

# Biology 257 Immunology Fall 2005

## Final Exam

### Instructions

This exam is designed to take ~2.5 h. The relative number of points should give you a rough idea of how much time to spend per question. The **space** that has been left between questions **roughly approximates** how long your answers will be, depending on the size of your handwriting. **Aim for CLEAR, CONCISE, COMPLETE answers--most questions on this exam only require 1-3 sentences max per section.**

**Hand in all sheets** before you leave and **make sure your name is on this front page.**

### Helpful hints

#### Before starting to write:

**Read** through the **entire question** (all sections).

**Think carefully** about **what exactly each section** is asking.

**Highlight ideas and jot down relevant notes** to yourself such as: "GK1.5 = anti-CD4"

#### When writing your answer:

**Make sure you answer** the **question that was asked.**

**Avoid** adding **irrelevant** information.

**Diagrams are great** if they help you explain your answer.

#### Before turning in the exam:

**Read through all your answers** to make sure that:

**Each answer is complete.**

**Each answer addresses the specific question asked.**

#### After turning in the exam:

**Good luck on the rest of your finals.**

**Have a great break!**

**1. (35 pts total)** In this month's issue of the Journal of Clinical Investigation, J. Kolls and coworkers describe the development of DNA vaccines to help reduce opportunistic infections in immunosuppressed patients. Their vaccines are designed to fight infection by the fungal pathogen *Pneumocystis* (PC), which can cause severe pneumonia. In fact, *Pneumocystis* pneumonia is one of the clinical conditions used to diagnose AIDS (acquired immunodeficiency syndrome).

**a) (4 pts) Why does HIV infection cause severe immunodeficiency? Make sure to include which cell type(s) are affected and why these cell(s) are crucial to a robust immune response.**

Kolls and coworkers have developed two DNA vaccines. One plasmid encodes the PC protein kexin, which they have shown to be a major PC antigen recognized by mammalian immune systems. The second plasmid has both the PC kexin gene and a mouse gene that encodes CD40 ligand. Strong constitutive promoters drive the expression of both genes, so all cells that take up the DNA should express high levels of the gene(s) on the plasmid.

Their experimental protocol is shown schematically in **Figure 1**. Three intramuscular injections of the DNA vaccine are given at three-week intervals (Day 0-42). In their first experiment (Fig. 2), three days before starting vaccination they treat the mice with an anti-CD4 antibody raised in rats (GK1.5) or total IgG2b from a naïve rat (control Ab). After the third vaccination, they draw blood from the mice and measure the titer of anti-PC antibodies by ELISA, using plates coated with proteins from PC. These results are shown in **Figure 2**.

**b) (4 pts) Draw a three- or four-step diagram to show how their ELISA allows them to detect anti-PC antibodies. Which step is different when they are measuring IgG1 vs. IgG2a and why?**

**c) (4 pts) What effect would the injection of GK1.5 have on the immune system of the mice and why?**

**1d) (4 pts) When they use a plasmid containing only Kexin, what effect does GK1.5-treatment have on the titer of anti-PC antibodies (compare samples 2 and 3 in Fig. 2)? Explain why this result makes sense.**

When mice have been pre-treated with the GK1.5 antibody, the addition of CD40 ligand to the kexin DNA vaccine significantly increases the antibody titer (compare samples 1 and 2 in Fig. 2).

**e) (2pts) What is the normal role of CD40 ligand in the immune system?**

**f) (4 pts) Explain why adding CD40L to the kexin vaccine plasmid could increase the anti-PC antibody titer in GK1.5 mice.**

**g) (3 pts) Why do you think the positive effect of adding the CD40L gene is not as extreme when mice were treated with the control antibody (compare samples 3 and 4 in Fig. 2)?**

**h) (2 pts) Why would the result described in (f) be particularly exciting to HIV+ individuals?**

To test the efficacy of the vaccine, after the three rounds of vaccination mice were infected with live PC cells and their lung tissue was tested for levels of PC a month later. PC levels in Kexin-only vaccinated mice were only slightly lower than those in unvaccinated control mice, whereas PC levels in Kexin/CD40L vaccinated mice were close to 1000 times lower than in control mice!

Next they tested how effective the antibodies elicited by the vaccine are in an *in vitro* assay for PC killing. Their experimental design is described on the next page and their results are shown in **Fig. 3**.

1. PC cells were incubated with serum from unvaccinated or vaccinated mice.
2. Macrophages were isolated from untreated mice and incubated with the PC cells from step 1.
3. Living PC cells were quantified after 16 h to determine the percent PC cells killed by the macrophages.

**1i) (4 pts) Draw a picture that explains what is happening in this assay including at least 3 key molecules. Given that serum from vaccinated mice increases macrophage killing of PC, what can we hypothesize about where the kexin protein is normally located?**

**j) (4 pts) How would the kexin/CD40L vaccine compare to a killed whole PC vaccine? Give at least one advantage each type of vaccine has over the other type.**

**2. (15 pts)** You are fascinated by the possibility of using antibodies to address medical challenges including allergies and autoimmunity. You decide to tackle these issues in alphabetical order.

First you produce a monoclonal IgG1 antibody that recognizes the rabbit Fc $\epsilon$  receptor (Fc $\epsilon$ R). You take a portion of the antibody stock, digest it with papain and separate Fc and Fab fragments. You then inject whole anti-Fc $\epsilon$ R, Fc or Fab into a rabbit allergy model. You find that one sample alleviates allergic reactions, another has no effect and the third in fact worsens the allergic reaction.

**a) (5 pts) Which treatment (whole anti-Fc $\epsilon$ R, Fc, Fab) do you expect to have which effect (alleviating, no effect, worsening)? Explain your prediction (pictures may help).**

Next you purify IgE molecules from a rabbit, digest a portion of *this* antibody stock with papain, separate Fc and Fab fragments and perform the same experiment. Yet again, you find that one sample alleviates allergic reactions, another has no effect and the third in fact worsens the allergic reaction.

**2b) (5 pts) Which treatment (total IgE, Fc, Fab) do you expect to have which effect (alleviating, no effect, worsening)? Explain your prediction (pictures may help).**

A friend with a severe autoimmune disease says that her doctor mentioned a new treatment involving anti-IL-2 receptor antibody, but she is wondering whether this treatment might have severe side effects.

**c) (5 pts) Briefly explain why anti-IL-2R treatment might help a patient with an autoimmune disease but why such a treatment could have severe side effects.**

**3. (38 pts) In the introduction to a paper in 2004, Arjona and Sarkar wrote:**

"Acute and chronic ethanol consumption impairs the immune system by causing specific defects in the cellular components of the innate immune response and by creating increased risk and susceptibility to infections and cancer."

To understand better the connection between ethanol and innate immune system function, they used their rat system to compare levels and activity of key components of the immune system in the spleens of ethanol-fed animals or control animals. For two weeks before spleen harvest, animals were fed one hour before the beginning of the dark cycle.

The results of two of their first experiments are shown in **Fig. 1**.

**a) (2 pts) Which cell type(s) in the spleen would express granzyme B?**

**3b) (5 pts) Briefly describe how they would detect levels of granzyme B protein in the spleen (4-5 steps).**

**c) (3 pts) Briefly describe how they would detect levels of granzyme B mRNA in the spleen (~3 steps).**

To test the cytolytic activity of NK cells in the control and ethanol-fed rats, they harvested splenocytes and incubated them with lymphoma cells that had been loaded with  $^{51}\text{Cr}$ . The results are shown in **Fig. 2**.

**d) (2 pts) What do they monitor to detect NK cytolytic activity (i.e. what are the raw data that they convert to "lytic units")?**

**e) (5 pts) How does this assay differ from other  $^{51}\text{Cr}$  assays we have discussed? Explain one hypothesis for why this assay should be specific for NK cell-mediated cytotoxicity.**

**f) (4 pts) The time of the peak in the control animals in this experiment is closer to the peak for granzyme B protein levels than for the granzyme mRNA peak. Does this result make sense? Why or why not?**

**g) (3 pts) What effect does ethanol have on granzyme expression? On NK cell activity?**

**3h) (5 pts) The effect of ethanol on perforin expression was the same as the effect on granzyme expression. Describe one hypothesis for why the effect of ethanol on NK cell activity is slightly different than the effect on the expression of these proteins.**

Another group studying the effects of alcohol on immune system function found that ethanol treatment of cultured cells inhibited proteasome function.

**i) (3 pts) What is a main function of proteasomes in immune system function?**

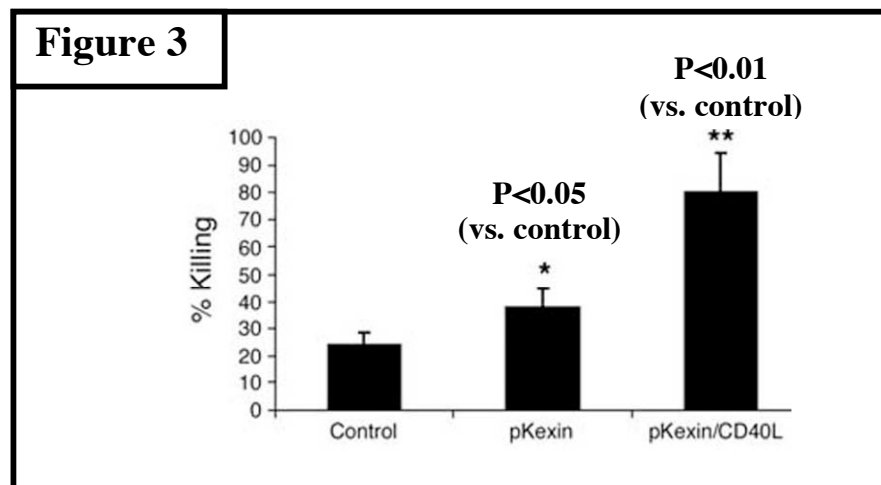
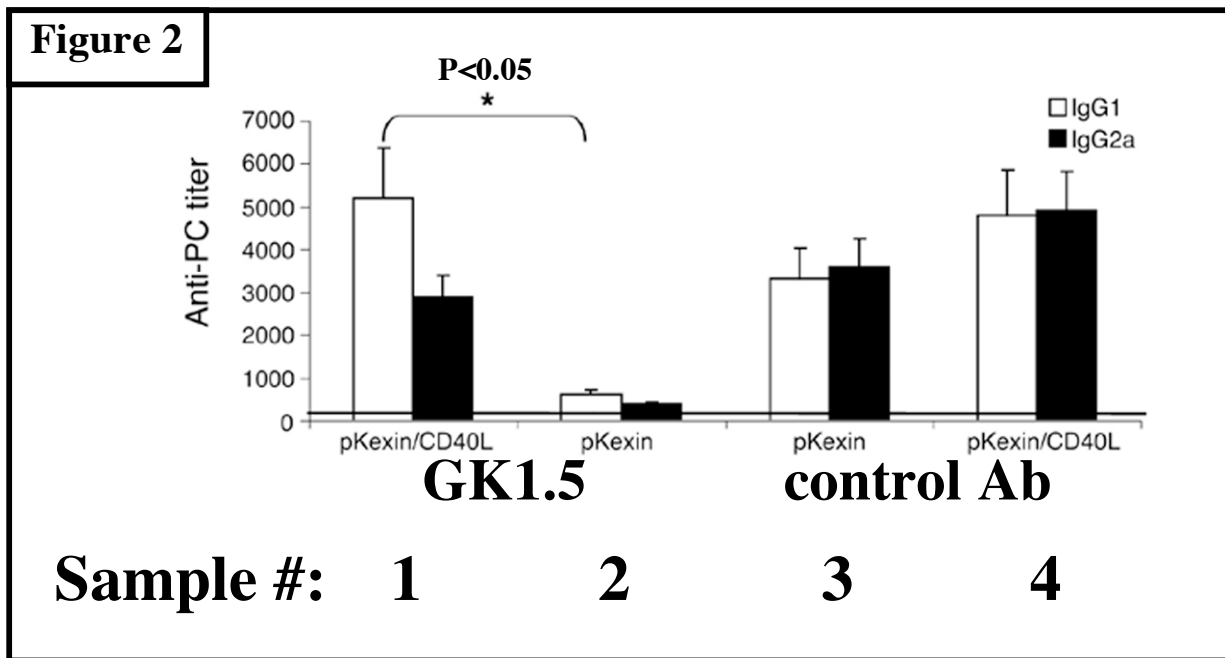
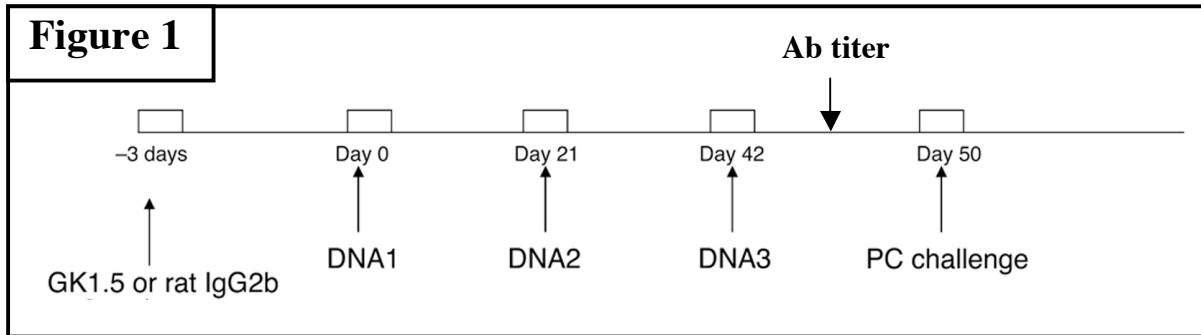
**j) (6 pts) Given your answer in (i), explain how ethanol inhibition of proteasome function could also be involved in "creating increased risk and susceptibility to infections and cancer."**

**1. Infections**

**2. Cancer**

# 2005 Bio257 Immunology: Final Exam Figures

## Question 1



# Question 2

For all experiments

\*  $P < 0.05$  significant difference from lowest value in the same group

#  $P < 0.05$  significant difference between groups

