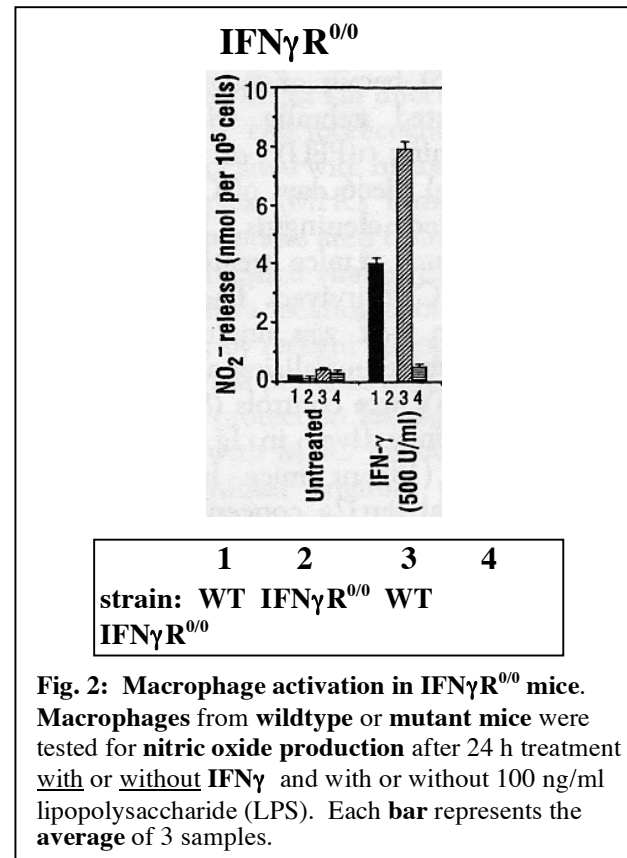
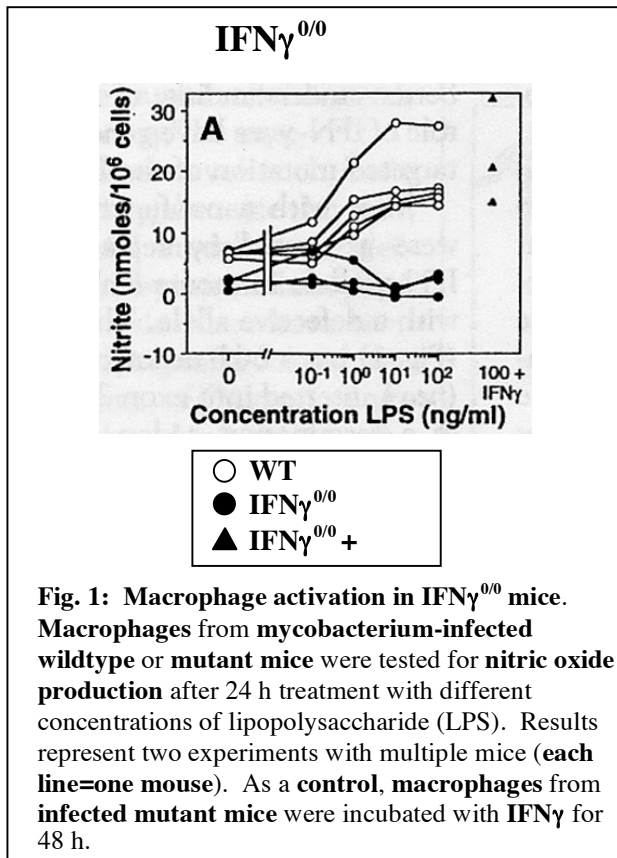


Bio257 Immunology Practice Questions #4,5 key

4.1. Cytokines are crucial to immune system function. In class we discussed data from papers that support the importance of **interferon γ** : **1) IFN γ knockout mice (IFN $\gamma^{0/0}$) were killed by normally sublethal doses of mycobacteria and 2) IFN γ receptor knockout mice (IFN $\gamma R^{0/0}$) were unable to fend off *Listeria monocytogenes*, another intracellular bacterium.**

The authors of these papers also designed experiments to address which aspects of immune system function were compromised in the different knockout mice. One of the **toxic molecules produced by activated macrophages, nitric oxide (NO)**, can be **detected** by incubation with a reagent that converts **NO into nitrite (NO $_2^-$)**. Results from experiments to **test levels of NO produced by macrophages from each type of mouse** are shown below.



a) Name one type of immune cell that expresses IFN γ and two types of immune cell that express the IFN γ receptor.

IFN γ : **helper T cell (T_H1 subset)** IFN γ receptor: **macrophage, B cell (T_H2, cytotoxic T cell)**

b) How does each IFN receptor-bearing cell respond to the presence of IFN γ ?

When IFN γ binds to IFN γR on MACROPHAGES, these phagocytes are activated such that they perform greater phagocytosis, increase presentation of peptides on MHC class II molecules, secrete more toxic molecules like NO and more IL-12 to activate further T_H1 cells.

The binding of IFN γ to IFN γR on B CELLS, the B cells are induced to undergo a class switch from IgM to IgG2a, which facilitates opsinization of bacteria for phagocytes or complement targeted destruction. (continued on next page)

The binding of IFN γ to IFN γ R on CYTOTOXIC T CELLS helps activate Tc cells so that they can start secrete perforin and granzymes to kill target cells, as well as form memory Tc cells.

The binding of IFN γ to IFN γ R on T_H2 CELLS inhibits the proliferation of this T_H subset, which downregulates expression of cytokines involved in a humoral response (IL-4, IL-5, IL-10).

c) Describe the **major result** shown in **Fig. 1**. Does this result **make sense**? **Why** or **why not**?

Fig 1 shows that deleting the IFN γ gene in mice causes their macrophages to produce less NO than macrophages from wildtype mice. Thus, IFN γ is necessary for activation of macrophages. Both IFN γ and LPS are necessary for optimal macrophage NO production. This result does make sense, since IFN γ is a T_H1 cytokine that helps activate macrophages.

d) Describe the **major result** shown in **Fig. 2**. Does this result **make sense**? **Why** or **why not**?

Fig 2 shows that only macrophages that express the IFN γ R show a significant response (NO production) to treatment with IFN γ (second set of bars) with (bar 3) or without (bar1) LPS. This result does make sense: if macrophages don't have IFN γ R (bars 2,4), they won't be able to bind to IFN γ to stimulate signaling pathways leading to macrophage activation and NO production.

e) If you **quantitated** levels of the **following cytokines**, would you expect levels in the **IFN γ knockout mice** to be **lower, similar, or higher** than in **wildtype mice**? **Explain** your reasoning.

IL-10

Higher levels in IFN γ knockout than in WT. Since IFN γ negatively regulates T_H2-type cells and positively regulates T_H1-type cells, one would expect to see helper T cell responses biased toward a T_H2 response in the absence of IFN γ . IL-10 is a T_H2 cytokine.

IL-12

Lower levels in IFN γ knockout than in WT. Upon activation, macrophages produce more IL-12 to help stimulate T_H1-type responses (positive feedback). Since there is less macrophage activation in IFN γ knockout mice, macrophages should secrete less IL-12.

f) What **type of immune response(s)** are these **mutant mice missing**?

Delayed-type hypersensitivity (DTH), T_H1-type responses.

The **experiments** shown in **Figures 1 and 2** are quite **similar**, yet if you look at nitrite production at the **highest LPS** concentration (100 ng/ml) and in the **absence of added IFN γ** , macrophages from **wildtype mice** in **Fig. 1** show a **significant amount** of nitrite production (**highest points on curves**) while **macrophages** from **wildtype mice** in **Fig. 2** show **very little** nitrite production (**untreated #3**).

g) Assuming that the **same strain** of **wildtype mice** was used in the two experiments, **explain** this **difference** in nitrite production.

The macrophages used in Fig. 1 were isolated from mice that had been infected with mycobacteria. Since mycobacterial infection elicits a DTH response and T_H1-type cytokines, infected wildtype mice will have made IFN γ and activated macrophages before the macrophages are removed. This prior activation of macrophages allows them to respond more strongly to LPS in culture. In Fig. 2, the mice were not infected. Therefore the macrophages had not been activated before removal and additional IFN γ would be required to activate them.

4.2 Viruses depend on the host cell for much of the macromolecular machinery required for viral replication (e.g. ribosomes). Although viruses usurp many host proteins, they also occasionally make their own, slightly different versions of host proteins. Epstein-Barr virus (EBV--a real virus, which causes mononucleosis) makes a protein that is a viral homolog of interleukin-10 (vIL-10). Why would it be beneficial to EBV to produce vIL-10? Explain the benefit of vIL-10 to the virus in terms of the cells and molecules involved.

IL-10 is a T_H2-type cytokine that inhibits secretion of IL-12 by macrophages. IL-12 activates T_H1-type helper T cells to stimulate a cell-mediated response, such as activation of cytotoxic T cells and macrophages. Therefore, production of an IL-10-like molecule by EBV will reduce the activation of virus-specific cytotoxic T cells and protect virus-infected cells (and therefore the virus) from elimination via a cell-mediated immune response.

5.1 In the early 1980s a group of immunologists was interested in identifying **the cell-surface receptor for type I interferons (α , β)**. 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>³H-uridine incorporation</u>
+IFN	-	-
-IFN	+	+

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

	+Virus	
	<u>CPE</u>	<u>³H-uridine incorporation</u>
+IFN	+	+
-IFN	+	+

- a) Why did they use **whole fibroblasts rather than a protein lysate to immunize the mice?**

Since they wanted to elicit a B-cell response specifically against cell-surface proteins (given that the type I interferon receptor is located on the surface), they used intact fibroblasts, which only expose cell-surface proteins to naive B cells. If they had lysed the fibroblasts and used the proteins in the lysate, antibodies would have been raised against many intracellular proteins as well.

b) Why did they **clone individual hybridoma** cells?

They were seeking a monoclonal antibody that only recognized the interferon receptor. If they had used pools of hybridoma cells, there could have been separate B-cell hybridomas with different heavy and light chains represented in the population. These pooled cells therefore would have expressed a number of antibodies, some of which might recognize the interferon receptor and some of which might recognize other cell-surface proteins.

c) In **Step 1**, why did they use the **supernatant** from the **hybridomas** ?

Antibody is secreted by B-cell hybridomas (like plasma cells) into the culture medium, so the supernatant from the hybridoma would contain antibodies that might bind to the interferon receptor on the fibroblasts.

d) Explain the **difference** between the 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**.

When cells are infected with VSV, the virus both kills the cell (as seen by CPE) and incorporates ³H -uridine into its RNA. As seen in the control sample, the presence of a type I interferon protects cells from viral infection. This effect is caused by the binding of interferon to the receptor, which initiates a signaling cascade that leads to the shutdown of protein synthesis in the cell, blocking replication of the virus (because no viral proteins are made). However, if an antibody that binds to the interferon receptor is present in the supernatant, the interferon will not be able to bind to the cell and elicit the anti-viral response and the cell will succumb to viral infection.

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

	+Virus	
	<u>CPE</u>	<u>³H -uridine incorporation</u>
+IFN	+	-
-IFN	+	-

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

1. It is strange that the two assays for viral infection--CPE and ³H -uridine incorporation do not agree with each other: why are the cells dying without the virus incorporating nucleotides into its genome?

2. It is odd that even in the absence of interferon there is no viral genome replication.

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.

The best candidate for a cell surface molecule recognized by this antibody is Fas. Fas is a cell-surface molecule that, when bound to a ligand, sends a signal for the cell to undergo apoptosis. Normally, Fas ligand on the surface of cytotoxic T cells will bind to Fas on a target cell and initiate the cascade for apoptosis. Sometimes binding of an antibody to a cell-surface molecule can cause activation of the downstream signaling pathway (e.g. anti-CD28--see answer for question 2a above). Therefore, anti-Fas antibody binding to Fas can also signal apoptosis. "Cytopathic effect" could be a sign of the fibroblast undergoing apoptosis if the supernatant contained an anti-Fas antibody.

g) Why is this molecule is expressed by **many different cell types**?

The main job of the cytotoxic T cells is to kill infected cells and T cells use the Fas ligand-Fas interaction as a major killing mechanism. Since many different cell types can be infected, it is crucial that many cell types express the Fas antigen so that they can be eliminated efficiently by Tc cells.

*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? **FS-7-associated cell-surface antigen***

5.2 After reading about the IFN γ R knockout mice, you are curious to know if there are any **humans** with **mutations** in the **IFN γ R gene**. You search PubMed and find that last month a group from Italy published a paper describing an **IFN γ R^{0/0}** 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**.

1. **helper T cells**
2. **macrophages**

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.

I would expect this patient to be more susceptible to HIV infection than a wildtype sibling. CCR5 is a cell-surface chemokine receptor that is highly expressed on macrophages which acts as a coreceptor for HIV entry into target cells. The binding of HIV gp120 protein to CD4 and CCR5 allows entry into macrophages. With more receptors to bind to, entry of "M-tropic" HIV will be more efficient and the IFN γ R^{0/0} patient should therefore be more susceptible to HIV. In addition, the lack of IFN γ signaling would limit the cell-mediated response to viral infection.

c) Would your answer in (b) be the **same for all strains of HIV**? **Why** or **why not**?

Strains of HIV (often called "T-tropic" strains) with forms of gp120 that bind to a different chemokine receptor, the CXCR4 protein, should not show increased infectivity unless CXCR4 is also upregulated in the IFN γ R^{0/0} patient.

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).

B-cell activation requires cross-linking of BCR on a single B cell; two BCRs need to bind to a single antigen. The small size of haptens (not much bigger than the side chain on some amino acids) makes it highly unlikely that two antigen-binding sites on neighboring antibodies could contact the hapten simultaneously. However, if multiple hapten molecules were attached to a single carrier protein, the haptens could be far enough apart to allow this cross-linking.