Immunology English Practical Session 3 Correction

Exercise 1:

1. Molecular structures recognized by the innate immune system

The receptors of innate immunity and the structures recognized by these receptors were identified much later than the receptors of adaptive immunity. In 1989, Charles Janeway proposed that discrimination between self and non-self could be largely operated by the innate immune system, distinguishing "infectious non-self" through receptors he called PRRs (Pattern Recognition Receptors). Janeway proposed that these PRRs recognize molecules expressed by microorganisms, which he called PAMPs (Pathogen Associated Molecular Patterns). These molecular patterns, characterized later, are not exclusively expressed by pathogens but by all microorganisms, so the term MAMPs (Microbe Associated Molecular Patterns) is today more appropriate than the term PAMPs (Figure 1).

In 1994, Polly Matzinger introduced the concept of "Danger Signal," proposing that the immune system recognizes not only products from microorganisms but also products from the host under stress. These latter compounds have been called DAMPs (Damage Associated Molecular Patterns) or "alarmins" (Figure 1). This "danger hypothesis" helped explain why the immune response observed following an infection could be similar to that appearing after sterile tissue damage. Indeed, it has been shown that the recognition of MAMPs or DAMPs by PRRs can trigger the activation of the same signaling pathways. It is important to note that the detection of MAMPs and DAMPs is the first step in activating any immune response, whether innate or adaptive.

2. Molecular structures recognized by the acquired immune system

The receptors of innate immunity are conserved throughout evolution, invariant within the same individual. These receptors have been selected during evolution to recognize conserved molecular patterns within microorganisms. The receptors of acquired immunity, i.e., the receptors of B and T lymphocytes, are not yet determined to recognize a given signal when they are generated (at the beginning of lymphocyte differentiation). The so-called constant part of these receptors is conserved between species and very similar between individuals, while the so-called variable part is randomly generated, which gives lymphocytes, as a whole, the ability to recognize an infinity of molecular structures, called antigens.

Receptors of acquired immunity cells for pathogens: receptors of T and B lymphocytes.

- B Cell Receptor (BCR): these are immunoglobulins (glycoproteins) of type:
    - IgM and IgD on the surface of naive B cells (have not yet encountered the specific antigen)
    - Other than IgM on the surface of non-naive B cells (memory cells)
- T Cell Receptor (TCR) is a glycoprotein formed of 2 types of chains:
    - TCR α/β (TCR-2) (95% of T lymphocytes)
    - TCR γ/δ (TCR-1) (05% of T lymphocytes)

3. An antigen is a molecule of any nature (organic or not) that can be recognized by an antigen receptor of adaptive immunity. These receptors are of two types: the B lymphocyte antigen receptor (BCR, for B-Cell Receptor), and the T lymphocyte antigen receptor (TCR, for T-Cell Receptor).

   We actually produce several million different molecules of each of these two types. The property of binding the antigen to the various receptors gives it its antigenicity. While BCRs recognize all forms of antigens in their native state, TCRs recognize only protein antigens in peptide form, and when they are

associated with Major Histocompatibility Complex (MHC) molecules. Only antigens that provoke an adaptive immune response are qualified as immunogens.

Note: Haptens are non-immunogenic antigens, they are salts of heavy metals (nickel, chromium, mercury), plant quinones, synthetic molecules (drugs, dyes, etc.), or natural molecules (peptide or steroid hormones). These substances, with a molecular weight less than 1 kDa, have antigenic properties but are not immunogenic. They become immunogenic when they are stably coupled to a carrier molecule that is immunogenic. The development of allergies to haptens requires such coupling.

4. An epitope: Antibodies, BCRs, and TCRs do not recognize antigens in their entirety. They recognize a small region of the antigen termed antigenic site, antigenic determinant, or epitope. The region of the antibody, BCR, or TCR recognizing the epitope is called the paratope. Antigens generally carry several different epitopes. The number of identical epitopes in an antigen determines the valence of that antigen. If the same epitope is represented several times, the antigen is said to be multivalent. Epitopes recognized by antibodies or BCRs are called "B epitopes," and epitopes recognized by TCRs are called "T epitopes."

5. Factors influencing immunogenicity:

    1. Chemical nature of molecules
    2. Biomolecules with a molecular weight greater than 1 kDa are generally immunogenic.
    3. Protein and peptide antigens, which are numerous and varied, and which can provoke a T lymphocyte response in addition to B lymphocyte activation, are often immunogenic.
    4. Polysaccharides are quite immunogenic due to their size and also varied structures. However, unlike proteins, epitopes found on polysaccharides are sequential, repetitive, and each composed of the same sequence of five to six sugars.
    5. Lipids, like nucleic acids, are weakly immunogenic.

    Factors extrinsic to the antigen:

    6. The anatomical site of encounter with the antigen and its quantity are also important. Thus, in the case of artificial immunization, the antigen dose, the injection route used, or the frequency of administration can increase immunogenicity.
    7. The presence of danger signals detected by innate immunity will also favor immunogenicity. In the case of many vaccines, the addition of exogenous molecules, called adjuvants, is paramount to increase the immunogenicity of an antigen. Adjuvants act on the mode of antigen diffusion or on innate immunity, by respectively favoring the capture of the antigen by dendritic cells or the maturation of these cells.

Exercise 2:

Complete the following table and indicate the essential roles of each of the cells in the innate immune response:

Identified Cell	**Origin**	**Cellular Roles**
A: Neutrophilic polymorphonuclear cells	HSC from Bone marrow	- They are phagocytes- They are early markers of inflammation
B: Eosinophilic polymorphonuclear cells	HSC from Bone marrow	- They are pro-inflammatory cells- Play an important role in antiparasitic response- Also contribute to chronic allergic pathologies

C: Basophilic polymorphonuclear cells	HSC from Bone marrow	- They play a central role in certain pathological conditions such as allergies and parasitoses
D: Lymphocytes	HSC from Bone marrow	- B lymphocytes produce antibodies to neutralize microbes and toxins- T lymphocytes destroy infected cells by direct contact
E: Macrophage	HSC from Bone marrow	- Ensures phagocytosis

Exercise 3:

1. The finger injury caused an opening, allowing bacteria to cross the skin, one of the body's natural barriers. In addition, an internal temperature of about 37°C and a fairly humid environment remain very favorable for their rapid development. This explains the presence and abundance of bacteria on the wound.
2. The presence of bacteria in the wound alerts neutrophilic polymorphonuclear cells, which attack them in order to capture and kill them (thanks to their microbicidal activities). To do this, they adhere to the bacteria, envelop them, and lock them in a cytoplasmic pocket. This explains the presence of bacteria inside the neutrophilic polymorphonuclear cells. This phenomenon is called phagocytosis.

Exercise 4:

Natural killer (NK) lymphocytes recognize infected cells (particularly by viruses) or modified cells (for example, tumor cells). These lymphocytes are part of innate immunity because they do not express an antigen receptor like TCR or BCR. However, they express activating or inhibitory receptors that are specific to them and release cytokines like IFNγ or cytotoxic proteins contained in their granules.

General characteristics of NK cells:

- NK cells have the morphology of large granular lymphocytes, with a cytoplasm rich in lytic granules.
- They are characterized by the expression of CD56, CD16 (FcγRIIIA), and NKp46 molecules, and by the absence of CD3 expression, which distinguishes them from T lymphocytes.
- NK cells are present:
    - In the blood circulation, where they represent 5 to 15% of lymphocytes.
    - In lymphoid organs (spleen, tonsils, peripheral lymph nodes).
    - In certain tissues (liver, lung, placenta...) where they act as sentinels.
- Their renewal in the blood is about two weeks.

1. Recognition of target cells NK cells are capable of rapidly eliminating abnormal cells (e.g., tumor cells or cells infected by an intracellular pathogen), while respecting healthy cells. This ability results from a dynamic balance between different activating and inhibitory signals transmitted by membrane receptors (Figure 2). NK receptor genes are in germline configuration and do not undergo genetic rearrangements unlike genes encoding TCR or BCR.

NK cells recognize:

- missing self (absence of MHC class I molecules) through inhibitory receptors
- "stress" molecules on abnormal cells through activating receptors.

These receptors exert their function (inhibitory or activating) when they recognize their ligands on target cells. The integration of the sum of activating and inhibitory signals transmitted by the receptors regulates the activation of the NK cell following its interaction with a target cell. The resultant of these signals determines whether the NK cell will exert its cytotoxicity and/or cytokine secretion functions towards the target cell.

2. Functions of NK cells NK cells are "sentinel" cells whose purpose is to rapidly eliminate abnormal cells (tumor or infected), while respecting healthy cells. The establishment of NK functions is rapid because, unlike that of T and B lymphocytes, it does not require a proliferation or differentiation step.

A. Cytotoxicity The direct cytotoxicity of NK cells towards abnormal cells is their best-known function. It can be exerted by different mechanisms, generally similar to those employed by CD8 T lymphocytes, such as perforin-dependent cytotoxicity. Following recognition of the target cell, the NK cell degranulates: it releases the contents of its cytoplasmic granules at the synapse, in particular perforin, which forms nanometer-sized pores in the membrane of the target cell, favoring the entry of granzyme molecules. Proteins of the granzyme family are enzymes capable of inducing cell death by cleaving regulatory molecules of apoptosis such as caspases (programmed cell death process).

Other cytotoxic mechanisms leading to the death of the target cell by apoptosis are possible:

- Receptors for the Fc fragment of IgG: NK lymphocytes have other receptors on their surface that recognize the Fc fragment of IgG. The interaction of these receptors with IgG specifically combined with a cell activates NK lymphocytes.
- CD95 (Fas) interactions on the target - CD95L (Fas ligand) on the NK cell;

B. Cytokine production NK cells produce in particular IFN-γ, but also TNF-α, IL-10, and chemokines. These cytokines participate in:

- Regulation of the inflammatory response, for example by the recruitment and activation of macrophages and dendritic cells;
- Direct control of viral replication by the production of IFN-γ;
- Control of the type of adaptive response (Th1/Th2/Treg orientation). Rather than confining them to innate immunity, NK cells are now considered to occupy a key position at the interface between innate and adaptive immunity.

Exercise 5:

1. Complete the captions: An injury breaks the natural barrier of the skin
2. What is the role of sentinel cells, which are the first to come into action? Sentinel cells, resident in tissues or circulating, monitor the integrity of the organism. Mast cells, dendritic cells, and macrophages have receptors on their surface capable of recognizing the antigenic patterns of foreign elements that have entered the organism. They are the ones that will initiate the immune response.
3. Complete the captions: The binding of a sentinel cell on a bacterium.
4. What is the consequence of this binding? Give an example After recognition, cells produce chemical mediators including:

- Example: histamine → (vasodilation + permeability of vessels that allows the passage of circulating cells to tissues)
- Cytokines → attract macrophages
- Prostaglandins → stimulation of nerve fibers → pain

5. What are the characteristic clinical signs of the inflammatory reaction and their causes?

The characteristic clinical signs of the inflammatory reaction and their causes are summarized in the following table:

Manifestations	**Causes**
Redness	A dilation of capillaries (vasodilation: dilation of the blood vessel), the vessel is so dilated that it becomes transparent
Heat	Slowing of blood flow and activity of the different cell types involved
Edema (swelling)	Plasma exudation (passage of plasma fluid) and diapedesis (passage of leukocytes) into the tissue
Pain	Excitation of nerve endings due to: - Mechanical pressure due to edema - Mediators: prostaglandins and leukotrienes, etc.

6. Which cells intervene next? How? The cells that intervene next are phagocytes (resident macrophages, neutrophilic polymorphonuclear cells, and dendritic cells) attracted by the released chemokines. They will phagocytose the antigens and present antigenic determinants associated with MHC, which will result in recruiting the actors of the adaptive response: lymphocytes.