Chapter 7: The Immune Response

- **Document 1: The Non-specific Immune Response**

Introduction:

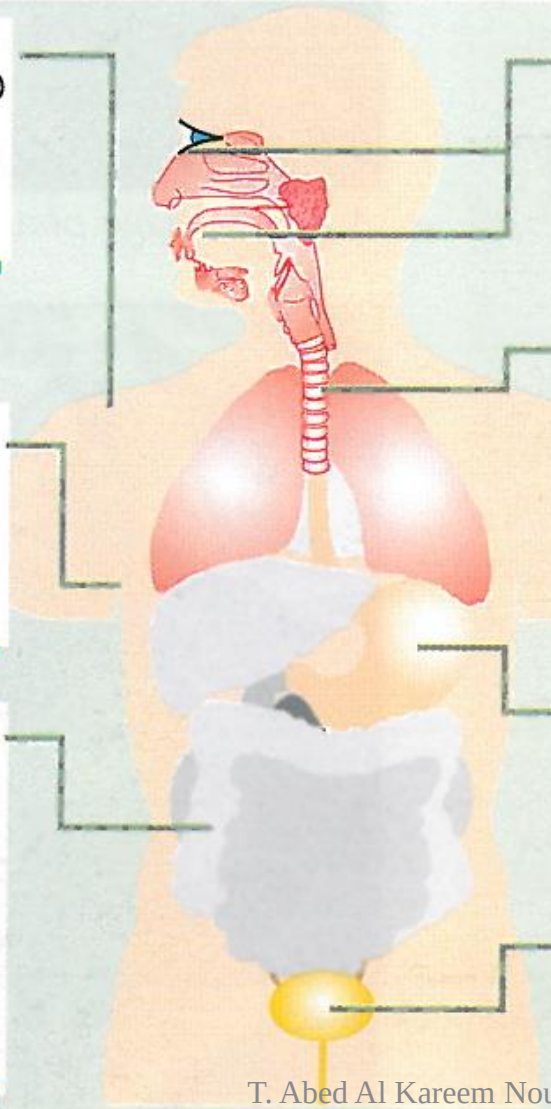
- when a pathogen **infects the host** (crosses a natural barrier and replicates in the tissues of the host), it triggers the host defense mechanism **within only few hours**. The first phase of defense after the natural barrier is called **non-specific immune response**.

1. Natural barriers

The skin is virtually impermeable to foreign substances and micro-organisms.

Sweat is acidic (pH=3.5) and can destroy chemically micro-organisms that colonize the skin .

Non-pathogenic bacteria which live on the surface of the mucosa and are called "commensal flora", compete with harmful bacteria and make the internal medium unsuitable for their implantation .



Mucosal secretions, tears and saliva contain lysozyme, an enzyme that can destroy many bacteria .

Mucus in the nasal and bronchial secretions protects the respiratory tract by trapping inhaled particles and microbes. These are subsequently expelled from the body by the movement of the ciliated cells that line the mucosa.

The extreme acidity of the gastric juices (pH=1-2) destroys most ingested toxins and microbes chemically.

Vaginal secretions and semen contain anti-microbal substances.

1. **Natural barriers**

Mechanical	**Chemical**
Skin	Sweat (acidic)
vibrating cilia of mucosal cells	Gastric secretion (acidic)
Mucosa	Lysozymes from most secretion: tears; saliva
	Vaginal secretion and semen contain anti-microbial substances
	Commensal Flora: Non-pathogenic bacteria which lives in the body (ex: on skin and intestinal tract) without causing any harm for body. They compete with other pathogenic bacteria.

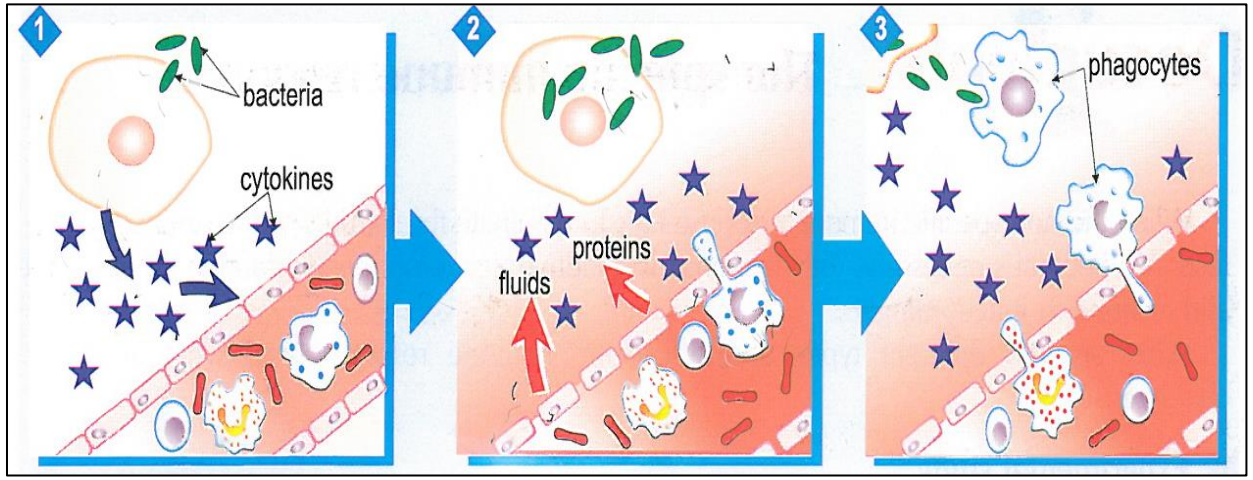
Manifestation of Non- specific Immune Response

- Upon crossing the natural barriers and infection leads to immediate non-specific immune response demonstrated by **local inflammatory Reaction.**

- **Signs (symptoms) of inflammatory Reaction:** Redness, swelling, heat and pain

What happens in the inflamed wound?

- When microbe enters a tissue, the infected cells, the macrophage and other leukocytes release **cytokines** having **local and systematic effects** inducing the:
- **Vasodilation:** Increase the diameter of the blood vessel to increase the flowing of blood into the infected area **(causing redness and heat)**
- Increase vascular permeability and the leakage of plasma from blood vessels to the infected area. **(causing edema)**
- Attraction of phagocytes (macrophages) **(chemotactic effect)** ,
- Increase the squeezing of phagocytes through blood vessels to site of infection by process movement called **(diapedesis)**



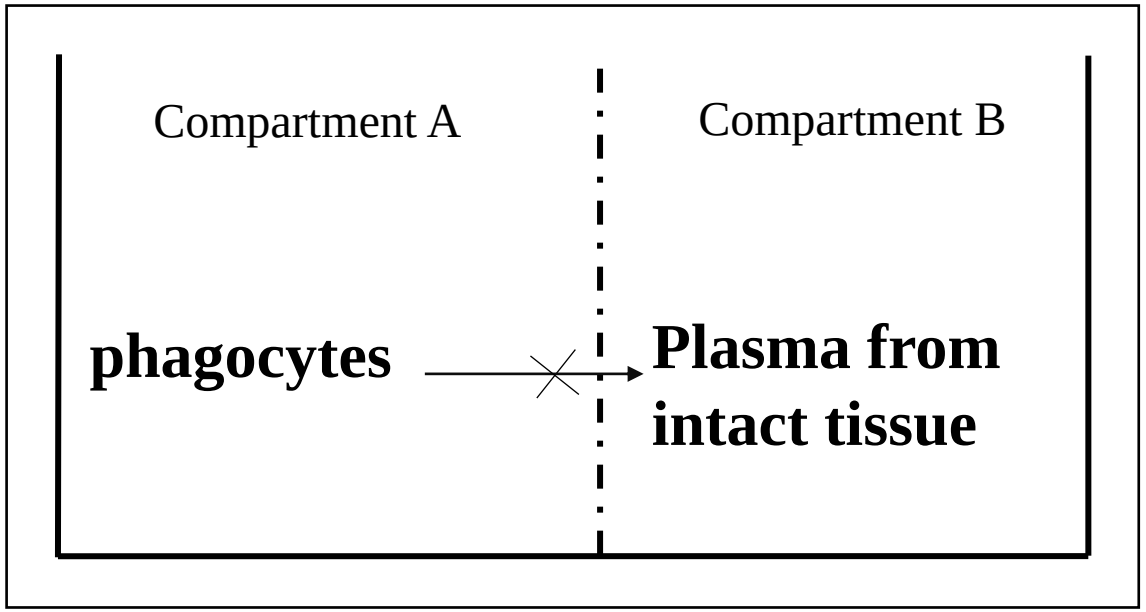
Note: Macrophages are monocytes that have migrated from the bloodstream into any tissue in the body

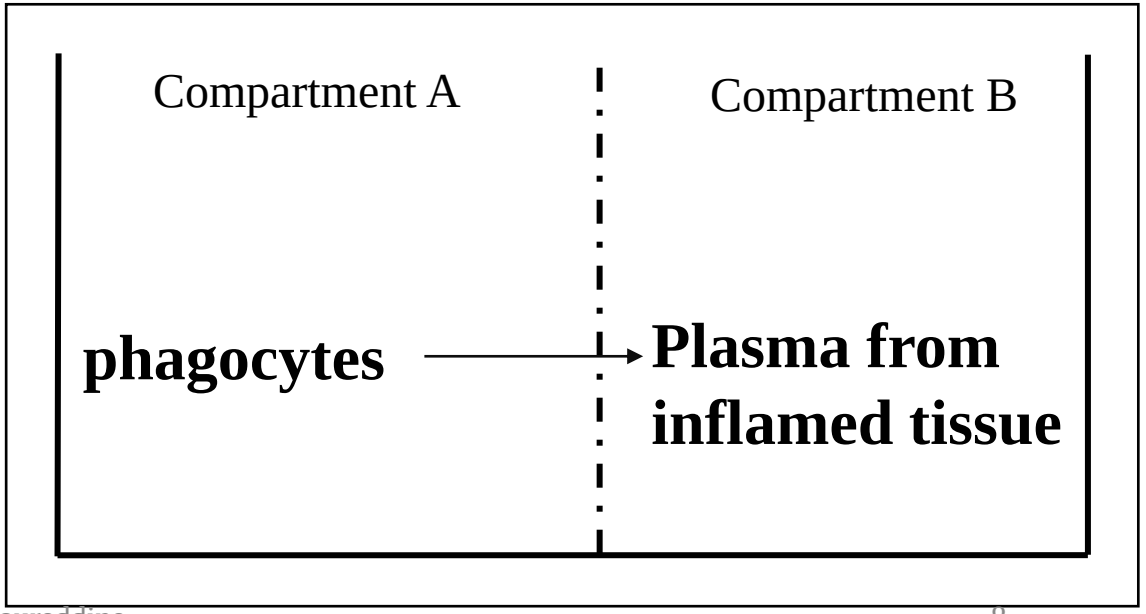
- **Note:**
- Pain is due to the stimulation of the pain nerve ending (Receptors) by the accumulated plasma and microbial secretions.
- Some cytokines have **chemotactic effect on phagocytes** (ex: monocytes and some granulocytes). In other words, they have the ability to **attract them**.

Application:

- An apparatus is made up of 2 compartments separated by a porous filter (filter containing pores). The first compartment contains phagocytes and the other contains plasma either from normal or from an inflamed tissue. Results show movement of phagocytes from their compartment toward inflamed tissue plasma

1. **Analyze the experiment:**
2. **What can you conclude regarding inflamed cells?**
3. **Explain how macrophages reach the infected tissue.**





1. Interpret the experiment:

Separating by a porous filter phagocytes and plasma from an inflamed tissue leads to the movement of phagocytes and plasma from an inflamed tissue leads to the movement of phagocytes toward the compartment that contains the plasma. On the contrary, where phagocytes didn’t move toward plasma from intact tissue.

2. What can you conclude regarding inflamed cells:

We conclude that inflamed cells secrete chemotactic substances that attract phagocytes

3. Explain how macrophages reach the infected tissue.

Upon infection, the infected cells, the macrophage and other leukocytes release cytokines having local and systematic effects inducing vasodilation of blood capillary in the area, which increase the blood flow. In addition, some cytokines have chemotactic effect, which attract monocytes to the site of infection by chemotaxis. The monocytes then cross the blood vessel by diapedesis where they transform into macrophage.

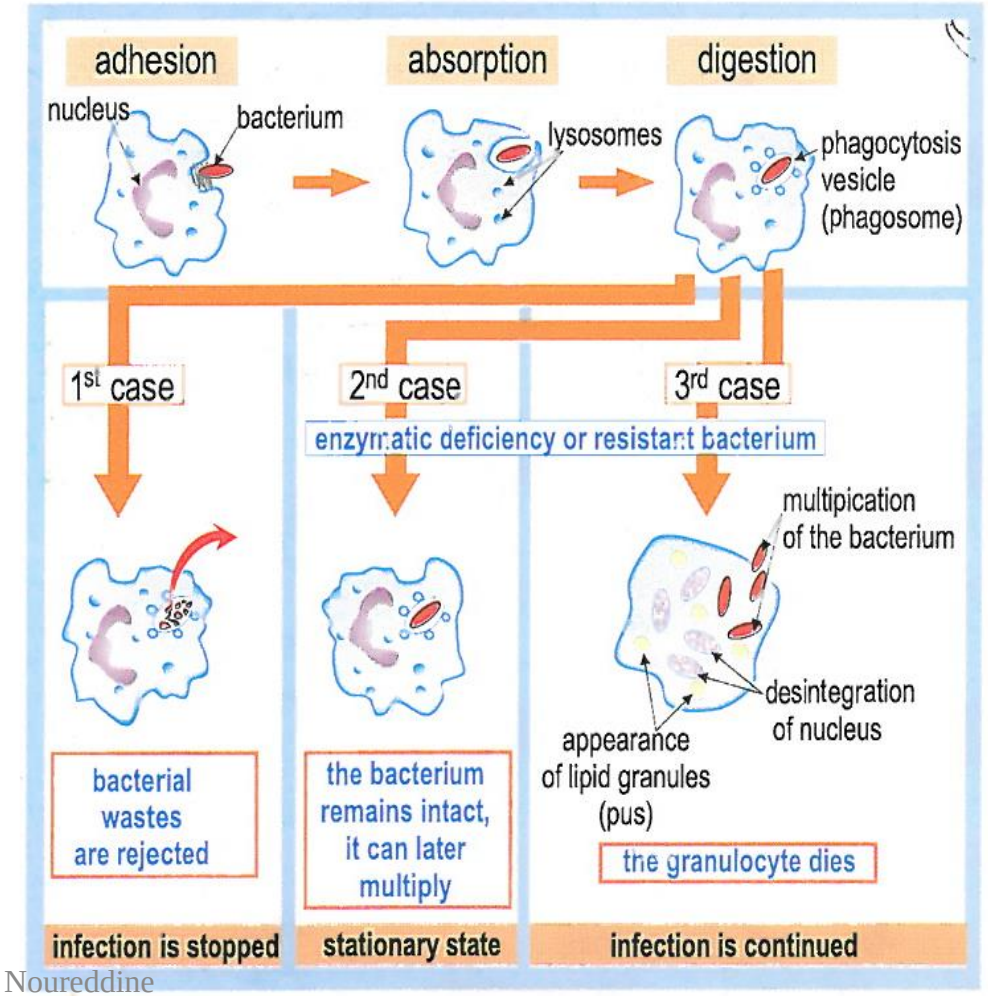
Phagocytosis:

- **Steps of phagocytosis: Doc. c p:139**

1. **Adhesion:** the macrophage gets in contact to the bacteria due to the interaction of surface molecules of both macrophage and bacteria
2. **Absorption:** The macrophage engulfs the intruder which become enclosed in a vesicle (phagosome)
3. **Digestion:** lysosomes are added to the phagosome to digest the bacteria.
4. If the macrophage manages to eliminate the bacteria, the disease will **regress.**

- **Failure of phagocytosis:** (case 2 and 3 doc: c p: 139)
- In some cases, the macrophage can’t digest the microbes due to:
  - bacterial resistance to phagocytosis
  - absence of hydrolytic enzymes in macrophage.

- In these cases, the bacterium may:
  - Multiply later (this is the stationary phase).
  - Multiply leading to destruction of macrophage.



Characteristics of non-specific immune response

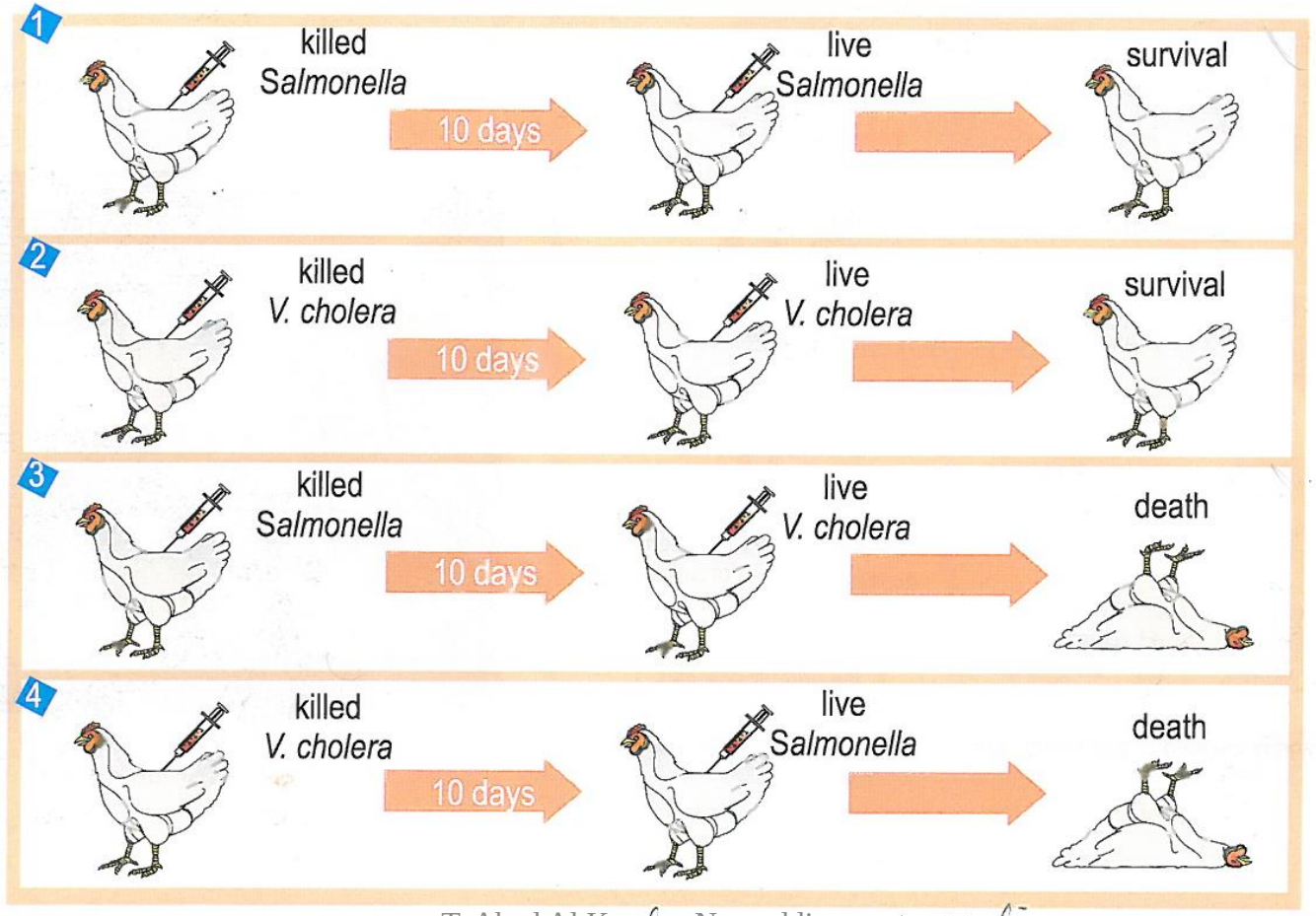
- No latency period: starts after only few hours
- Non- specific: because the macrophages phagocytes any intruder regardless of its identity (phagocytes done have specific receptors for specific antigens)
- The phagocytic cells (mainly macrophage) are the **effector cells** in this response where their **mechanism of action** is by **phagocytosis**

Document 2: The Specific Immune Response:

- If the non-specific immune response fails to eliminate the intruder, the specific immune response becomes necessary. The different types of specific immune response and their characteristics will be demonstrated in the following activity.

- **Experimental Study:** Induction of specific Immune Response:

1. Interpret document
2. What can you conclude about the triggered immune response?



1. Interpret doc. a

- **Exp 1 and 3:** The injection of live salmonella to a hen leads to its survival 10 days after being injected by killed Salmonella whereas the injection of live cholera to a hen leads to its death 10 days after being injected by salmonella. **This indicates that** the killed salmonella protects **(immunizes)** hens against live Salmonella and not against live Cholera.
- On the other hand, the injection of live cholera to a hen leads to its survival 10 days after being injected by killed cholera where as the injection of live salmonella to a hen leads to its death 1- days after being injected by killed cholera. **This indicataes that** killed cholera protects **(immunizes)** hens against live cholera and not against live salmonella

2. What can you conclude about triggered immune response?

- We conclude that the triggered immune response is specific.

3. Why the live antigen was injected after 10 days of injection the killed antigen?

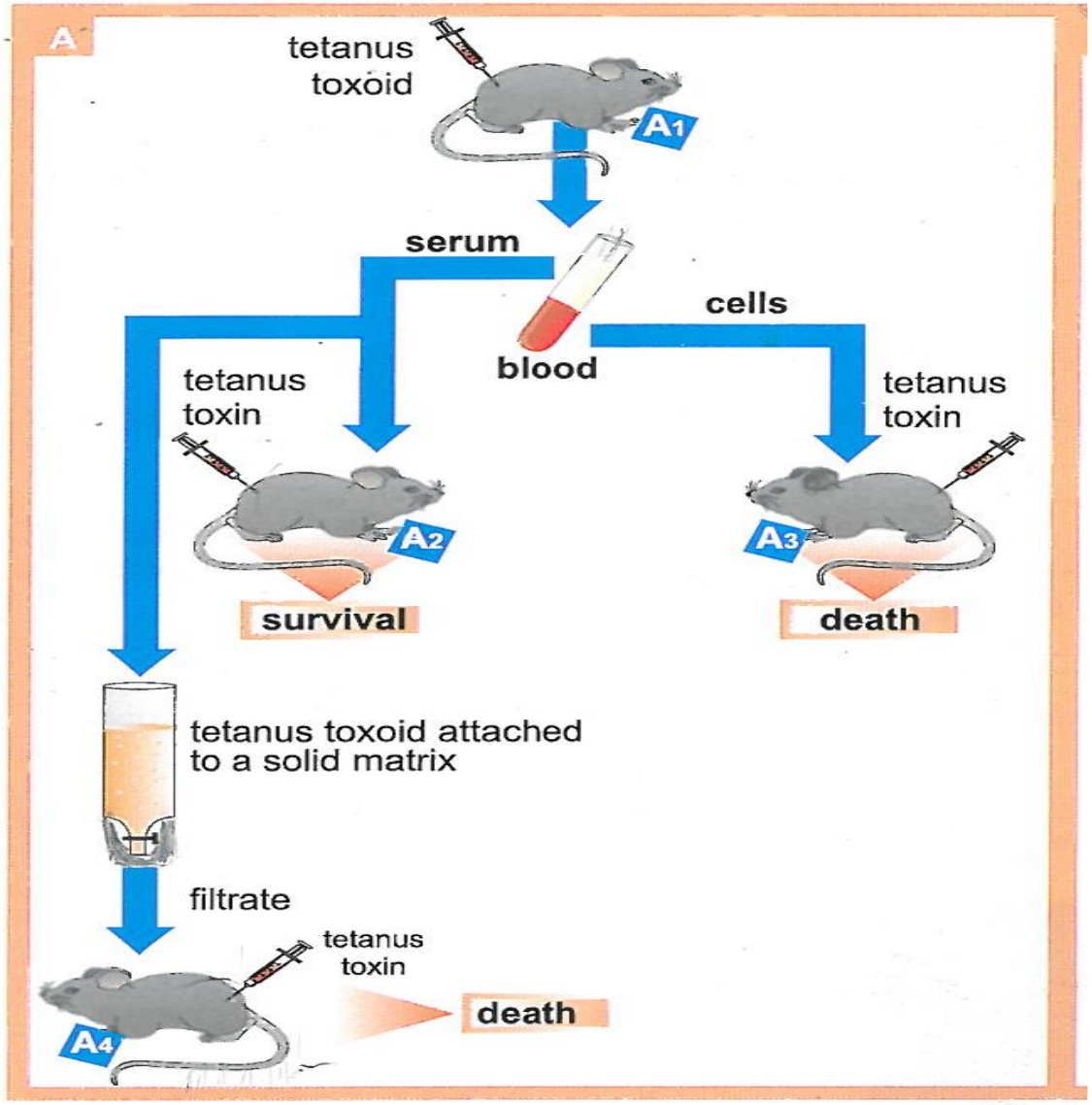
It is the time needed to trigger the specific immune response.

Types of Specific Immune Response:

- There are 2 types of specific immune response:

1. **Humoral mediated immune response:** where the immunity is due to serum (antibodies) secreted by plasma cells.
2. **Cell Mediated Immune response:** where the immunity is due to Tc lymphocytes.

Humoral mediated immune response:



1. **Interpret the results**
2. **Conclude the type of immune response**

- **Remark:**

1. **<u>The filtration column</u>** contains tetanus toxoid attached to a solid matrix. After adding the serum that contains a mixture of antibodies only the anti-tetanus antibodies attach to the matrix while the others remain free and leave the column as filtrate. So the filtrate contains all the antibodies except those specific to tetanus toxoid
2. **<u>Toxoid:</u>** is attenuated toxin: the harmful effects of toxin are eliminated but antigenic determinants (epitopes) remain intact

1. Interpret the results

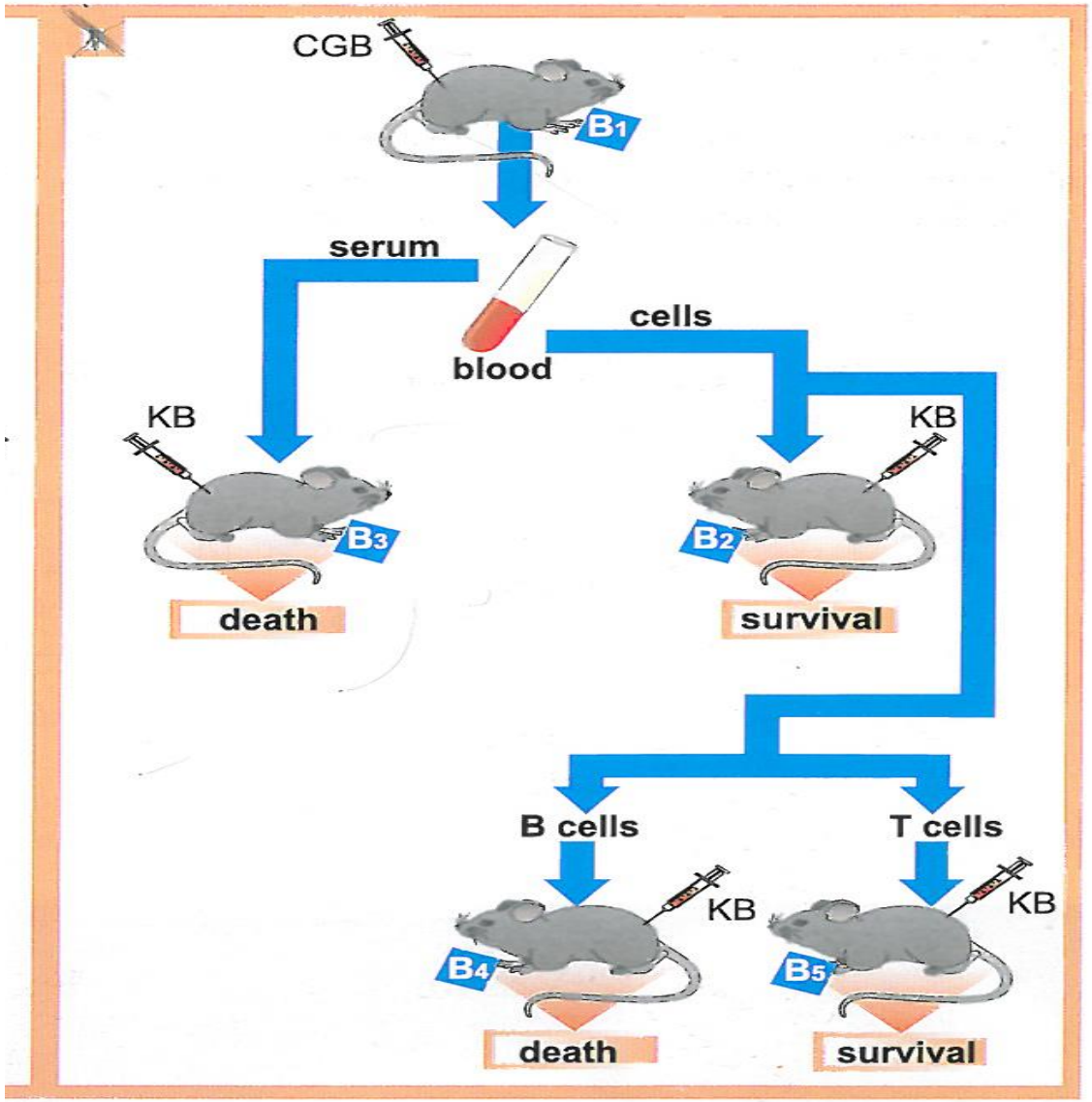
The injection of blood cells, taken from a mouse A1 injected with tetanus toxoid, into mouse A3 followed by injection of tetanus toxin, leads to the death of this mouse (A3). **This indicates that** the blood cells do not transmit immunity from A1 to A3. However, the injection of blood serum, taken from mouse A1 that was injected with tetanus toxoid, into mouse A2 followed by injection of tetanus toxin leads to the survival of the mouse A2. **This indicates that** the serum transfers immunity from A1 to A2.

Moreover, the injection of the filtrate taken from the circulation of the serum of mouse A1 in a solid support containing tetanus toxoid, into a mouse A4 followed by the injection of tetanus toxin, leads to the death of this mouse A4. This indicates that the serum contains effectors that are specific against tetanus toxin.

2. What can you conclude?

We conclude that the immune response triggered against tetanus toxin is humoral immune response by secretion of antibodies (effectors) in serum.

Cell mediated immune response:



1. **Interpret Results of Exp B of doc. b**
2. **Conclude the type of immune repsonse**

- **Remark:** CGB is a harmless bacterium that shares common antigenic determinants with KB

1. Interpret Results of Exp B of doc. b

The injection of blood serum, taken from a mouse B1 injected with CGB, into a mouse B3 followed by injection of KB, leads to the death of this mouse (B3). **This indicates that** the serum does not transmit immunity from B1 to B3.

However, The injection of a part of blood cells, taken from a mouse B1 injected with CGB, into a mouse B2 followed by the injection of KB, leads to the survival of this mouse (B2). **This indicates that** the blood cells transmit immunity from B1 to B2.

Moreover the injection of B cells, take from a mouse B1 injected with CGB into a mouse B4 followed by injection of KB leads to the death of this mouse B4. **This indicates that** the B cells do not transmit immunity from B1 to B4.

On the contrary, the injection of blood T cells, taken from B1 injected with CGB into a mouse B5 followed by injection of KB causes the survival of this mouse B5

2. **What can you conclude**

We conclude that the immune response against KB is cell mediated whose effector is T cell.

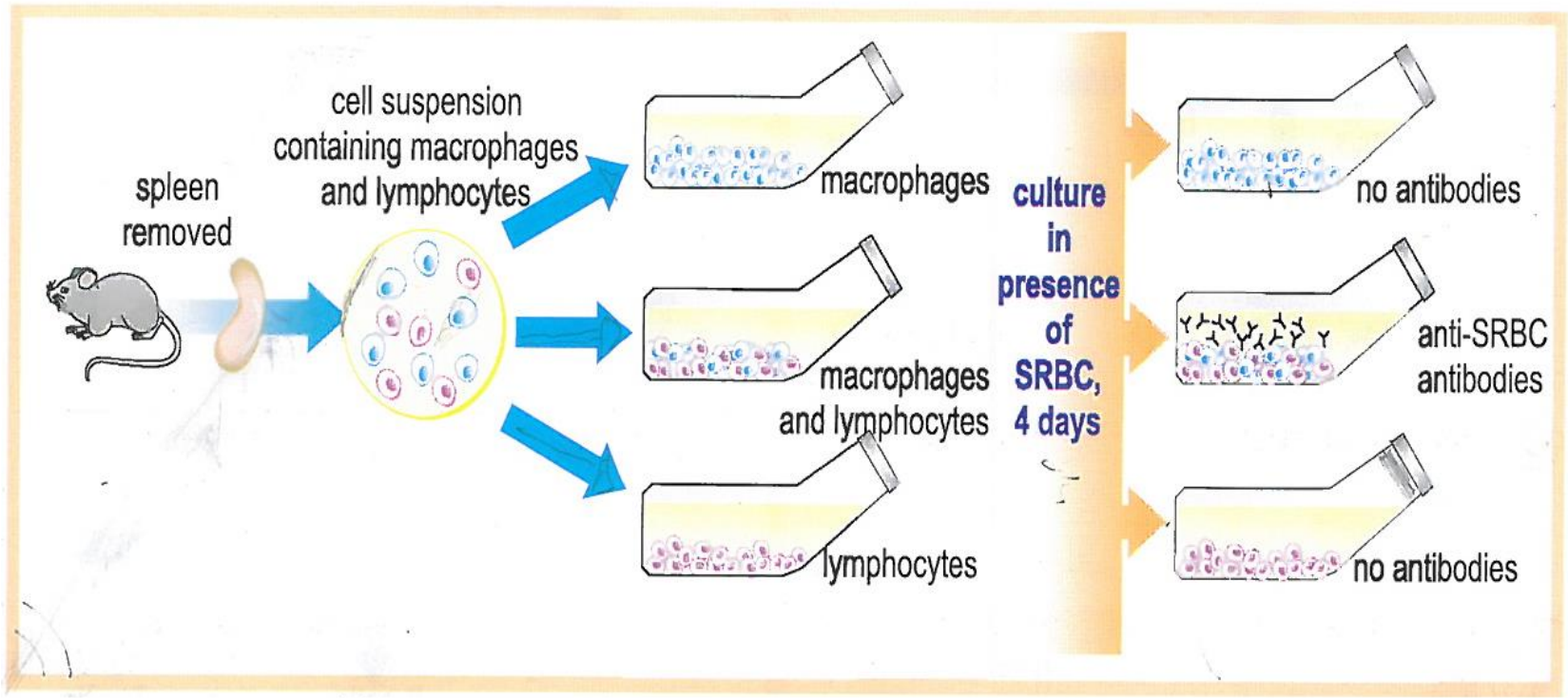
Document 3: Induction of the specific immune response

- The mechanism of specific Immune response include 3 phases:

1. **Induction** (Document 3 p: 142)
2. **Activation** (Document 4 p: 144)
3. **Effector Phase** (Document 5 p: 146 + Document 6 p:148)

How does the induction of the specific immune response take place?

I- Mosier Experiment:



1. **Interpret doc. a p:142**
2. **Explain how specific immune response is triggered by non-specific immune response**

1. **Interpret doc. a p:142**

The culturing of macrophages alone or lymphocytes alone, extracted from the spleen of a mouse with SRBC's, does not provoke secretion of anti SRBC Ab's after 4 days This indicates that neither macrophage alone nor lymphocyte alone are capable of secreting antibodies.

On the contrary, culturing both lymphocytes and macrophage extracted from spleen of a mouse with SRBC's provokes the secretion of anti SRBC Ab's after 4 days. This indicates that antibodies can not be produced unless macrophage and lymphocyte are present together (cooperation between macrophage and lymphocytes in presence of antigen) .

3. Explain how specific immune response is triggered by non-specific immune response

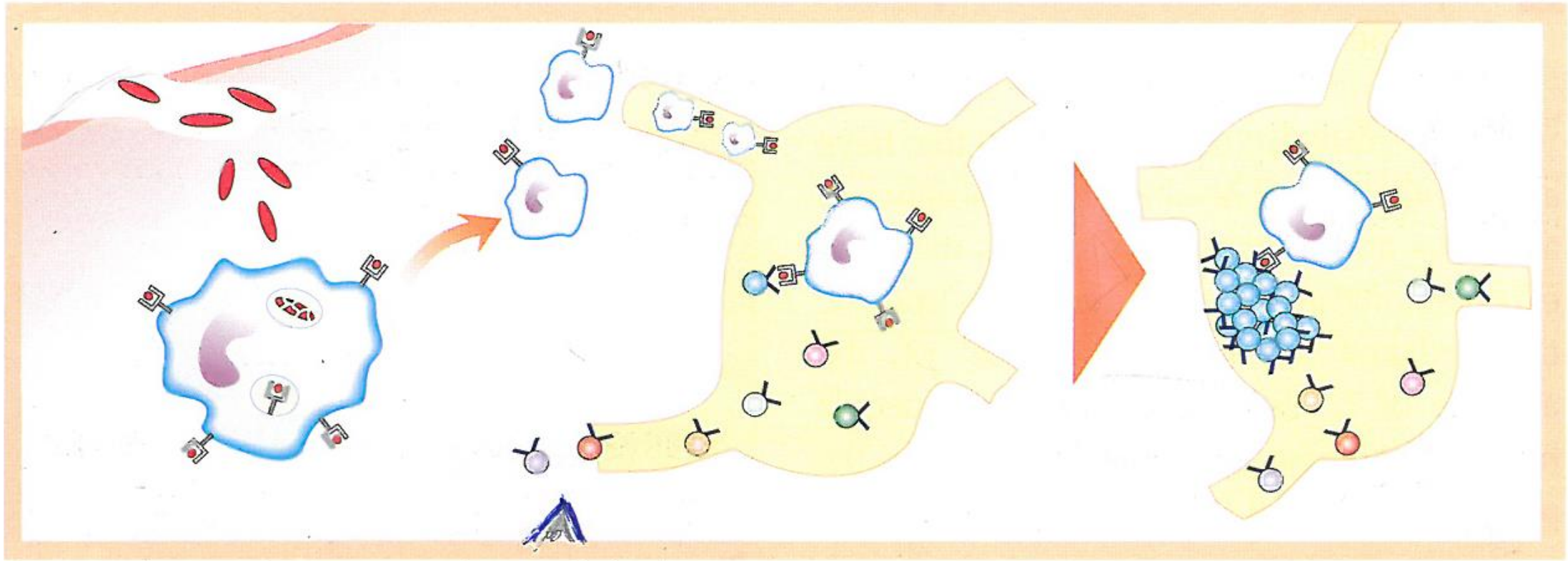
- The macrophages are the effector cells of the non-specific immune response while lymphocytes are the effectors of the specific immune response, and without macrophage, the lymphocytes can not produce antibodies.

II- Macrophage’s role in the induction phase

The macrophage phagocytes the non-self-antigen, it associates its peptides to HLA-II on its membrane surface.

Macrophage migrates to the closest lymph node where it becomes APC (Antigen Presenting Cell)

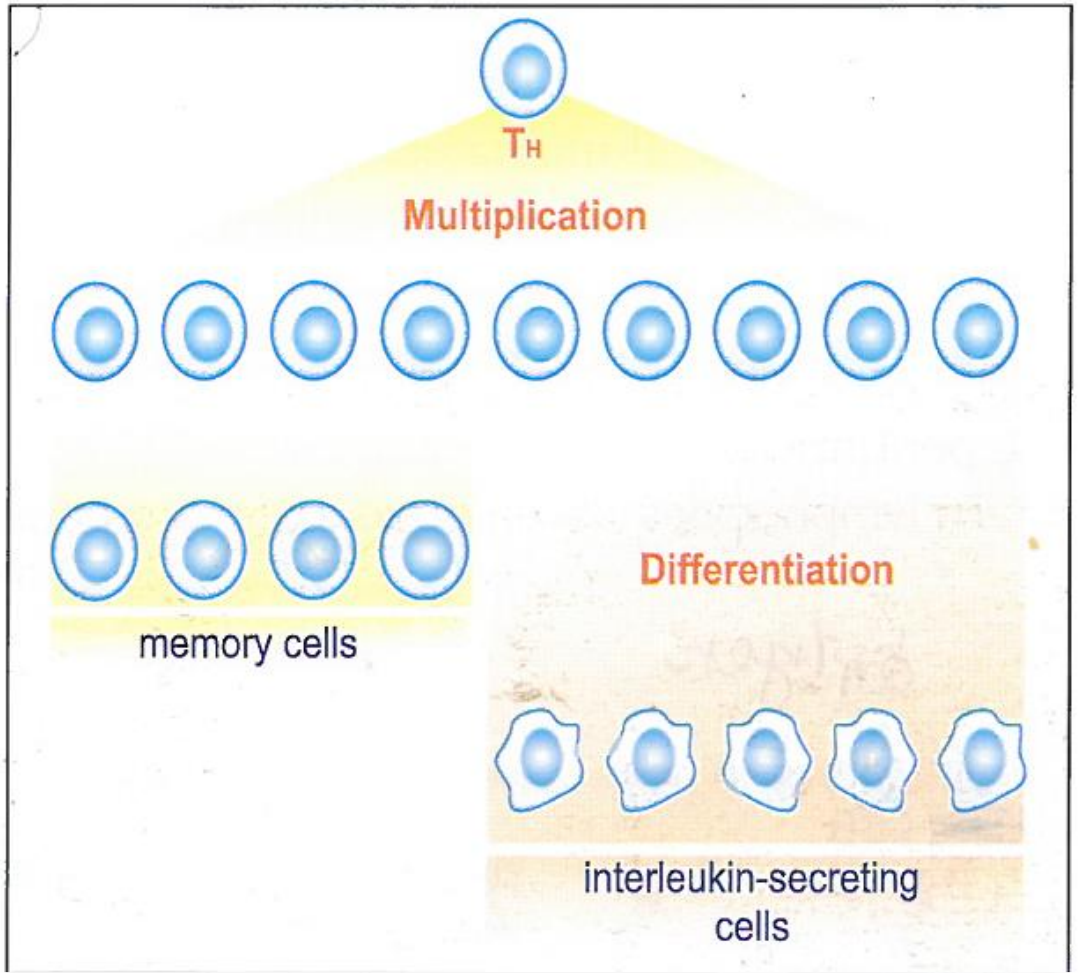
When **antigen specific** T4 arrives, it binds on the HLA-II non-self-peptide complex by double recognition with its TCR leading to its activation. This is the “**clonal selection”**



Doc.c *Selection of antigen-specific TH cells by the APC.*

III- Fate of activated T4

- **Clonal Selection:** the macrophage due to double recognition selects the specific T4
- **Multiplication:** only the activated T4 cells multiply by mitosis giving many identical T4 having identical TCR.
  Some of these cells remain as **memory cells .**
  However, the others undergo differentiation.
- **Differentiation:** Most of these T4 differentiate to become interleukin secreting cells that secrete cytokines called interleukins (IL).
- **Remark:** memory cells are partially differentiated

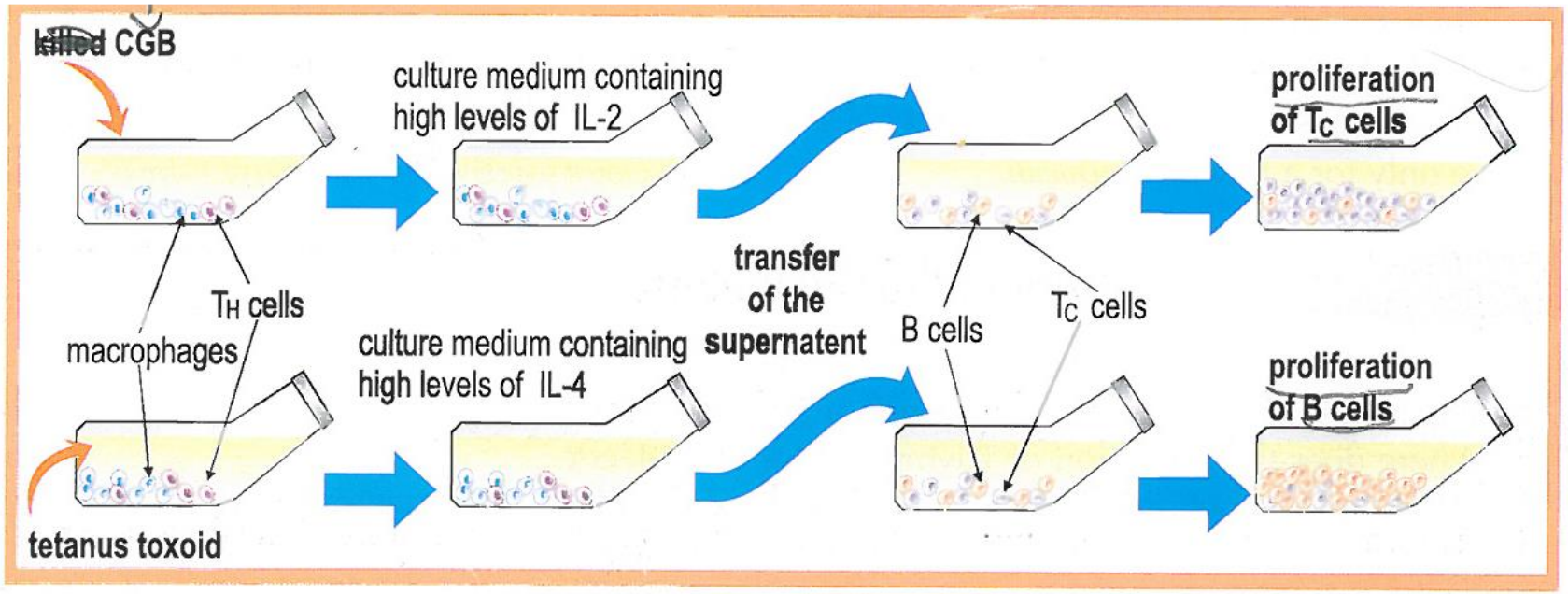


Document 4: Role of TH cells in the specific immune response

- **Introduction:** In the previous Document (3), document (a) showed that cellular cooperation between macrophages and lymphocytes to trigger immune response. Moreover, documents c and d demonstrated the role of macrophage in induction of immune response by activating antigen-specific T_4 cells which some of the cells produced upon multiplication after clonal section differentiate into interleukin secreting cells. A problem arises: **What is the role of interleukins in immune cells activation? What is the fate of the activated cells?**

Importance of T_H cells in the specific immune response:

- In order to understand the role of T_H in determination the type of immune response, experiment of **doc. b** was done:



1. **Interpret the obtained results**

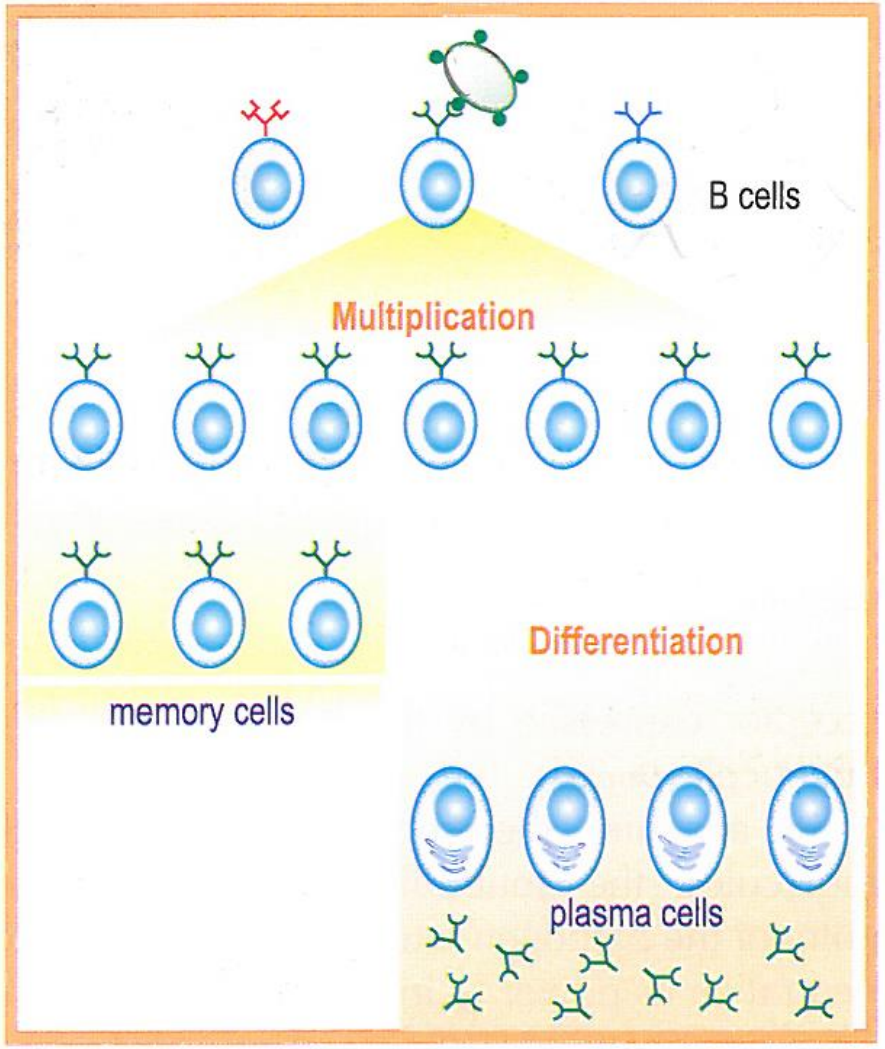
1. Interpret the obtained results:

The transfer of $IL_{2,}$ obtained from the culture medium containing macrophages, T_H cells and killed CGB, into a culture medium containing B cells and T_C cells, provokes the proliferation of T_C cells only. **This means that T_H secretes IL_2 to activate T_C cells.**

While upon transfer of IL_4, obtained from the culture medium containing macrophage, T_H cells and tetanus toxoid, into a culture medium containing B cells and T_C cells, provokes the proliferation of B cells only. **This means T_H secretes IL_4 to activate B cells.**

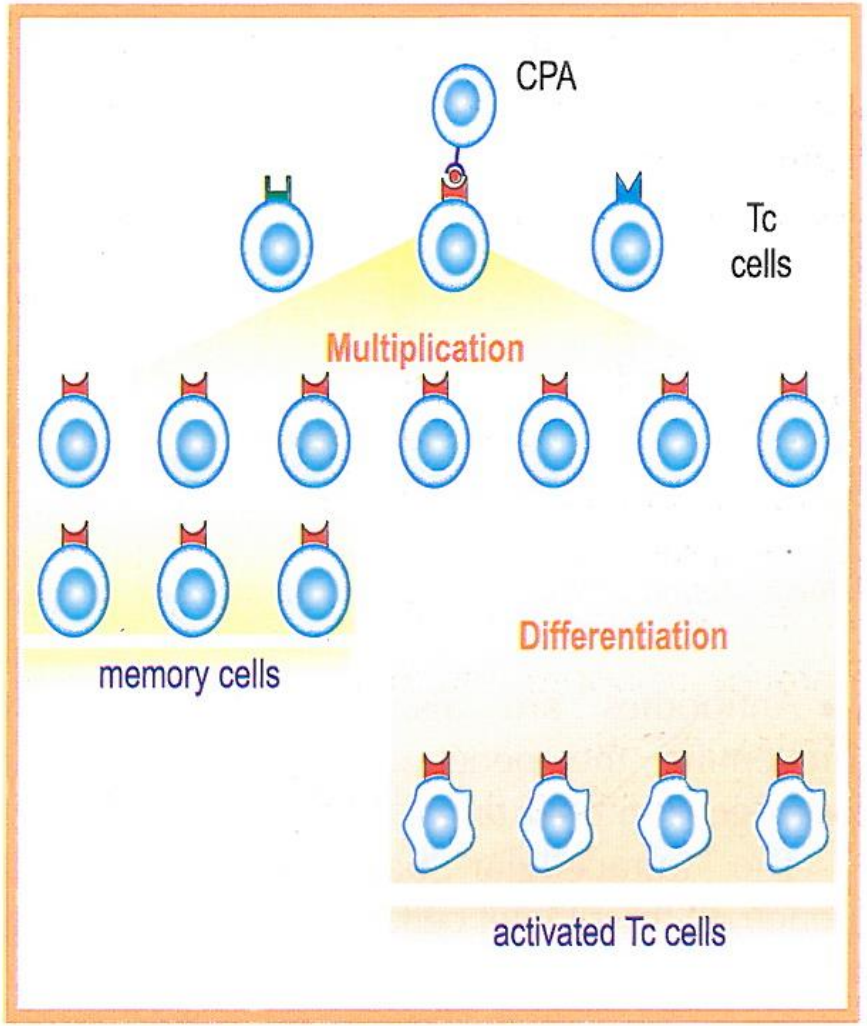
Fate activated cells (B lymphocytes and T_C lymphocytes)

- **Fate of activated B lymphocytes (doc. c, p:145)**
- **Clonal Selection:** Only B lymphocytes that **already had encountered** and bind to **free** non-self-antigen by their membrane bound antibody will be activated by IL4 and thus undergo multiplication
- **Multiplication (Proliferation):** the selected BL multiplies by mitosis producing a clone of BL having identical membrane antibodies. Some of these cells produced will remain as **memory cells.** The others will undergo differentiation
- **Differentiation:** The remaining differentiate into **plasma cell** that **secretes Ab** specific to the encountered antigen.



Fate of activated T_C lymphocytes (doc. d, p: 145)

- **Clonal Selection:** Only T_C lymphocytes that already had encountered and bind to non-self-peptide on **HLA-I** of **infected cells** by their TCR will be activated
- **Multiplication (Proliferation):** the selected T_C multiplies by mitosis producing a clone of T_C having identical TCR. Some of these cells produced will remain as **memory cells.** The others will undergo differentiation.
- **Differentiation:** The remaining differentiate into cytotoxic T_C that are able to kill infected cells



Note about characteristics of memory cells and effector cells

- Memory Cells
  - Long life span (few months to dozens of years)
  - Can proliferate
- Effector cells (differentiated)
  - Short life span (few days)
  - Cant proliferate.

- **Nude mice (doc. a p:144) are born without thymus; they have no cell- mediated immune response and no humoral mediated immune response. Explain why.**
- In the absence of thymus, T_L cannot be mature so there is absence of T_C leading to no cell-mediated specific immune response. Moreover, there is no mature T_4 which cannot be activated to secrete interleukin 4 so cannot activate B-lymphocytes. Therefore, humoral mediated immune response will not be activated

Comparative Table between activation of T_H, T_C and B lymphocytes

	T_H	T_C	B lymphocytes
Clonal Selection	Only cells that are specific for the antigen are activated		
Activation	Proliferation followed by differentiation leading to production of memory cells and effector cells		
Antigen Nature and Antigen presentation	Recognize antigenic peptides that are associated to MHC II	Recognize antigenic peptides that are associated to MHC I	Recognize epitopes of native antigen
The need of IL	Produce Interleukins	Needs IL_2 to be activated	Needs IL_4 to be activated

Document 5: Specific Humoral Immune Response

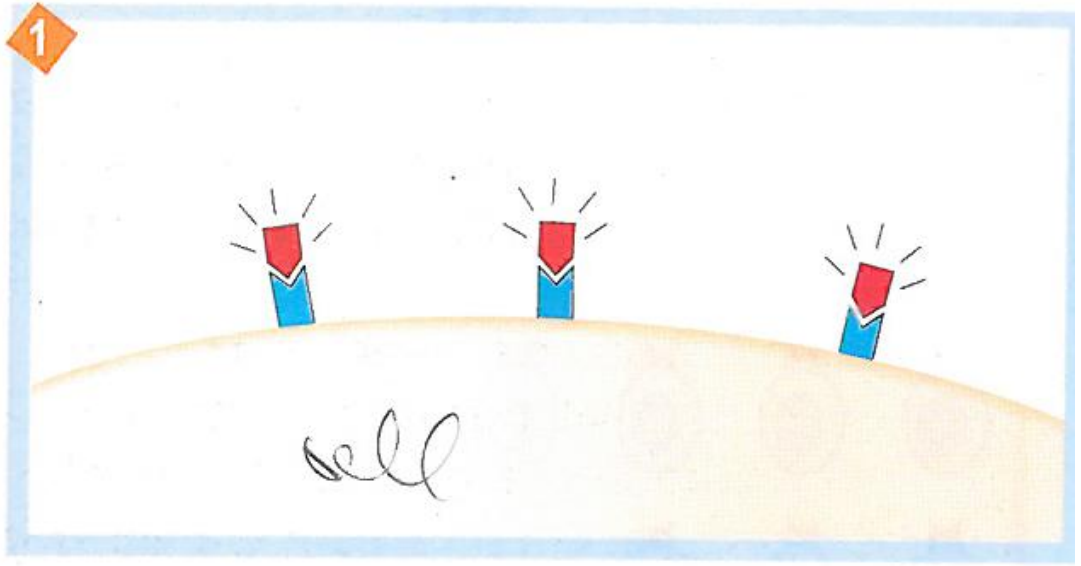
Introduction:

- Antibodies secreted by the plasma cell helps in elimination of antigens. **How?**
- First the antibody **neutralizes** the Anitgen by binding to it, then it leads to its **destruction** by macrophage **(opsonization) or** by activation of **complement cascade**

- **Remark:** Neutralization is the inhibition of toxin or microbe pathogenic effect simply by interacting with its active site.

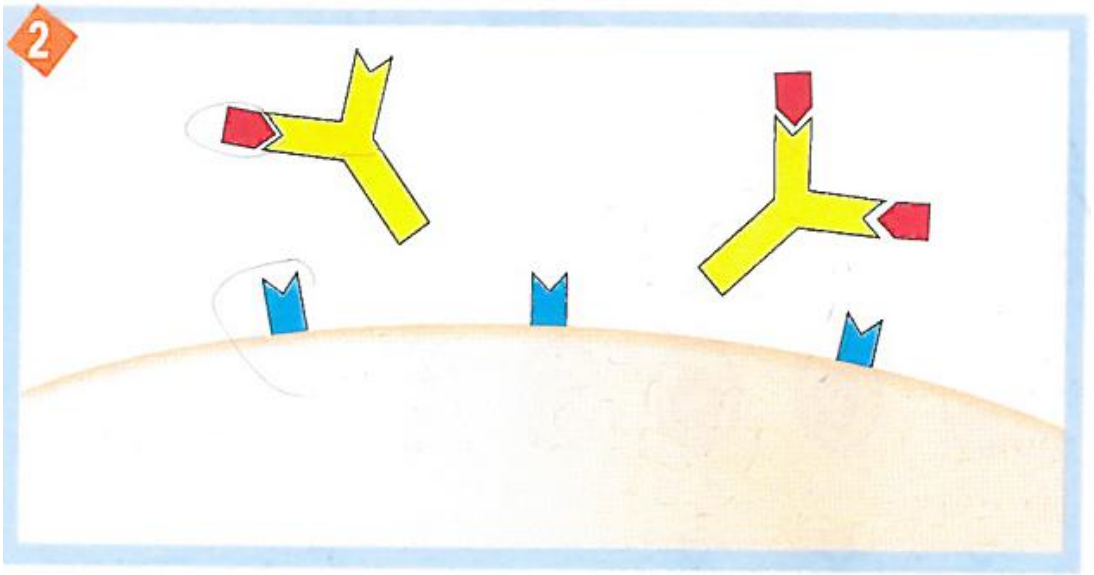
Neutralization of Toxin and viruses:

- **How does a toxin act?**
- To harm a cell, the toxin binds, using its attachment site, to membrane receptors of target cell.



- **Neutralization of toxin**

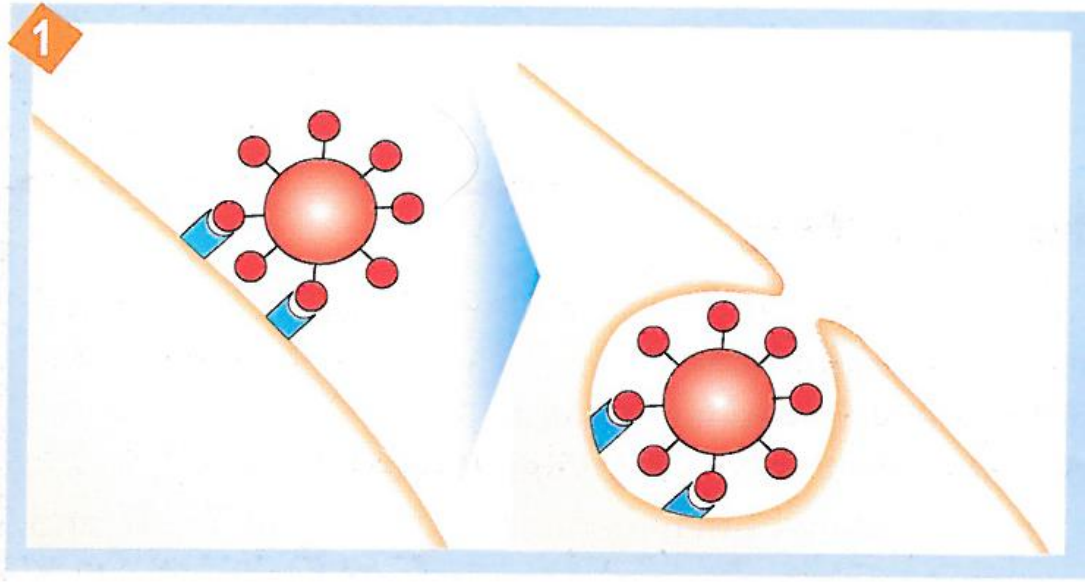
antibody that is specific to the attachment site of toxin binds to it preventing them to bind to the receptors of the target cell, so it is neutralized (loses its harmful



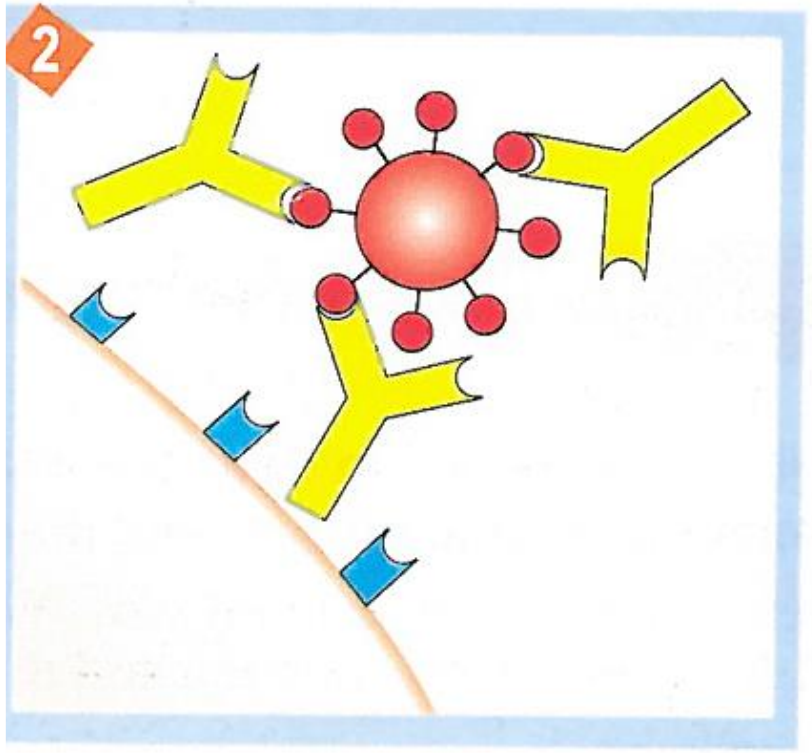
Neutralization of Viruses and intracellular bacteria

- **How do viruses and bacteria infect target cells?**

Viruses and intracellular bacteria infect target cells by binding their antigen on some specific membrane receptors on target cells.



- **Neutralization of intracellular bacteria and viruses by antibody:**
- Binding of antibodies specific to these antigens prevent them from binding to the specific receptor thus preventing infection of cell.

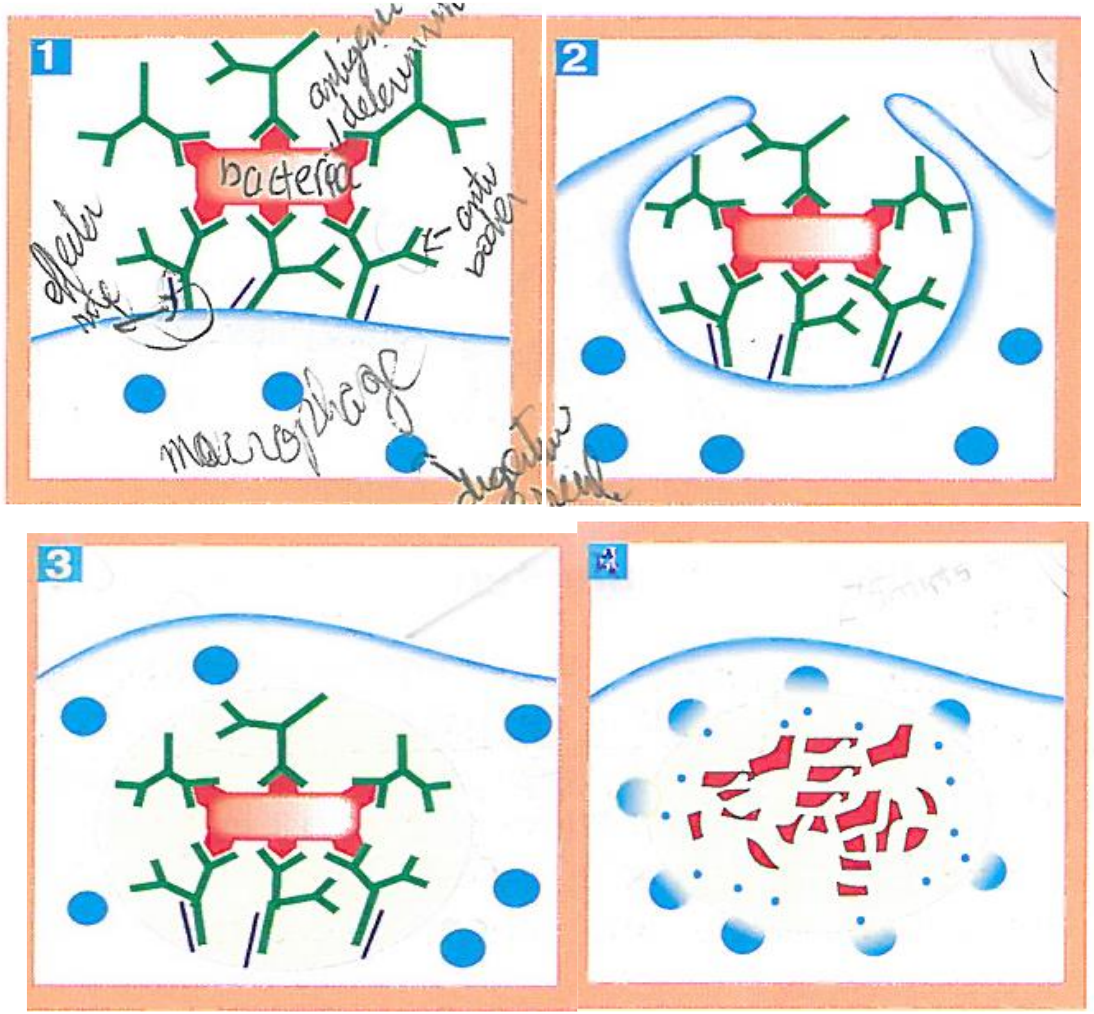


Elimination of the formed immune complex:

- The elimination of immune complex can be done by:
  - **Opsonization**
  - **Activation of complements enzymatic cascade**

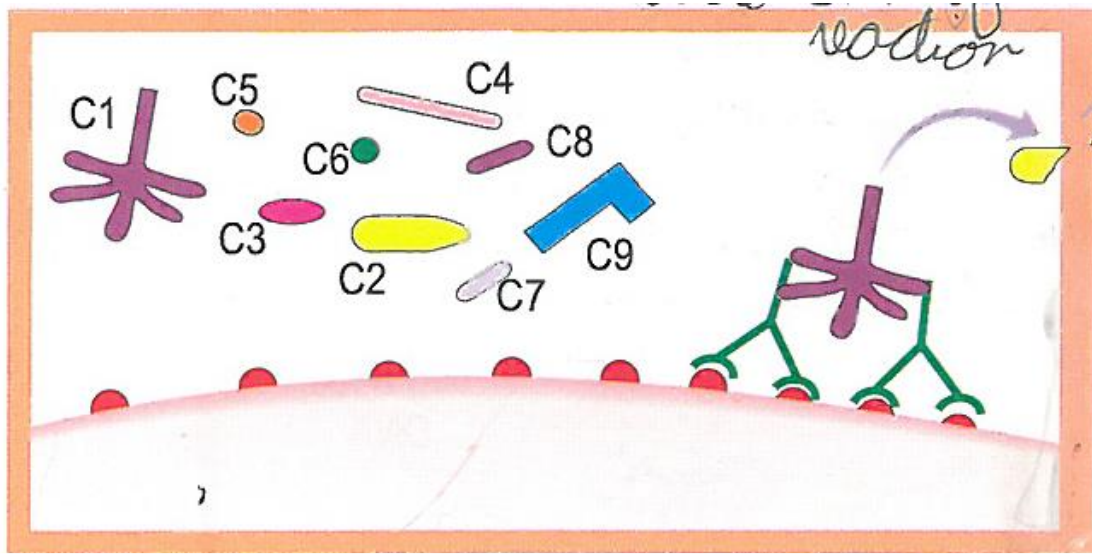
By opsonization:

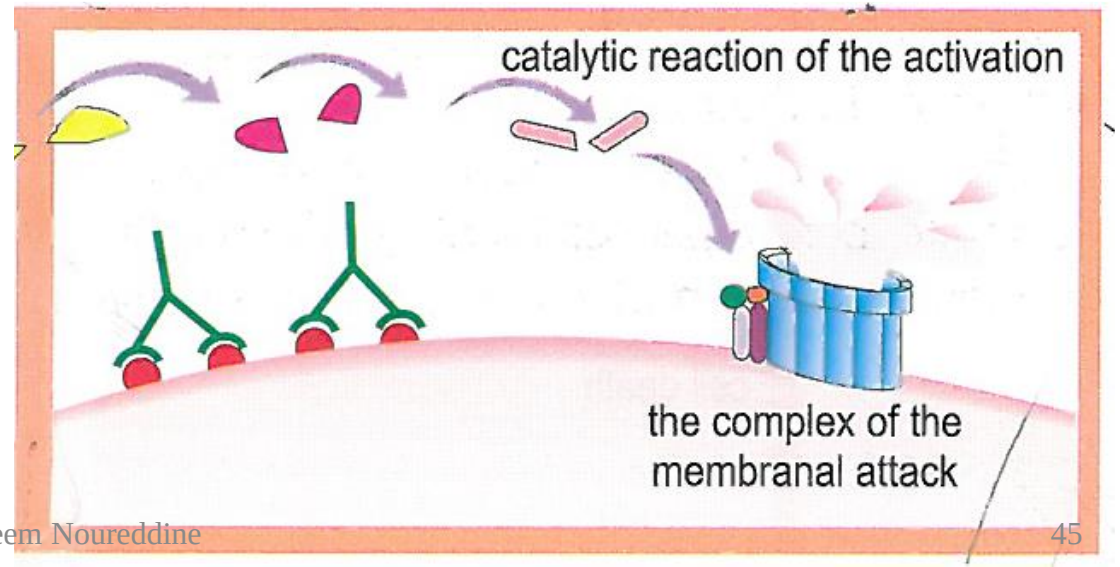
- After binding of the antibodies using their Ag binding sites of variable region to the epitope of the antigen, **the antibody can bind to membrane receptors of macrophage through its constant region making a molecular bridge between antigen and the phagocyte that facilitates adhesion**. **This is opsonization**. The macrophage phagocytes the antibody with antigen bound to it in order to eliminate them.
- **Therefore, Specific immune response (by Ab) facilitates the phagocytosis (Non-specific IR).**



Activation of complement enzymatic cascade:

- Upon the binding of antibodies by their variable region on the bacterial antigens, **a complement protein C1, may bind to constant region and become activated**. This activation leads to complement cascade activation from C1 to C9 that **forms at the end a membrane attack complex (MAC)** on surface of the bacteria that **perforate** the membrane of this cell **destroying** it



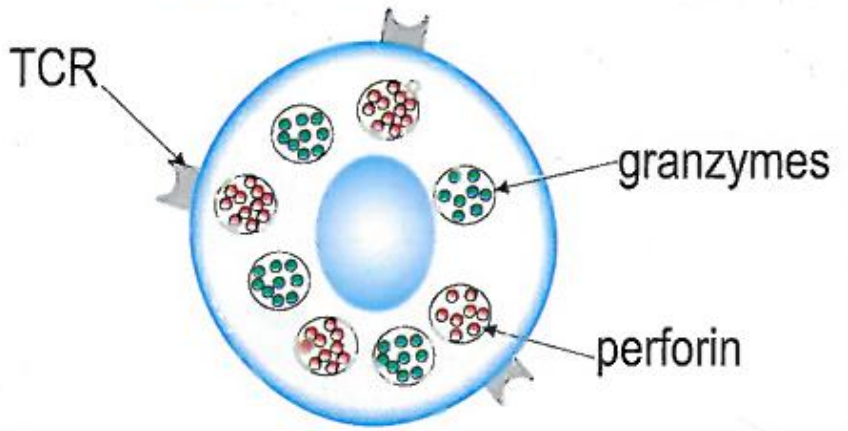


- **Note: Complements are not specific for species. The complement of any specie can act in the blood of any other species (Example: complement of human can work in rabbit).**

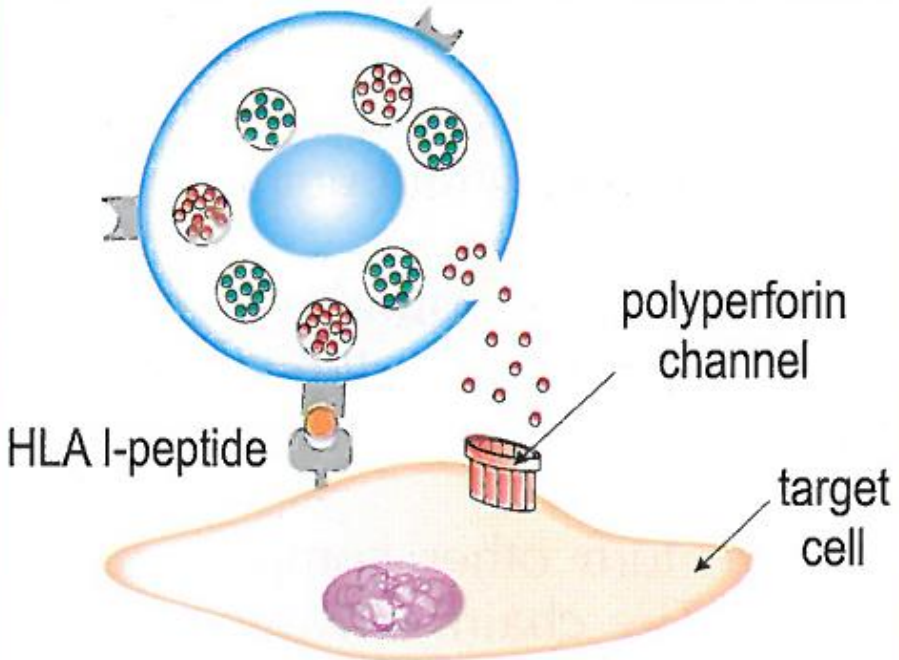
Document 6: Specific cell-mediated immune response:

- **How TC does eliminated infected cell, modified self or allograft?**

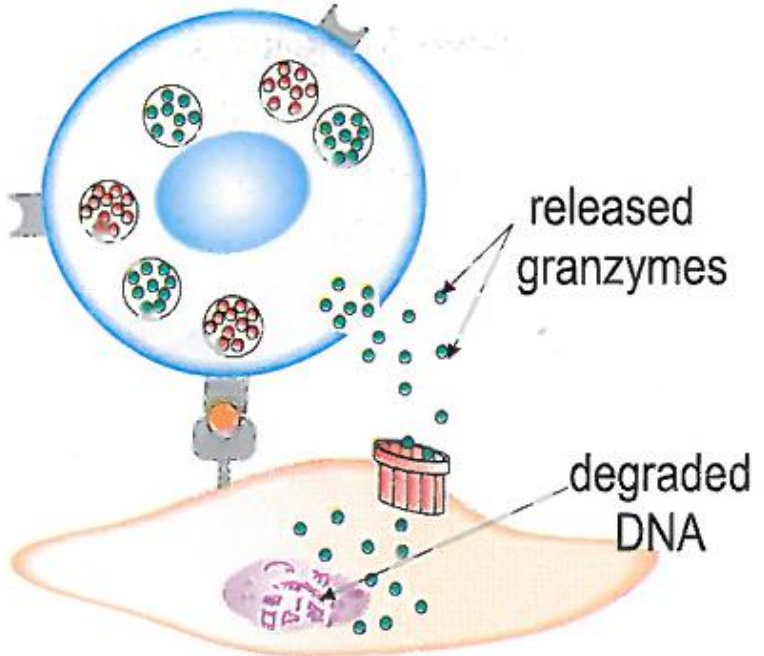
1- The T_C cell has cytoplasmic granules which contain toxic substances called cytotoxins, able to kill infected cells. There are two types of cytotoxins: perforin and granzymes.



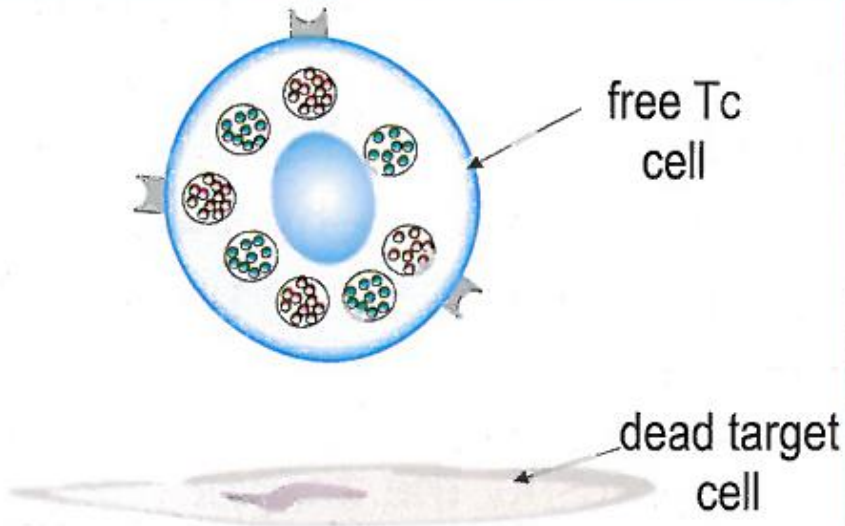
2- When a T_C cell recognizes an infected body cell and binds to the HLA I-peptide complex on the target cell membrane through its TCR, it releases its perforin content. Perforin assembles into polymers that form a hollow channel through the target cell membrane.



3- The Tc also release granzymes that penetrate into the target cell through the polyperforin channels. Granzymes trigger an enzymatic chain reaction within the cell, leading to DNA degradation: this causes cell death.



4- The Tc cell detaches from the killed target and recirculates. It is again ready to kill other targets carrying the same HLA I-peptide complex .



1. Tc lymphocytes recognizes non-self-peptide HLA-I complex by its infected cell by its TCR by double recognition.
2. Then it releases perforin that forms polyperforin channels through the cell membrane of the infected cell
3. then it releases granzymes that enters through these channels and trigger an enzymatic reaction in the infected cell that leads to degradation of its DNA and thus, the death of infected cell
4. Tc cell detaches from the killed target cell and recircultes. It is ready to kill other targets carrying the same HLA I- peptide complex.

Cancer and Immunity:

- **Definition of cancer:** caused by an uncontrolled division of abnormal cells in a part of the body.
- **What is the cause of <u>uncontrolled</u> cell division? :** Due to accumulation of genetic mutations (alteration in genetic program).
- **How immune system can recognize cancer cells?**

The cancer cells synthesize modified proteins from mutant DNA. Peptide of this protein binds to HLA I molecules to form a new complex (HLA I- modified self-peptide) which will be recognized by T_C by their TCR.

- **Type of immune response triggered against cancer:** Cell-mediated specific immune response since cancer cells present on their surface non-self-peptide associated with self-MHC-I, which is recognized by TC cells that are the effector cells in cell, mediated specific immune response.

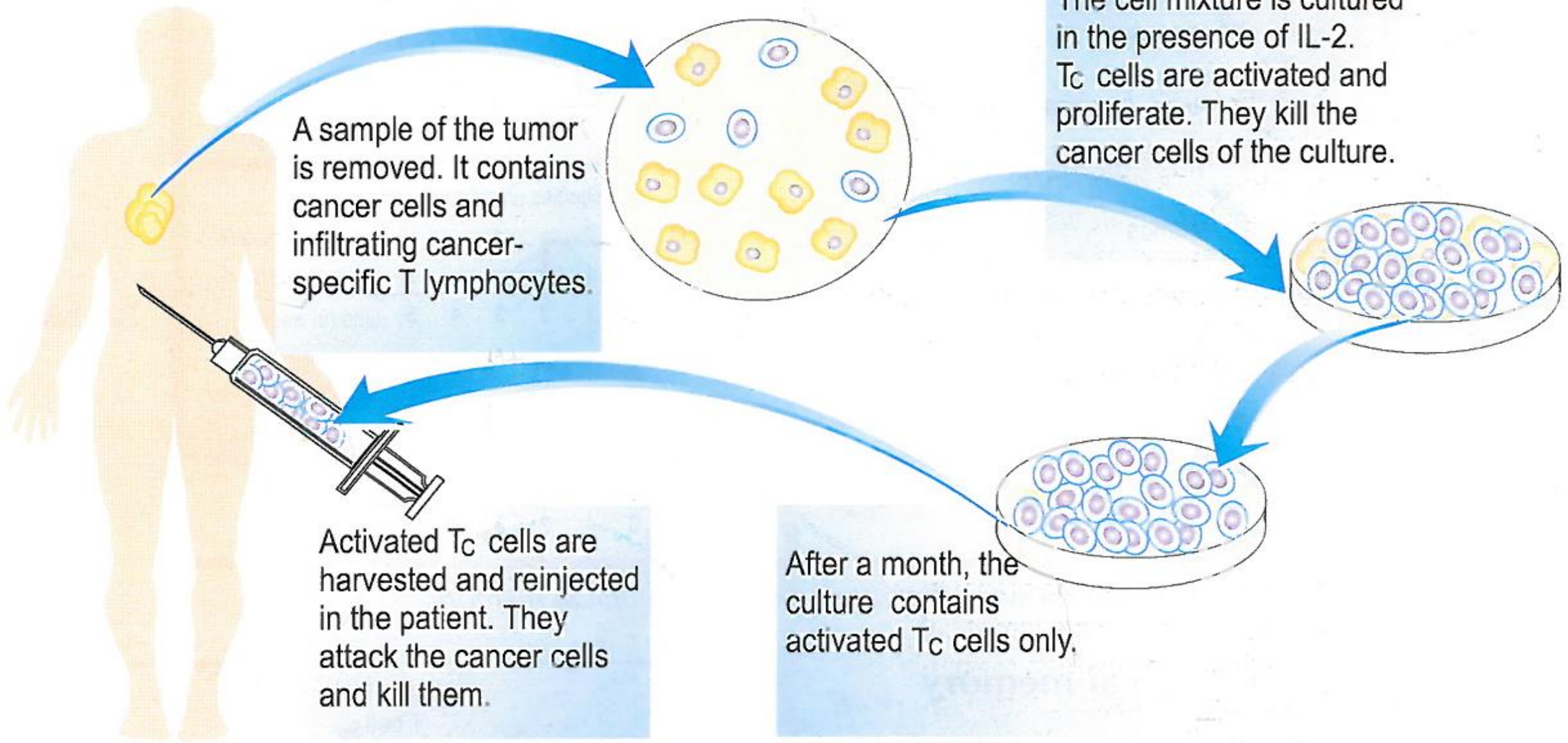
- **Why does immune system fail in case of cancer?**

It is very difficult for the body to eliminate cancer cells because they proliferate very rapidly and overwhelm the immune system.

Treatment of Cancer:

Radiotherapy	Chemotherapy	Immunotherapy (doc. b p:149)
Expose tumor to radiations that destroy DNA of tumor cells but it also destroys normal cells	Administration of chemicals that stop multiplication of cancer cells and normal cells of the patient especially bone marrow so causes weakening of immune system.	Is the best choice because it is the most specific. It selectively kills tumor cells and thus theoretically should have fewer side effects than chemo and radiotherapy

Immunotherapy



Document 7: Immunological Memory

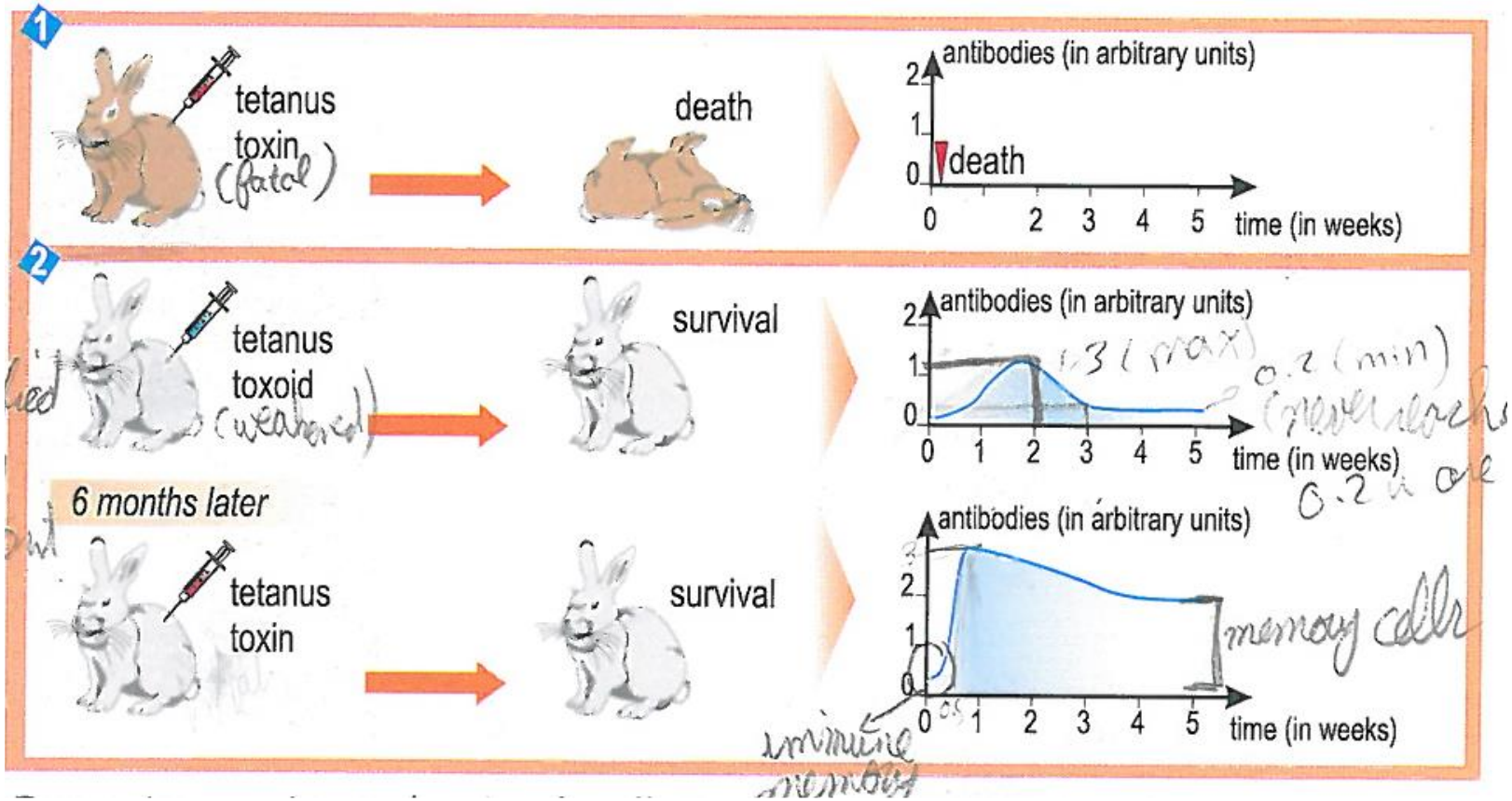
- **Introduction:** The encounter of immune system with the antigen for the first time triggers specific immune response (primary immune response). Upon activation, the antigen-specific immune cells proliferate where some of them become **memory cells**. These memory cells **play a role in the protection of the body during the next infection of the same antigen.**

Characteristics of primary and secondary responses:

- **Definitions:**

  - **Primary specific immune response:** the immune response induced during the initial contact with a given antigen
  - **Secondary specific immune response:** the immune response induced after the second contact with the same antigen injected during the primary immune response

Characteristics of secondary immune response:



1) **Interpret the adjacent experiment**

2) **Conclude the characteristics of 2^{ary} immune response**

3) **Justify the characteristics of secondary specific immune response**

1) Interpret document (a) p:150 (important)

- In the first experiment, the injection of tetanus toxin tot a rabbit leads to its death, and the level of anti-tetanus toxin antibodies is 0 a.u. This indicates that tetanus toxin is **pathogenic and lethal**, and has not induced an immune response. **While,** in the second experiment the injection of tetanus toxoid to the rabbit leads to its survival and to the increase of antibodies after a week to reach maximum value of 1.5 a.u after two weeks then it decreases till about 0.3 a.u to remain constant till t= 5 weeks. **On the other hand,** the injection of this same rabbit with tetanus toxin 6 months later leads **also to** survival and to increase of the antibodies from a **higher** initial level = 0.3 a.u at t= 0 weeks till reaching **a higher maximal** = 3 a.u (2x higher than that of upon first contact) within **less time** (1 week) (2x less time). Then **it also decreases but <u>slower</u>** until reaching a **higher** level= 1.8 a.u after 5 weeks.

- **This means that** the tetanus toxoid protects the animal against tetanus toxin and the immune response triggered upon second contact is faster, more intense and more lasting.

2) Conclude: secondary immune response is **faster, more amplified and long lasting (more persistent)**

3) Justify the characteristics of secondary specific immune response:

- **Faster:** latency (time between injection of antigen and secretion of antibody) is shorter than that of primary (maximum reached 1 week in secondary less than that of primary 2 weeks)
- **More intense:** more efficient by secreting larger amount of antibodies (maximum 3 a.u> maximum of first contact 1.5 a.u)
- **Long Lasting:** more persistent, the antibodies remained high even after 5 weeks (2 a.u> 0.3 a.u in case of primary response)

Primary Vs. Secondary Immune Response

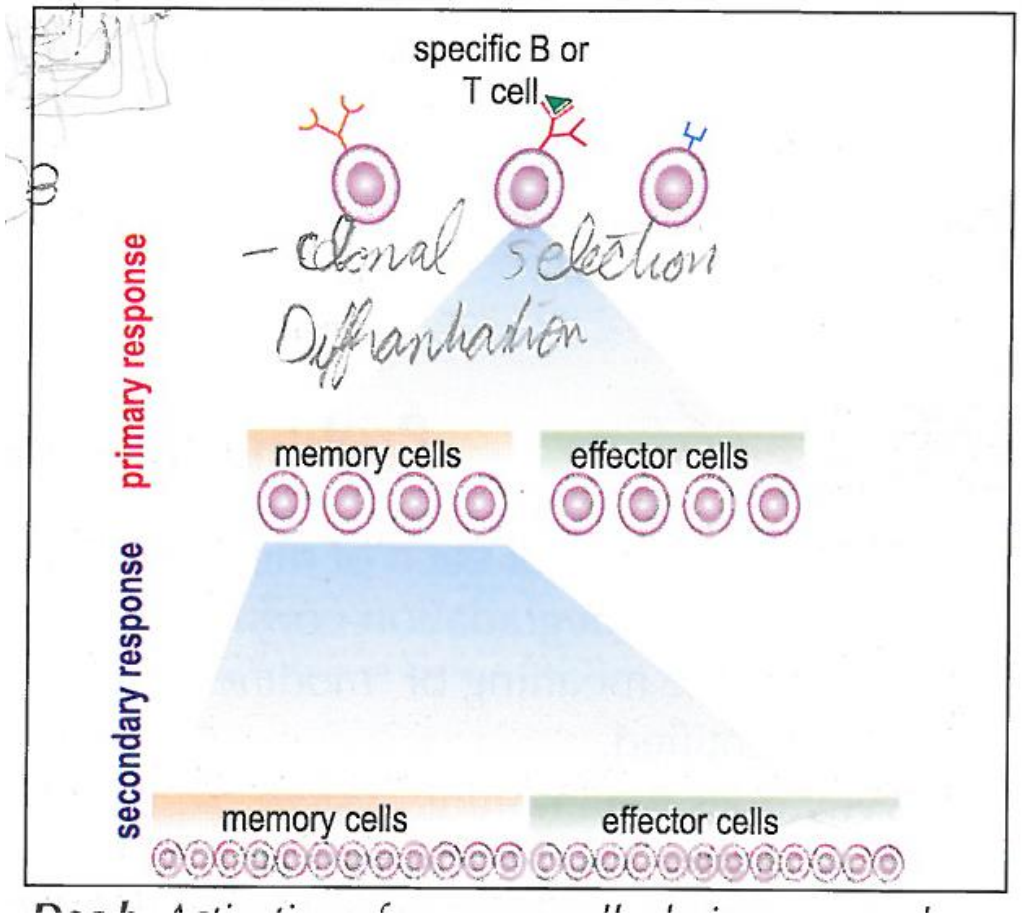
Primary Immune Response	Secondary Immune response
Less amplified	More amplified
Slower, has greater latency time	Faster, has shorter latency time
Less persistent	More persistent

NSIR Vs SIR

Non specific immune response	Specific immune response
Does not lead to immunological memory	Lead to immunological memory
Not amplified following repetitive contacts with the same antigen	Amplified through repetitive contacts with the same antigen
Non specific: The reaction is not dependent on identity of antigen (non specific)	Specific: the immune response triggered depends on identity of antigen.

Bases of immunological memory (Refer to doc b p: 150)

- The primary specific immune response leads to **production of memory cells** that are **partially differentiated**, which are then **activated in secondary specific immune response**.
- The **partial differentiation** of these cells, prior to the 2nd injection of antigen, **explains the shorter latency**.
- The **higher number of memory cells** compared to stimulated cells in primary response in 2ary immune response r**leading to higher number of effector cells** results in **amplification** of the response and its **persistence** for a longer duration.



Vaccination Vs. Serotherapy

- **Vaccination: (Preventive method)**

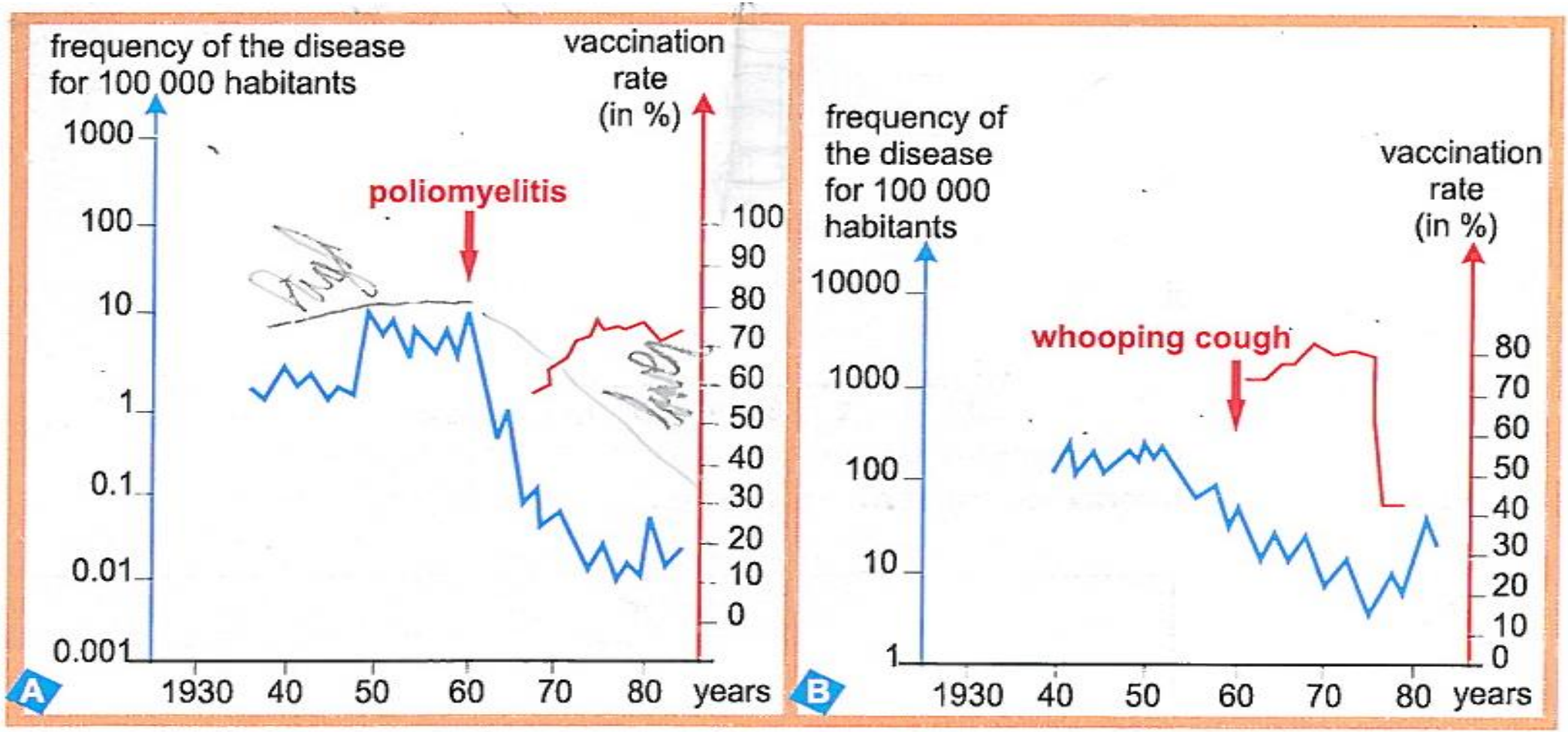
It is the injection of a killed or attenuated microbe or microbial products (vaccine), **inducing immune memory** able to protect the body from subsequent specific immunity against the pathogen.

- **Serotherapy: (Curative method)**

it is the injection of serum containing antibodies.

	Vaccination	**Serotherapy**
Nature of the injection	Killed or attenuated antigen	Serum **containing** antibodies
Origin of antibodies	Endogenous	Exogenous
Latency period	10 days	few hours
Duration of protection	Several years	Two weeks
Nature of immunity acquired	Active	Passive

Importance of vaccines:



- **Analyze Doc. C (a) p: 151**

Before the introduction of the poliomyelitis vaccine, that is, between the years 1935 and 1960, the frequency of the disease ranged between 1 case and 10 cases per 100000 individuals. But it started to decrease after the introduction of the vaccine in the year 1960 and as the rate of vaccination increased to reach 72%, this frequency decreased to reach about 0.01 cases per 100000 individuals in the year 1980. The frequency showed a slight increase following the slight decrease in the vaccination rate, after which the frequency and rate resume their original variation following the year 1982.

- **Analyze Doc. B (b) p:151**

Before the introduction of the W.C vaccine, that is, between the years 1940 and 1960, the frequency of the disease ranged (fluctuated) between 7 case and 140 cases per 100000 individuals. But it started to decrease after the introduction of the vaccine in the year 1960 and as the rate of vaccination increased to reach 80%, this frequency decreased to become less than 10 cases per 100000 individuals in the year 1975. Then as the vaccination rate decreased, the frequency increased to reach about 40%.

- **What can you conclude?**

We can conclude that vaccination protects the body from pathogen that diminishes the frequency of disease.

Document 8: Diagnostic Applications of the antibody properties.

- **Introduction:** The high specificity of antibody characteristic make them valuable tools to use for detection of particular molecules. For that, **using antibodies allows us to detect whether an individual is infected or not by a certain pathogens**, to **diagnose a cancer** and even to **detect the presence of certain antibody in serum of an individual**

Tests

1. **Serological Tests:**
   a) **Agglutination Reaction**
   b) **Immunodiffusion on a gel**

2. **Immunomarking tests**
3. **Immunofluorescence**

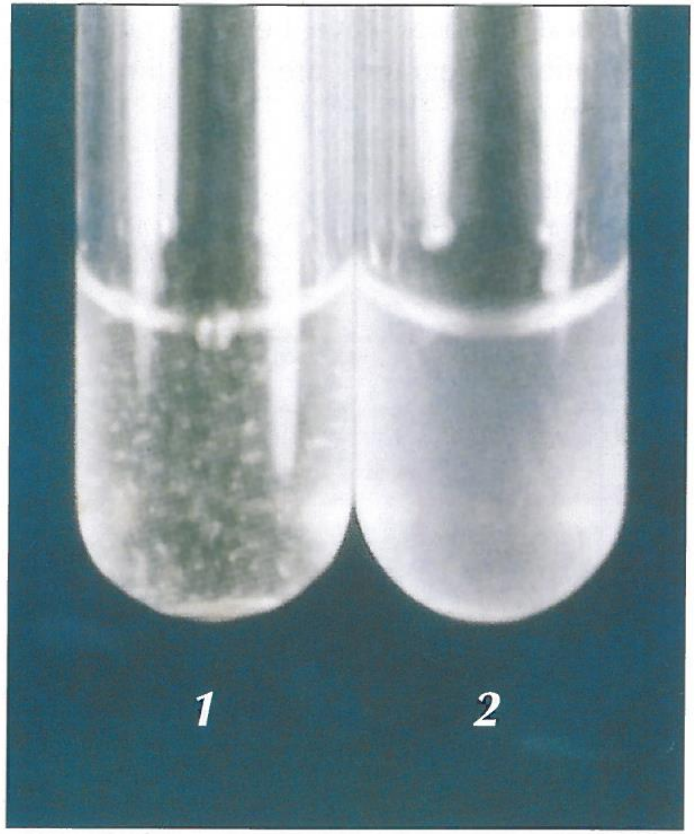
Serological Tests:

- **Agglutination Reaction:**

Agglutination of a microbe by plasma of an individual indicates that this individual has specific antibodies against this microbe; thus, he is affected

Agglutination Reaction:

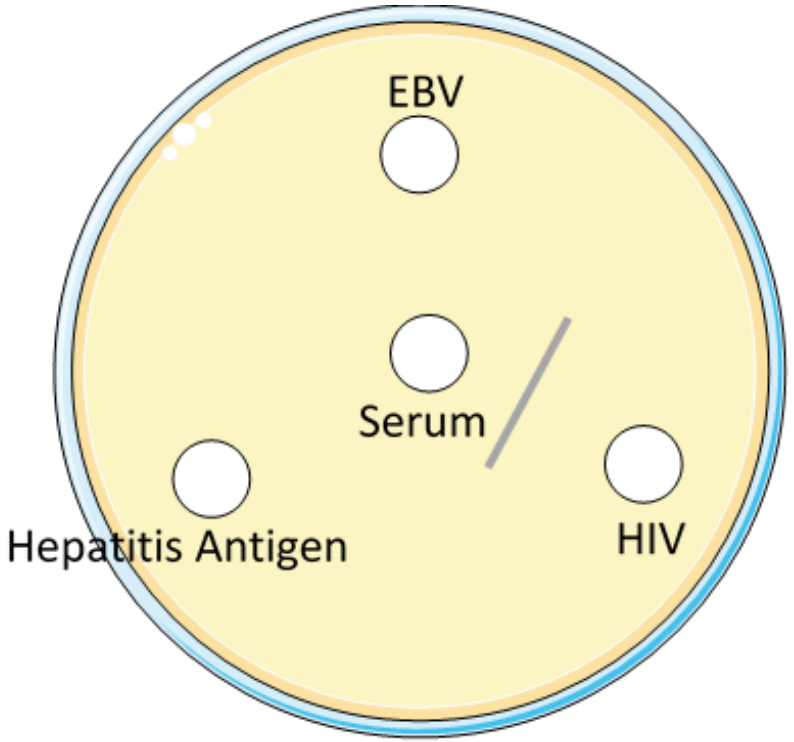
- Tube 1 **shows agglutination**, so the individual's serum co**ntains anti-salmonella antibodies** (seropositive); therefore, the **individual is infected with salmonella**
- Tube 2 shows **no agglutination**, so the individual's serum **does not contain anti-salmonella antibodies (seronegative);** therefore, the individual is **not infected with salmonella**



Doc.a *salmonella agglutination tests: positive test (1), negative test (2)*

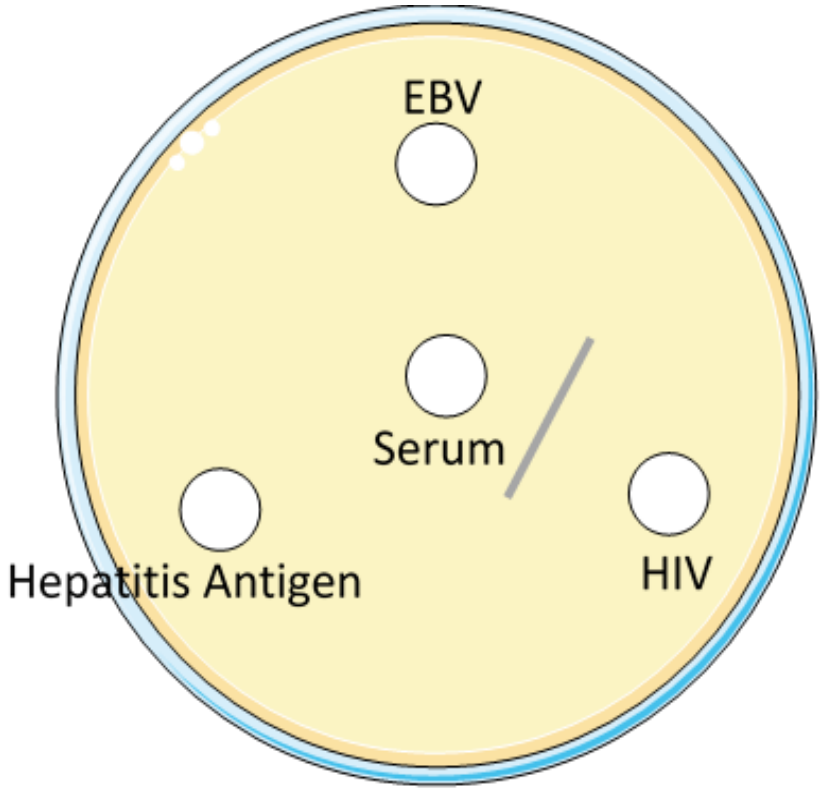
Immunodiffusion on a gel:

- This technique is used to detect antibodies in serum using many antigens
- **Technique:** In this test, serum is applied in a central well in a gel and known antigens in surrounding wells. With time, both antigens and antibodies diffuse in all directions. When the antibody meets its specific antigen, a precipitation arc will appear



Immunodiffusion on a gel:

- Referring to the adjacent figure:



- **Interpret the obtained results:**

A precipitation arc is formed between central well and that containing HIV while no precipitation arcs are formed between this central well and other wells. This means that the serum contains only anti-HIV antibodies and no Ab for the other antigens.

- **Conclude:**

We conclude that the patient is infected with HIV.

Immunomarking tests

- **ELISA Test: E**nzyme **L**inked **I**mmunosorbent **A**ssay
- It is used to detect presence of antibodies specific against an antigen.
- More sensitive than agglutination and immunodiffusion in gel
- It can be used **qualitatively** (presence or absence of antibody) in addition to **quantitatively** (determining the concentration of antibodies) and thus duration of infection by comparison with control cases

Immunofluorescence (doc. d p: 153)

- It is used to test for cellular antigens as tumor antigens:
- **Technique:**

1. Add fluorescent antibodies (antibodies couple to a fluorescent molecule) on sample of cells
2. Wash the sample (to remove non-specifically bound antibodies).
3. Submit the cells to ultraviolet light beam,
4. **Result:** the fluorescent substance emits colored light that can be observed under fluorescent microscope