

## Bio257 Immunology Problem Set #2 Answer Key

1. What is the advantage of a B cell undergoing **somatic hypermutation** after it binds to an antigen? Would the change involve a change to another **isotype, allotype or idiotype**? **Explain** your choice.

Somatic hypermutation is the process by which a **B-cell introduces mutations primarily** into the **variable regions** of the antibody **heavy and light chain genes**. These mutations may result in antibody molecules that have a **higher affinity for the antigen** and are therefore **more effective at eliminating** the antigen. By limiting somatic hypermutation to a time after antigen binding, the body produces a **large population of very similar B-cells only** when one of the resulting cells is **likely to be selected for use** (i.e. the body is only using the energy to keep many similar B cells alive when such a population may prove useful). Somatic hypermutation usually involves a **change to a different idiotype**, since the **majority of mutations** are in the **variable region**.

2. Although we predominantly think of viruses as intracellular pathogens and bacteria as extracellular pathogens, there are many types of bacteria that live and multiply within cells. While escaping the dangerous world outside host cells can be advantageous for intracellular bacteria, there are also immune challenges once a bacterium has taken up residence in a host cell.

a) Early in infection, intracellular and extracellular pathogens face similar challenges from the innate immune system.

**Describe two (2) innate immune defenses** (other than phagocytosis) that bacteria face upon entering a host. Make sure to include what makes these **defenses effective against bacteria**. You can include innate immune defenses that cooperate with the adaptive immune system.

**Physiological defenses** include the **low pH of the skin and stomach** (many bacteria are **unable to survive under acidic conditions**), **tears** (which contain **enzymes** such as **lysozyme** that can **digest the cell wall of bacteria**), and **high body temperature** (e.g. **fever--some bacteria cannot survive at high temperature** in part because their enzymes cannot function at elevated temperatures).

The **binding of antibodies** (resulting from adaptive immunity) to **bacteria** can not only target them for phagocytosis by macrophages, but can also lead to the **deposition of complement proteins** at the **bacterial surface**. A **terminal step** in the complement cascade is the **insertion of a multi-protein complex** (termed a membrane attack complex) **through the cell wall of the bacterium, disrupting the osmotic balance of the bacterium and leading to its lysis**.

**Inflammation** at the site of a wound **raises the local temperature** (see above) and involves the **release of chemokines** to **attract phagocytes** to the site of infection (phagocytes can then ingest and digest bacteria).

b) *Shigella flexneri* is an intracellular bacterium that causes dysentery and it enters host cells through phagocytosis by the host cell. At low pH, however, *Shigella* has the ability to disrupt membranes and escape into the cytoplasm.

**Why** is it **advantageous** for *Shigella* **to escape from phagocytic vesicles** (i.e. what would happen if the bacterium remained in the vesicle)? **How** would an **inability to escape** the vesicle affect *Shigella* **bacteria that have not yet entered host cells**? Describe the **cells and molecules** involved.

**Phagocytosis** is the process whereby macrophages or other phagocytes **surround and internalize** foreign material such as bacteria. Within the cell, **phagocytic vesicles** can **fuse with endosomes and lysosomes, exposing bacteria to digestive enzymes and low pH** that can cause severe damage. Therefore, by escaping from phagocytic vesicles, *Shigella* can **avoid being digested by the phagocyte**.

**Endosomes** are also the **compartment in which MHC class II molecules bind to peptide antigens**, which in this case would result from the digestion of bacterial proteins. The **movement of MHCII with antigen to the surface** of the phagocyte allows this **antigen-presenting cell to interact with and activate helper T cells** that have **T-cell receptors that specifically recognize both the MHCII molecule and a bacterial peptide**. Since **activated helper T cells** will play a key role in **defending the body against other *Shigella* bacteria** (e.g. by activating **B-cells** to make ***Shigella*-specific antibodies**), the ability of a *Shigella* bacterium to escape from the phagocytic vesicle will protect the phagocytosed bacterium from digestion and **other, extracellular bacteria from helper T cell responses**.

2c) You discover a bacterium, *S. boydii*, that can enter **directly** into the **cytoplasm** of host cells. Interestingly, you find that *S. boydii* secretes a protein called **TBP** that **binds tightly to the TAP protein and blocks its function**.

**How would TBP help protect *S. boydii* from the host immune system? Describe the cells and molecules involved.**

. The **TAP protein** (transporter associated with antigen presentation) is present on the **membrane of the endoplasmic reticulum (ER)**. When the **proteasome digests proteins in the cytoplasm**, **TAP transports** the resulting peptides into the **lumen of the ER** and **helps direct the peptides to bind to MHC class I molecules**. MHC I molecules bound to peptide are then **displayed on the surface of the cell**. Binding of this complex to **cytotoxic T cells with T-cell receptors** that interact specifically with **both the MHCI molecule and the peptide antigen** leads to the **death of the MHCI-bearing target cell**. Therefore, by blocking TAP protein function, *S. boydii* can **prevent peptides derived from its own proteins from being displayed on MHCI molecules** and therefore **prevent its host cell from being targeted for killing by cytotoxic T cells**.

d) You delete the TBP gene from *S. boydii* in the hopes that this mutant bacterium will have lowered virulence. You test the mutant (TBP-) and wildtype bacteria in **two inbred mouse strains A and B** and find that while the wildtype bacteria kill both types of mice, the **mutant bacteria only kills the A strain**.

Based on what you know about immunology, give a **hypothesis** for why **strain B is resistant** and **strain A is sensitive** to the **TBP- bacteria**. Explain your hypothesis in terms of the **cellular and molecular interactions** in the **infected mouse strains**.

One main **difference** that is frequently found **between inbred mouse strains** (and within any mammalian population) is the **specific alleles** present at the **MHC loci**. The **binding of peptides to MHC molecules** can be **influenced** by the **exact amino acids in the MHC peptide-binding groove**. If a **mouse does not** have MHCI molecules that **bind effectively** to *S. boydii* peptides, these peptides will **not be presented to cytotoxic T cells**, even if the peptides are imported into the lumen of the ER by TAP (as would occur during infection by TBP- bacteria). Therefore, **mouse strain A may have MHCI molecules that do not bind effectively to *S. boydii* peptides**, in which case **mouse A could not mount a robust immune response** and would be susceptible to the TBP- *S. boydii*. On the other hand, the MHCI molecules of **mouse B would be able to present *S. boydii* peptides**.

Another possibility is that the **TCR gene segments available in strain A cannot be recombined to form a TCR that binds specifically to *S. boydii* peptides** (in spite of junctional flexibility and the addition/removal of nucleotides at V-D-J junctions). This gene segment difference would not necessarily lead to an overall higher susceptibility to infection the way the deletion of the TAP gene or other genes involved in general antigen presentation would.

e) To try to help strain A mice, you decide to use **irradiation to kill the T-cells in strain A** and to **replace them with T cells from a strain B mouse**. You make sure that the **donor strain B mouse** has been **immunized with *S. boydii* proteins**.

Will the *S. boydii*-specific T-cells from strain B help **protect strain A** from subsequent *S. boydii* infection? Why or why not (explain in terms of cells and molecules involved)?

The *S. boydii*-specific T-cells from strain B will **NOT help protect strain A** from subsequent *S. boydii* infection. Given the above hypothesis, the **immune system defect** in strain A is at the **level of peptide presentation**. Therefore *S. boydii*-specific T-cells from strain B would **not have any presented antigen to recognize in strain A** and the bacteria could live happily in the susceptible host with no fear of lysis by B-strain **cytotoxic T cells**. In addition, even if some bacterial peptides were presented, **T-cell receptors are MHC-restricted**, meaning that they **will only bind to a certain peptide presented in a self MHC molecule**. Since **strains A and B contain different MHC alleles**, their MHC molecules will be different and the T cells from strain B should not be able to recognize peptides presented in MHC I from strain A. Similarly, if the susceptibility of strain A to *S. b.* is at the **level of TCR genes**, in order for **strain B T-cells to work in strain A**, these two mouse strains must have the **same MHC alleles**.

3. The availability of technology to disrupt genes in mice has allowed researchers to test the importance of individual genes for proper immune system function. One possible phenotype for the disruption of genes involved in immunity is **severe combined immunodeficiency (a SCID phenotype)**. Whereas **deletion of gene(s) that encode certain protein enzyme(s) of the V(D)J recombinase** leads to a SCID phenotype, **deletion of other V(D)J recombinase gene(s) does not result** in such a severe phenotype.

a) What are the **four key enzymatic proteins** involved in V(D)J recombination?

**RAG1**

**RAG2**

**terminal deoxynucleotidyl transferase (Tdt)**

**double-strand break repair enzyme**

b) **What cell types** will be **affected by deletion of the genes** for these proteins and **why**?

Deleting **RAG1, RAG2 and Tdt** genes would result in **problems in B- and T-cell development**, because these genes are **specifically involved in somatic recombination of antibody and T-cell receptor genes**. Deleting **double-strand break repair enzyme genes** would affect **many cells in the body**, because these **enzymes are important** in the **general repair of DNA damage** (as by UV irradiation). **B-cell and T-cells** would be **among the cells affected**, since this enzyme is also involved in somatic rearrangement of antibody and TCR genes.

c) For **each protein**, predict whether the **deletion of the gene** encoding that protein **will lead to a SCID phenotype** and **explain** your prediction.

Deletion of either **RAG1 or RAG2**, which **cooperate to select and cleave immunoglobulin and TCR gene segments**, should lead to a **severe combined immunodeficiency phenotype**. If **immature B- and T-cells cannot make cuts at the V, D, or J gene segments**, they will **not be able to produce antibodies or T cell receptors** and therefore will have **no adaptive immune system** and will have to **rely solely on innate immunity to fight infection**.

Similarly, since **double-strand break repair enzymes perform a key function in joining Ig and TCR gene segments**, deletion of these genes should also lead to a **SCID phenotype**, since the B- and T-cells in DSBR mutant mice will **not be able to make antibodies or TCRs**. However, if there are

**multiple DSBR genes** that can perform the joining function in T- and B-cell development, **the deletion of a single gene may only lead to a partial defect.**

In contrast, **Tdt is responsible for generating diversity at V, D, and J junctions by adding additional nucleotides during heavy chain antibody and T-cell receptor gene rearrangement.** This enzyme is **NOT required for the formation of complete, rearranged Ig or TCR genes**, therefore deletion of this B- and T-cell specific gene would **not** necessarily lead to a **SCID phenotype.**

d) **For each gene deletion** that you believe will **NOT have a SCID phenotype**, predict **what the effects of the gene deletion will be** and **explain** your prediction.

**Tdt deletion** is likely to cause a **slight decrease in the diversity of Ig or TCR** generated by the mouse. The mouse will **still have different V, D, and J segments to choose from**, but there should be **slightly less variability in the third complementarity determining region**, which is **encoded by the VDJ junction area**, and which is **involved in antigen binding.** Therefore this mouse may be **\*slightly\* more susceptible to infectious diseases.** (Note: in fact, Tdt knockout mice are hardly immunocompromised at all!).

4. Immunologists have constructed a strain of mice in which both alleles of the gene for  $\beta_2$ -microglobulin have been deleted. If one of these mice is infected with Theiler's virus (a mouse relative of poliovirus), its immune system has a hard time fighting the infection.

A. Explain why lacking  $\beta_2$ -microglobulin causes these mice to be susceptible to viral infection. What cells would normally be involved in fighting infection and how are they disabled in these mice?

**The cytotoxic T cell is a major component of the adaptive immune system that is involved in fighting viral infections. The T-cell receptor (TCR) on a Tc cell recognizes a viral antigen (from inside the cell) presented by an infected cell on MHC class I molecules. Since  $\beta_2$ -microglobulin is one of the two MHC class I subunits, the lack of this protein will block the presentation of peptides to Tc cells. Therefore, Tc cells won't be able to kill virus-infected cells and the mice will be susceptible to Theiler's virus infection.**

B. You believe that the immune system of these mice would be able to defend them from infection with the bacteria *E. coli* or *Staphylococcus aureus*. Explain this hypothesis. What cells and molecules are involved in fighting such extracellular bacteria?

**The T-cell response to extracellular bacteria requires that macrophages or other phagocytic cells envelope the bacteria, digest its proteins into peptides, and present those peptides on MHC class II molecules to activate helper T cells. Since MHC class II molecules do NOT use  $\beta_2$ -microglobulin as a subunit, presentation to helper T cells should still function in the  $\beta_2$ -microglobulin knockout mice. Also, an antibody response can still occur in these mice (to target bacteria for phagocytosis or lysis by complement), since B-cell activation requires signals from helper T cells.**

Question from old textbook: a) 5, b) 1, c) 6, d) 2, e) 3, f) 7, g) 4