

## Bio257 Immunology Practice Questions #7 key

1. (30 pts) 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) (3 pts) **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).

**The first injection allowed presentation of cryptococcal peptides by professional APCs, initial activation of MP98-specific helper T cells (as well as T cells specific for other cryptococcal proteins), and formation of CD4+ memory T<sub>H</sub> cells with the same specificity. On the second injection, the MP98-specific T cell populations were expanded to increase levels of *Cryptococcus*-specific T cells by day 28.**

b) (3 pts) **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**?

**The BW cell line must be missing the gene for the alpha chain of the TCR (as well as the gene for the beta chain). If the BW cell line already had TCR genes, not all TCRs on the surface of the hybridoma would bind to presented MP98 peptides, since there would be some hybrid TCRs.**

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:

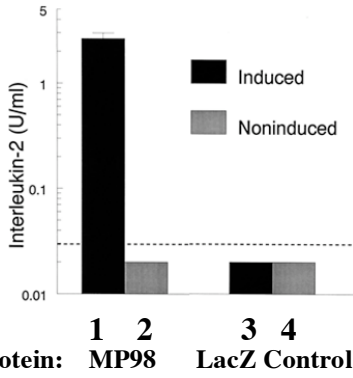
1. **Induce expression of MP98 protein** (or a control bacterial protein) **in *S. cerevisiae***
2. **Disrupt yeast cells** and **extract all proteins**
3. **Remove and gamma irradiate splenocytes** from **C57BL/6 mice** to **prevent their proliferation** (**other cellular processes still occur**)
4. **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**.

c) (3 pts) **What** is the **function** of the **splenocytes** in this assay?

**The splenocytes serve as antigen-presenting cells (APCs) to take up, process and present MP98 peptides (containing the appropriate epitope) on MHC class II molecules to the P1D6 T cells.**

d) (3 pts) **Why** did they use **splenocytes specifically** from **C57BL/6 mice**?

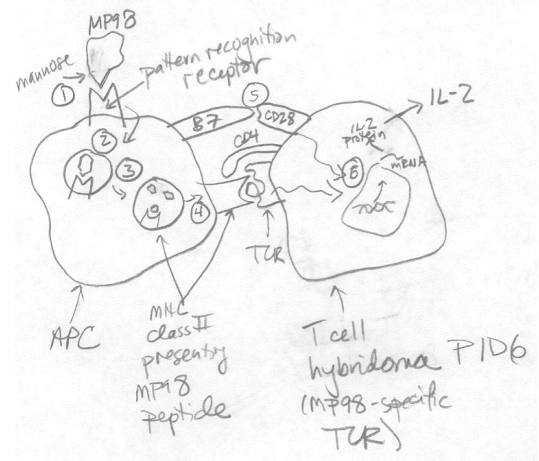
**The MP98-specific TCR was already positively selected during T cell development in the thymus to recognize H-2<sup>b</sup> MHC molecules. Therefore, for effective MP98 peptide presentation, the peptide must be displayed on a H-2<sup>b</sup> MHC class II molecule.**



**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.

e) (9 pts) 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).

1. Binding of MP98 to the mannose receptor
2. Internalization of MP98 by phagocytosis
3. Digestion of MP98 into peptides in phagosome
4. Presentation of peptide to T<sub>H</sub> cell
5. Co-stimulatory signals
6. Signal transduction into the nucleus for IL-2 transcription, translation and secretion



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

If IL-2 levels had been the same when LacZ was expressed, this result would suggest that another *S. cerevisiae* protein that was induced under the same conditions could be presented to activate P1D6 cells and that P1D6 was therefore not specific for the *Cryptococcus* MP98 protein.

2. (14 pts) Although **Fc receptors** play a large role in **helpful immune effector responses**, we often think of Fc receptors in the context of **hypersensitive reactions**.

a) (4 pts) Name **two cell types** that have **Fc receptors** on their surface.

**Macrophage, mast cell (basophil, natural killer cell, neutrophil, B cell, dendritic cell, eosinophil)**

b) (4 pts) Describe the role of **Fc receptors** in an **allergic (Type I hypersensitive) response**.

**Fc receptors on mast cells bind to IgE molecules, sensitizing the mast cell. When the IgE molecules are crosslinked by ligand (allergen) binding, a signal is transduced within the mast cell causing release of histamine and other inflammatory molecules.**

c) (3 pts) Which subset of **helper T cells** is most important for **Type I responses** and why?

**T<sub>H</sub>2 cells, since these cells produce IL-4. IL-4 is critical both for class switching to IgE (the isotype that binds to mast cells) and for mast cell activation.**

d) (3 pts) Describe the role of **Fc receptors** in a **Type II hypersensitivity response**.

**In a type II hypersensitivity response, the body makes antibodies against cell-surface proteins. The binding of the Fc portion of the antibody to macrophages (or neutrophils, natural killer cells, eosinophils) causes complement activation and antibody-dependent cell-mediated cytotoxicity (ADCC), either through phagocytosis of the target cell or through the secretion of toxic molecules.**

3. (14 pts) 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.

a) (3 pts) Give one reason why **haptens** are **not immunogenic**.

**Haptens are small organic molecules that are not immunogenic because:**

a) They **cannot crosslink anti-hapten B cell receptors** because of their **small size** and likely **presence of a single epitope**.

b) They are **not effectively presented on MHC molecules (not being peptides)**

b) (3 pts) Give one reason why **DNA** is **not immunogenic**.

**DNA is not immunogenic because it is not effectively presented by MHC molecules (not being a peptide).**

c) (8 pts) 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!

**If multiple hapten molecules are conjugated to a carrier protein, the conjugate can crosslink BCRs that recognize the hapten. Internalization of the conjugate can lead to digestion and presentation of a peptide from the carrier protein, which can be recognized by T<sub>H</sub> cells and cause production of cytokines to activate the anti-hapten B cell.**

**If DNA is bound to a non-self DNA-binding protein, crosslinking of anti-DNA BCRs can lead to internalization of the complex and presentation of a peptide from the DNA-binding protein. This presented peptide can be recognized by T<sub>H</sub> cells, which can then activate antibody production by the anti-DNA B cell.**

**4. (30 pts)** 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:

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

a) (6 pts) **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).

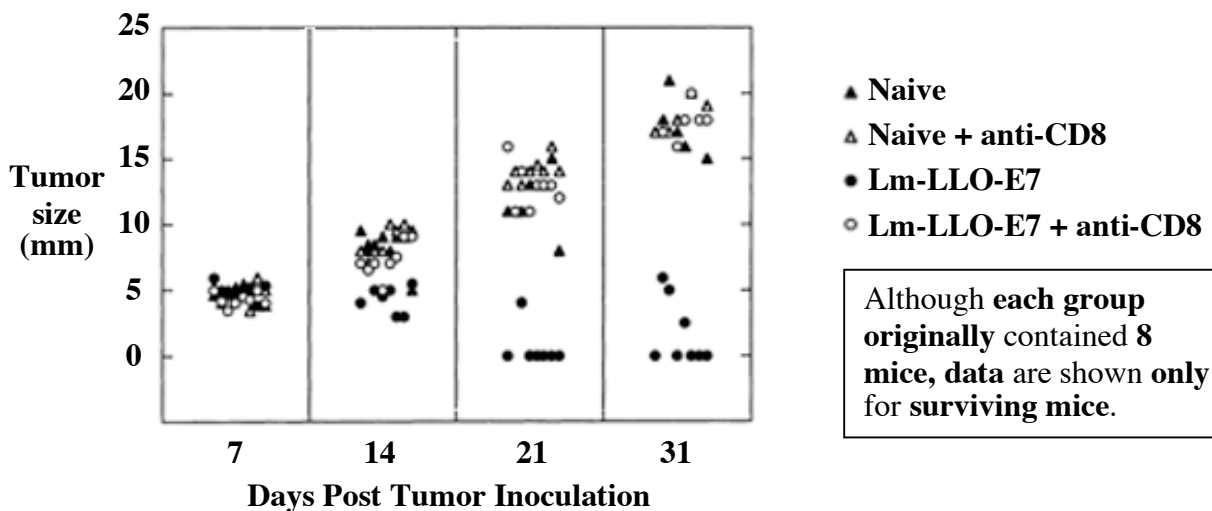
1. **Lm-LLO-E7 bacteria are phagocytosed by macrophages**
2. **In the phagosome, Lm-LLO-E7 starts expressing and secreting E7.**
3. **Some E7 peptides are presented on MHC class II molecules to T<sub>H</sub> cells.**
4. **Other E7 in the cytoplasm may be digested and presented on MHC class I molecules to T<sub>c</sub> cells.**
5. **T<sub>H</sub> cytokines help activate macrophage and T<sub>c</sub> cells**
6. **T<sub>c</sub> cell can then kill other E7-expressing cells.**

b) (5 pts) **What is the major effect of anti-CD8 antibody addition on lymphocyte populations? Describe two different mechanisms responsible for this effect.**

**Anti-CD8 antibody decreases the population of CD8+ T lymphocytes (mostly cytotoxic T cells) either through ADCC (opsinizing CD8+ cells for phagocytosis as described in question 2d) or by fixing complement to the CD8+ cells, causing the formation of membrane attack complexes in the T cell membrane and their rapid demise.**

c) (2 pts) **Hypothesize why mice were treated with anti-CD8 antibody at such frequent intervals. Antibody is consumed in the process of ADCC and may not be stable in circulation in the host animal. In addition, T cells are constantly being produced in the thymus and need to be destroyed.**

The results of these experiments are shown in the figure on the next page.



Although each group originally contained 8 mice, data are shown only for surviving mice.

d) (5 pts) Briefly describe the major result shown in this figure. What cell type(s) is important for the immune response in these mice?

The only mice whose tumors showed significantly smaller sizes are those mice that received Lm-LLO-E7 but were NOT treated with anti-CD8 antibody. Even when mice had been immunized, removal of CD8+ T cells prevented the diminution in tumor growth, demonstrating the importance of cytotoxic T cells in tumor growth control. One can also see this importance by the small number of naive, anti-CD8 Ab-treated mice surviving at day 31 (≤4).

e) (4 pts) Would you expect to have similar or different results if the mice were treated with anti-CD4 antibody in step 3? Explain your reasoning.

Similar results. Activation of cytotoxic T cells requires cytokines from T<sub>H</sub> cells. Therefore, removal of CD4+ helper T cells should severely reduce the activation of T<sub>c</sub> cells and their efficacy in killing the tumor.

f) (4 pts) 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.

C57BL/6. If a non-MHC matched tumor line (Balb-C) was used, the H-2<sup>d</sup> MHC molecule would be recognized as foreign by host T cells and the tumor cells would likely be rejected (as an allotransplant) by day 14. No tumor would therefore be present to test the effects of the Listeria vaccine.

g) (6 pts) Why is Lm-LLO-E7 considered as a possible model for vaccination against cervical cancer? Include three advantages to this system.

1. Cervical cancer is frequently associated with HPV-16 infection and E7 is a viral oncogene. Therefore, targeting E7-expressing cells for destruction could help control tumor growth. By targeting E7-expressing cells specifically, the non-specific immunosuppressive effects of other cancer treatments could be avoided.
2. By using an attenuated bacterial strain one should be able to minimize harmful effects of the infection. By allowing reproduction of the bacteria and more widespread E7 expression and presentation, one may also eliminate the need for a booster.
3. By introducing E7 into a host APC through the phagocytosis pathway, it should be possible to elicit both T<sub>H</sub> and T<sub>c</sub> responses.