Examination in Immunotechnology, 1 june 2010, 8-13

1 Each question can give 5p, with a total of 10 questions (i.e. 50 points in total).

2 Write name and personal number on ALL pages (including the cover).

3 Use a NEW paper for each question.

4 Fill out and HAND IN the course evaluation (2 separate forms).

Exam in Immunotechnology, 1 june 2010, 8-13

Each question gives a maximum of 5 points.
(<25 p = not passed, ≥25p = passed)

NB! Use a New paper for each question **NB!**
WRITE NAME AND PERSONAL NUMBER ON EACH PAPER!
(including the cover)
Do not forget to fill out the COURSE EVALUATION! (2 forms)

QUESTION 1
You work as a doctor at Skånes University hospital in Lund. Suddenly, you are called to the emergency room to resolve a tricky issue with a patient that is infected by an unknown antigen.

1A) When you examine the patient, you observed increase levels of IFNα/β and altered levels of MHC class I. Briefly explain what is going on and what this tells you about the unknown pathogen? (1.5p)

1B) Briefly describe the mechanisms used by the immune systems to generate T cell receptors with different specificities. (1.5p)

1C) Mechanisms for maintaining tolerance towards self-antigen prevent the generation of self-reactive lymphocytes and thus the initiation of autoimmune diseases. Describe briefly how the immune system prevents self-reactive mature T cells from being generated. (2p)

QUESTION 2
Major histocompatability (MHC) molecules play a central role in the immune system.

2A) Normally, we have both MHC class I and class II molecules. Why? (1p)

2B) Describe the two mechanisms used by the MHC molecules to generate diversity with respect to binding specificity. (1.5p)

2C) Briefly explain why the immune system have to potentially make $>5x10^{13}$ different antibodies, but only ≤ 14 MHC molecules. (1.5p)

2D) Somatic hypermutation is not a mechanism used by the T cell receptor to generate diversity. Give one immunologically reasonable explanation to why. (1p)

QUESTION 3
You work at a leading biotech company (Vaccinate AB) focusing on developing vaccines as well as other novel ways of utilizing the immune system for protecting the population.

3A) In one case you have come up with the great idea of offering the population protection against a bacterial infection, Bug23, through passive immunization. Initially this is a blockbuster but the market drops like a rocket when people realize that the product only offers a relatively short-lived protection. Give a reasonable explanation to this limited protection. (1p)

3B) To circumvent the issue in 3A, you chop up the bacteria Bug23 in pieces using a digestive enzyme and then use these pieces to formulate a vaccine. Although this vaccine mounts a specific antibody response, it does not offer any protection against Bug23. Give one immunologically relevant explanation to why based on these facts. (1p)

3C) Recommend a solution how to circumvent the problem in 3B). (1p)

3D) What is an adjuvant and what does it do? (1p)

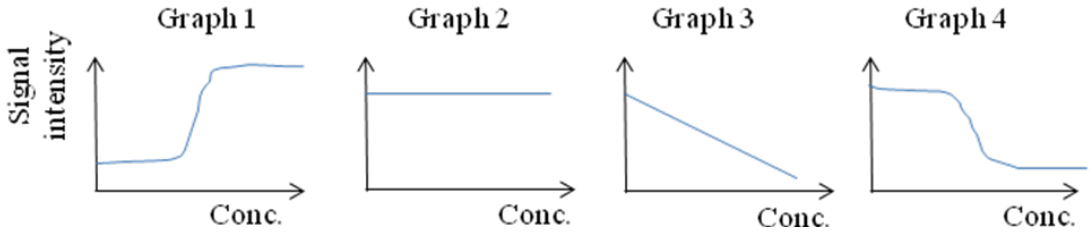
3E) Define what a conjugated vaccine is? (1p)

QUESTION 4

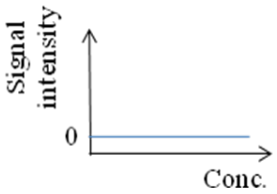
You are a senior scientist at BioFind Inc, and are responsible for running their bioassay group.

4A) One of your co-workers has just run a very important test, targeting OX19, a small, hapten-like bacterial toxin, with the aim to determine the concentration of OX19. By mistake he mixed up the data and the reagents used. By reviewing the material below, please describe (motivate) which graph is his and which of the reagents he probably used. (1.5p)

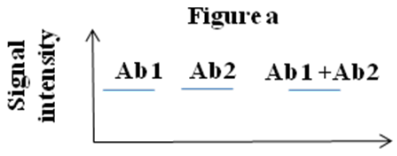
Reagents at hand
Pure OX19
Pure, fluorescently labeled OX19
Polyclonal rabbit anti-OX19 antibodies
Monoclonal mouse anti-OX19 antibodies
Polyclonal goat anti-mouse Ig antibodies
Fluorescently labeled horse anti-goat Ig antibodies
Fluorescently labeled horse anti-rabbit Ig antibodies



4B) Your colleague confirms that he used the reagents you just defined in 4A) and ran the assay accordingly. For logistical reasons, he then had to make a new batch of reagents, involving a new supplier of pure OX19 and a new dye to label OX19 with. The reactivity of the antibodies against OX19 was confirmed by Biacore analysis. He then tried to reproduce the results above, but he then got a completely different graph, see figure to the right. Give a reasonable explanation to why it could like this. (1p).



4C) Recently, you have produced two mouse monoclonal antibodies against another antigen, Big44, which is a large glycoprotein. You test the binding of each antibody, either alone or together, to reassure that they bind antigen (see figure a). Based on this test you are "happy" with the antibodies and design a sandwich ELISA. You use one antibody as capture Ab and the other as detector Ab. The ELISA is developed by adding a fluorescently labeled rabbit-anti mouse Ig antibody. When you run the assay you get a high and constant signal intensity. Give a reasonable explanation to why? (1p)



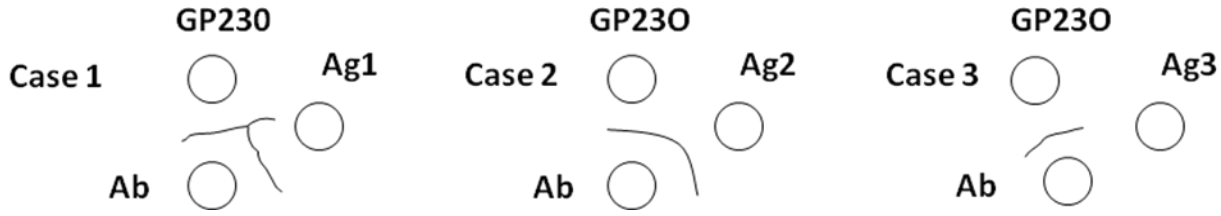
Ag immobilized, you add Ab in excess and determine the amount of bound Ab. You add 50 nM Ab1, 50 nM Ab2, or 50 nM Ab1 + 50 nM Ab2

4D) You would like to increase the sensitivity of the assay in 4C, and you are asked to incorporate Tyramide signaling amplification (TSA). To this end, you biotinylate one of the monoclonal antibodies (used as detector antibody, and the other is immobilized and used as capture ab). You then add perxoidase labeled streptavidin, biotinylated Tyramide etc according to the instructions of the TSA assay. But to your surprise, you do not observe any signal intensity at all. Based on the information in 4C and 4D, give two reasonable explanations to why. (1.5p).

QUESTION 5

You are head of the antibody facility at Bioprotection Inc, and have just developed several batches of antibodies against GP23O (a large protein) by immunizing rabbits.

5A) You then analyze the reactivity of one of the polyclonal antibodies against GP23O as well as against various other antigen preparations (denoted Ag1 to Ag 3) using double immune diffusion and observe the following. Briefly, explain the observed patterns of precipitation. (1.5p)



5B) Briefly describe a gel-based assay that can be used for quantifying GP23O, and outline the reagents required? (1p)

5C) By mistake, you mix-up the tubes of two of your polyclonal antibodies with a tube of a mouse monoclonal antibody (also against GP23O). Briefly, describe 3 potential ways how you could go about to determine which of the tubes that contains the monoclonal antibody using different gel-based approaches / methods. (1.5p)

5D) In a similar fashion, you would like to generate rabbit antibodies against the bacterial toxin AJ3 (a hapten). While reading the lab manual you see that using a carrier protein in the immunization process is vital. Briefly explain why. (1p)

QUESTION 6

You were out working in the garden, and cut yourself on a dirty knife. Later on you suspect that you got a bacterial infection through the wound.

6A) Initially, you got an inflammation in your wound. Briefly, describe what an inflammation is and give the main characteristics. (1.5p)

6B) Your immune system then starts to produce antibodies against the invading bacteria and after a while it will try to eliminate the antigen. Describe one way how the bacteria can be eliminated involving both antibodies and complement proteins? (1p)

6C) You isolate the bacteria, immobilize it on beads and then use this reagent in order to isolate T helper cells specific for the bacteria from your own blood. However, you fail to find these T helper cells. Give one reasonable immunological explanation to why you failed? (1p)

6D) Briefly describe what is required to generate activated T helper cells? (1.5p)

QUESTION 7

You have just caught an infection, and you are at the doctor's office.

7A) The doctor examines you and notices that your lymph nodes are swollen. Briefly, explain why? (1p)

7B) During the course of the infection, the immune response develops and somewhat later, critical B cell events take place in the Germinal Center (GC). Where are the GCs located and briefly describe these events taking place in the GC? (2p)

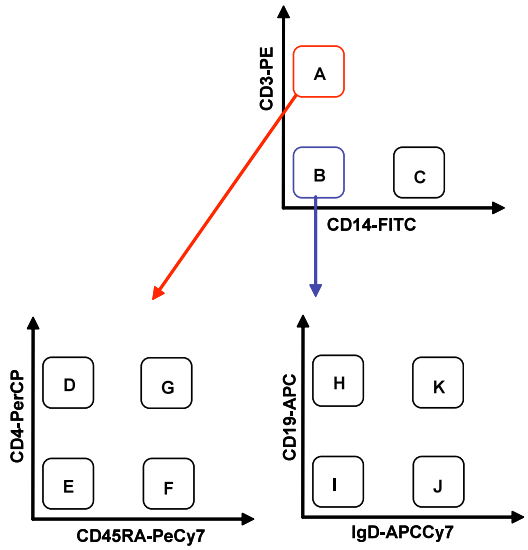
7C) The immune response also involves CD4 positive T cells. It is well-known that CD4 positive T cells can differentiate into distinct subsets of armed effector T cells. Briefly outline these sets and describe their role. (2p)

QUESTION 8

Your department have just recently purchased and installed new equipment for flow cytometry analysis of cells (i.e. not sorting). Your are responsible for this equipment, and your co-workers have a lot of questions.

8A) Outline 4 properties of the cells that can be studied using a flow cytometer? (1p)

8B) One of your co-worker shows you some of her data, and asks for your help to interpret the data, see figure. Motivate in which gate (C to K) i) naïve T cells, ii) naïve B cells, and iii) monocytes end up in? (1.5p)



8C) You would like to perform a multiplex assay to profile a number of cytokines in a single experiment. You review your options, and considering the instrumentation at hand, you decide to go with a cytometric bead assay (CBA). Briefly outline how a CBA assay works. (1.5p)

8D) You have generated an antibody specific for a novel candidate cell surface protein biomarker, but still do not know the identity of the marker. Briefly outline how you would go about to determine the specificity of the antibody? (1p)

QUESTION 9

You teach undergraduate students at a course in immunology. At one session you start talking about some specific parts of the human immune response. Help the students to explain the issues outlined below.

9A) A person has a defect which which impairs his CD8 positive cells. Still, you notice tha he has some protection against (some) viruses. Could you give two reasonable immunological explanations to this protection. (2p).

9B) The membrane attack complex is a component of the immune response. Briefly describe what it is. (1p)

9C) Define which components that belong to the i) central lymphoid system and ii) the peripheral lymphoid system. (1p)

9D) Sensitization is a term used within allergy. Briefly define this term. (1p).

QUESTION 10

Sheila is analysing 4,000 monoclonal antibodies established after immunization of mice with the monomeric protein gp124. The mouse strain used for immunization had been genetically engineered and was incapable of producing lambda light chains. Each antibody was evaluated using two different methods.

The first assay was a conventional ELISA in which she adsorbed the recombinant antigen onto microtiter plates. After a wash she added monoclonal antibodies diluted in phosphate-buffered saline solution containing 0.05% Tween 20 and BSA. After washing, she detected bound antibodies using an enzyme-labelled rabbit antibody preparation specific for mouse IgG. This rabbit antiserum had been treated so as to remove all antibodies binding to other mouse isotypes using affinity chromatography.

The second test was a Biacore-based analysis. Swine antibodies recognizing the constant domain of mouse kappa light chains were immobilized onto the sensorchip. One mouse antibody was injected. After saturation of the binding sites on the chip, the antigen was injected. Finally, the chip was regenerated by injection of 20 mM glycin buffer pH 2.8 containing 500 mM NaCl, a treatment that was well tolerated by the immobilized swine antibodies. The change in signal (RU) was recorded.

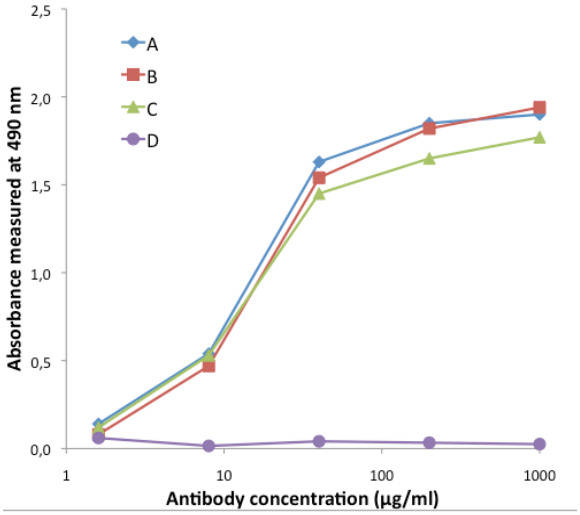


Fig. a. Examples of results of assay number 1 using 4 different antibodies (A, B, C, D)

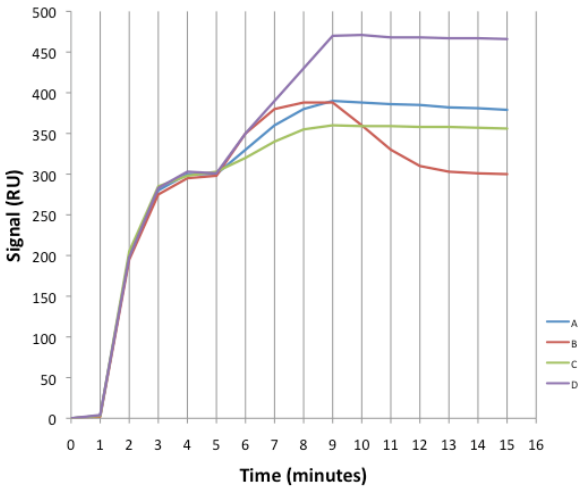


Fig. b. Examples of results of the Biacore assay (assay no. 2) using 4 different antibodies (the same as those shown in Fig. a). The monoclonal antibody was injected on the chip between 1 and 5 minutes. The antigen was injected between 5 and 9 minutes. Dissociation was followed from 9 minutes until 15 minutes. The regeneration cycle was always successful but is not shown in this illustration.

10A) She noted that 20 % of the monoclonal antibodies did not produce a signal in test 1 (exemplified by sample D in the Fig. a). They were obviously functional as they bound antigen in test 2 (Fig. b) Please suggest two reasons that could explain this finding. Also suggest one immunoassay-based test to your colleague that could be used to confirm one of these possibilities. You have at you disposal a wide range of antibodies specific for mouse immunoglobulins to test this idea. (2p)

10B) When comparing the results of test 1 and 2, she noted that some antibodies (in fact, about 800) gave very similar signals in test 1 to other antibodies that were positive in that test, but they performed differently in test 2. Suggest a likely explanation for the fact that e.g. antibody B was so different from antibody A and C in test 2 but not in test 1. (2p)

10C) Next, you would like to explore the possibility of using one of these mouse monoclonal antibodies for therapeutic purposes in humans. In order to avoid HAMA, the antibody is humanized by generating a chimeric antibody. Briefly, describe how this is accomplished. (1p)

Good Luck!