

## Bio257 Immunology Practice Questions #5

1.. In the early 1980s a group of immunologists was interested in identifying the cell-surface receptor for type I interferons ( $\alpha$ ,  $\beta$ ). As a first step, they decided to raise monoclonal antibodies against human cell-surface proteins. They immunized mice with whole human fibroblasts, which were known to respond to type I interferons. They then fused the spleen of these mice with myeloma cells and cloned individual hybridoma cells.

To test whether antibody produced by each hybridoma recognized a type I interferon receptor, they performed the following experiment:

1. Supernatant from each hybridoma (the liquid medium the cells were grown in) was incubated with human fibroblasts in the presence or absence of type I interferons.
2. The fibroblasts were infected with an RNA virus.
3. Viral growth was measured by:
  - a) cytopathic effect (CPE--changes in cell morphology caused by the virus killing the cell)
  - b) incorporation of tritiated uridine into viral RNA

In the absence of supernatant they expected the following result:

		+Virus
	<u>CPE</u>	<u><math>^3\text{H}</math>-uridine incorporation</u>
+IFN	-	-
-IFN	+	+

They expected antibodies against the interferon receptor to show the following results:

		+Virus
	<u>CPE</u>	<u><math>^3\text{H}</math>-uridine incorporation</u>
+IFN	+	+
-IFN	+	+

- a) Why did they use whole fibroblasts rather than a protein lysate to immunize the mice?
- b) Why did they clone individual hybridoma cells?
- c) In Step 1, why did they use the supernatant from the hybridomas ?
- d) Explain the difference between the actual and expected results shown above. Why should the addition of supernatant alter viral growth in the presence of interferon? Explain in terms of the molecules involved and their interactions.

Much to their surprise, one of the supernatants showed the following results:

		+Virus
	<u>CPE</u>	<u><math>^3\text{H}</math>-uridine incorporation</u>
+IFN	+	-
-IFN	+	-

- e) What is surprising about these results? Include at least two oddities.

Further tantalizing evidence was revealed when the supernatant was incubated with fibroblasts in the absence of virus. The supernatant killed the cells on its own!

- f) What cell-surface molecule do you think might be recognized by the antibody produced by this hybridoma? Explain the normal role of this molecule and why you chose this molecule as a likely antigen for the antibody.
- g) Why is this molecule is expressed by many different cell types?

*Extra credit: The fibroblast cell line used to inoculate the mice was called FS-7. Can you guess how the cell-surface molecule got its name?*

2. After reading about the IFN $\gamma$ R knockout mice, you are curious to know if there are any humans with mutations in the IFN $\gamma$ R gene. You search PubMed and find that last month a group from Italy published a paper describing an IFN $\gamma$ R<sup>0/0</sup> patient. They cloned individual CD4+ cells from this patient, grew them in culture, and detected higher than normal levels of CCR5 in these cells.

- a) Name two types of cells that could be present in their cultures.
- b) Would you expect this patient to be more or less susceptible to HIV infection than a wildtype sibling? Explain your answer in terms of the cells and molecules involved.
- c) Would your answer in (b) be the same for all strains of HIV? Why or why not?

### **Reworded questions from earlier problem set**

A hapten is a small organic molecule that does not elicit an immune response on its own (i.e. it is not immunogenic), yet when it is conjugated to a larger antigen and the conjugate is injected into an animal the polyclonal antiserum includes both antibodies to the larger antigen and anti-hapten antibodies (i.e. the hapten is antigenic). Explain why this phenomenon might occur. Think carefully about what is required to elicit an immune response vs. to be recognized by an antibody (think about all the molecules involved).

### **Question from the book**

Ch. 18      #1