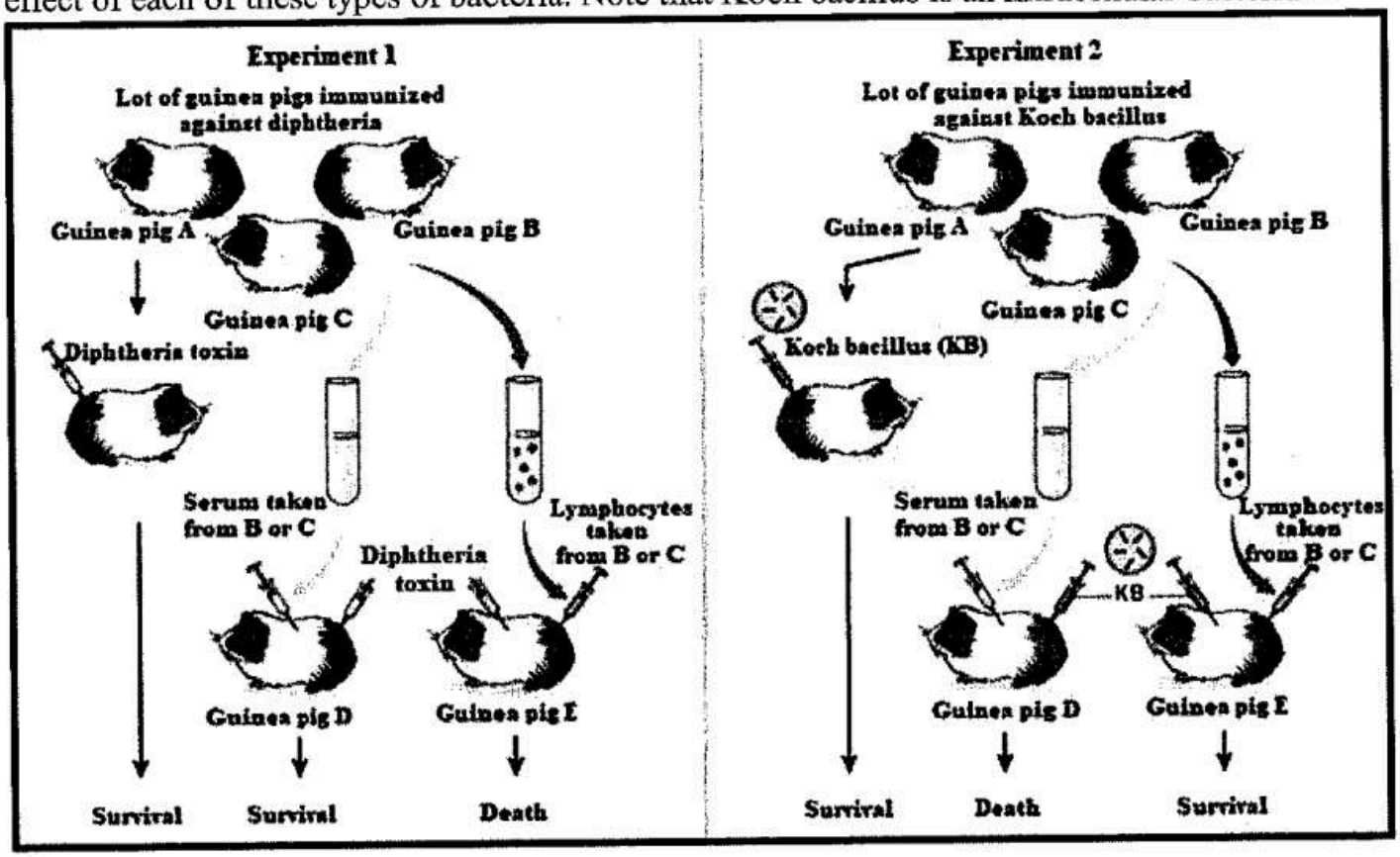
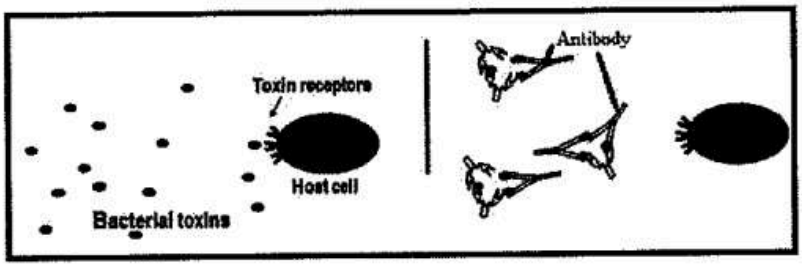
EXERCISE 1 Specific immune response

You cannot escape bacteria, they are everywhere! The good thing is that very few bacteria are harmful. Some of them are so because they are intracellular, they are able to harm the body cells by multiplying inside them, others are extracellular but toxic, they produce toxins that perturbate the function of some body cells by binding on their membrane receptors.
The following experiment is achieved in order to understand how the immune system neutralizes the effect of each of these types of bacteria. Note that Koch bacillus is an intracellular bacterium.



Document 1

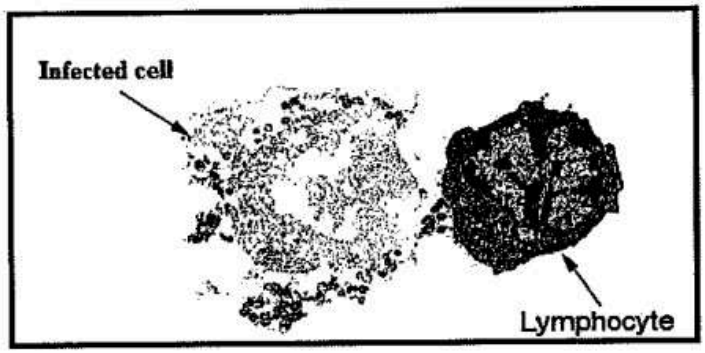
1. Pick out the problem studied by this experiment.
2. Compare these two experiments.
3. Deduce the elements of blood implicated in the immune response against each type of bacteria.
4. Specify the type of immune response involved against each bacterium.



Document 2

Documents 2 and 3 show two different mechanisms of the specific immune response.

5. Match each of the two documents 2 and 3 to the corresponding experiment of the two experiments 1 and 2 in document 1. Justify your answer.
6. Explain the mechanism of action of the immunity in each of the two cases in documents 2 and 3.



Document 3

Exercise 1 Specific immune response

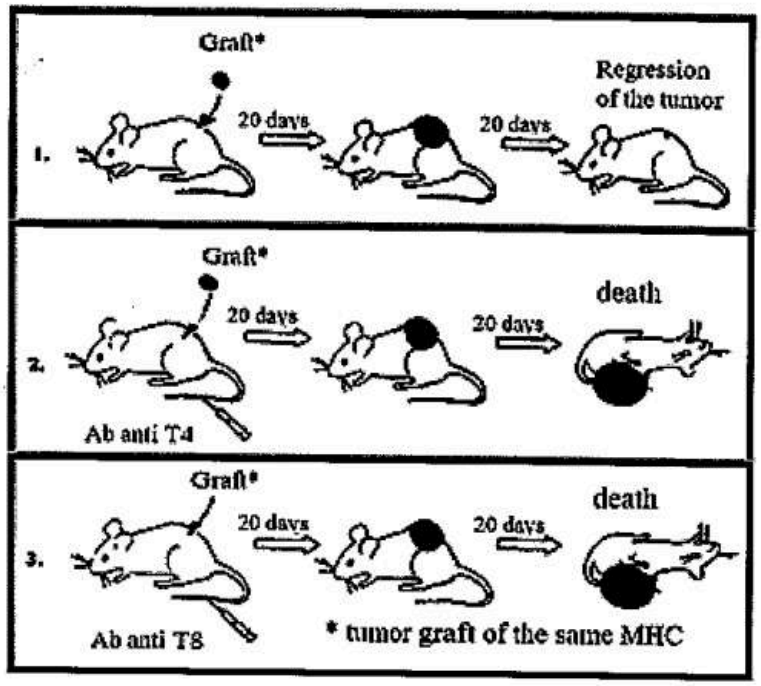
1. How does the immune system neutralize the effect of different types of bacteria?
2. In both experiments, a lot of guinea pigs is immunized against a pathogen, which is the agent of diphtheria in experiment 1 but it is the Koch bacillus in experiment 2.
   In both experiments, the serum and the lymphocytes are taken from the immunized guinea pigs and each injected into a different guinea pig which is then injected by the same active agent of the one that has immunized the initial lot, also in both cases an immunized guinea pig is injected again by the same active agent as the immunized one agent.
   In experiment 1, the guinea-pig receiving the lymphocytes dies whereas the other two guinea-pigs survive, whereas in experiment 2 the guinea-pig receiving the serum dies, whereas the other two guinea pigs survive.
3. The guinea pig A immunized against diphtheria or KB survives following its injection respectively by diphtheria toxin or KB. But as only guinea pig D injected by the serum of B or C immunized against diphtheria survives following its injection by fatal diphtheria toxin, whereas the guinea pig E which has received B or C lymphocytes dies. Thus, a chemical substance of the serum is involved in the immune response against diphtheria toxin.
   Whereas in experiment 2 it is only the guinea pig E which received the lymphocytes of B or C immunized against KB followed by an injection by the KB survives, but the guinea pig D injected by the serum of B or C followed by an injection by the KB dies. Thus, the lymphocytes are involved in the immune response against KB.
4. It is a specific humoral immune response against diphtheria toxin because it is the serum antibodies that protect the guinea pig and not the lymphocytes, the antibodies of the serum are the effectors of the specific humoral immune response.
   It is a specific cell-mediated immune response against BK because it is the lymphocytes that protect the guinea pig and not the serum antibodies, some lymphocytes are the effectors of the specific cell mediated immune response.
5. Document 2 corresponds to the neutralization of an antigen by the antibodies whereas it is a specific humoral mediated immune response corresponding to experiment 1.
   Document 3 corresponds to the lysis of an LTc-infected cell, whereas it is a specific cell-mediated immune response corresponding to experiment 2.
6. The mechanism of immune action in doc 2:
   the toxins attack the target cells by binding to membrane receptors. When the antibodies bind to the toxins they cover their attachment sites on the target cells; and prevent the toxin from acting, this is the neutralization.
   The mechanism of immune action in doc 3:
   The cytotoxic cells recognize the infected cell and bind to the HLA-I- non-self peptide by the TCRs and release perforin that make polyperforine channels through the plasma membrane. then the Tc cell releases the granzymes that enter the polyperforine channel and triggers within the infected cell a chain of enzymatic reactions that lead to the degradation of the infected cell's DNA and leads to its death.

EXERCISE 2 Immunity and treatment of tumor

Much has changed in our understanding of cancer since Hippocrates, in around 400BC, described a tumor, most likely of breast tissue, as resembling a crab and named it a 'cancer' (which is Latin for crab).

In the aim to study the immune response induced against the tumors (cancers) several experiments are achieved. These experiments aim to determine the elements of the body responsible for the immune responses against cancer. Document 1 shows one of these experiment.

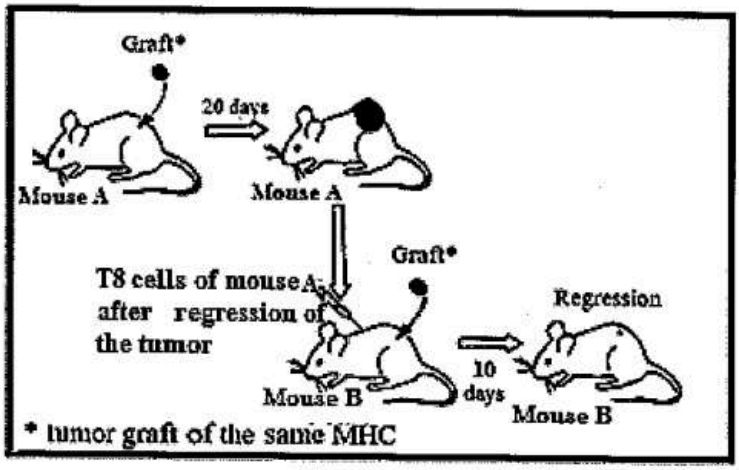
1. Formulate, by referring to the text, the problem studied by this experiment.
2. Describe this experiment.
3. Explain the necessity for the transplant to be of the same MHC of the recipient mouse to permit for the experiment to answer the posed problem.
4. Interpret the results of this experiment.
5. Explain the results of mouse 2.



Document 1

Document 2 shows an experiment of transfer of T8 of a normal mouse, after the regression of a tumor, to a mouse having received a tumor graft at the same time of injection of T8.

6. Explain the results of this experiment.



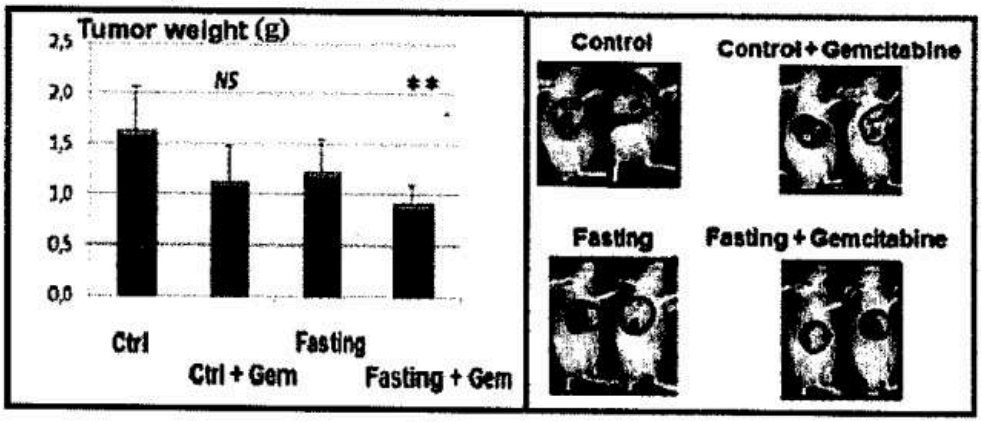
Document 2

The results of these two experiments require the lysis of the tumor cells by some immune cells. These tumor cells express new peptides that are considered as non self.

7. Explain the mechanism of lysis of the tumor cells by the suitable immune cells.

Aiming to study the effect of fasting combined or not with a drug called gemcitabine on tumor, four groups of NOD mice totally immunodeficient are manipulated differently. The performed manipulations and the obtained results are given in document 3.

8. Show starting from these experiments that fasting and gemcitabine are two factors of inhibition of tumor independent from the action of T4 and T8 shown above.



Document 3

Exercise 2 Immunity against tumor

1. What are the body elements responsible for immune responses against cancer?

2. A tumor transplant of identical MHC develops 20 days after grafting in a mouse 1, the next 20 days shows a regression of the tumor. The same transplant carried out in a mouse 2 injected by the anti-LT4 Abs and in a mouse 3 injected by the atni-LT8 Abs, show the same development of the tumor after 20 days and develops in the following 20 days to end by the death of these mice.

3. If the tumor is of different MHC, it can be rejected due to the different MHC marker but not because of its tumor character. On the other hand, a grafted tumor from a different MHC mouse presents for the experiment two variable factors that do not allow us to draw the conclusion proper to the problem.
4. Tumor grafts identical MHC are grafted in three mice of the same strain, these grafts take the same aspects developed after 20 days but at the end of the following 20 days a regression of the tumor is observed only in the first mouse showing that the normal mouse is able to fight against the tumor, but the second injected by the anti-T4 Ab as well as the third mouse injected by the anti T8 Ab shows a significant development of the tumor after 20 days, to end in the death of two mice. This shows that the presence and cooperation between LT4 and LT8 is essential to fight against tumors.

5. Antibodies are proteins that have specific binding sites on another molecule. The anti-LT4 antibodies will therefore bind to a molecule present on the surface of T4 lymphocytes and thus agglutinate them. They will be neutralized and can't accomplish their role. It is a specific cell-mediated immune response whose effectors are LT8s that attack and destroy cancer cells but the LT4 activated by the macrophages (APC) must secrete IL2 to activate LT8, they remain inactive and no lysis of the cancer cells is obtained.

6. This experiment confirms the results of the first experiment by showing that LT8 are responsible for tumor regression and that LT8 should be activated by LT4 already activated by APC, hence in the first experiment the regression of the tumor is observed only after 20 days, time necessary for the activation of the LT8 whereas in the second experiment the injected LT8 contain the selected and activated clone. This is why the regression of the tumor is faster 10 days instead of 20.

7. The cytotoxic cell recognizes the cancer cell and binds with the TCR on the HLA-I - non-self peptide complex to its membrane, then it releases perforin that make a polyperforine channel across the plasma membrane; then the Tc cell releases the granzymes that enter the polyperforine channel and triggers within the infected cell a chain of enzymatic reactions that lead to the degradation of the infected cell's DNA and leads to its death.

8. The tumor weight is 1.6 g in the control mouse, it decreases to 1.2 g with fasting and decreases more to 1.1 g with treatment by gemcitabine, however this weight decreases much more with the combination of fasting with treatment by gemcitabine to 0.9 g. This indicates that fasting and gemcitabine help in the regression of the tumor. But since the experimented mice are totally immunodeficient so the action of fasting and gemcitabine is not in relation with the activity of the immune system, then it is independent from the action of T4 and T8.

EXERCISE 3 The secretion of antibodies

In order to study the effect of a secondary immune response in the elimination of an antigen from the body, two series of experiments are done.

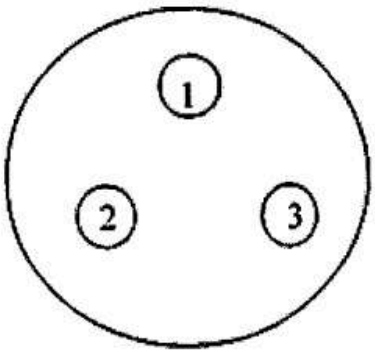
1st series of experiments: We inject in mice A by intravenous route a suitable amount of an antigen, the bovine serum albumin (BSA), which causes the appearance of antibodies that we measure during the 7 weeks which follow this injection. Mice B also receive an injection of BSA; this is followed, eight weeks later by a second injection of another antigen, teta to mice A and BSA to mouse B.
Two weeks after the injection either of BSA to the mice A or of antigen teta to the mice B, the serum is taken and we test its capacity to cause the agglutination of antigens BSA and teta by a test of immunodiffusion on gel. The results of measurements of the rates of antibodies of the mice A and B are shown in document 1.

Injection of BSA (mice A and B) ↓ — Injection of antigen teta to mice A and BSA to mice B ↓

Time (weeks)		2	3	4	5	7	8	9	12	13	14	15
Rate of antibodies a.u.	mice A	1	10	15	105	2	1	1	80	10	3	2
	mice B	1	9	12	100	2	1	60	300	250	200	150

Document 1

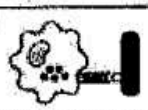
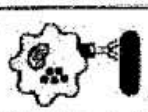
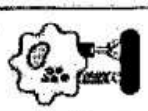
1. Draw the graph showing the evolution of the rate of antibodies in the mice A and B shown by document 1.
2. Analyze the results shown by the table.
3. Draw out the differences between the primary and the secondary immune responses.
4. Explain the origin of the secondary immune response's characteristics.
5. Draw the results of the test of immunodiffusion on gel for the mice A at times 5 and 12 weeks while using Document 2 where: 1: BSA, 2: teta, 3: serum of mice A.



Document 2

2nd Series of experiments:
The complement cascade is activated directly by the bacteria, but it is highly activated by the constant regions of the antibodies bound on them; this activation will lead to the cleavage of the components of the complement among which the component C3 that is cut into two fragments C3a and C3b, we decide to study the effect of C3b fragment on the process of phagocytosis made by the macrophage. The figure in document 3 summarizes the experimental setup and the obtained results.

6. What can you deduce from document 3?
7. Explain, based on all what preceded, the fast elimination of antigens during the secondary immune response.

Content of the medium	Adhesion and phagocytosis
1  Macrophage Bacteria	±
2  Macrophage Bacteria Fragment C3b	++
3  Macrophage Bacteria Specific antibodies	++
4  Macrophage Bacteria Fragment C3b Specific antibodies	++++
+: Arbitrary speed of phagocytosis -: Decrease of the speed with time	

Document 3

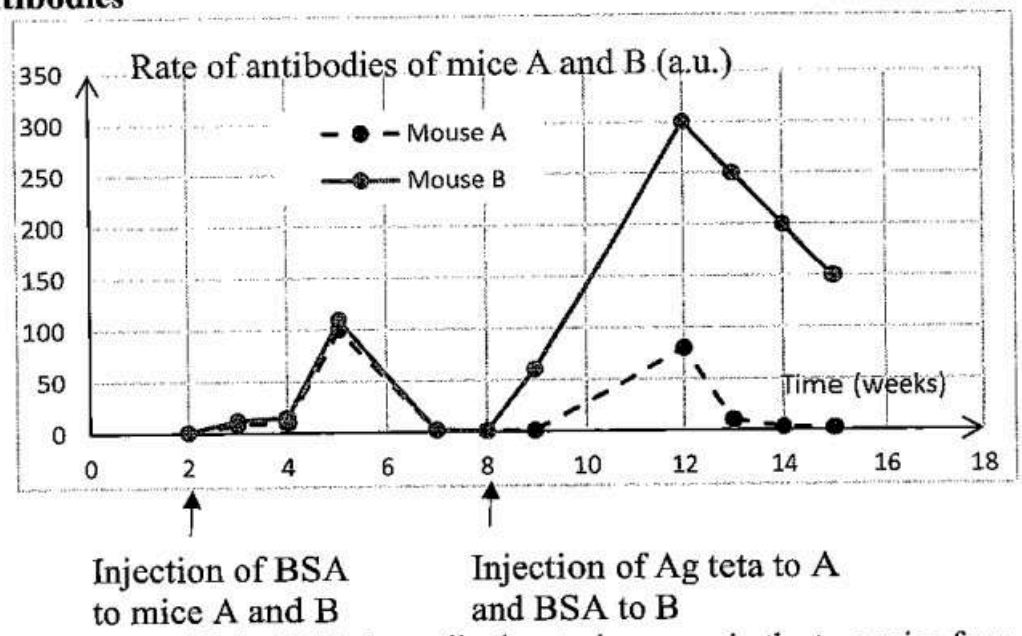
Exercise 3 The secretion of antibodies

1. Graph showing the variation of the rate of antibodies of the mice A and B after injection of antigens BSA and teta during time.

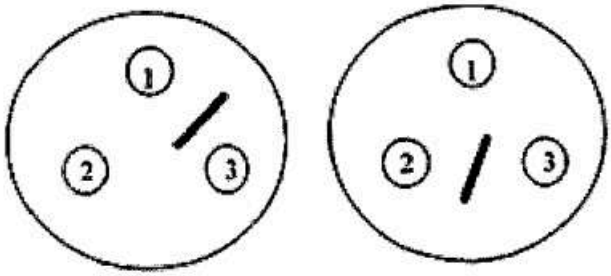
Scale:

50 a.u.

2 weeks



2. Following the Injection of two Mice A and B by BSA the antibody rate increases in the two mice from 1 to 100 a.u. in the mouse A, and from 1 to 105 a.u. in the B mouse when the time passes from 2 at 5 weeks but beyond this time the antibody level decreases in the two mice, to become 1 a.u. at the 8th week, whereas when the mouse A is injected by the antigen teta the antibody level increases up to 80 at the 12th week but decreases rapidly until 2 at the 15th week but in the mouse B injected for a second time with the BSA antigen it increases rapidly up to 300 a.u. at the twelfth week a value 3.75 times higher than A but beyond this time the antibody level decreases to 150 a.u. and remains 75 times higher than that of A at the fifteenth week.
3. The secondary immune response is faster, more amplified and more persistent.
4. When an antigen enters the body, it causes a clonal expansion of the B cells specific to it. Each clone gives, on the one hand, short-lived plasma cells and, on the other hand, long-lasting memory cells that will be activated during the secondary response. The partial differentiation of these cells, prior to the second introduction of antigen, explains the short latency (faster). The high number of effector cells obtained from the activation of memory cells, results in an amplification of the immune response and a longer persistence of the response.
5. Results of the gel immunodiffusion test for mouse A at times 5 and 12 weeks.

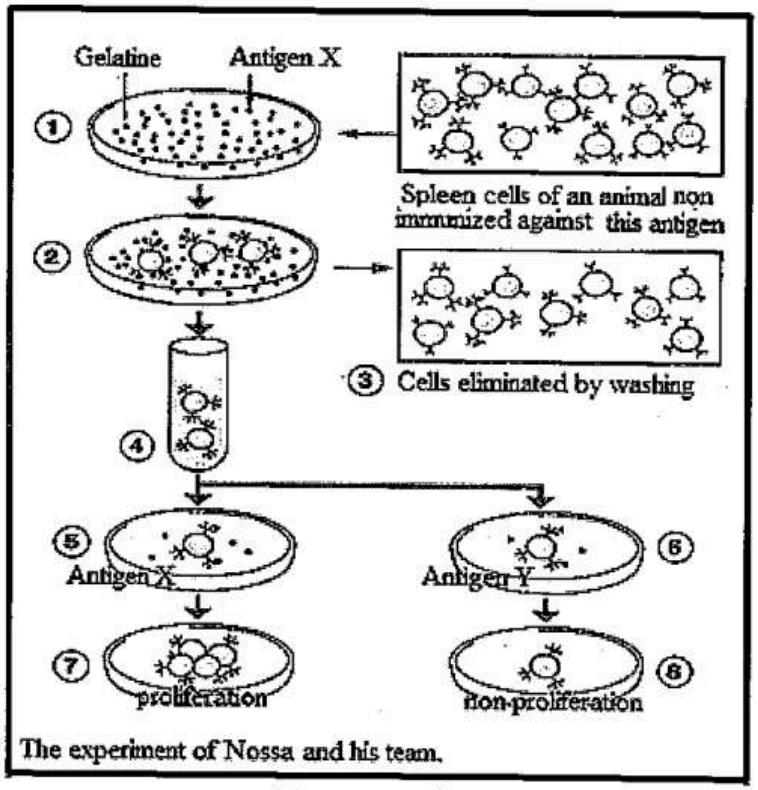


5th week **12 week**

6. Since adhesion and phagocytosis of bacteria are observed in low speed in the presence of macrophage only with a decrease with time, while they are with higher speed (double) in the presence of C3b fragment of the complement with no decrease with time, the same in the presence of the specific antibodies this means that C3b and antibodies amplify and keep adhesion and phagocytosis for longer time. Moreover, this speed increases again to the double when specific antibodies and C3b fragment are present together with no decrease with time. Thus, there is a cooperation between C3b fragment and specific antibodies in the amplification and the persistence of the speed of adhesion and phagocytosis.
7. The binding of antibodies to a bacterium activates the component C1 of the complement, which in turn activates the enzymatic cascade of complement, this cascade leads to the cleavage of C3 to give C3b fragment that amplify and keep adhesion and phagocytosis persistence. Since in the secondary immune response amount of antibodies is much rapid and higher than that of the primary, then the secondary immune response accelerates and amplifies the activation of the complement and the process of phagocytosis that permit a very fast elimination of the non self antigens.

EXERCISE 4 Role of T4 in the induction of the immune response

The immune system is able to recognize and defend all the types of non self antigens that are introduced to the body, this means by its ability to differentiate all the types of lymphocytes that are directed against these antigens. In order to search if a given animal is able to produce specific lymphocytes against an antigen before encountering it (making contact with it), Nossal and his team excuted the experiment schematized in document 1.

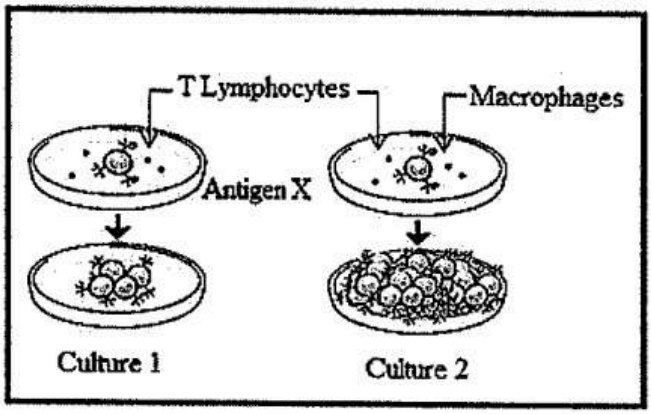


Document 1

1. Describe this experiment in few lines.
2. 1. Pose the problem studied by this experiment.
2. 2. Formulate a hypothesis that aims to answer it.
3. Justify that the lymphocytes used in this experiment are BL.
4. Show that the tested hypothesis is validated.
5. Explain the mechanism of the production of specific lymphocytes before immunization.

After the proliferation made in the medium (7) of this experiment, macrophages and T lymphocytes are used in order to study some characteristics of the activation of B lymphocytes. The experiments made are represented in the document 2.

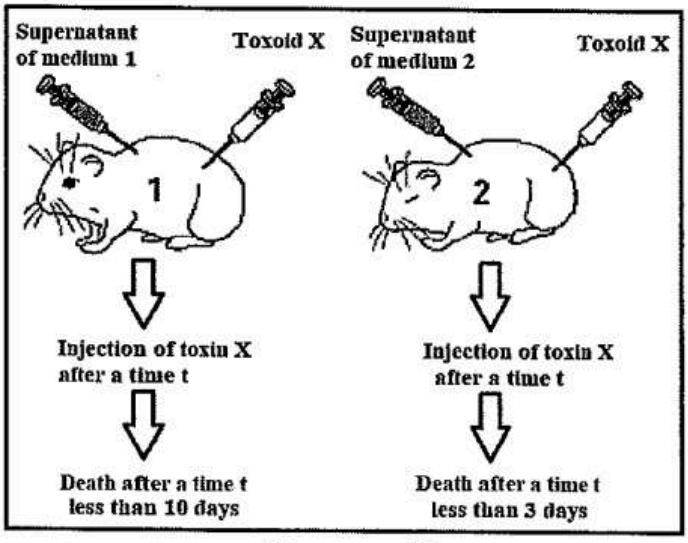
6. Interpret the results of this experiment.



Document 2

The antigen X is a toxin; this toxin is used with the supernatant (the serum taken from the culture) of the culture media 1 and 2 before the formation of plasma cells, in the experiment schematized in document 3.

7. Explain the obtained results to show the role of T4 cells in the induction of the immune response against the toxin X.



Document 3

Exercise 4 Role of T4 in the induction of the immune response

1. Antigens X placed in a medium containing gelatin are mixed with spleen cells of an animal not immunized with this antigen and then a number of cells are removed by washing, then the remaining cells are brought into contact with antigen X or in the presence of an antigen Y. With X there is a proliferation, with Y no proliferation.

2. 2-1. Is a given animal able to produce specific lymphocytes against an antigen before contact with it?
   2-2. Hypothesis: The body is able to produce specific lymphocytes against an antigen before contact with it.

3. The lymphocytes bound by the antigens X are B lymphocytes because they are bound by free antigens since only B lymphocytes have membrane antibodies which are receptors capable of binding to free antigens.
4. Since some immune cells, of the non-immunized animal against antigen X, bind to this antigen, these immune cells proliferate in the presence of this antigen and do not proliferate in the presence of another antigen, this shows that the antigens bind cells that are specific to it. This validates the hypothesis tested that before the immunization against a given antigen there are immune cells that are specific to it.

5. During the production of lymphocytes, the bone marrow produces all the varieties of B lymphocytes each having membrane antibodies specific for a given antigen (rather for an epitope), these lymphocytes comprise autoreactive BL. During the maturation process, the bone marrow removes auto-reactive B-cells by recognizing them by self antigens, while the other lymphocytes directed against non-self antigens are preserved.

6. The culture of TL with BL in a medium containing antigens X gives very slight proliferation of BL, whereas this proliferation is very high in a medium containing BL, TL and macrophages. This means that the cooperation between TL and macrophages leads to high proliferation of BL.

7. Macrophages are responsible for the induction of the humoral immune response by activation of T lymphocytes which in turn activate B lymphocytes by the secretion of interleukins; in fact, macrophages (APC) present non-self peptides after their association with HLA-II to T4 lymphocytes which recognize them by TCR, then proliferate and differentiate into interleukin-secreting cells, interleukin-4 is able to activate B lymphocytes having recognized the antigen by their membrane receptors which are specific antibodies for the antigen. This will happen in both mice 1 and 2 in document 3, but this process needs 10 days to be accomplished in mouse 1 where the culture medium 1 supernatant is injected having no IL-4; whereas, after injection of supernatant of medium 2 containing IL-4, the process of activation of BL is induced directly and leads to a fast secretion of antibodies. These antibodies are able to protect the mouse 2, starting from day 3 earlier than mouse 1, by neutralizing the toxins.