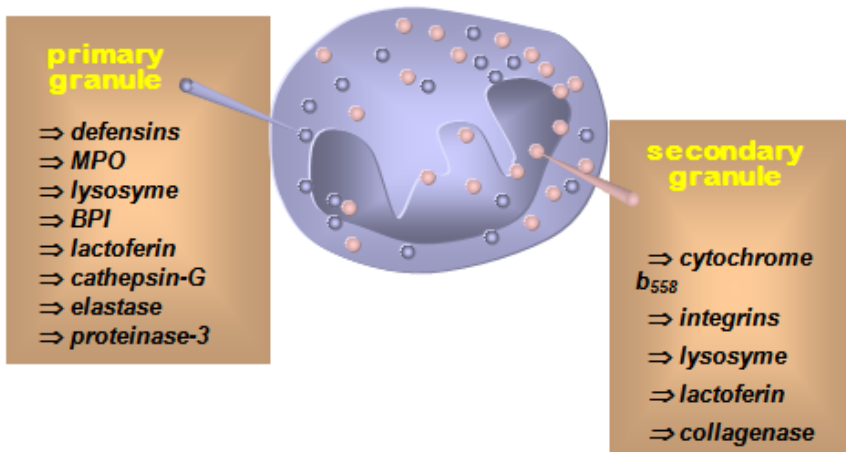


Figure 7

We talked about the macrophages and their function, now what about the natural killers?

Generally, natural killers do not have the same function as the macrophages. They are a type of lymphocytes, important in the specific immune system, important in killing the infected cells by direct contact. They contain certain granules and with the T- lymphocytes are involved in the immune surveillance, which means they continuously scan the cells for any infected cells or tumor cells and destroy it by direct contact.

What will happen in direct contact?

Say a cell is infected by a virus, the natural killer will recognize this cell and a signal will be sent inside. We said the natural killers have a certain type of granules which contain at least two molecules, perforin which will produce holes on the cell, and integrins which destroys the cell upon entrance.

Cytokines and NK cells combine to provide early defense against virus infections

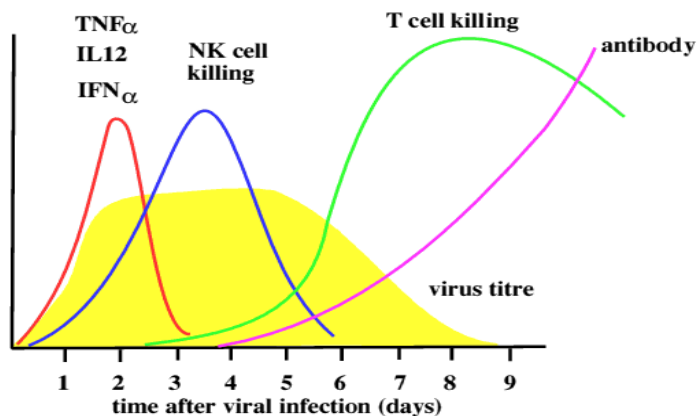


Figure 8: Immune Response

So natural killer will produce two things, porforins which will produce holes and through them the other molecules will go and destroy the cells.

Again, in the non specific immune response, there are molecules which are important fluid products. After the infection by 2 hours, you will see

TNF α , IL12 and IFN α go up and after that we will see the natural killers. After the natural killers, we'll start to see the T cell killing and later on we will see the antibody go up. The natural immune response is the one which acts immediately.

The white blood cells and the macrophages they have receptor for antibody. That receptor will react with the antibody in an area where the antigen will not react.

Antibody has specific arms for recognition; one arm receptor is for white blood cells. When the antibody reacts with a cell through the other part, the antigen binding site will be free but it is bound on the cell membrane. When a bacterium enters, if the antibody can recognize this microorganism specifically it will bind to it and by this, it increases the phagocytosis process. This killing is called Antibody-Dependant Cellular Cytotoxicity. Foreign substance will react with the antibody present on the target cells and recognize them, then activate the natural killer cells, porforins and integrins which will finish off the foreign cell.

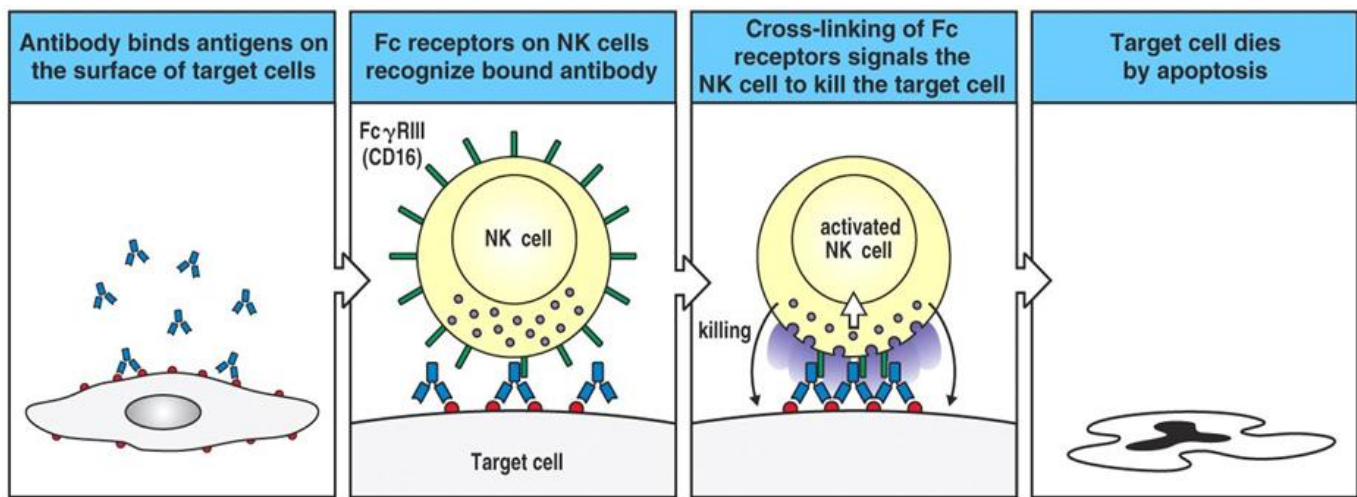


Figure 9: Antibody-Dependent Cellular Cytotoxicity

So the killing of the foreign body can be direct or by antibody.

We have another cell, which is non specific in response, the B- lymphocyte. This B-lymphocyte is different from the normal one that has to do with memory. Each B-Lymphocyte has a receptor on its membrane. The normal B-lymphocyte has a receptor composition made of α and β chains, the immunoglobulin. While the other B-lymphocyte, found in specific locations, has a receptor made of δ . This cell has no memory; they'll act towards the microorganism exactly as a first time. These B- lymphocytes, the ones with no memory, are called B-1 cells, and they're important but are non specific, just like natural killers. So the innate like lymphocytes, which are non-specific, shown in table 1. All of them have non-specific response, and kill directly.

Innate-like lymphocytes		
B-1 cells	Epithelial $\gamma:\delta$ cells	NK T cells
Make natural antibody, protect against infection with <i>Streptococcus</i>	Produce cytokines rapidly	Produce cytokines rapidly
Ligands not MHC associated	Ligands are MHC class IB associated	Ligands are lipids bound to CD1d
Cannot be boosted	Cannot be boosted	Cannot be boosted

Table 1

The antimicrobial proteins are interferons [its main function is to give resistance to the non-infected cells by inducing certain molecules.] So we have the first site of infection, they'll produce γ -interferon which will activate the non-specific cells. If you have an interferon and you add a virus to it, the virus will not do its function.

And the complement system, we have about 13 different molecules found in the body, inactive normally. But, if antigen-antibody complex was made (bacteria entered and the immune system made a response) then the complement system will be activated. It works as a cascade and if the whole cascade was activated, it will produce holes in the target cells (bacteria or infected cells) which will destroy the cell. The intracellular pressure is much higher than the extracellular, and so if you make a hole in it, it will burst and its entire component will be lost causing cellular death.

When we activate the complement system during the reaction of the immunoglobulin and the microorganism, we end with the destruction of the target cell.

To summarize:

The function of the innate system will cause inflammation (We won't talk about it here). At the end of the inflammation we'll have fever, which is a good response against microorganism. The early "earlies?" response will be seen within 0-4 hours, the early response up to 6-9 hours, there will be infection, phagocytosis and its production, removal of microorganism and if they can't remove there will be inflammation. If microorganism couldn't be removed, then we'll talk about the adaptive system.

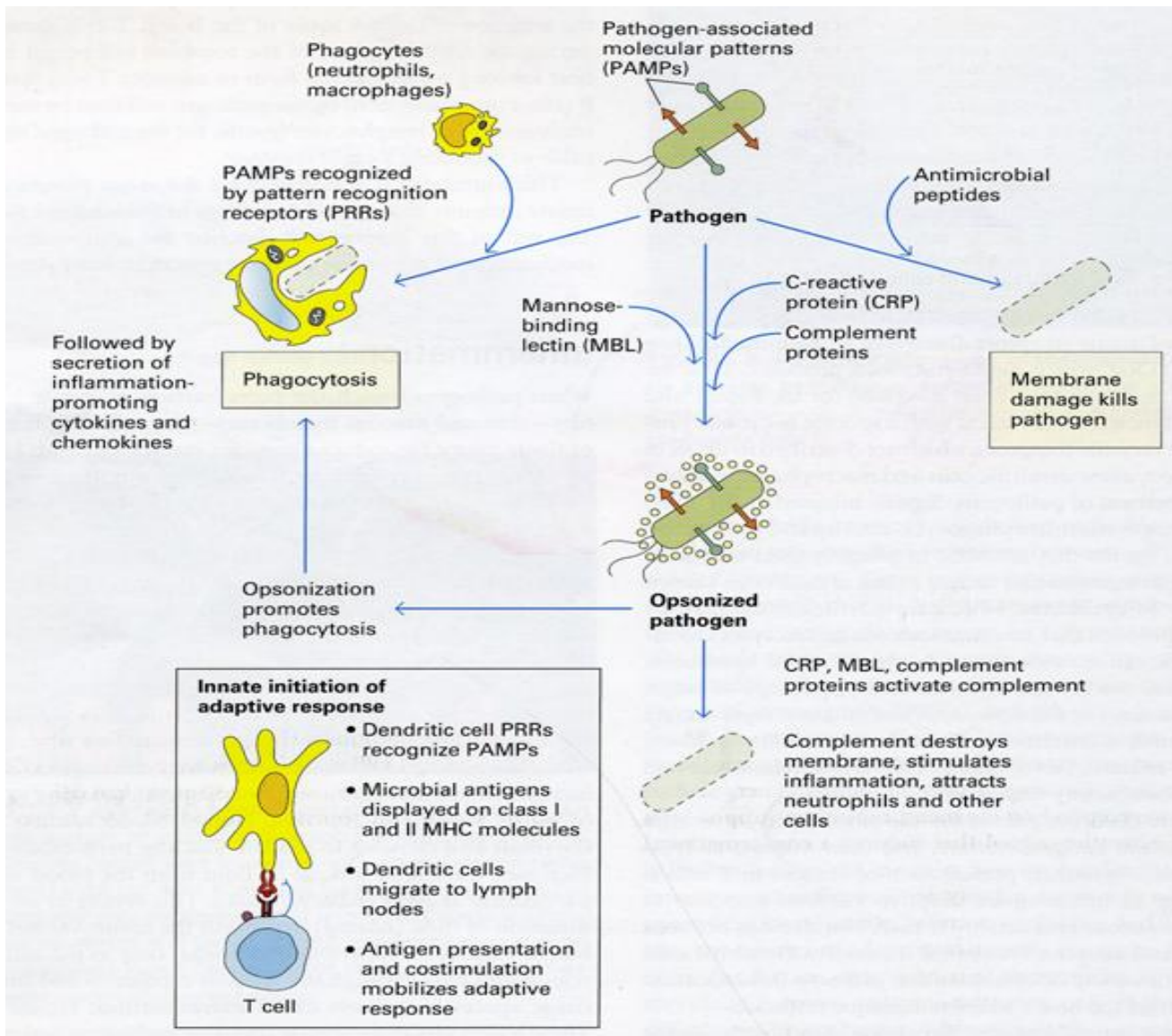


Figure 10: Summary and Review of Innate Immune Responses

p.s. I used last year's slides'; they aren't that different.