

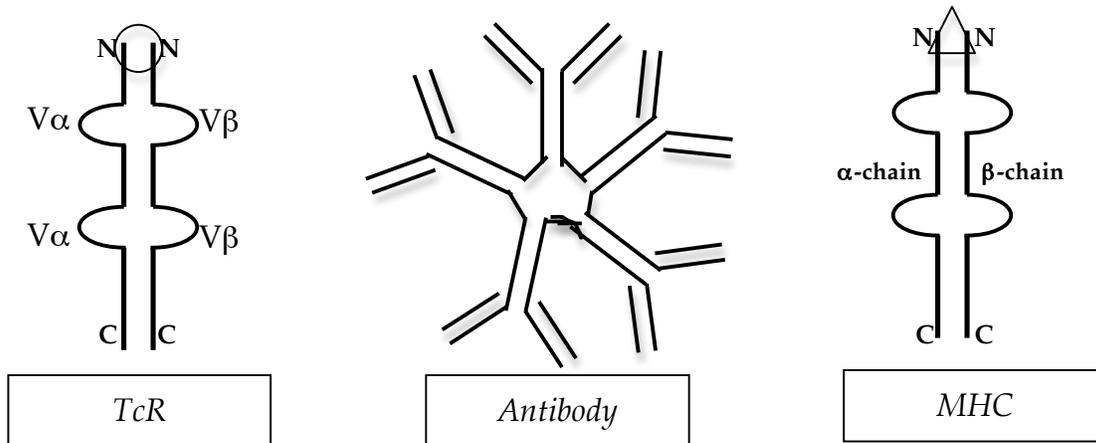
Solution key- 7.013 Problem Set 6 - 2013

Question 1

a) Our immune system is comprised of different cell types. Complete the table below by selecting all correct cell types from the choices provided.

Cells types that...	Choose from mast cells, macrophages, helper- T (T_H), cytotoxic- T (T_C), memory T_H and T_C , memory B and plasma B cells or ALL
Participate in the innate immune response .	<i>Mast cells, macrophages</i>
Bind directly to the heat-killed antigen, circulating in the blood stream.	<i>Memory B cells</i>
Secrete large amount of antibody in response to an infection.	<i>Plasma cells</i>
Provide adaptive immunity against second exposure to the same virus .	<i>Memory T_H, memory T_C and memory B cells</i>
Present the antigens to T_C cells during the T_C - mediated (CTL) immune response.	<i>ALL</i>
Show rearrangement of specific gene(s) and do not have the same genome unlike other cell-types in the body.	<i>T_H, T_C memory B cells (and plasma cells)</i>

b) The following is a schematic of the structure of specific **cell surface molecules**.



i. Identify the above molecules as an antibody (Ab), T cell receptor (TcR) or major histocompatibility factor (MHC) by filling in the boxes.

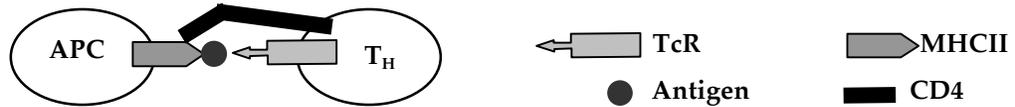
ii. Which of the above cell surface molecule(s) ...

- Can **directly** bind to a circulating antigen? *Antibody*
- Can present the antigen to a T_C or a T_H cell **after the antigen has been processed** either within an infected cell or by an antigen- presenting cell (APC)? On the schematic, show the antigen-presenting site of this molecule by drawing a **triangle**. *MHC*
- Can recognize the antigen **after it has been presented** on the surface of an infected cell or an APC cell? On the schematic, **circle** the antigen- binding site(s) of this molecule. *TcR*

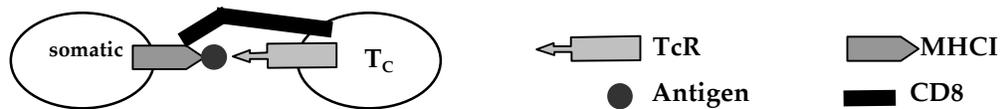
Question 1 continued

iii. Diagram the interaction of the molecules above with the antigens on...

- An APC cell that is interacting with and activating a T_H cell. *Note:* Include the relevant molecules on your schematic (choose from antigen (Ag), TcR , MHC-1, MHC-II, CD4 and CD8).



- A virus infected somatic cell interacting with and activating a T_C cell. *Note:* Include the relevant molecules on your schematic (choose from antigen (Ag), TcR , MHC-1, MHC-II, CD4 and CD8).



c) The diverse array of TcR and the antibodies is generated by DNA rearrangement. However this diversity is further enhanced by the function of enzymes like terminal transferases. Briefly **explain** how the activity of terminal transferase enzymes further contributes to antibody diversity.

This enzyme can add bases at the point of joining of the V, D and J segments of the antibody gene. This contributes to further diversity.

d) Although the immune system has the IgG and the IgM antibody molecules with the antigen-binding site against the same antigen these two classes of antibody have very different structure.

- Name the process that contributes to the variation between the structure of IgG and IgM molecules that are specific to the **same antigen**.

Alternative splicing

- Circle** the cells types that produce and secrete IgG (choose from *mature B cells, memory B cells, Plasma cells*).
- Circle** the cells types that produce IgM (choose from *mature B cells, memory B cells, Plasma cells*).

e) Very rarely, the immune system in some individuals may produce cells that can recognize "self-antigens". These individuals therefore develop autoimmune diseases such as lupus, rheumatoid arthritis, diabetic mellitus and multiple sclerosis. Briefly describe how the self-reacting T or B cells are eliminated during the development of immune system in a normal healthy individuals.

These cells with self-reacting antigens are contained during development and eventually eliminated by clonal deletion/ negative selection. So they will not be able to reach and attack other tissues.

f) Organ transplant is required in various clinical conditions. One has to consider various criteria in order to reduce the chances of transplant rejection.

- Name the surface molecule(s) that is critical in preventing transplant rejection. *MHC-I*
- Is this molecule(s) expressed on the surface of **ALL** nucleated cell (Yes/ No)? **Explain** your choice.

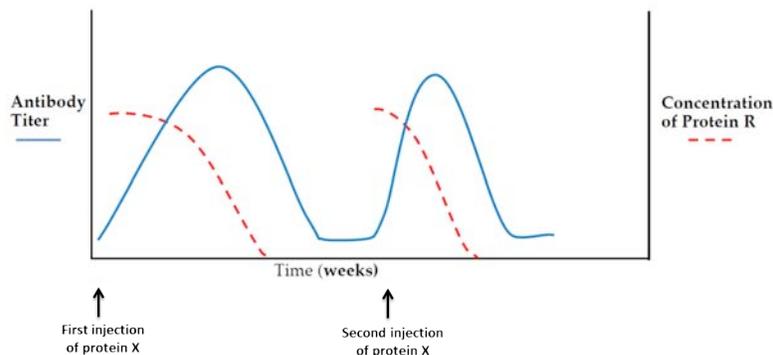
Yes, since different cell pathogens can infect different cell types, which can present the antigens through their MHC-I.

Question 2

You purify a **novel viral protein** (Protein R) and intend to further characterize it. Accordingly, you develop antibodies against Protein R. You inject Protein R into a rabbit, draw out some blood from the rabbit after a month and confirm the presence of Protein R specific antibody in the rabbit's blood. You wait for two months and then re-inject Protein R into the same rabbit. You observe a stronger secondary immune response developing within a few days against Protein R.

a) On the graph below...

- Draw the primary and secondary immune responses as a **solid line (-)** specific to Protein R.
- Draw the alteration in the concentration of Protein R as a **dashed line (-----)** during the primary and secondary immune responses.



Note: please look at SLIDE 9 of Immunology –II lecture

- Why is the primary immune response **slower** and **weaker** compared to the secondary immune response?

During the primary immune response, the memory B cells, against the specific antigen, are generated by the proliferation of the mature B cells that are specific to that antigen. These B cells express surface IgM molecules against the specific antigen. Furthermore, they can also proliferate to form more memory B cells and also the plasma cells that produce and secrete the IgG antibody to counteract the antigen infection. During the secondary immune response, the memory B and memory T-helper cells generated during the primary immune response immediately start proliferating to generate more of their own kind and also make the plasma cells to counteract the viral infection thus making the response faster and stronger compared to primary response.

- c) **Circle** all the correct options from the following choices. The **innate immune response**...
- Occurs **only** in response to **first injection**.
 - Occurs **only** in response to **second injection**.
 - Occurs in response to **both injections**.
 - Is **non-specific** unlike the adaptive immune response.

d) Briefly describe **two major mechanisms** by which the secreted antibodies destroy a virus circulating in the blood stream.

*The following are the three major mechanisms for antigen disposal: **Neutralization:** The antibodies bind to the antigens located on the surface of the virus and neutralize it by blocking its ability to bind to a host cell.*

***Opsonization:** The antibodies bind to the antigens on the bacterial surface to promote the phagocytosis of the bacteria by macrophages. **Activation of complement system:** The antibodies bind to an antigen located on the surface of a foreign cell to activate the complement system, which then forms a membrane attack unit. This unit forms pores in the membrane of the foreign cells allowing water and ions to rush in. The foreign cell therefore swells and ultimately dies (**But this was not covered in the lecture and you are not responsible for this**).*

Question 2 continued

e) Your friend decides to inject an **attenuated form of the virus** into a rabbit instead of injecting only Protein R derived from this virus.

- i. What additional immune response will be observed in this rabbit compared to your experimental rabbit?

This will also show the T_C mediated killing of the infected cells along with the humoral immune response.

- ii. How does this additional immune response in part (i) destroy the virus- infected cells?

The activated T_C will secrete perforin proteins that can make holes in the membrane of the infected cells leading to its killing. They may also activate granzymes, which causes the apoptosis of the infected cells.

Question 3

a) You come across an article online titled “How Carrot-Chocolate Shakes Improve Memory?” The summary reads: “After drinking Carrot-Chocolate shake mix, mice show an immediate (within 10 seconds) increase in memory of where treats are located. The authors of the study claim that the shake...

- i. Increases the amplitude of the action potential in hippocampal neurons (involved in memory) from +55mV to +90mV.
- ii. Changes the threshold potential from -50mV to -40mV.
- iii. Furthermore, it works by targeting the metabotropic AMPA receptors.

For each of the claims made, indicate whether the claim is valid and **explain** your answer.

The amplitude of the action potential and the threshold potential is always constant for a type of neuron and it does not change. It is only the frequency of action potential that can change based on factors such as the intensity of the stimulus or the amount of neurotransmitter etc. Hence Claim 1 and Claim 2 are invalid. The article claims that the ingredient of this shake targets the metabotropic receptors. These receptors by definition as slow acting and hence their activation cannot explain the sudden increase in the memory (within 10 sec). Thus one can argue that Claim 3 is also invalid.

b) On the same site, you read the following.

- i. Feeding mice the protein called the “ Na^+K^+ ATPase pump” improves their memory.
- ii. This pump is essential for transmitting a signal along a neuron.
- iii. It is not used anywhere else in the body.”

For each of the claims made, indicate whether the claim is valid and **explain** your answer.

Each cell type in the body has a resting membrane potential that is established by the action of Na^+K^+ ATPase pump and open channels. The resting membrane potential value may vary between different cell types but is fixed for a specific cell type. So Claim 3 and Claim 1 are invalid since Na^+K^+ ATPase pump is an inherent part of the plasma membrane of ALL cell types and is needed for maintaining their resting membrane potential. It however does not contribute to the generation of an action potential or transmitting a signal across the length of a neuron Hence Claim 2 is also invalid.

c) The depolarization phase of the action potential is generated by the passage of ions through...

- i. The resting ion channels
- ii. **Voltage-gated ion channels**
- iii. G-protein coupled receptors that are associated with a ligand gated K^+ channels.
- iv. The sodium potassium ATPase pump

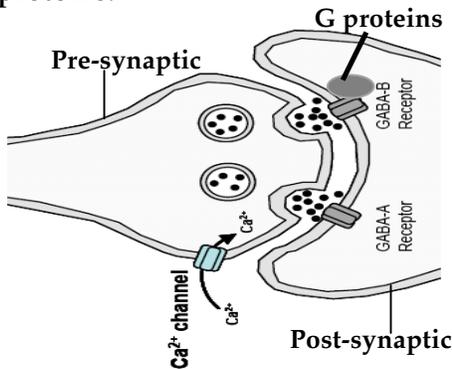
Question 3 continued

d) Complete the following table for the two channels/pumps that play a critical role in establishing and maintain the resting membrane potential.

Channels/pumps	Direction of movement of ions (choose from into the cell or out of the cell)?	Default state (choose from open, closed or always on)?	Is this an example of active transport or diffusion?
Na ⁺ K ⁺ ATPase pump	Na ⁺ : out & K ⁺ : in	Always on	Active transport
Open K ⁺ channel	K ⁺ : out	Always on	Diffusion

Question 4

GABA is a major inhibitory neurotransmitter in central nervous system (CNS). It acts by binding to GABA-A receptors that are chloride channels and GABA-B receptors that activate K⁺ channels via G proteins.



a) In response to GABA, would you expect both types of receptors to activate ion flow with the same time course? **Explain.**

No. In response to GABA, since the GABA-B receptor first has to activate G proteins before the associated ion channel can be opened. This takes more time than directly opening the ion channel of the GABA-A receptor.

b) K⁺ concentration is high inside the neuron, while Ca²⁺, Na⁺ and Cl⁻ ion concentrations are high outside. Passage of Na⁺ into the neuron is responsible for an action potential.

- i. In what direction will Cl⁻ ions flow when the GABA-A receptor is activated – *in or out* of the neuron?

The Cl⁻ ion will move inside and shift the membrane potential even further away from threshold.

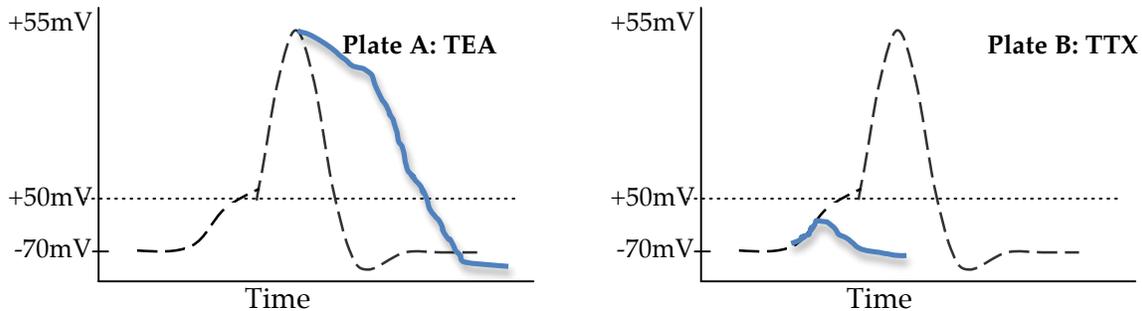
- ii. How does this flow alter the likelihood of an action potential in the post-synaptic neuron? **Explain.**

Due to the flow of Cl⁻ ions from outside to inside of the neuron, the inside of the neuron will have a membrane potential further away from the threshold. Therefore it will be less likely to activate an action potential.

Question 4 continued

c) You culture a GABAergic neuron (i.e. one that produces GABA) in the presence of the following neurotoxins in two separate petri-plates (Plate A & Plate B).

- **Plate A:** Neuron is treated with tetraethylammonium (TEA), which **inhibits voltage gated K^+ channels**.
- **Plate B:** Neuron is treated with tetrodotoxin (TTX), which **inhibits voltage gated Na^+ channels**.



A normal action potential in a GABA secreting neuron that has been stimulated **in the absence of any neurotoxin** has been drawn in each panel above. Sketch the alteration in the action potential following the treatment of the neuron with each neurotoxin. *Note: If there is no change please write "NO CHANGE" on the graph.*

d) Give two possible mechanisms by which GABA is removed from the synapse?
It may either be take back by the pre-synaptic neuron (re-uptake) or it may be degraded.

e) Consider the following synapse between two neurons.

- **Neuron 1** when stimulated secretes epinephrine. This neuron expresses the GABA-A and GABA-B receptors.
- **Neuron 2** when stimulated secretes GABA.

If neuron 2 is pre- synaptic and neuron 1 is post- synaptic would you expect Neuron 1 to secrete epinephrine? **Explain** your choice.

No, since GABA will bind to its receptor and move the membrane potential away from threshold.

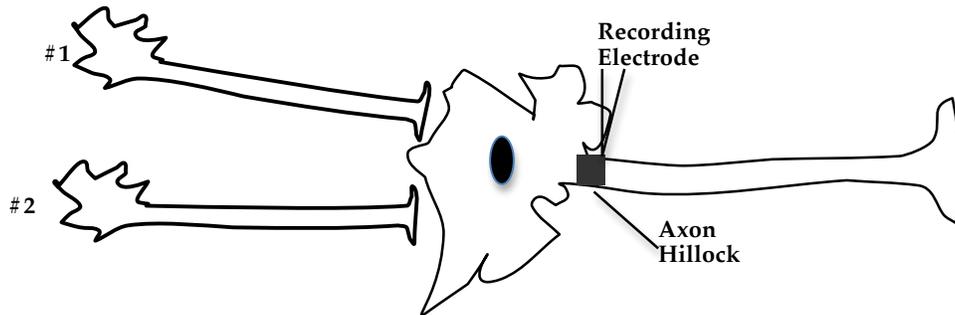
f) A functional neuron may receive both excitatory and inhibitory signals from multiple neurons at the synaptic junctions. In a post- synaptic neuron, where are the signals from all the pre- synaptic excitatory or inhibitory synapses integrated and the decision to fire an action potential made? **Circle** the correct options from the following choices and **explain** why you circled this option.

Cell Body **Axon Hillock** **Myelin Sheath** **Synaptic Cleft**

The depolarization of membrane depends in related to the opening of voltage gated Na^+ channels, which are absent in cell body and located at the axon starting from axon hillock.

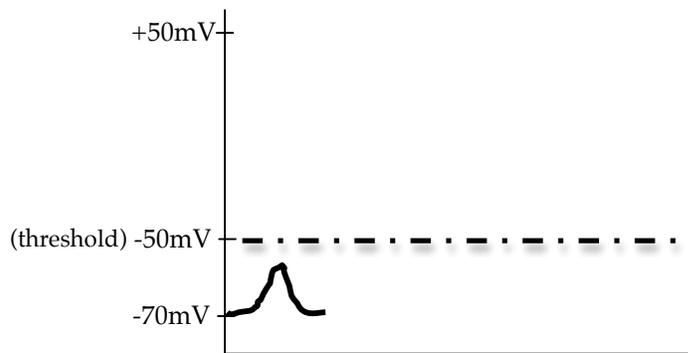
Question 4 continued

g) The following schematic shows **two excitatory pre-synaptic neurons** that independently converge on a post-synaptic neuron. The two pre-synaptic neurons can be stimulated individually. In the absence of any stimulation, the recording electrode in the post-synaptic neuron measures the membrane potential as -60mV .

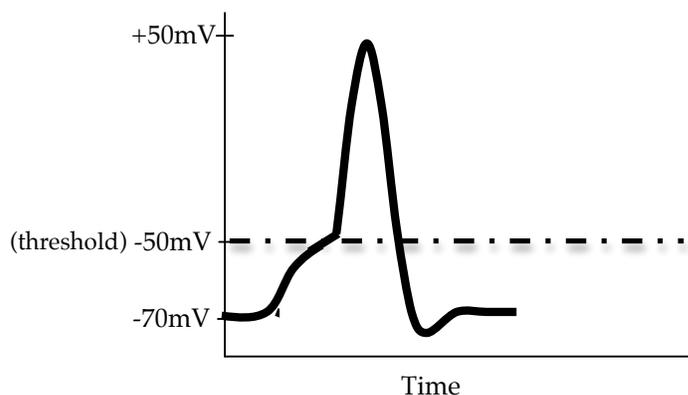


If **only one** excitatory pre-synaptic neuron is stimulated, you record a deviation from -60mV with the recording electrode in the post-synaptic neuron, but you do not record an action potential. If **both** the excitatory pre-synaptic neurons are stimulated, you record an action potential in the post-synaptic neuron.

- i. On the graph below sketch the changes in the post-synaptic neuronal membrane potential, as measured by the recording electrode, when **only one** excitatory pre-synaptic neuron is stimulated.



- ii. On the graph below sketch the changes in the post-synaptic neuronal membrane potential when **both** the pre-synaptic neurons are stimulated.



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