This sheet will include only the extra notes that the Dr. mentioned
** It will start with slide number 7 from lecture 4

Today we’ll discuss the non-specific immune response and their mechanisms talking about the major barriers that are a very important part in non-specific immune mechanism, the receptors utilized in non-specific immunity and utilized cells.

**Slide 7:**

- Initial protection is provided by mechanical and physiological barriers such as skin and mucous membranes of the different systems.

- The continuity of the skin is an effective barrier and it is resistant to penetration by organisms, this is the reason why skin infections usually follows trauma, injections, wounds that disturb the continuity of the skin.

There are some organisms that can penetrate intact skin such as spirochetes.

- Skin and mucous membranes can produce substances that creates unfavorable environment for the growth of organisms like the secretions of sebaceous gland (lactic acid, different fatty acids that creates an acidic pH), some antibacterial molecules like β-defensins

- The mucous membranes of the respiratory, GI, genitourinary systems are thick

**Slide 8:**

- Normal flora occupies a space that prevent the organisms from growing by competing with them over site and nutrient, and by producing antibacterial substances (E.coli is an example, it produces coliseum that is toxic to organisms other than itself)

- Mucosal surfaces are protected by several mechanisms:
A. Thick secretions that impede the movement of organisms, its role in inhibiting the infection appear in preventing the contact of the organism with the body surface that initiates the infection.

B. The presence of enzymes (lysozymes) that can digest the cell wall of gram positive bacteria, some microbicidal substances (α-defensins and cryptedin)

C. Hair and cilia that work as filters (to clean air from particles including micro-organisms) and entrap micro-organisms, its function is completed by coughing and sneezing that discharge the organisms.

**Slide 9:**

The Dr. mentioned every point written in the table and added few notes:

- Epithelial cells joined by tight junctions that are resistant to penetration by organisms.
- Longitudinal flow of air and fluid across epithelium that remove micro-organisms and not giving them the opportunity to establish contact.
- Fatty acids: change the pH
- Low pH (in stomach): most viruses are inactivated by acidity an exception: enteroviruses, hepatitis A. bacteria are also destroyed by the acidity of the stomach, exception: helicobacter pylori which protect the mucosa of the stomach by producing substances that creates an alkaline environment.

**Slide 10:**

If the first line fails to do their function then cellular mechanisms starts

The receptors of innate immunity:

1. Pattern recognition receptors (PRR): the most important receptor, for phagocytic cells, neutrophils and macrophages,
2. Chemotactic receptors: macrophages know that a pathogen is invading a tissue by the release of chemotactic factors, by the organism, which are recognized by these receptors.
3. Mannan binding receptors: these receptors recognize the organisms that release this substance.
4. Scavenger receptors
5. LPS binding protein: gram negative bacteria which have LPS in their cell wall are recognized by macrophages.

**Slide 11:**

Toll-like receptors (they are the PRR) are inherited receptors, they enable the cells to recognize foreign substances.

They interact with molecules on the pathogen known as pathogen associated molecular patterns.

They are 13 in number, 3 of them are newly discovered.

**Slide 12:**

This slide shows a table that lists 11 if the toll-like receptors with their ligands.

The Dr. read the information about TLR-1, TLR-5 and TLR-9

**Slide 13:**

- The toll-like receptors are expressed on the surface of the organism so it can recognize the different PAMPs of the organism and as a consequence they activate the expression of many nuclear factors like NF-κB which can lead to activation of inflammatory cytokines.
- Other toll like receptors can activate interferons that activate interferon pathways, which can activate IRF7 pathway that can also lead to interaction at nuclear level with the expression of NF-κB that lead to activation of inflammatory cytokines.
So a complex process of interaction takes place as a consequence of recognizing an organism by macrophages and neutrophils that will lead to the expression of inflammatory cytokines.

**Slide 14:**

This shows what happens after binding of a pathogen, the picture shows the following sequence of events that lead to production of IL-1..., etc.

**Slide 15:**

If we have an immature dendritic cell and this cell is exposed to a pathogen, the toll-like receptors on the cell will recognize the organism which leads to phagocytosis. This leads to activation of the dendritic cell and expression of pseudopodes, many nuclear factors are activated, and the digestion of the antigen will be followed by its presentation via MHC II to the naïve T cell (attracted to the site of reaction).

The naïve T cell will not be activated by this signal alone it requires another signal, it needs a co-stimulatory signal that will be provided by the binding to other molecule and the production of IL-12, it causes differentiation of naïve T cell into T helper 1 cell.

After recruitment of T helper 1 a new immune response starts (adaptive immune response).

So this is non-specific in nature and is followed by an activation of specific immune response, which is why it’s very appropriate to call these cells as the gate keeper for adaptive immune response.

**Slide 16:**

Macrophages initiate an immune response after binding to a foreign substance via their utilized receptors such as toll-like, scavenger, mannan, and others. That can be utilized by macrophages which can engulf the organism. Following its binding to the receptor the organism is surrounded by pseudopodes and the phagosome pinch off into the cytoplasm where it
binds to lysosomes to form phagolysosome where digestion takes place. The organism is presented to a specific T cell via MHC I if it’s a virus or MHC II if it’s an extracellular substance like bacteria.

**Slide 17:**

The process of phagocytosis is forced by chemotaxis; it’s mediated by certain molecules that attract macrophages or neutrophils. The chemotaxins is followed by the formation of pseudopodes that surround the pathogen that’s attached to the receptor, then the formation of phagosomes, followed by the binding to lysosomes to form phagolysosomes. Then degradation of the organism will occur followed by antigen presentation.

**Slides 18, 19:**

The most efficient cells in acute infections are neutrophils and they act in response to chemotaxic stimuli, the stimulus is followed by trafficking and homing (expression of selectins and binding to the endothelium in the vascular bed then leaving it).

Neutrophils promote necrosis and inflammation as they release the content of their granules to the extracellular environment.

During phagocytosis a metabolic process known as respiratory burst activates a membrane-bound oxidase that generates oxygen metabolites, which are toxic to ingested microorganisms. Two oxygen-dependent mechanisms of intracellular digestion are activated as a result of this process:

- NADPH oxidase reduces oxygen to super oxide anion, which generates hydroxyl radical and hydrogen peroxide, which are microbicidal
- Myeloperoxidase reduces oxygen to super oxide and chloride ions to produce hypochlorite (the active ingredient in household bleach), which is microbicidal.
In addition, the lysosomal content of phagocytes contains oxygen independent degradative materials:

- Lysozymes: digest bacterial cell walls by cleaving peptidoglycan.
- Defensins: circular peptides that form channels in bacterial cell membranes.
- Lactoferrin: chelates iron.
- Hydrolytic enzymes.

**Slide 22:**

Macrophages are the second important phagocytic cells

They differ from neutrophils:

1- The first and most important difference is that they are antigen presenting cells, neutrophils can’t present antigens but macrophages are professional antigen presenting cells

2- Killing by neutrophils doesn’t lead to the activation of specific immune response (adaptive immune response) but killing by macrophages is followed by the activation of the adaptive immune response

Macrophages are present in the blood stream as monocytes then they are present in tissues, lymph nodes... etc as fixed macrophages.

*the reference for this information is Kaplan
You can find the same information in the record (which isn’t clear) at 28:30*

**Slide 23:**

Oxygen dependent killing takes place although it is less efficient than neutrophils because the burst is smaller than neutrophils but they also mount a response via the production of nitric oxide because the LPS that is
recognized by CD14 binds to macrophages and this activates the enzyme nitric oxide synthetase leading to the synthesis of nitric oxide which is one of the mediators of inflammation (Efficient inflammatory mediator that promotes inflammation) and some of the side effects of severe inflammatory response that accompany infection is the failure of certain organs like (heart, kidney, liver.. failure) that is seen in severe sepsis.

Severe sepsis is associated with the production of nitric oxide and other weapons that interfere in the perfusion of organs and this can lead to cell death and multiple organ dysfunction or single organ dysfunction depending on the severity of the infection.

**Slide 25:**

Macrophages and neutrophils especially macrophages can produce substances which compete for certain growth factors or elements necessary for the growth of organisms. They have lactoferrin which binds to ferrous so they can deplete the environment from ferrous. Many organisms require ferrous for example Corynebacterium diphtheria can’t produce the toxin unless they have ferrous. Ferrous is an element necessary for the production of their toxin so toxin production is ferrous dependent. If it is depleted, organisms can’t produce their toxin.

Vitamin B12 can act as growth factor meaning that if you provide the organism with everything and that factor is not present it can’t grow. so it determines the growth of the organism.

So vitamin B12 binding proteins can be produced by macrophages and this can deplete the substance and as a consequence the bacteria can’t grow.

Now the activation of macrophages is followed by the release of many mediators which act on different organs to promote an inflammatory response and the cytokines that are produced in addition to enzymes include (IL-1, IL-6, IL-8, IL-12, and TNFα).
The production of these affects other organs in different manners leading to an inflammation against the organism in addition to attraction of other cells.

**Slide 28:**

So there are local effects mediated by these cytokines which also affect the whole body (at a systemic level) in the form of fever, inflammation and sometimes shock.

**Slide 29:**

- IL-1, IL-6, TNF-alpha affect the hypothalamus which increases the body temperature. And body temperature elevation is unfavorable for the growth of bacteria and viruses, (this is a defense mechanism by the body)

- The process of activation of phagocytes like macrophages by a foreign invader is very a complex process which leads to production of cytokines which act on different sites of the body and initiate an adaptive immune response against infections

**Slide 30:**

- The complement system is one of the components of the non-specific innate immune response.

- The complement is not a product of the immune response.

Complement proteins are naturally present, there are 9 major proteins, many are regulatory components in addition to the 9 major proteins, and they are activated by antibodies or other substances. They are usually activated in three pathways (we will discuss this when we come to the complement). And the activation of the complement leads to the production of many cleavage products that are pro-inflammatory as well as opsonins.

The activation of the complement leads to:
1- The release of C3B, which is a major opsonin, it bridges the target cell (foreign body) to the phagocytic cell.

- The other opsonin is IgG. (IgG and C3B are the only opsonins utilized in the body)

2- Fixation of a giant molecule composed of C5B, C6B, C7B, C8B, and C9B. A complex which has a molecular weight of 1 million Daltons, and this large molecule digs holes in the cell membrane of the target cell, allowing the entrance of fluids, and this will cause swelling of the cell until it dies. (Death of the cell occurs by apoptosis).

3- The cleavage products like C5, C3A, C5A, and C4B can induce inflammation.

- The complement is activated by an immune response, so it acts like an amplifier of the specific immune response. But the complement is not specific! (It’s not part of the specific immune response; it only acts as an amplifier, its non-specific in nature).

**Slide 31:**

- Natural killer cells don’t attack bacteria or viruses directly, instead they attack:
  
a. Virus infected cells
  
b. Or a cell that expresses a new antigen-like tumor cells

- They become activated when they recognize an antigen on the surface of the infected cell.

- Perforin is similar to the complement giant molecule (1million Dalton molecule) that digs holes in the cell membrane of the target cell and kills it.

**Slide 32:**

- C3b can act as an opsonin.
Lecture 6

- Heat shock protein and adhesion molecules can be expressed on tumor cells or on virus infected cells.

**Slides 33, 34:**
- The most important determinant in the killing mediated by natural killer cells is MHC class 1 molecules.

Natural killer cells are inhibited by normal MHC class 1 molecules. Meaning that if the cell has normal levels of MHC class 1 the killing will be inhibited. If the cell does not express MHC class 1 then it will be killed by natural killer cells.

- NKG2D recognizes components produced by MHC class 1, (MICAs and MICBs), these are molecules present on cells and are invariable (the same). Natural killer cells recognize these molecules via their killer activation receptor. So they bind to the cell, and if the cell expresses normal MHC, a second receptor called killer inhibitory receptor will come into play, if it detects sufficient MHC a signal will be provided to stop killing, but if the cell does not express enough MHC the killer inhibitory receptor will not act and killing will proceed.

So MHC is the most important determinant in killing by natural killer cells.

**Slide 35:**

A natural killer cell with the killing activation receptor (NKG2D) that will bind to a ligand (MICA or MICB) has a second receptor which is killing inhibitory receptor which will bind MHC, and if this takes place then there won’t be any killing, whereas if the inhibitory receptor is free (does not bind to its ligand) then killing will proceed, and the natural killer cell will produce its Perforin which will destroy the cell and activate apoptosis.

**Slide 36:**
The yellow molecules are MHC ++, so the NKG2D binds to its ligand, the killer activation receptor detects sufficient amounts of MHC--> no killing takes place. (The 2 pictures above)

In the pictures below, the cell lacks MHC all together, so the killer activation receptor binds but the killer inhibitory receptor does not bind and as a consequence the killing continues.

**Slide 37:**

There are substances that are not truly non-specific and not truly specific, so we have components of the immune system that are in between the innate and the adaptive immune system.

- These include:

  1- The gamma delta T lymphocytes that do not mature in fat, these are present in intra epithelial sites.

  So they are lymphocytes with T cell receptors that recognize antigens but do not mount an adaptive immune response.

  2- CD5 + B lymphocytes:

  B cells are classified as CD5 + or CD5 -.

  CD5 +cells produce Ig1 so they produce primary immune response, they don’t switch to IgG, although they are B cells and are lymphocytes they are not considered true adaptive immune response.

  3- Natural antibodies, they are antibodies present without any stimulation, they have low affinity, they are of the IgM class, they are produced by CD5 B cells and they are not part of the adaptive immune system.

**Slides 38, 39:**
It is very important to mention that chemokines, cytokines act via receptors, and their receptors are present in different cells that are involved in killing...

And these two tables summarize the two classes of chemokine receptors: CC, CXC.

**Slide 40:**

This slide summarizes the whole lecture.

The first step is phagocytosis, followed by activation of neutrophils and macrophages with the production of many molecules that activate antigen presentation and processing that terminate the activation of T lymphocytes with mounting of an adaptive immune response.

Sorry for any mistakes, and for being late

*Good Luck*

*Special thanks to: Sarah Qawasmeh, Lubna Hamdan.*

*Your colleague: Maram Abu-Halaweh.*