Instructions: This exam consists of 160 points. On questions with choices, all of your attempts will be graded and you will receive the highest grade. You may answer any question with a diagram, just be sure that it is adequately labeled. Use the space provided, or the back of the preceding page.

1. (6 pts) Pick any two (2) of the following proteins and describe the consequence of a deficiency in this protein on the function of the innate or acquired immune system.
   
a) C3  No C3 convertase of the alternative pathway, and no C5 convertase at all. Difficulty with opsonization (no C3b), and inflammation (no C3a or C5a), MAC complex can't be formed.

b) C4 or C1q  No lectin (C4) or classical pathway (C4 and C1q) since C4 is part of the C3 convertase of those pathways. Difficulty with opsonization (but still possible).

c) TLR3  No detection of dsRNA in endosome, trouble fighting dsRNA viruses.

d) TLR4  No activation of macrophages by lipopolysaccharides - poor inflammatory response.

e) INFα or INFβ  No antiviral state in un-infected cells, difficulty in activating NK cells, poor response to viral infections.

f) INFγ  Poor activation of macrophages, difficulty fighting intracellular infections.

g) histamine receptor  Poor response to histamine released by mast cells, no increase in vascular permeability, reduced inflammatory response.

h) Fas/Fasl  Poor killing of virally infected cells and cancer cells, loss of immunoprivileged status.

i) Perforin  Can't form pores to allow granzymes in to cause apoptosis. Poor killing of virally infected cells and cancer cells, loss of immunoprivileged status.

j) Invariant chain  Involved in preventing internal peptides from loading on the class II MHC. If absent class II MHC would also present internally synthesized peptides. May prevent loading of externally derived peptides.

2. (6 pts) What is the function of the Rag1/Rag2 proteins in a normal individual, and how would a deficiency of these proteins affect the function of the humoral (Ab) and cellular immune system?

   These enzymes are responsible for VDJ joining of DNA segments to form antibodies and the TCR. (3 pts)
Since it would not be possible to generate antibodies or TCR, there would be negative selection for both B and T cells and none would be produced. The individual would have a severe combined immunodeficiency.

3. (6 pts) Please do one of the following choices:
Choice A: An individual appears to have normal levels of IgM and what appears to be fully functional B and T cells, yet they cannot produce high affinity antibodies of other classes. What is the most likely immunodeficiency that would cause this condition? Briefly justify your answer.

Choice B: An individual cannot mount an effective immune response against viruses, yet they appear to have normal T-cells. What is the most likely immunodeficiency that would cause this condition? Briefly justify your answer.

Choice A:
- It could be a general problem in B-cell activation - producing an effect that is similar to T-cell independent antigens. B-cell could be missing complement receptor, MHC II, B7, CD40 - anything that would affect activation.
- Both affinity maturation and class switching require AID, activation induced cytosine deaminase. The lack of AID would also explain these results.

Choice B:
Anything that might interfere with antigen presentation on class I - missing peptide transporter or MHC I on the cell. Missing CD8 on the Tc cell. Other molecules (b7, CD24) are used by both Tc and Th cells, so they are probably not involved.
4. (10 pts) The response to parasitic infections is very similar to an allergic response. Briefly discuss the common features of the two processes and then indicate the differences between the two. Your answer should discuss T-cell subsets and what other cells and molecules are involved. You should also clearly define the events that occur during each phase (sensitization, activation, response).

The allergic response is a type I sensitivity - the only difference to parasitic infections is the nature of the antigen.

**Similarities:**

- **Sensitization:**
  - Exposure to the antigen in the mucosal region - IL4 rich environment due to basophil, so antibody response is IgE.

- **Activation:**
  - IgE are bound to the surface of mast cells via IgE Fc receptors. Antigen causes crosslinking of receptors, causing activation and degranulation of mast cells. Mast cells produce histamine and TNFα.

- **Response:**
  - Histamine released causes: i) vascular permeability, ii) smooth muscle contractions, causing diarrhea, vomiting, etc. Long term inflammatory mediators can intensify the response, producing mucus. Eosinophil are activated and secrete peroxidases, collagenases, neutrotoxic peptides.

**Differences:**

- Allergens are not harmful – therefore an inappropriate and potentially damaging response.
- Allergens can lead to a systemic response, causing narrowing of the airways – asthma.

5. (10 pts) Please do one of the following choices:

**Choice A:** Many drug allergies result in the destruction of red blood cells.
- i) Briefly describe how the immune system becomes sensitized to the drug.
- ii) Provide one mechanism that leads to the destruction of red blood cells.
- iii) Why does the process stop when the drug is no longer given to the patient.

**Choice B:** A patient is being treated with a mouse monoclonal antibody as part of their anti-cancer therapy and they develop serum sickness. What is serum sickness and how could it have been avoided?

**Choice A:**
- i) The drug forms a covalent complex with cell surface proteins on the RBC.
  - Protein-drug complex is recognized by B-cells as foreign
  - Peptide-drug complex is presented on class II MHC and recognized by TH cells
  - B-cells become activated, plasma cells produce Ab.
- ii) Ab bind to modified proteins on RBC - ADCC by NK cells, complement activation, phagocytosis by macrophages, neutrophils.
- iii) Antibodies only recognize the drug complex, generally do not cross react with original protein.

**Choice B:**
- Serum sickness starts with the formation of immune complexes between the monoclonal antibody and the patient’s antibodies that have been generated against the mouse monoclonal.
- The immune complexes activate complement, causing mast cells to degranulate, and attracting neutrophils.
- Neutrophils release reactive species that cause tissue damage.
- This could have been avoided by using chimeric or humanized antibodies, making it less likely to generate an immune response in the patient.
6. (10 pts) Rejection of solid transplanted tissue occurs by the same mechanism as type IV hypersensitivity.
   i) Briefly discuss differences in sensitization, including the nature of the antigen.
   ii) How does the immune response lead to rejection of the transplanted tissue?

   i) In the case of type IV hypersensitivity the antigen is a modified peptide presented to T\(_H\) cells that activates the T\(_H\) cell. In the case of transplantation, the T-cells become activated in the following two ways:
      - Presentation of peptides derived from donor MHC on host APCs
      - Presentation of any peptide on donor APCs to host T\(_H\) cells, the combination of the donor MHC and the peptide mimic the presentation of foreign peptides on host MHC.
   ii) The activated T\(_H\) cells migrate to the site of transplantation and secrete INF\(\gamma\). Macrophages get activated (inflammatory response) leading to killing of the transplanted tissue.

7. (2 pts) In transplantation of solid tissues it is important to ensure that ABO blood groups are correctly matched. Why?

   Otherwise a type II hypersensitivity reaction will occur. Pre-existing antibodies against the wrong blood group will bind to the AB antigens on the surface of the vessel endothelium in the transplanted tissue. The endothelium will then be destroyed by complement, ADCC, macrophages. Without a vascular supply the transplanted tissue dies.
   (1 pt if they talk about blood transfusion problems)

8. (8 pts) Your friend is getting a kidney transplant. Serotyping indicates that a donor kidney is a perfect match for transplantation. However, the mixed lymphocyte assay shows a high level of radioactive counts when the donor cells are mixed with the recipient cells.
   i) Is the data from serotyping consistent with the data from the mixed lymphocyte assay? Briefly justify your answer.
   ii) Should the transplant proceed? Why or why not?

   i) No, the high radioactivity means that the host cells were activated by MHC on the donor tissue. These MHC alleles were not detected by serotyping, perhaps the antibodies crossreacted with the different alleles.
   ii) No, there will likely be a strong rejection. The mixed lymphocyte assay is a much better predictor of transplantation success.
9. (4 pts) Your friend’s (previous question) new kidney transplant is showing signs of rejection, what could the transplant surgeon do to stop this process – give one possible treatment.

- Raise FK506 levels to further reduce activation of T-cells by interfering with signaling.

  OR

- Increase the levels of antibodies against the α-chain of the TCR. This chain is only produced by activated T-cells.

  OR

- Try another transplant of donor stem cells to induce tolerance in the host T-cells.

10. (6 pts) In bone marrow transplants, it is necessary to have a good match between the donor and the recipient, even though the recipient cannot mount an immune response because their immune system is destroyed prior to the transplant. Why?

- The transplanted cells generate B, T, and APCs - all with the MHC of the donor. In particular the APCs have the MHC of the donor.
- The T-cells, although from the donor, are trained to recognize the MHC of the host in the thymus (the thymus is the host’s - we did not transplant the thymus).
- The naïve T-cells, to be functional, require the same MHC on the APCs, this will only be the case if the MHCs are similar for both the donor and the host.

11. (6 pts) Please do one of the following choices.

Choice A: Some individuals are resistant to infection by HIV. What is different about their genotype and how does deficiency interfere with the progression of the disease.

Choice B: What types of retroviral inhibitors are currently used to treat HIV infected individuals and why are these inhibitors generally effective.

Choice C: An individual discovers that they are HIV positive and begins treatment with retroviral inhibitors. Unfortunately, even though they were compliant with respect to the drug treatment, they still develop AIDS. Provide one reason why this might occur.

Choice D: What cells are infected by the HIV virus in the lymph node? List three ways by which these infected cells are killed.

Choice A:
They are lacking the receptor for CCR5 - this is required by M-tropic viruses to infect macrophages (and dendritic cells). Since macrophages cannot be infected at the site of entry (mucosal membranes), the infection does not progress to T-tropic viruses developing in the lymph node.

Choice B:
HIV protease inhibitors (competitive) and two types of reverse transcriptase inhibitors (competitive and allosteric). This are effective because the drugs are excellent inhibitors and these two enzymes are absolutely required for reproduction of the virus.

Choice C:
1) the infection is in the late stages and there has been sufficient damage to the lymph nodes such that they are no longer functional. This impedes B and T cell activation, effectively causing an immunodeficiency.
2) They have developed mutations in the HIV protease or reverse transcriptase that have resulted in drug resistant viruses - although unlikely, this is still possible.

Choice D:
1. Viral replication
2. Recognition of anti-HIV antibodies on the surface - ADCC, complement
3. T_{CTL} activity due to HIV peptides being presented on the MHC I on the infected Th cell.
12. (4 pts) Please do one of the following choices:
Choice A: Why are vaccines relatively ineffective against pathogens that show disease symptoms quickly, say within 2-3 days?
Choice B: How does an adjuvant increase the effectiveness of a vaccine – give one reason.

Choice A:
All vaccines protect against the symptoms of the disease by generating memory cells, so that when the person is infected with the actual pathogen they will produce a rapid secondary response. 2-3 days is not enough time to generate a secondary response, so the person will still have the symptoms, even though they have been vaccinated.

Choice B:
1. Slow release of the antigen - more time for the immune system to respond and make memory cells.
2. Irritation - mimicking an inflammation- therefore APCs will be activated, more likely to develop memory cells.

13. (4 pts) There have been recent outbreaks of measles, in spite of the fact that there is an effective vaccine for this disease. What is the cause of these outbreaks?

Loss of herd immunity. Too few people are vaccinated, so the virus has a high probability of being spread from one unvaccinated person to another. If enough people are vaccinated, an infected individual will only be in contact with vaccinated people.

14. (10 pts) Vaccines can be divided into two broad classes, those that consist of non-living material and those that consist of living material. Give one example of each of these classes and briefly discuss why one form of vaccine may be better than another.

Non-living material (3 pts)
- Toxins
- Cell surface carbohydrates
- Killed bacteria, viruses

Living material (3 pts)
- Attenuated viruses - natural, or passaged on non-human cells.
- Inactivated viruses - missing key proteins required for infection
- Recombinant viruses - antigen is incorporated into the genome of a virus and will be expressed when the virus replicates.

In general, the living vaccines are better because they will generate antigen for a longer time, increasing the probability of generating memory cells.
It is also acceptable to talk about safety issues - i.e. non-living materials cannot be infectious and potentially cause disease. (4 pts)
15. (8 pts) State the steps in T-cell development starting with where cells originate, migrate to, and the requisite four checkpoints they undergo before migrating to lymph nodes. What might be the end result if a T-cell escapes the last checkpoint?

- 1pt: Thymocytes originate in the bone marrow but migrate to the thymus for development.
- 1pts: 1st chkpt: \( V(D)J \) rearrangement of \( \beta \)-chain occurs. **Surrogate a-chain** is paired with the \( \beta \)-chain to construct the TCR. Successful rearrangement of \( \beta \) causes 1) allelic exclusion of other \( \beta \)-chain, 2) CD4 & CD8 expression, 3) clonal expansion \( \rightarrow \) lots of the same cell
- 1pts: 2nd chkpt: VJ rearrangement of \( \alpha \)-chain. **Allelic exclusion insures that only one allele is successfully rearranged.** T-cells that cannot produce functional \( \alpha \)-chain die by apoptosis
- 2pts: 3rd chkpt: **Positive selection** by non-lymphoid epithelial cells eliminates T-cells with low-to-no affinity for self-MHC; TCR must have moderate affinity for self-MHC to receive survival and proliferation signals.
- 2pts: 4th chkpt: **Negative selection** by thymic DCs eliminates T-cells that recognize MHC+self-peptides complex with high affinity. Self peptides expressed due to AIRE.
- 1pt: If peripheral tolerance does not remove these reactive cells, then autoimmune disease likely will develop due to T-cells attacking various self-tissues due to strong recognition of self-peptides in the periphery.

Grading note: they do not have to give all of the detail at each checkpoint – this is included for future study purposes.

16. (2pts) Briefly describe the role of **AIRE** (autoimmune regulator) in negative selection of T-cell development. What might happen if someone was missing AIRE due to an inactivating mutation?

- AIRE: transcription factor allows expression of large \# of organ-specific antigens by epithelial and dendritic cells in thymus. Does so via chromatin remodeling (epigenetic gene expression). Without AIRE \( \rightarrow \) person would develop autoimmune disease (do not have to mention autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome (APECED) but 1 bonus pt if they do)

17. (2pts) Why is CTLA-4 such an important possible therapeutic target in cancer? What biological therapy are researchers developing to target CTLA-4 in the clinic?

- **CTLA-4** binds to B7 on DCs and shuts down T-cell activation. If we can block CTLA-4, the hope is that the immune system can mount a stronger, longer lasting attack against cancer cells. Monoclonal antibodies
18. (6 pts) List at least two examples for each of the following innate mechanisms of immune defense against extracellular bacteria:

   Physical: skin, mucus, biofilm of host commensal bacteria

   Physiological / Chemical: pH of sweat, stomach acid, tears; lysozyme

   A. Cellular: macrophages, neutrophils

19. (6 pts) Briefly describe some of the key characteristics and attributes of gut-associated lymphoid tissue (GALT), and how these play a role in immune defense?

   - Host non-pathogenic bacteria set up biofilm that prevent access to epithelial cells
   - Host mucus layer secreted by goblet cells in gut lining
   - DCs can “taste” the gut environment and present antigens to T-cells in GALT
   - Activated B-cells secrete IgA, which dimerizes and crosses epithelial cells to bind to pathogens → agglutinates pathogens which are removes via peristalsis of gut
   - M cells engulf bacteria, do not process, but present to DC inside, which activate T-cells in peyer’s patches (LN of GALT)

20. (2 pts) Briefly describe the consequences of when your doctor, although well intentioned, gives you antibiotics for your viral GI infection?

   The antibiotics rapidly kill of commensal host bacteria, and with them, goes their protective biofilm, protective natural antibiotics, impaired mucus layer, thus affording access to your gut epithelium to any pathogenic bacteria that might be present in small numbers. Now pathogens can proliferate and infect.

21. (8 pts) Describe the concepts of Antigenic Shift vs. Antigenic Drift. In doing so, provide an example for each and briefly describe the possible impact on human health.

   **Antigenic Shift:** Large genetic changes in Ags. Some pathogens repeatedly change antigenic composition of their surface. Ex: pili on *Neisseria gonorrhoeae* OR major shuffling in influenza A virus genes, such as H1N1, due to mixing in intermediate host. The potential impact is a major epidemic that could kill millions of people because the immune system can be overwhelmed by the novelty of the pathogen.

   **Antigenic Drift:** Small (single nucleotide) genetic changes alter the structure of surface antigens, leading to loss of recognition of antigen. Ex: Human Immunodeficiency Virus: capsid protein OR single mutations in Influenza type A & B: Hemagglutinin and Neuraminidase surface proteins. These smaller changes demand that we produce new vaccines each year but the threat is small.
22. (6 pts) Answer one of the following (do not give antigenic shift or drift as a possible answer to this question)
   **Choice A:** List at least three mechanisms that bacteria employ to invade host cells or evade host immune detection or destruction.
   **Choice B:** List at least three mechanisms that viruses employ to evade host immune detection or destruction

**Choice A:**
- **Degradation of Secretory IgA:** Secretion of a protease that cleaves IgA, rendering it ineffective
- **Attachment to host cells:** Bacteria have specialized structures that enhance the ability to attach to host cells.
- **Interfere with Complement:** *N. gonorrhoeae* produces an outer-membrane protein that inactivates MAC.
- **Bacteria possess surface structures that inhibit phagocytosis:** secretion of variant polysaccharide capsule e.g. *Streptococcus pneumoniae*
- **Intracellular growth:** Formation of granuloma (TB, leprosy). Chronic T-cell stimulation of Mø with intracellular bacteria that survive → granuloma formation by blocking phagolysosome formation. Necrotizing granulomas and fibrosis (scarring) → tissue damage.

**Choice B:**
- **Inhibition of Ag Processing and Presentation:** can block at various steps in Ag processing and MHC loading.
- **Reduction in Antigen Presentation:**
- **Latency state:** viruses such as Herpes, EBV, and CMV hide in the CNS
- **Production of immunosuppressive cytokine-like molecule:** EBV – produces IL-10 like molecule

23 (6 pts) In general terms, briefly compare and contrast central versus peripheral tolerance in lymphocytes. Also, what is anergy and what role does it play in tolerance?

- **Central tolerance** – process of eliminating self-reactive lymphocytes in the bone marrow, if B-cells (and the lymph node too) and in the thymus, if T-cells.
- **Peripheral tolerance** – process of eliminating self-reactive lymphocytes in the LNs or other peripheral immune organs.
- **Anergy** results from insufficient lymphocyte activation where the 1st signal (MHC II to TCR) is present but the 2nd signal (B7 to CD28) is not, thus inducing a state of quiescence

24. (6pts) Of the following three activating mechanisms for autoimmunity, select and describe two, providing details of what happens and how reaction to self occurs. 1) Molecular mimicry; 2) Release of normally sequestered antigens; 3) Bystander activation.

1) **Molecular mimicry:** antibodies produced against foreign antigens cross react against antigens in heart, joints or kidney due to high similarity in antigenicity of self-peptides. Rheumatic fever

2) **Release of normally sequestered antigens:** minor tissue damage exposes proteins normally sequestered from immune cells. These antigens are processed and presented to lymphocytes and reacted to as “foreign” resulting in inflammatory response → further damage to tissue
3) **Bystander activation:** presence of microbe trigger activation of APC; however, it somehow alters MHC presentation and APC activation, leading to presentation of self-peptides along with co-stimulatory signal #2 → activation of lymphocytes against self-peptides.

25. (5pts) Pick **ONE** of the following and briefly describe how the autoimmune disease presents (1-2 symptoms) and the underlying immunological mechanism of the disease:

- a) Graves disease
- b) Diabetes mellitus
- c) Myasthenia gravis
- d) Multiple sclerosis
- e) Hashimoto’s thyroiditis
- f) Sympathetic ophthalmia

**GD:** Hyperactive thyroid gland → increased metabolism, intolerance, nervousness, weight loss, insomnia. Autoantibodies act as agonist for TSH receptor and mimic stimulating action of TSH.

**MG:** Severe muscle weakness → difficulty chewing, swallowing and breathing → respiratory failure. Autoantibody acts as antagonist for ACh receptor on post-synaptic nerves. Cannot propagate action potential b/c ACh-activated ion channels don't open to allow Na+ entry.

**HT:** TH1, B-cells, Macs destroy thyroid → hypothyroidism. Weight gain, brittle nails, dry skin, cold intolerance, lower metabolism, depression, fatigue. Goiter formation.

**DM:** no insulin production due to autoimmune destruction of Beta islet cells. Increased glucose in the urine → excessive urination.

**MS:** TH1 cell → IFN-γ → recruits Macs (microglia), neutrophils and mast cells → damage myelin sheaths on nerve axons in CNS (oligodendrocytes). Impaired motor control → muscle weakness, spasms, coordination and balance issues, visual impairment, speech, depression and cognitive impairment.

**SO:** damage to vision, potentially both eyes. Damage to eye releases sequestered ocular Ags. APCs pick up Ags, migrate to cervical LNs, and present Ag to CD4+ TH1 cells → cellular immune response targets other eye.

26. (5 pts) Answer **ONE** of the following questions:

**Choice A:**

i) Briefly describe at least two of the four categories for cancer antigens discussed in class.

ii) List at least one example of an actual cancer antigen.

**Choice B:** List at least three examples of reasons for failed immune response to tumors.

**Choice A:**

- Unique structure not found on normal cells
- Common to normal and malignant but only unmasked on malignant cells
- Structurally similar to fetal/embryonic proteins but not exp’d by normal adult cells
- Over-expressed (oncogenes)

**Choice B:**

- Lack of epitope
- Deficient Ag processing
- Deficient tumor Ag presentation by APCs
- Masking of tumor Ag
- Altered MHC
- Lack of co-stimulatory mol expression
- Production of inhibitory molecules (IL-10, TGF-β)
27. **Questions Based on Presentations** (6 pts). You can answer up to 3 questions, 2pts each. Two additional choices can be answered for bonus points. **Please indicate the five that you would like graded, otherwise the first five of your answers will be graded. Please answer on the back of the previous page.**

1. How does childbirth by Cesarean section affect the immune system?  
   Changes the type of bacterial species that are present in the infant, potentially affecting the interaction between the commensal bacteria and the immune system.

2. Briefly describe how cancer immunotherapy using antibodies results in killing of the tumor cells.  
   Direct tumor killing by Ab binding to a receptor. Immune mediated killing (ADCC, complement, Macrophages).

3. Discuss a genetic origin of rheumatoid arthritis, or the most likely auto-antigen.  
   Certain HLA alleles of DR4 can increase incidence. Citrullinated proteins.

4. Why is the blood cell stage of the malarial life cycle a better target for vaccine development?  
   The parasite moves into the liver too fast to be detected by the immune system.

5. Why is the rabies virus undetectable during the early stages of incubation?  
   Grows in muscle cells, apparently hidden from the immune system.

6. Henipavirus infects horses. What is the basis of vaccine development for this virus?  
   Incorporation of antigen into virus like particles, or recombinant viruses.

7. What is the relationship between a high fat diet and insulin sensitivity?  
   A high fat diet reduces insulin sensitivity – by reducing inflammation.

8. Antibodies against hCG provide a means of establishing contraception. How is the hCG made to be immunogenic?  
   Added a carrier protein to increase immunogenicity, also altered the length of peptide used to immunize.

9. What type of vaccine is most effective for individuals with IgA deficiencies?  
   Mucosal vaccines.

10. How are chimeric antigen receptors used to treat cancers?  
    The Fab regions of antibodies are grafted to the signaling domain of the TCR, causing activation of T-cells by tumor antigens.

11. What cancer can be effectively controlled by vaccination?  
    Cervical cancer, by vaccination against HPV.

    IL2 signaling

13. What do Ebola and Dengue have in common?  
    The infectivity of both is enhanced by antibodies attached to the virus.

14. How is the vaccine against anthrax made?  
    Attenuated, non-encapsulated bacteria.

15. Discuss one way to generate a catalytic antibody.  
    Use transition state as antigen. Make antibodies against enzyme, then antibodies against those antibodies – giving an image of the active site of the original enzyme. Make antibodies against peptide competitive inhibitor.

16. How is the vaccine against TB made?  
    Attenuated strain of TB bacteria

17. How do cancerous lymphocytes evade the immune system?  
    Do not express cancer antigens of class I, CD47-CD47L escape macrophage destruction.

18. Genetic resistance against the HIV virus also provides resistance against what other pathogen?
19. What are the two types of polio vaccines?
Sabin (attenuated strain), Salk - killed virus.