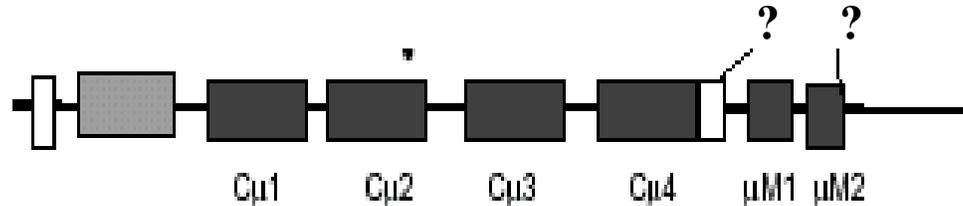


## HST 176 Problem Set

1. This is a schematic view of a rearranged  $\mu$  chain.

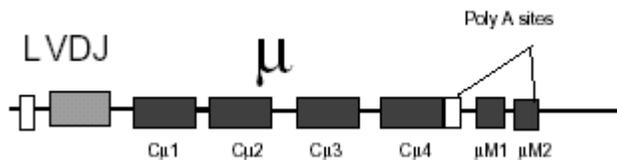


- a. What is coded by the unfilled box at the left? What does the second box from the left in light gray code for? What regions are at the locations of the two question marks? Leader exon or signal sequence exon; the V, D, and J segments after rearrangement; polyadenylation sites
- b. In which box will the first amino acid of the mature protein be located?  
Second box from the left (after cleavage of the signal peptide)
- c. What function is mediated by the protein sequences in the unfilled box on the left? Describe how these sequences are critical and what they might bind to in the cell.  
N-terminal signal peptide for translocation into the ER; Binds to a protein component of SRP or Signal Recognition Particle, which docks with SR (the SRP Receptor) in the rough ER membrane prior to translocation of the nascent peptide through the sec61 channel into the lumen of the ER
- d. How does the above protein contribute to opsonization? Describe what structural region of the protein may contribute to this process.  
There are no Fc receptors for IgM. Opsonization is mediated through the classical complement pathway: the constant domain C $\mu$ 3 binds to C1q and activates the complement cascade. This causes the antigen-antibody complex to be coated with C3b (an opsonin with receptors on phagocytes).
- e. What is the functional role of the second unfilled box on the right?  
Encodes the tailpiece for secretory IgM, which forms disulfide bridges with the J chain and with other IgM monomeric units to form pentameric IgM.
- f. Which portions of the above protein are associated with light chains?  
Variable domain (VDJ) and the first constant domain C $\mu$ 1
- g. Name the proteins that are physically associated with this protein in pre-B cells.  
Surrogate light chains ( $\nu$ preB and  $\lambda$ 5), Ig $\alpha$ , and Ig $\beta$

- h. In one of the forms of the  $\mu$  protein, some portion of the protein must be involved in stopping the transfer process across the ER membrane. Where in the protein is this sequence? What does it look like?**

The actual transmembrane domain is thought to signal the stopping of the translocation process through the ER membrane along with positively charged amino acids in the cytoplasmic tail just adjacent to the transmembrane domain (KVK, remember that lysines are positively charged)

- i. Put together the required boxes from the above figure to recreate what a cDNA for the secretory form of the  $\mu$  heavy chain may look like. Where will the untranslated regions be? Where do you think the transcriptional and translational start sites will be located on your figure?**



The cDNA is a direct reflection of the mRNA sequence. Within the white box on the left, there are three different landmarks. The beginning is the **transcriptional start site**. This is followed by the **5'-UTR** (untranslated region) which is followed by the **ATG** start codon for translation. After the ATG are the codons coding for the signal sequence (which will get cleaved off once the protein is translocated into the ER).

Within the white box on the right, there is the **stop codon** followed by the **3'-UTR**, which is then followed by the polyA tail.

- 2. The affinity of CTLA-4 is much higher than that of CD28 for B7. Signal One induces CTLA-4 in T cells, but not to high enough levels to sequester all the B7 on an activated APC.**

- a. How does CD28 cooperate with Signal One?**

One example in which this may occur is in the regulation of IL-2 transcription: TCR signaling (Signal One) activates the Ras/Erk MAP Kinase pathway and contributes to the induction of c-fos, which is one component of the AP-1 dimer. The other component is c-jun, which must be phosphorylated by the Jun Kinase (JNK) in

order to be active. JNK activation occurs downstream of CD28 (Signal Two). C-fos and C-jun dimerize to form AP-1, which is a transcriptional activator for IL-2.

**b. What is the role played by CTLA-4 in the induction of an immune response?**

CTLA expression at low levels sets a threshold for T cell activation – requiring higher levels of B7 on the APC (i.e., via a danger signal) in order for the T cell to engage CD28 and respond positively.

**c. As a consequence of the cooperation between Signal One and Signal Two, a cytokine is produced that signals for the proliferation of T cells. Name an immunosuppressive drug that interferes specifically with this cytokine signaling event. Which other drug binds to the same protein as this inhibitor of cytokine signaling? Which pathway does this other drug inhibit?**

IL-2 signaling may be inhibited by rapamycin. The mechanism of action of rapamycin is to bind to FKBP (i.e., in T cells) and the rapamycin-FKBP complex then binds to another cellular protein called the mammalian target of rapamycin (MTOR). MTOR may be a regulator of a protein kinase that participates in cell cycle control. In this way, rapamycin inhibits IL-2-driven proliferation and cell cycle progression. FKBP (as its name implies) also binds to the drug FK506. The FK506-FKBP complex (like the cyclosporine-cyclophilin complex) works in a different manner by inhibiting calcineurin. Calcineurin is a calcium-dependent serine/threonine phosphatase that normally dephosphorylates NFAT (“nuclear factor of activated T cells”). Dephosphorylated NFAT enters the nucleus to activate the transcription of IL-2 and other cytokine genes. Thus, FK506 and cyclosporine inhibit IL-2 gene transcription by keeping NFAT in the inactive, phosphorylated state.

**3. Describe the term transcytosis and explain two important examples of this in the immune system.**

Transcytosis is the movement of a molecule from one side of a cell to the other. As opposed to paracellular movement in which a molecule moves in between the junctions of cells (i.e., the tight junctions of epithelia), transcytosis involves movement inside the cell and usually occurs via vesicular trafficking. It is often discussed in the case of epithelial cells since they are polarized, in which molecules move from either apical to basolateral sides or vice versa. There are many examples of transcytosis in the immune system but two major ones are: (1) in mucosal immunity, in which IgA is secreted by plasma cells (i.e., residing in the lamina propria of the gut or in the mucosa of the respiratory system). These IgA are in dimer form and bind to the poly-Ig receptor on the basolateral surface of epithelial cells. The complex is endocytosed and moves in vesicles to the apical surface where the extracellular domain of the poly-Ig receptor is cleaved. Thus, the IgA is released into the lumen. This IgA release also occurs into bile, milk, sputum, and saliva and is thought to protect epithelial cells from bacterial adherence. (2) In neonatal immunity, maternal milk contains both IgA and IgG. Once ingested, the IgG is taken up specifically in the gut of the neonate via an

IgG-specific Fc receptor called the neonatal Fc receptor (FcRn). Thus, the FcRn is involved in the transcytosis of maternal IgG antibodies from the apical to the basolateral surface of the gut epithelium. FcRn is also responsible for maternal IgG transport across the placenta. (The IgA in maternal milk is thought to remain in the neonatal gut to prevent bacterial colonization.)

**4. Fc receptors come in many flavors and have many different functions. List three different Fc receptors with different functional roles in the immune system.**

(1) FcRn – specific receptor for IgG important for neonatal immunity and transport of maternal IgG across the neonatal gut epithelium as well as across the placenta.

(2) The poly-Ig receptor is also considered an Fc receptor (see answer to #3).

(3) CD16 (aka FcγRIIIA) – An Fc receptor found on macrophages and NK cells. For NK cells, this Fc receptor is important for Antibody-Dependent Cell Cytotoxicity (ADCC). When a cell is infected with a virus and expresses viral proteins on the cell surface, IgG antibodies can recognize these foreign proteins and coat the surface of the cell. The Fc portions of these IgG's are recognized by CD16 and signals the NK cell to kill the infected cell via granzyme/perforin release. This process is ADCC.

(4) FcγRI (aka CD64) is expressed on macrophages and is important for phagocytosis of IgG-coated bacteria.

(5) FcεRI is expressed on mast cells, basophils, and eosinophils. This receptor signals for cell activation and degranulation, thus causing histamine release in an allergic response (Type I – immediate type hypersensitivity) mediated by IgE antibodies.

There are many other examples of Fc receptors as well. One important point to remember is that there are no Fc receptors for IgM antibodies.

**5. Describe the process of making monoclonal antibodies in the lab.**

**First, mix together:**

- spleen cells from a mouse that has been immunized with the desired antigen with
- myeloma cells.

Use an agent to facilitate fusion of adjacent plasma membranes. Even so, the success rate is so low that there must be a way to select for the rare successful fusions. So,

**Use myeloma cells that have:**

- lost the ability to synthesize any antibody molecules of their own (so as not to produce a hybridoma producing two kinds of antibody molecules).
- lost the ability to synthesize hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) or thymidine kinase (TK).

HGPRT enables cells to synthesize purines using an extracellular source of hypoxanthine as a precursor (called the “salvage pathway”). Also, cells can make thymidylate from extracellular thymidine via the TK enzyme. Ordinarily, the absence of HGPRT or TK is not a problem for the cell because cells have an alternate (“de novo”) pathway that they can use to synthesize purines and thymidylate.

However, when cells are exposed to aminopterin (a folic acid analog), they are unable to use this de novo pathway and are now fully dependent on HGPRT and TK for survival (as well as hypoxanthine and thymidine in the medium).

How are HGPRT- or TK- myeloma cells selected for? Substrates can be added to cell cultures that yield lethal products when HGPRT or TK are active.

Figure removed due to copyright restrictions.

The second property above is exploited by transferring the cell fusion mixture to a culture medium - called HAT medium because it contains:

- hypoxanthine
- aminopterin
- the pyrimidine thymidine

The logic:

- Unfused myeloma cells cannot grow because they lack HGPRT.
- Unfused normal spleen cells cannot grow indefinitely because of their limited life span. However,
- Hybridoma cells (produced by successful fusions) are able to grow indefinitely because the spleen cell partner supplies HGPRT and the myeloma partner is immortal.

Clinical Correlates

6. A family undergoes pedigree analysis for a disease in which affected individuals lack the Ig peak in serum electrophoresis tests (usually < 100 mg/dL). Also, affected family members have experienced recurrent infections with pneumococcus and streptococcus. Only males are affected with the disease. Identify a likely candidate gene for the mutation and explain the mechanism of the disease. What other gene mutations could cause the same phenotype?

This is X-linked agammaglobulinemia (Bruton's disease) with a mutated Btk gene. No Ig's are present because Btk is an important mediator for downstream signaling of the pre-BCR and is required for maturation beyond the pre-B cell stage. Thus, affected individuals lack mature B cells. Other mutations that could cause the same phenotype are mutations in pre-BCR signaling:  $\nu$ preB,  $\lambda 5$ ,  $Ig\alpha$ ,  $Ig\beta$ , Syk. However, these mutations are not observed in human diseases.

7. A 56-year-old male patient presents to you with recurrent bacterial infections of *Staph. aureus*. Laboratory analysis reveals to you that he is anemic, is hypercalcemic, and has large amounts of proteinuria. Creatinine and BUN levels are also elevated. Within the past few months, the patient has noted back pain. X-ray examination reveals a punched-out osteolytic lesion of the spine.

- a. What diagnosis are you suspicious of? How would you confirm the diagnosis?

We are suspicious of multiple myeloma (see explanations in part b). For diagnosis, we would look for the electrophoresis "M spike" in the plasma consisting of Ig molecules, which is observed in 80% of patients. The other 20% of patients only make excess light chains, which could be detected as Bence-Jones proteins in the urine.

- b. Describe the mechanisms for the recurrent bacterial infections, the proteinuria, elevated creatinine/BUN, and the osteolytic lesions. What

**important cytokines are thought to be involved in the pathogenesis?  
What are the major concerns for cause of death?**

The infiltration of bone with plasma cells as well as excess cytokine production (i.e., IL-6 and IL-1 $\beta$ , which increase osteoclast activity) most commonly results in pain, osteolytic lesions, and bone fracturing. The areas most often involved are the pelvis, spine, ribs, and skull. Hypercalcemia (excess calcium in the plasma) is often a result of the increased bone resorption and may result in neurological problems such as weakness and confusion. A reduced humoral response (due to the monoclonal plasma cells overwhelming the bone marrow and other B cells, etc.) leads to increased vulnerability to infection with bacteria (such as *Staph. aureus*, *Strep. pneumoniae*, and *E. coli*). Infections are the major cause of death for patients with multiple myeloma. Renal failure is the second most common cause of death and can be a result of the toxicity of Bence-Jones proteins on tubular epithelial cells of the kidney as well as amyloid deposits. The elevated creatinine/BUN are indicative of renal failure. These light chains can also pass through the glomerulus, leading to proteinuria. Anemia is also commonly found in these patients since plasma cells are overcrowding the bone marrow and also due to renal damage (i.e., EPO is made by the kidney).

**c. How would you treat this patient?**

Prednisone is used for controlling hypercalcemia (can block cytokine production), while blood transfusions and recombinant erythropoietin (EPO) are commonly used to treat the anemia. Bisphosphonates are being investigated to prevent fractures because they inhibit bone resorption. High dose chemotherapy is given to treat the root of the disease, most commonly melphalan or cyclophosphamide (alkylating agents). Autologous peripheral stem cell support has largely replaced bone marrow transplantation for myeloma patients undergoing myeloablative chemotherapy.

**8. Describe the clinical symptoms one would see in a patient with deficient expression of  $\beta 2$  integrins (i.e., which includes LFA-1). How would you treat such a disease?**

Any process that depends on the homing of cells via integrin adhesion is defective in this disorder. These processes include adherence to endothelium, neutrophil aggregation and chemotaxis, phagocytosis, and cytotoxicity mediated by neutrophils, NK cells, and T cells. Patients would present with recurrent infections, impaired wound healing, progressive soft tissue necrosis, and leukocytosis. This disease is called Leukocyte Adhesion Deficiency Type I and is autosomal recessive. Treatment consists of vigorous antibiotic therapy and also bone marrow transplantation.

9. A 24-year-old female patient complains of a red rash on her cheeks that she has had for a few months that becomes worse if she has too much sun exposure. Lately she has been feeling pain in her joints and some shortness of breath upon exertion. Lab analysis reveals that she has hemolytic anemia and proteinuria.

a. What is your diagnosis and how would you confirm this diagnosis? Explain the mechanisms by which the joint pain, anemia, and proteinuria develop.

Based on the symptoms of a malar rash that is photosensitive, arthritis (usually a nonerosive type with tenderness and swelling), persistent proteinuria, and hemolytic anemia, a diagnosis of systemic lupus erythematosus (SLE) can be made. This can be further confirmed by testing for anti-nuclear antibodies (ANA's) or anti-DNA antibodies, which is the most common indicator of SLE.

The symptoms of SLE suggest some broad level breakdown in tolerance. SLE patients can exhibit antibodies against both nuclear and cytoplasmic components of the cell as well as cell surface antigens of blood elements (i.e., leading to hemolytic anemia). These antibodies are not only important in the diagnosis of the disease but also directly lead to the clinical symptoms of SLE, such as the immune complex-mediated disease of the glomerulus (i.e., antigen-antibody complexes deposit within the glomerular membrane and lead to an inflammatory response in this tissue; kidney filtration is impaired). The arthritis is thought to be caused by immune complex deposition followed by neutrophil exudation and mononuclear infiltrates into the synovium.

b. Compare and contrast the mechanisms of pathogenesis for this disease with that of rheumatoid arthritis.

SLE is thought to involve a widespread breakdown in tolerance and it has been suggested that patients exhibit an intrinsic hyperactivity of T cells and B cells. There is thought to be an environmental trigger to SLE as well. Rheumatoid arthritis is thought to depend on exposure of the host to an arthritogenic microbial antigen that initiates an acute arthritis that continues on as an autoimmune reaction to joint components. RA is localized to the joints whereas SLE has a broad systemic signature.

10. Describe the process by which HIV enters into a T cell. Give the names of the important viral proteins as well as the host receptor proteins.

On the viral envelope, soluble **gp120** is bound to the transmembrane glycoprotein **gp41**. Binding of the exterior envelope protein gp120 of HIV to the receptor **CD4** on helper T cells allows viral attachment. The gp120-CD4 complex causes a conformational change in gp120 that allows it to bind to a member of the **chemokine receptor** family (**CCR5** for primary macrophage-tropic viruses, **CXCR4** for T-cell tropic viruses). Upon binding of the chemokine receptor, gp41 mediates fusion of the viral membrane to the target cell membrane.

**11. Severe Combined Immunodeficiency (SCID) represents a group of disorders in which both B and T cell populations are affected. In X-linked SCID, patients are observed to have a marked decrease in T cells and impaired function of B cells (although the numbers of B cells may be normal).**

- a. Describe the mutation causing X-linked SCID and how this results in T cell deficiency. What is a salient feature of lymphocytes in females carrying this mutation?** X-linked SCID is caused by a mutation in the common  $\gamma$  chain of cytokine receptors such as IL-2, IL-4, IL-7, IL-9, and IL-15, thus preventing effective cytokine signaling. T cells require IL-7 signaling for development from immature thymocytes and, thus, are lacking in this disease (T cell deficiency is not due to improper IL-2 signaling, since IL-2 only acts in the proliferation of *mature* T cells.) Due to X-inactivation in females, all of the mature T lymphocytes present in carrier females will have inactivated the X chromosome carrying the mutation. Those cells that inactivated the normal X chromosome do not develop.
- b. What is the status of natural killer cells in these patients?** These patients are also deficient in NK cells since IL-15 signaling is required for their proliferation. (Note: IL-15 also plays an important role in maintaining CD8<sup>+</sup> CTL populations.)
- c. What is another mutation in humans that has been shown to cause the same disease phenotype as X-linked SCID, but is found in both males and females?** A Jak3 mutation since the common  $\gamma$  chain signals via Jak3.
- d. What mutations can cause a lack of both T cells and B cells, but do not affect other cell types?** Mutations in those proteins that are only required for the development of B and T cells such as Rag-1, Rag-2, DNA-PK, Ku70, Ku80 (i.e., proteins involved in VDJ rearrangement).