

Bio257 Immunology Practice Questions #7

1. In their work to investigate **potential immunogens for vaccination against cryptococcosis**, Levitz and coworkers wanted to **create a T-cell hybridoma** that was **specific for a *Cryptococcus neoformans* protein**. To create the hybridoma, they used the following procedure:

1. **Disrupt *C. neoformans* yeast** and **mix the protein** extract with an **adjuvant**.
2. **Inject the protein/adjuvant** mixture into **C57BL/6 mice** on **day 0** and **day 21**.
3. **Sacrifice the mice on day 28**, **remove T cells** and **fuse them with BW cells** (from an **immortalized T-cell line**).
4. **Isolate individual T cell-BW fusions** and grow these **T cell hybridomas** in culture.

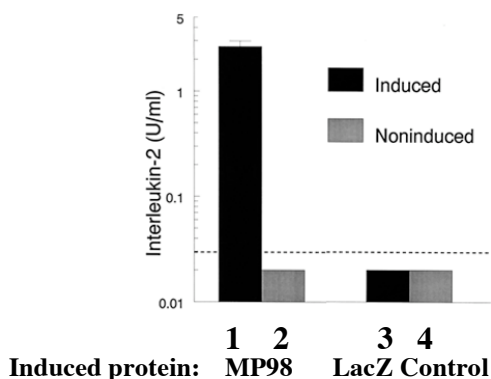
They found one **CD4+ hybridoma** that specifically recognized a **98 kDa mannoprotein (MP98)** from *C. neoformans* and called this hybridoma **P1D6**.

a) Why were the mice given two injections in step 2? In general terms, explain what happened in the mice after each injection (on a cellular level, no molecular details, please).

b) Name one gene that must have been missing from the BW cell line to allow production of a MP98-specific hybridoma. Why must this gene have been deleted from BW before step 3?

Thinking about mass production of MP98, Levitz et al. **cloned the MP98 gene from *Cryptococcus neoformans*** and **inserted it into a plasmid that allows inducible gene expression** in the **non-pathogenic yeast *S. cerevisiae***. They next wanted to **test whether this recombinant form of MP98** would be **recognized by the P1D6 hybridoma** so they performed the following procedures:

1. **Induce expression of MP98 protein** (or a control bacterial protein) in *S. cerevisiae*
2. **Disrupt yeast cells and extract all proteins**
3. Remove and **gamma irradiate splenocytes** from **C57BL/6 mice** to **prevent their proliferation** (other cellular processes still occur)
4. **Incubate these splenocytes** with the *S. cerevisiae* protein extract and the **P1D6 hybridoma**
5. **Monitor production of IL-2** by P1D6 using an ELISA assay.



Stimulation of P1D6 by MP98 expressed in *S. cerevisiae*. Expression of proteins was induced or not induced in *S. cerevisiae* and then the yeast cells were disrupted and all proteins were extracted. The protein extracts were then tested for their ability to stimulate the P1D6 hybridoma to produce IL-2 in the presence of gamma-irradiated splenocytes. The dotted line denotes the lower limit of the IL-2 bioassay, 0.03 units/ml. Values below this lower limit are arbitrarily assigned a value of 0.02 units/ml.

c) What is the function of the splenocytes in this assay?

d) Why did they use splenocytes specifically from C57BL/6 mice?

e) Draw a **diagram** of the **molecular and cellular interactions** that lead to the production of **IL-2** in **sample 1**. Include at least **3 cell-surface molecules** for each cell type involved. To help clarify the specific steps, you may include an **accompanying list** (5-7 steps).

A protein extract from *S. cerevisiae* transformed with a plasmid expressing an *E coli* protein was used as a negative control (**LacZ Control**). If **IL-2 levels in sample 3** had been as high as those in **sample 1**, this paper would never have been published.

f) What **conclusion** would you have drawn if **IL-2 levels of samples 1 and 3 had** been the same?

2. Although **Fc receptors** play a large role in **helpful immune effector responses**, we often think of Fc receptors in the context of **hypersensitive reactions**.

- Name **two** cell types that have **Fc receptors** on their surface.
- Describe the role of **Fc receptors** in an **allergic (Type I hypersensitive) response**.
- Which subset of **helper T cells** is **most important** for **Type I responses** and **why**?
- Describe the role of **Fc receptors** in a **Type II hypersensitivity response**.

3. Neither **haptens** nor "**naked**" **DNA molecules** (DNA without associated proteins) are **immunogenic**, yet **anti-hapten antibodies** can easily be produced in animals and **anti-DNA antibodies** are found in humans with systemic lupus erythematosus.

- Give one reason why **haptens** are **not immunogenic**.
- Give one reason why **DNA** is **not immunogenic**.
- Explain how a **humoral response against each of these antigens can be elicited**. Notice that the mechanisms for raising the anti-hapten and anti-DNA antibody responses are very similar!

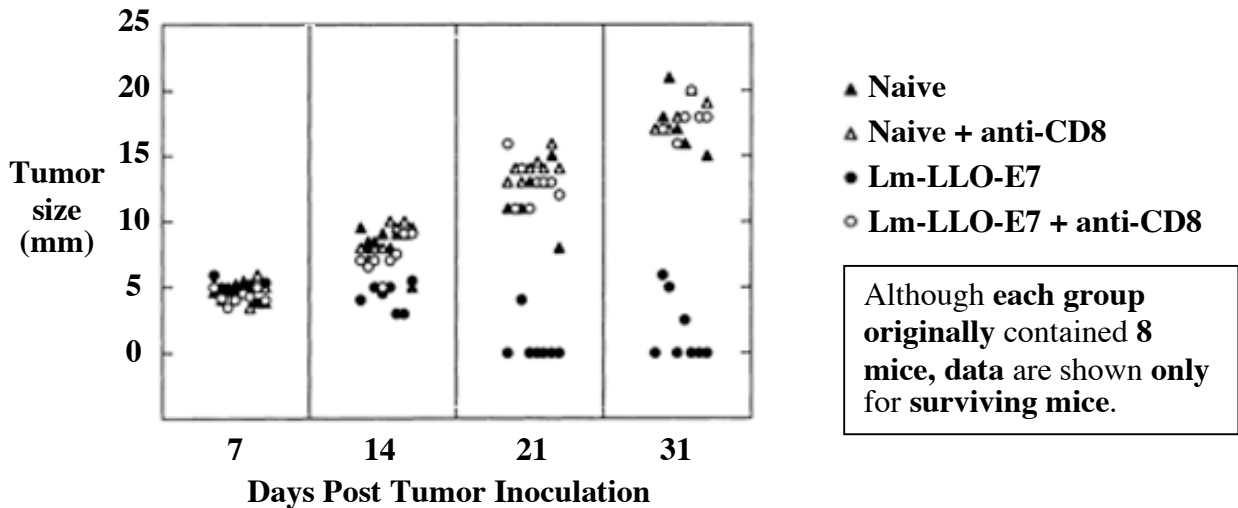
4. Cancer is an enormous health concern in the U.S. and around the world. Although there are no magic bullet cures for this devastating disease, people are working hard to develop new therapies to treat cancer. Whereas chemotherapy, irradiation and surgery to remove tumors have traditionally dominated cancer treatments, possibilities for **anti-cancer vaccines** have started to emerge.

In **one model system** we discussed in class, an **attenuated strain of *Listeria monocytogenes*** was transformed with a plasmid that allowed it to **express a hybrid protein** in which the *L. monocytogenes* protein **listeriolysin (lacking the C-terminus)** was **fused** to the **E7 protein of human papilloma virus-16**. This **bacterial strain** was called **Lm-LLO-E7**. The **results** of experiments that involved **injection of this strain into mice with early stage tumors** provided hope for such **anti-cancer vaccines**.

To understand the immune response of the immunized mice more fully, the authors performed the following experiment:

- C57BL/6 mice** were **injected** with a **tumorigenic T-cell line** that **expresses E7 (day 0)**.
- Mice were either **left untreated (naive)** or **treated on day 7** with **Lm-LLO-E7**.
- On **days 6, 7, 8, 10 and 12 after tumor injection**, mice were **treated (or not)** with an **anti-CD8 antibody**.
- Tumor size** was **determined at different times** after step 1.

- a) Describe what is occurring in mice when they are injected with Lm-LLO-E7. Include the major cells and molecules involved (feel free to use a picture if you like).
- b) What is the major effect of anti-CD8 antibody addition on lymphocyte populations? Describe two different mechanisms responsible for this effect.
- c) Hypothesize why mice were treated with anti-CD8 antibody at such frequent intervals.



- d) Briefly describe the major result shown in this figure. What cell type(s) is important for the immune response in these mice?
- e) Would you expect to have similar or different results if the mice were treated with anti-CD4 antibody in step 3? Explain your reasoning.
- f) Do you think the tumorigenic T-cell line was derived from a C57BL/6 or a Balb-C mouse? Explain your thoughts and what would have happened if a tumor line from the other type of mouse had been used.
- g) Why is Lm-LLO-E7 considered as a possible model for vaccination against cervical cancer? Include three advantages to this system.

From the textbook:
 Ch. 15: Analyze the data a-d, f, g
 Ch. 16: 8, 9, 14a,c
 Ch. 17: 3, 6