

Bio257 Immunology Practice Questions #3 Answer Key

1. **X-linked hyper-IgM (XHM) syndrome** is a heritable **immunodeficiency** syndrome with a number of **B-cell phenotypes**. **B cells** from XHM patients:

Produce **high levels** of **IgM** antibodies

Produce **no IgG, IgA** and **IgE** antibodies

Can be **activated** by **mitogens** such as **LPS**, but **not** by **specific antigens**.

Also, **XHM** patients **frequently** suffer from **recurrent infections** by opportunistic pathogens. XHM is caused by a **mutation** in the **gene** that **encodes CD40 ligand (CD40L)**; XHM patients therefore do **not** produce **functional CD40L**.

a) What is the **normal role** of **CD40L** in the **adaptive** immune system?

The **CD40 ligand** found on the **surface of helper T cells** binds to **CD40** on the **surface of B cells**. This binding is one of **several cell-surface molecular interactions** that is **important for signaling B cell activation** (through the release of **cytokines** from the **helper T cells**).

b) Why would a **defect** in **CD40L** result in the **absence** of **IgG, IgA** and **IgE** antibodies?

Helper T cell activation of B cells is **required** for **class switching** of B cells. B cells **start** by producing **only IgM**, but **upon activation** can undergo **further somatic rearrangement** to **exchange constant segments** to make **other antibody isotypes**. Therefore a **defect in CD40L** will lead to a **defect in B-cell activation**, which will result **no class switching** and the **absence of IgG, IgA and IgE antibodies**.

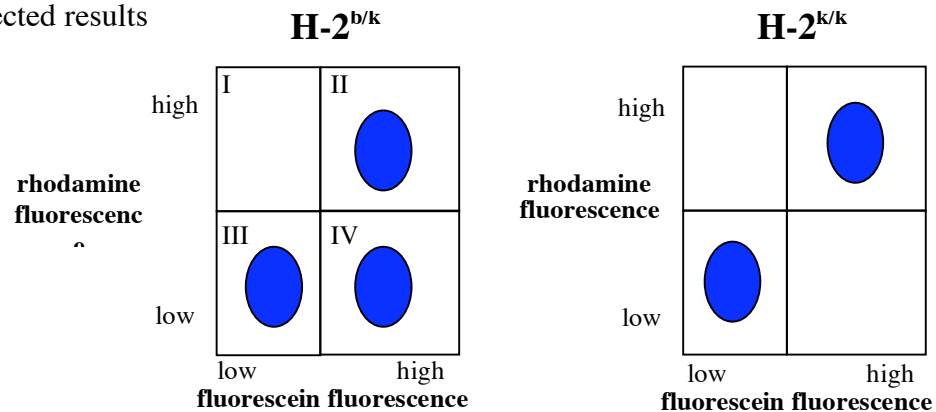
c) Why would **XHM** patients suffer from **recurrent infections**?

Helper T cell activation of B cells is also required for **B-cell differentiation** to form **memory B cells**. In the **absence of memory B cells**, XHM patients will **not** be able to **elicit a memory response** to **fight infectious agents** they have been **exposed to previously**.

2. You are interested in studying **T-cell development** in mice. Having read about the **H-Y antigen** (which is encoded on the Y chromosome), you decide to get the **gene** for the **H-Y-specific TCR** used by von Boehmer and Kiselow. They cloned this gene from a **cytotoxic T cell line** originally **derived** from an **H-2^{b/b}** mouse. You make **two transgenic mouse lines** bearing the **H-Y TCR gene**--one line is **H-2^{k/b}** and the other line is **H-2^{k/k}**. Previous work has shown that in **H-Y TCR transgenic mice**, T cells bearing the **transgenic TCR** vastly outnumber other T cells.

You isolate **thymocytes** from **transgenic female mice**, **stain** the cells with **fluorescein-conjugated anti-CD8** antibody and **rhodamine-conjugated anti-CD4** antibody and perform **FACS analysis**.

a) **Sketch** your expected results



b) For the **H-2^{k/b}** FACS profile, indicate the **type of cells** that are found in **each quadrant** and explain **why** you **do** or **don't** expect to see cells in **that quadrant**.

I: CD4+CD8- cells, mostly helper T cells, are found in this quadrant. There should be no/very few cells in this quadrant; since the TCR was derived from a cytotoxic T cell line, the mature T cells should recognize MHC class I molecules and after negative and positive selection CD4 should be downregulated.

II: CD4+CD8+ double positive cells. At this stage in T-cell development in the thymus, the TCR β chain has successfully been expressed and both CD4 and CD8 are expressed. Since the transgenic TCR contains a properly rearranged β-chain gene, there should be cells in this quadrant.

III: CD4-CD8- double negative cells. When T cells arrive in the thymus from the bone marrow, they do not yet express either the α or β TCR chain nor CD4 or CD8. Since the CD4 and CD8 genes are not turned on until β chain expression has been checked, there should be some cells in this quadrant.

IV: CD4-CD8+ cells, mostly Tc cells (cytotoxic T cells and their mature precursors), are found in this quadrant. There should be many cells in this quadrant; since the TCR was derived from a cytotoxic T cell line, the mature T cells should recognize MHC class I molecules and after negative and positive selection only CD8 should remain on the surface.

c) Explain the **difference(s)** between **the two profiles** shown in (a). What **process** do your results reflect?

The TCR was cloned from a cell line derived from a homozygous H-2^{b/b} mouse. Therefore the TCR must be specific for a H-2^b MHC class I molecule. The H-2^{b/k} mouse does have one H-2^b allele that should allow binding to the transgenic TCR and prevent death of the T cell during POSITIVE SELECTION (due to MHC restriction of TCRs) in the thymus. T cells will thus become mature CD4-CD8+ cells. However, in a mouse with only H-2^k class I molecules, T cells bearing the transgenic TCR should be deleted and no mature CD4-CD8+ cells should be seen.

3. The regulation of gene expression is crucial for cellular differentiation and organismal development. Whereas most cells have exactly the same genes, which genes are expressed vary with time, place, and cell type. The bone marrow contains many different cells types, some of which express RAG1 and RAG2 and others of which do not express these genes.

a. Name the two main types of cells in the bone marrow that we have discussed.

B cells (including many stages of development) and stromal cells

b. Which cells express the RAG genes in the bone marrow and why?

B cells express the RAG genes in the bone marrow. The RAG1/RAG2 proteins are responsible for recognizing immunoglobulin V, D, and J gene segments and cutting them apart. The RAG genes play an essential role in B cell development in the bone marrow by allowing Ig gene rearrangement, which results in B cells with complete B-cell receptors (antibodies) on their surface.

c. Which cells do NOT express the RAG genes in the bone marrow and why?

Stromal cells do not require gene rearrangement, since these cells are responsible for presenting self-antigens to B cells in the bone marrow, rather rearranging Ig genes themselves.

d. Some cells in the bone marrow do not express RAG genes when they are in the bone marrow, but turn on their RAG genes elsewhere in the body. What cells are these and where and why do they express RAG genes outside the bone marrow?

T cells turn on the RAG genes in the thymus to allow TCR gene rearrangement. The environment of the thymus allows for positive selection of cells with TCRs that bind self MHC molecules and negative selection of cells with TCRs that recognize self antigens.

4. You discover a new virus that infects T cells and you name it Ursavirus. Interestingly, Ursavirus produces high quantities of a protein called ULLP (Ursavirus Lck-like protein) that acts exactly like Lck except that it is constitutively active (enzymatic activity is always on). You express ULLP in a cultured T-cell line and you test IL-2 production by the cells under different conditions and get the following results:

<u>Fixed APCs (no B7 expression)</u>	<u>purified B7 in solution</u>	<u>IL-2</u>
-	+	+
+	-	-
+	+	+

a. Explain why APCs (antigen-presenting cells) are not needed to induce IL-2 expression when ULLP is expressed. Describe the molecules involved.

Since ULLP is constitutively active, it will be able to phosphorylate the zeta chain of CD3 *without* receiving a signal through the TCR/MHC/CD4 complex. ZAP70 will then be able to bind to CD3, be phosphorylated by Lck and activate downstream molecules in the signaling pathway, eventually leading to transcription factor activation and transcription, translation, and secretion of IL-2.

b. Explain why soluble B7 is required to induce IL-2 expression in this system. Describe the molecules involved.

Activation of T cells requires not only binding of the TCR/MHC/CD4 complex leading to the downstream effects described in (a), but also the interaction of B7 with CD28 on the T cell. Since B7 is not supplied by APCs in this experiment, either soluble B7 or an anti-CD28 antibody needs to be used to stimulate signaling pathways downstream of CD28 to allow IL-2 expression.

c. If you deleted the CD45 gene (which encodes the phosphatase that acts on Lck) from the T cell line would you expect to get the same results after expression of ULLP? Why or why not? If not, indicate the results you would expect for the conditions shown in the table.

Yes, you would expect to get the same results. Since ULLP is constitutively active, it does *not* need to be dephosphorylated to signal to downstream molecules (whereas Lck *does* need to be dephosphorylated).

d. If you deleted the ZAP70 gene from the T cell line, would you expect to get the same results after expression of ULLP? Why or why not? If not, indicate the results you would expect for the conditions shown in the table.

No, if you deleted the ZAP70 gene you would expect to see no IL-2 expression in any of the experimental conditions. Since signaling through ZAP70 is required for IL-2 expression, removal of ZAP70 will prevent any synthesis of IL-2.

5. We have discussed a few reasons why haptens are not immunogenic. Given what you now know about the process of B-cell activation, can you think of another reason why haptens would not be immunogenic?

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.