Examination in Immunotechnology, 30 May 2011, 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, 30 May 2011, 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

Our protective immune response is divided into an innate and an adaptive part, which both are essential for our survival.

1A) The innate immune system utilizes several different strategies to protect us from a wide range of pathogens. Give three examples of processes involved in innate immunity. (1.5p)

1B) Antigen-presenting cells, such as dendritic cells, act as the link between innate and adaptive immune responses by activating naïve T cells. What are the three kinds of signals required for mounting antigen-specific T cell-mediated immunity? (1.5p)

1C) Naïve CD4+ T cells can differentiate into a number of different effector T cells with a variety of functions. What are the main four subclasses of effector T cells and their key functions? (2p)

QUESTION 2

2A) From what organ/fluid do immune cells enter the spleen and what is the major function (in relation to immune surveillance) of the spleen? The spleen can be removed from adults without severe complications, why is that? (1p)

2B) Name and describe the function of the circular structure indicated by the red arrow in Figure 1. (1.5p)

2C) The immune system has an intricate system in place to make sure that only antibodies directed against foreign antigens are produced. Briefly explain this system. (2.5p)

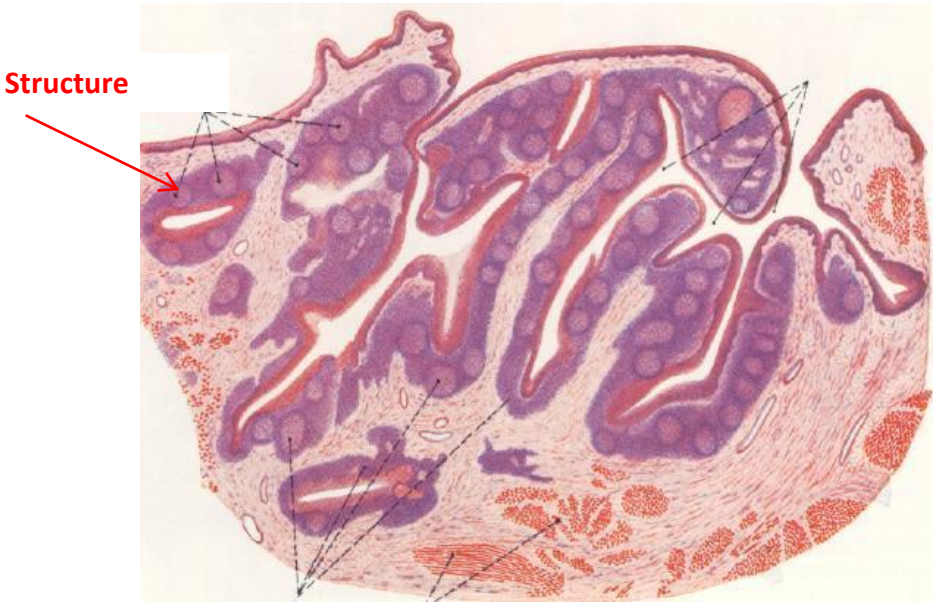

Figure 1. Mucosa-associated lymphoid tissue with crypt epithelium

QUESTION 3

MHC class I and class II molecules are structurally and functionally homologous. Still, they have different pathways of assembly and delivery to the cell surface.

3A) What cell types in the human body express MHC class I and class II molecules. (2p)

3B) Describe the differences in assembly and delivery to the cell surface of the class I and class II molecules, and how this relates to their respective functions (2p).

3C) What is cross-presentation? (1p)

QUESTION 4

As a project leader in a biotech company you have been given the task to set up an antibody facility in order to make human antibodies to be used as therapeutics. The potential target list that you are provided with contains a wide range of antigens including cancer biomarkers, cytokines, hormones and snake venom. You are asked to set up a facility based on phage display or on hybridoma. After a thorough literature search you finally settle for phage display as the core technology for generating antibodies.

4A) Briefly describe the principles behind hybridoma technology (1.5p).

4B) Briefly describe the principles behind phage display and how to select antibodies with the desired specificity (1.5p).

4C) Why would phage display be a good choice in this case? Give examples of two advantages of this method compared to hybridoma technology? (1p)

You start the practical work by constructing a large (>10^{10}) combinatorial scFv phage library that you use to generate binders against the breast cancer marker, BC-X. During the screening process it turns out that the scFv also bind the highly similar protein, H-X, which is highly expressed in healthy breast tissue.

4D) How would you design your selection strategy in order to minimize this un-wanted reactivity? (1p)

QUESTION 5

Axel, 4 years, experiences his first spring with itchy and red eyes, sneezing, congestion and a runny nose. These are common are symptoms of the effector phase in type I hypersensitivity.

5A) Describe the sensitization phase that has occurred in Axel, i.e. the initial immunological mechanisms that characterize the adverse immune responses towards protein allergens. (2p)

5B) Degranulation of mast cells and basophils in Axel's airways lead to release of mediators that induce his symptoms of allergy. What are the mechanisms that trigger degranulation? (1p)

5C) Current standard treatment for type I hypersensitivity today is performed with drugs that target and inhibit histamines (so-called anti-histamines) in the airways. These drugs treat the symptoms and not the underlying immunological causes of disease. Describe one existing (or future) way of treating allergic diseases before degranulation has occurred, and how this way affects the immune response. (2p)

QUESTION 6

You recently bought a rabbit polyclonal antiserum specific for human FSH from a commercial provider. FSH is a heterodimer, that is, it is composed of two different domains in a complex. One of these domains is identical to one of the two domains found in another protein, TSH. The other domain in FSH is different from the one found in TSH. According to the datasheet this antiserum has been shown to

"be specific for human FSH and not to recognize human TSH or mouse FSH or mouse TSH, as determined by immunoprecipitation (double immunodiffusion".

You are now going to use this antibody in a competitive ELISA assay format to determine the concentration of human FSH that you are to administer by injection to mice. You get a very nice standard curve when you assay FSH diluted in buffer (phosphate buffered saline (PBS)

containing 1% bovine serum albumin). When your colleague Stig makes some additional tests he notices that the assay you have created also detects the presence of human TSH, mouse FSH (but not mouse TSH) diluted in the same buffer (Figure 2). Unfortunately, this creates a problem in your experiment as your assay must not show cross-reactivity to mouse FSH. All of these proteins are available to you in large quantities. He also tested the behavior of a rabbit monoclonal antibody that detects human TSH and he got standard curves of proteins diluted in buffer as outlined in Figure 3. He now comes to you to discuss this matter.

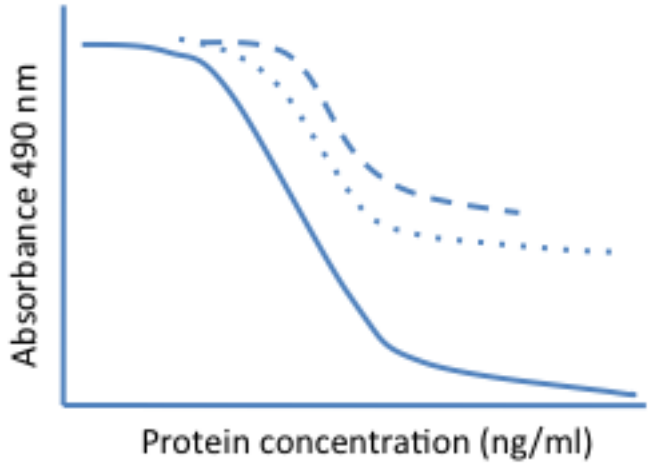

Figure 2. Standard curve obtained with the rabbit antiserum used in the assay for human FSH. The signal obtained in the presence of different concentrations of human FSH (solid line), human TSH (dotted line) and mouse FSH (dashed line) is illustrated. Mouse TSH does not affect the affect the signal in this assay setup.

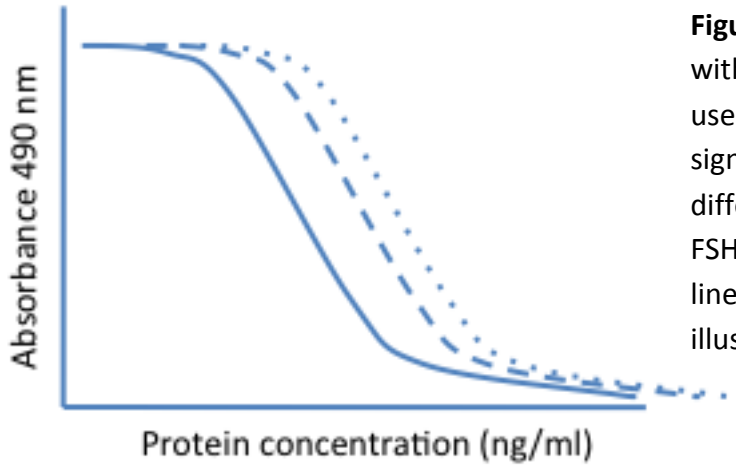

Figure 3. Standard curve obtained with the rabbit monoclonal antibody used in the assay for human FSH. The signal obtained in the presence of different concentrations of human FSH (solid line), human TSH (dotted line) and mouse FSH (dashed line) is illustrated.

6A) Outline briefly how you and Stig likely designed the assay for human FSH described above. (1p)

6B) Give Stig a reasonable explanation to why the assay seems to detect proteins that are not, according to the datasheet, recognized by the antiserum. (1.5p)

6C) Suggest a way in which you could get the antiserum (assessed in Figure 2) to be specific for human FSH in an assay like this. (1.5p)

6D) Stig is likely to ask you if he should try the same approach (as in **6C**) with the monoclonal antibody. What would be an appropriate response to such a question? (1p)

QUESTION 7

Antibodies are highly specific molecules generated by the immune system.

7A) Briefly explain the terms affinity, avidity and valency. (1.5p)

7B) Once the antibody has bound to its antigen, the formed complex should be eliminated. Describe two functions by which the antibody-bound antigens are eliminated by the immune system. (2p)

7C) You have generated a mouse monoclonal antibody against the hapten, oxxy4. Briefly describe how you would go about to validate the specificity of the antibody. (1.5p)

QUESTION 8

You want to investigate the cytokine production by human B cells after stimulation with bacterial antigen. For this purpose, you need to perform studies in vitro, i.e. in cell cultures in the laboratory, and you need to isolate B cells from human peripheral blood. You have already isolated the mononuclear cells through Ficoll-Hypaque density centrifugation. You have a well equipped cellular laboratory at your disposal, with a fridge full of antibodies and other reagents for cell isolation and cytokine analysis.

8A) Describe how you would isolate a pure population of B cells using magnetic beads? (1p)

After the isolation, you want to investigate the purity of your cells with flow cytometry. Unfortunately, it turns out you still have other types of cells in your sample. You decide to investigate with flow cytometry which cell types are responsible for the impurity.

8B) What information does the forward and side scatter plot provide you with? (1p)

8C) What are the cellular populations observed in red, green and purple in Figure 4 below? (1.5p)

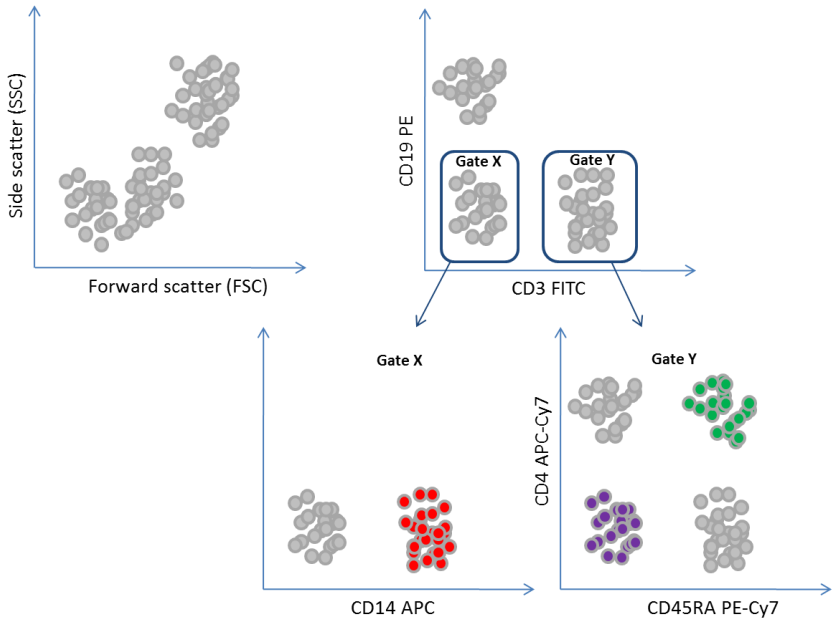

Figure 4. Flow cytometric analysis

8D) You repeat the isolation using magnetic beads and finally get a pure population of human B cells. You perform your stimulations and collect the supernatant (i.e. the cell medium, not the cells). Next you want to use multiplex cytokine analysis, profiling a number of cytokines in a single experiment. What method would you choose and briefly outline how this works. (1.5p)

QUESTION 9

Erik has isolated a novel antigen from a virus that causes disease in mouse and man. His colleague Doreen cloned the entire gene that encodes this protein. The gene has been cloned in a vector that allows production of this protein (without any tags) in mammalian (CHO) cells (vector pMS-eu5) and in a vector that allows for production of this protein in bacterial cells (vector pMS-pro5). She successfully produced and purified the intact, well-folded, full-length protein from both CHO cells and from a bacterium, *Escherichia coli*. An SDS-PAGE gel of the purified proteins is shown in Figure 5. Doreen gave these vectors and the recombinant proteins to Erik for further investigations. He immunized four mice with either of these proteins in Freund's complete adjuvant and received an efficient antibody response in all eight animals.

You obtained sera from these eight animals but in no case were antibodies in these sera able to prevent infection of cells in the test system (based on preincubation of the virus with diluted sera prior to infection of a mouse cell line) you had in your cell lab. You also noted that addition of complement to the test system did not allow the antibodies to prevent (neutralize) virus infection in the test system. However, by chance you noted that if you added any one of these sera together with lymphocytes purified from unimmunized mice to infected cells, these lymphocytes were able to kill infected cells.

Doreen had also sent one of her vectors to Cecilia who injected it (pMS-eu5) into mice on several occasions. She subsequently infected these mice with virus and observed effective protection against disease in these animals. This was in complete contrast to observations made after infection of mice immunized with recombinant protein in Freund's complete adjuvant, as these animals were not protected against disease. She also recovered some serum from these mice (into which she had injected the vector) but when you tested these sera (by themselves or together with complement) they were unable to prevent infection of cells in your cell culture system. Again, if you added lymphocytes from unimmunized mice together with these sera to infected cells you were able to kill infected cells. Importantly, Cecilia noted that also a mouse strain that were unable to produce B cells and antibodies (but in all other respects were normal) could be efficiently protected from disease by this immunization procedure (i.e. by injection of pMS-eu5). Her conclusion was that protection in vivo was mediated by a mechanism different from the one that prevented virus infection in the *in vitro* cell culture system that had been used.

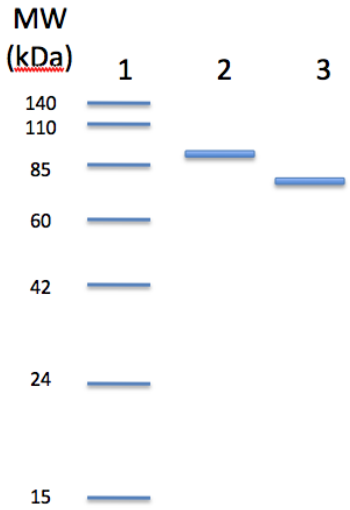

Figure 5. SDS-PAGE of recombinant protein produced in CHO-cells (lane 2) and in E. coli (lane 3). Molecular weight markers are shown in lane 1

You are now trying to write a report summarizing your findings to your research managers to give them some background that will facilitate their decision on whether or not to continue research on this protein.

9A) Describe a likely reason why the proteins in lane two and three differ in speed of migration. (1p)

9B) Describe a likely mechanism whereby your antisera together with lymphocytes from unimmunized mice were able to kill infected cells in culture. Describe this mechanism in brief. (2p)

9C) Describe a likely mechanism whereby mice were protected from disease after injection of the vector. Why were your recombinant proteins not able to efficiently induce this type of effect? (2p)

QUESTION 10

You are head of research at RapidBio and have been asked by a customer to design an assay against a large protein antigen, Prop2.

10A) The customer would like you to design a non-competitive ELISA. Briefly outline which assay reagents you would require and make a schematic drawing outlining how the assay signal vs analyte concentration would look like. (2p)

10B) The customer would also like you to design a gel-based method for determining the concentration of Prop2. Briefly describe one method for this purpose and motivate which reagents you would require. (2p)

10C) Finally, the customer would like you to enhance the assay signal for an already existing standard antibody array set-up using rolling circle amplification. Briefly outline how this signal amplification method works. (1p)

Good Luck!