Instructions: This exam consists of 100 points. You should allot ~1 min/point. 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. (5 pts) Please do one of the following choices:
   **Choice A:** Briefly discuss how the high diversity of the B-cell receptor or the T-cell receptor genes is generated. Why is this diversity important for the normal functioning of the immune system?
   **Choice B:** Briefly discuss DNA rearrangements/changes that occur after B-cell activation. Why are these changes important in the immune response?

   **Choice A:**
   Diversity is created by combining V D and J segments (HC or β-chain) and V J segments (LC or α-chain).
   Additional diversity is generated during the joining process – imprecise crossover, Nbase, Pbase addition.
   Lastly, allowing any HC-LC or β-α chain to pair increases diversity.
   In the case of Ab – the high diversity allows the immune system to generate B-cells that have BCR that can recognize a wide variety of pathogens (however one B-cell usually recognizes one antigen).
   In the case of TCR – the high diversity allows the generation of T-cells that can recognize many different MHC-peptide complexes (however one T-cell usually recognizes only one MHC-peptide combination).

   **Choice B:**
   After activation, class switching occurs where the exons for the heavy chain constant domains for IgM/IgD are replaced by others – this allows the tailor of the immune response by changing the biological function.
   Also, affinity maturation occurs, where random mutations in the V regions of both heavy and light chains are selected such that the affinity increases. A higher affinity means that the antibody will bind more effectively to the antigen.

2. (5 pts) Briefly describe the molecular (e.g. protein-protein) interactions that would occur during the normal activation of a B-cell. Comment on the specificity of the key interactions and why the level of specificity is important.

   i. BCR interacts with antigen, this is specific and ensures that the B-cell response is targeted to that antigen (1 1/2 pt)
   ii. Peptide presented on MHC – this is non-specific, allowing a single MHC to present many peptides (1 1/2 pt)
   iii. Peptide-MHC-TCR interaction, this is specific, so that only a small number of T-cells are activated. (1 1/2 pt)
   iv. CD40-CD40L interaction/ b7-CD28 interaction (1/2 pt)

3. (5 pts) Briefly discuss how the classical complement pathway is utilized in the defense against pathogens. You should discuss activation of the pathway by the innate and acquired system, the overall pathway, and how the products of the pathway lead to pathogen destruction. There is no need to discuss the detailed composition of protein assemblies, e.g. you don’t need to state that C3 convertase is made of C4bC2a.

   Activated by antibody-antigen complexes (acquired) or c-reactive protein (innate)
   Proteases are activated that cleave C2 and C4 to make C3 convertase.
   C3 convertase splits C3 to C3a (anaphylatoxin) and C3b (opsin)
   C5 convertase formed from C3 convertase
   C5 convertase splits C5 to C5a (anaphylatoxin) and C5b
   C5b + C6+C8+C9 make membrane attack complex (MAC)
   C3a/C5a = enhance inflammation, C3b enhances phagocytosis, MAC makes pores in membrane.
4. (7 pts) Compare and contrast antigenic shift, antigenic drift, and multiple serotypes. Given one example of one pathogen that employs any of the three to evade the immune system.

**Antigenic shift** = large change in the nature of the antigen/epitope, e.g. gene conversion, Flu, sleeping sickness

**Antigenic drift** = small change in the epitope, e.g. single site mutation, e.g. HIV

**Multiple serotypes** = multiple antigens that do not change or intercovert, e.g. staph, dengue virus.

2 pts for each definition, 1 pt for the one example.

5. (4 pts) Please do one of the following choices:

**Choice A:** Briefly describe one mechanism, besides those covered in the previous question, that bacteria utilize to evade the immune system.

**Choice B:** Briefly describe one mechanism, besides those covered in the previous question, that viruses utilize to evade the immune system.

**Choice C:** Briefly describe why the subsequent infection by a related serotype of the dengue virus can lead to a severe disease (dengue fever).

**Choice D:** An immune response to one serotype of the dengue virus prevents the efficient production of antibodies to a subsequent infection by a different serotype, why?

**Choice A:** Degrade IgA, pili that bind to mucosal membranes, inhibit phagocytosis, intracellular growth.

**Choice B:** Latent virus, reduce MHC levels, reduce peptide presentation on MHC.

**Choice C:** Ab increases infection of macrophages by binding to virus, but not inactivating it. Large inflammatory response.

**Choice D:** "Original antigenic sin" - the excess antibodies from the first infection binding to the virus from the second infection - these antibodies bind to the Fc receptor on B-cells and prevent them from being activated. The inhibited B-cell could produce better antibodies against the second virus, but are inhibited from doing so.

6. (8 pts) Describe the events that occur either during an antibody response to parasites or to allergens. Your answer should include information about the sensitization, activation, and response phases.

*Basophils produce a cytokine (IL-4) that enhances an IgE response to the antigen (1 pt)*
*IgE bind to Mast cells. (2 pt)*
*Antigen binding crosslinks IgE on mast cells, causing degranulation (2 pts)*
*Mast cells secrete:*

- **Histamine** - increase permeability of endothelium - fluid enters site. Smooth muscle contraction - expel parasite. (1 pt)
- **TNFα** - also increases fluid flow. (1 pt)
- **Proteases** - tissue remodeling/damage or Potent inflammatory mediators: leukotrienes, prostaglandins (1 pt)
7. (1 pt) Please do one of the following choices:
   **Choice A:** You are having dinner with some friends at a restaurant and during the meal one of your friends begins to have difficulty breathing. What has occurred, and how would you help your friend recover from this life-threatening situation?
   **Choice B:** Describe one mechanism by which allergy shots reduce the immunological response to allergens.

   **Choice A:** systemic anaphylactic shock – they have ingested an allergen that has travelled to their bronchial tubes, causing smooth muscle contraction. Epinephrine will reduce smooth muscle contraction and decrease fluid flow.

   **Choice B:** Causes the production of IgG which can reduce mast cell degranulation and compete with IgE for binding to allergen.

8. (6 pts) A person was successfully treated for a bacterial infection with an antibiotic. They become infected with the same bacteria and are treated with the same antibiotic. Please answer one of the following choices [Each of the choices is related to one of the hypersensitivities; it is more important that you describe what is happening than state the number (e.g. 1-4) of hypersensitivity.]
   **Choice A:** During the second antibiotic treatment they rapidly develop anemia and have traces of hemoglobin in their urine the day they start taking the antibiotic. Why is this happening?
   **Choice B:** During the second antibiotic treatment they develop a severe rash over their entire body within an hour of taking the first dose. Why is this happening?
   **Choice C:** During the second antibiotic treatment their small intestine becomes inflamed, but the inflammation only begins a few days after they started the second treatment. Why does this occur?

   **Choice A:** The antibiotic has modified a protein in the surface of the red blood cell and antibodies were produced against that protein. The antibodies bound on the surface of the RBC are causing complement activation and formation of MAC complex – leading to leakage of hemoglobin from the cell (type II hypersensitivity)

   **Choice B:** The antibiotic caused the production of antibodies on the first exposure. These antibodies are forming immune complexes in the tissues during the second exposure. The immune complexes activate complement, the C3a and C5a are producing an inflammatory response (type III hypersensitivity)

   **Choice C:** The antibiotic has modified some proteins and these modified proteins were presented to T-cells by macrophages (sensitization). On the second exposure the memory T-cells were activated by the same process, causing inflammation at the site. (type IV hypersensitivity)
9. (16 pts) A person receives a liver transplant from a related donor and then is treated with immunosuppressant drugs after the surgery. (In liver transplants they don’t transplant the entire organ, just a lobe, and the missing lobe is regenerated in the donor). Please answer all of the following questions:
   i) Briefly describe one method by which the donor tissue would have been tested to see that it was compatible with the host (4 pts).
   ii) Several weeks after the transplant, the transplant surgeon notices from a biopsy the infiltration of macrophages into the surgical site – what has happened to cause this? What events have transpired to cause the host to recognize the donor tissue as foreign? (5 pts).
   iii) What could the surgeon have done to detect the infiltration besides a biopsy? (1 pt)
   iv) The surgeon increases the dose of FK506 and anti-IL2 receptor antibodies (e.g. basiliximab). What is the purpose of increasing these drugs? Select one and describe how it prevents rejection (2 pts).
   v) What could the surgeon have done that may have prevented the rejection, besides increasing the dose of FK506 or basiliximab? How would this have reduced rejection? (Hint: He could have learned this technique in Pittsburgh) (2 pts)
   vi) In spite of the surgeon’s best efforts, the liver is rejected after several weeks and the patient is in critical condition. In desperation, the surgeon uses the same donor tissue in a second transplant. The transplanted liver is rejected almost immediately, why did this occur? (2 pts)

i) **Serotyping** – where antibodies against different MHC alleles would be used to see if the same MHC were on the donor and the recipient. The binding of the antibody would be detected by the activation of complement and the formation of the MAC complex that would allow a dye to enter the cells.

   **Mixed lymphocyte assay** – irradiated donor cells (irradiated so that they cannot proliferate) are mixed with recipient cells in the presence of radioactive thymidine. If there is a MHC mismatch the recipient T cells will become activated and radioactivity will be incorporated into DNA.

   ii) Peptides derived from the donor MHC was presented to the host T-cells by host APCs. The peptides would be considered foreign if there is a mis-match between MHC alleles. Also, donor MHC, bearing human peptides, can mimic host MHC-foreign peptide complex, activating host T-cells.

   iii) MRI, using macrophages labeled with iron particles to follow their movement.

   iv) Both of these drugs will reduce the activation of T-cells. FK506 interferes with intracellular signaling, Basiliximab binds to the α-chain of IL-2R, which is only found on activated macrophages.

   v) They could have injected donor bone marrow into the host. The donor stem cells will produce APCs that have the donor MHC – the low level of these throughout the body of the host will induce tolerance of the host T-cells.

   vi) Antibodies were produced against cell surface markers on the liver – (such as MHC). The pre-existing antibodies bound to the next transplant and destroyed it by complement activation. Note, it would have been unlikely that these are blood group antigens, since they would have caused rejection in the first transplant.
10. (2 pts) Describe one event that occurs in central tolerance that reduces the possibility of autoimmune diseases?

a) Anergy can be induced in B-cells that recognize soluble antigens.
b) Alternative light (or \(\alpha\)) chains can be used to remove the interaction to self-antigens
c) Self-reactive B or T cells are killed.

11. (4 pts) Please do one of the following choices:

Choice A: The interaction of B- and T-cells occurs frequently in the lymph node. However, in the absence of an active infection, this interaction typically results in tolerance of the B and/or T-cell. What events (or rather the absence of events) lead to tolerance?

Choice B: How do \(T_{REG}\) cells arise and what is their role in peripheral tolerance? How do they induce peripheral tolerance?

Choice A: The B-cell will not be fully activated – the binding of C3b to the CR on the B-cell is required for full activation – which will only happen during an inflammatory response from an infection. This means that the levels of MHC and b7 will be low on the B-cell and may cause it to become anergic. The weak activation of the T-cell would make it anergic.

Choice B: There are self-reactive T-cells that are not removed during the generation of T-cells. They express high levels of a transcription factor (foxP3) that converts them to T-reg cells. T-reg cells induce tolerance by:

i) direct suppression of auto-reactive T-cells with TNFβ

ii) interaction between CTLA-4 on T-reg and b7 on APCs cause the production of cytokines by T-reg that reduces the output of inflammatory cytokines from APCs

iii) Tregs have a IL2 receptor that reduces the amount of IL2 produced by T-cells, preventing their activation.

12. (5 pts) Please do one of the following choices related to autoimmune diseases:

Choice A: A female patient goes to her doctor complaining that she cannot hold her eyelids open. What is causing these symptoms?

Choice B: A patient goes to their doctor with vague systems related to metabolism. The patient is shown to have a high level of thyroid hormones. Why has this occurred?

Choice C: After a bacterial infection by a Strep species, a patient develops a heart murmur, due to scarring of the heart tissue. How did this occur?

Choice D: A patient comes to her doctor complaining of joint pain. The doctor also notices a rash on the face of the patient. What disease is she likely to have and why is it causing these symptoms?

Choice E: Your friend is in a car accident which causes severe damage to one eye. The surgeon states that although they could surgically repair the eye and restore limited sight they will remove the damaged eye – why?

Choice A: She has an autoimmune disease where Ab recognize acetylcholine receptors at the neuro-muscular synapse – this reduces the response of the muscles to acetylcholine and also depletes the amount of receptors.

Choice B: They are producing antibodies against thyroid stimulating hormone receptor. The antibodies activate the receptor such that the thyroid produces a number of hormones, interfering with metabolism.

Choice C: The antibodies produced against cell surface carbohydrates on strep also cross-react with carbohydrates on cardiac tissue, causing tissue damage.

Choice D: Autoantibodies against soluble antigens, such as DNA or nucleoproteins. These are lodging in the joints and skin – producing the symptoms.

Choice E: Sequestered (hidden) antigens in the eye are exposed to the immune system due to the injury. If an autoimmune disease is generated, the undamaged eye could also be compromised. So the damaged eye is removed before an immune response occurs.
13. (5 pts) Please do both parts of this question:
   i) One patient has SCID (severe combined immunodeficiency) and totally lacks B and T cells. What genetic
deficiency might they have and why would it have caused this condition?
   ii) Another patient has B and T cells, but can only produce IgM antibodies and no B-memory cells. What
genetic deficiency might they have and why would it have caused this condition?
   i) Rag1, Rag2, enzymes involved in nucleotide metabolism (2 ½ pts)
   ii) Almost anything that involves B-T cell activation - e.g. missing MHC, missing b7, CD40, CD40L,
etc. (2 ½ pts).

14. (5 pts) A bone marrow transplant is performed to treat a patient with SCID. There are no technical
   problems with the transplant and the transplanted stem cells produce B, T, and APCs, yet the patient’s
   immune system remains non-functional - in that the T-cells produced from the transplanted stem cells
   cannot be activated. Why?

   There was a mismatch in the MHC between the donor and recipient cells. During maturation of
   the donor T-cells, they are trained to recognize the recipient MHC because those are the MHC
   molecules that are expressed in the thymus.
   The APC that are produced by the donor stem cells have a different MHC, thus the T-cells
   cannot bind to those MHCs and cannot be activated.

15. (2 pts) Please do one of the following choices:
   Choice A: How does a deletion in the CCR5 cytokine receptor lead to immunity from AIDS?
   Choice B: A patient was infected with HIV several years ago and begins to receive anti-viral drugs when they
   begin to notice they have trouble fighting common infections. Although the patients T-cell count rises,
   they die of AIDS a few years later. Why?

   Choice A: The CCR5 cytokine receptor is required for the entry of M-tropic viruses into
   macrophages. Since this is the initial route of virus into the body, the virus is not infectious.

   Choice B: There was significant damage to the lymph nodes before drug treatment due to
   inflammation from the response to HIV infected T-cells. Consequently, normal T-cell activation
   cannot occur.
16. (4 pts) Pick any one of the drugs that is currently being used to treat HIV and briefly describe how it interrupts the lifecycle of the virus. There is no need to name the drug, just describe its general characteristics and how it interferes with the life cycle.

A) Reverse transcriptase inhibitors - 1st step in HIV replication - conversion of viral RNA to DNA. Inhibitors either bind at the active site (and terminate chain growth) or act as allosteric inhibitors and bind elsewhere on the enzyme.

B) Integrase inhibitors - prevent the integration of the viral DNA into host cell DNA.

C) HIV Protease inhibitors - required for cleaving a long pre-protein into smaller proteins that are required for viral assembly.

17. (2 pts) Please do one of the following choices:

Choice A: What is the most significant difference between passive and active immunization?
Choice B: What is an adjuvant and what is its role in immunization.
Choice C: Give two reasons why booster shots may be required for protective immunity?

Choice A: Passive does not generate memory cells.
Choice B: Adjuvant is something that increases the immune response by prolonging the release of antigen and by causing inflammation.
Choice C: Booster shots would be required if the antigen was not particularly antigenic, or it was desirable to have antibodies present at all times to ensure a very quick response to exposure to the pathogen.

18. (2 pts) A new political party is trying to get childhood immunizations banned because they claim that the vaccines are unsafe. In addition to putting their own children at risk, how are they putting other children at risk with this policy?

A reduction in vaccination rates may break herd immunity. Herd immunity occurs when most of the individuals have been vaccinated such that it is difficult for a pathogen to travel from an infected individual to another because there are so few individuals who are not immune.
19. (6 pts) The following is a list of vaccination strategies. Pick any one from list A and any one from list B and briefly describe how they confer immunity. Give the relative advantages or disadvantages of the two that you selected.

**List A**
- Denatured Toxins
- Attenuated virus
- Killed organisms
- Viral vector encoded (e.g. insertion of HepB genes into measles.)
- Carbohydrate+protein
- Plasmid DNA
- Capsid Proteins
- ISCOMs
- Virus like particles

**List B**
- Denatured Toxins
- Attenuated virus
- Killed organisms
- Viral vector encoded (e.g. insertion of HepB genes into measles.)
- Carbohydrate+protein
- Plasmid DNA
- Capsid Proteins
- ISCOMs
- Virus like particles

**List A** are non-reproducing antigens and thus will only be exposed to the immune system for a relatively brief period of time (2 ½ pts)

**List B** are methods that will continuously produce antigen, thus increasing the likelihood of developing an immune response (2 ½ pts)

1 pt

General problems with list A are the pathogens are not completely killed (or the components are not completely pure) and may contain infectious particles.

A problem with list B, (at least for attenuated viruses) is that they can revert to a more virulent virus.

20. (2 pts) Based on the typical immune response to HIV, why is a T-cell based vaccine likely to be more beneficial? Why have all vaccine trials failed at this point?

Normally there is a vigorous antibody response to HIV infection, which clearly fails. So vaccination to produce a B-cell response would be unproductive (1 pt). HIV vaccine trials have appeared to fail because of the high rate of antigenic draft with the virus due to its error prone reverse transcriptase (1 pt).
21. **Questions Based on Presentations** (4 pts). You can answer up to 4 questions, 2pts each. 2nd two choices are bonus points. Please indicate the four that you would like graded, otherwise the first four will be graded.

a) How does Gardasil or Cervarix provide protection against cervical cancer?  
*Cervical cancer is caused by a viral infection. Gardasil and Cervarix are vaccines that prevent infection by that virus (Papillomavirus).*

b) Ibalizumab is an monoclonal antibody that is used to interfere with HIV growth. What protein does it bind to and how might this prevent HIV growth?  
*It binds to CD4, which is a co-receptor for HIV entry into cells, somehow blocking viral entry.*

c) How is passive and active immunization combined to provide protection against the Procine Epidemic Diarrhea Virus (PEDV) in piglets?  
*Mother pigs are vaccinated against the virus, and the antibodies produced by the mother are given to the piglets during breastfeeding.*

d) How is the Newcastle disease virus, an avian virus, used to develop a vaccine for ebola virus?  
*This virus is a weak virus when infecting humans and usually does not cause any symptoms. Genes for the ebola virus have been incorporated into the Newcastle virus and are used to vaccinate people.*

e) What is one key limitation regarding the development of vaccines for birth control?  
*They cannot be easily reversed, or become reversed unexpectedly.*

f) How can hookworms be used to treat autoimmune diseases that involve antibodies?  
*They shift the immune response to a TH1 response, reducing the production of antibodies against auto antigens.*

g) What are two key properties of the antigen that is selected for the development of anti-cancer therapeutic antibodies?  
*They must be unique to the cancer cell, preferably overexpressed, and essential for growth of the tumor cell.*

h) Why might the protective antigen from Anthrax be the most useful target for vaccine development?  
*It forms pores that allow lethal factor and edema factor to enter the cell.*

i) What experiment was done to demonstrate the anti-cocaine catalytic antibodies are more effective in the treatment of cocaine than antibodies that simply bind cocaine?  
*Measured how much cocaine was required to induce seizures or death in the presence of either catalytic antibodies of antibodies that simply bound cocaine. The former were more effective.*

j) Why are patients who are infected with HIV more susceptible to tuberculosis?  
*Pathogen is usually eliminated by a T-cell response, since T-cells are killed by HIV, this becomes more difficult.*

k) Why is suppression of apoptosis by N. Gonorrhoeae a useful advantage for the bacterium?  
*They can grow intracellularly – so apoptosis would essentially destroy their living environment.*

l) What experiment was done to show the linkage between Rag1/Rag2 expression and the severity of immunodeficiencies?  
*Simply varied the amount of Rag1/Rag2 in mice that were deficient in the endogenous copy of these genes. Low dose = immunodeficiency (SCID), high dose = autoimmune disease.*