Instructions: This exam consists of 22 questions on 11 pages for a total of 200 points. On questions with choices, all of your attempts will be graded and you will receive the best score. Place your name on each page.

1. (10 pts) Compare and contrast central versus peripheral tolerance, both in terms of location and the general events that occur to generate tolerance to self-antigens.

Central tolerance occurs in the primary immune organs. In the case of B-cells, in the bone marrow. In the case of T-cells, the thymus.

Any B cells that recognize membrane bound self antigens are either killed or undergo receptor editing (new light chain) in an attempt to rescue the cell. B-cells also undergo a self-tolerance check in the lymph nodes. This is still considered to be central tolerance because the B-cells have not completed their development yet.

Any T cells that recognize self-peptides on self MHC are killed.

Peripheral tolerance occurs with naïve B- and T-cells, there are three general themes:

i) Poor activation of B or T cells leads to anergy - or a non-responsive cell. Poor activation can occur because the antigen is not opsonized, leading to weak stimulation of the B-cell, and therefore weak stimulation of the T-cell.

T-cells interacting with other APCs (macrophages, DC) that are not activated (e.g. no infection) will also become anergic. This is the case for potential food allergens that have become systemic.

ii) T_{reg} cells. These are found throughout the body, and there is a specialized set near mucosal membranes in the gut (T_{H3}). These cells suppress the activation of T-cells.

iii) Immunoprivileged sites. Eye, brain, reproductive organs. These tissue produce suppressive cytokines and can actively kill lymphocytes by FasL-Fas.

2. (10 pts) Please do one of the following choices.

Choice A: How do T_{reg} cells prevent autoimmune diseases.

Choice B: Briefly exchange why food allergies are relatively uncommon, what immunological mechanisms are at play to reduce the response to antigens in our food?

Choice A: T_{reg} cells can recognize self-peptides, but when they do so, they produce cytokines that inhibit the APC. They also have IL2R on their surface that will sequester IL-2 produced by activated T-cells.

Choice B: Specialize T_{reg} cells, called T_{H3} will suppress the activation of local T-cells, using the mechanism above for choice A. In addition, if food allergens become systemic, they will only induce an immune response if the APC is activated - i.e. due to an infection.
3. (10 pts) Pick two of the following diseases and state which type of hypersensitivity is responsible for the immune response. Briefly justify your answer with reference to the nature of the antigen and the immune response (Note: You may complete Q21 first. Feel free to reference that table in your answer below.)

i. Rheumatic fever
   - type II, carbohydrate antigens on bacteria induce antibodies that crossreact with similar antigens on cardiac tissue.
ii. Graves' Disease
   - type II, antibodies bind to, and stimulate thyroid stimulating hormone receptor, causing release of thyroid hormones.
iii. Myasthenia gravis
   - type II, antibodies bind to acetylcholine receptor at neuromuscular junction, prevent transmission of signal to muscle cells. Can also cause internalization of receptors.
iv. Lupus
   - type III, soluble immune complexes, antigen is DNA or nucleoproteins.
v. Diabetes – type I
vi. Multiple sclerosis
   - type IV, T-cells recognize self-antigens against myelin basic protein on nerve cells.
vii. Sympathetic ophthalmia
   - type IV, T-cells recognize sequestered antigens on eye after damage.
viii. Serum sickness
   - type III, antigen are injected antibody to treat a disease.
ix. Penicillin allergy
   - type II, antigen are membrane proteins that are modified by penicillin.

4. (8 pts) A transplant patient receives a new kidney, and organ rejection begins almost immediately.
   i) What mistake did the transplant team make?
   ii) What immunological processes are occurring to cause rejection of the kidney?

   i) They failed to check for a compatible blood type, e.g. transplanted from a type B individual to a type O.

   ii) Pre-existing antibodies in the host will bind to blood group antigens on the surface of blood vessels in the transplanted tissue. The presence of the antibodies will induce:

   i) complement activation – leading to opsinization of endothelium – attack by neutrophils. Also MAC complex can form, leading to cell lysis.
   ii) Antibody dependent cell killing, by NK cells. – perforin & granzymes lead to apoptosis of endothelium.
5. (12 pts) A second kidney transplant is attempted and the kidney is rejected after about two weeks.
   i) Briefly describe the most likely rejection mechanism, in particular how the host’s immune system became sensitized to the transplanted organ.
   ii) The transplant team neglected to run a simple test to determine if rejection was likely. What test was that and how would the test have informed the transplant team as to the success of the transplant.

   i) This is a type IV hypersensitivity – recognition of antigens by T<sub>H</sub>-cells and then migration of the activated T-cells to the transplanted tissue, recruitment and activation of macrophages by INF-gamma.

   The major sensitization is presentation of human (self) peptides on the donor MHC. The peptide-MHC complex mimics a foreign peptide on host MHC. In addition, host MHC can present peptides from the donor that are foreign, predominately peptides derived from MHC.

   ii) They could have performed either a serological test or a mixed lymphocyte assays.
   Serological test – utilize anti-MHC antibodies that are specific for an allele, detect whether the antibody binds by seeing if complement can be activated, leading to pores in the cell (MAC) that allows the uptake of a dye. A good match is indicated by the donor and the host having the same alleles.
   Mixed lymphocyte assay – irradiate donor cells, mix with host cells, detect proliferation of host by the uptake of radioactive thymidine. A good match is indicated by a low level of incorporation.

6. (8 pts) A third transplant is attempted, and this time the patient was treated with:
   i) Cyclosporine,  
   ii) An anti-IL2Rα antibody,  
   iii) An injection of the donor’s bone marrow.

   The kidney is not rejected. Briefly discuss how two of the above treatments prevented rejection of the kidney.

   i) Cyclosporine – inhibits the signaling pathway in T-cells, T-cells are more difficult to activate.
   ii) An anti-IL2Rα antibody – binds to IL2R and inhibits activation by IL-2. Selective for activated T-cells because the alpha-chain is only produced in activated T-cells.
   iii) An injection of the donor’s bone marrow. The donor stem cells will produce APCs with the MHC of the donor. These will be at a low level and will be distributed throughout the body, this tends to induce tolerance of the host T-cells to the donor MHC.
7. (5 pts) The transplant team carefully monitors the health of the transplanted kidney. What methods could they use to do so and what are the comparative advantageous of one method over another?

They can either take a sample of organ with a biopsy - this is invasive and has poor sampling. They can also use MRI imaging to monitor the migration of the macrophages and/or T-cells to the tissue. In this case it is necessary to label the cells with iron oxide particles, which make the cells appear dark on the MRI image.

8. (4 pts) The following molecules are involved in B-cell/T-cell signaling: CD40, CD40L, b7, CD28. How would the loss of any one of these, due to a genetic deficiency, affect the ability of someone to respond to pathogens that enter via the mucosal membrane?

All of these molecules are required for activation of B-cells. Without activation, class switching to IgA cannot occur. IgA is the antibody that is secreted across the mucosal membrane and provides protective immunity for that region.
9. (8 pts) Discuss the interplay between the innate and the acquired system using an example. What innate processes are essential for the acquired response and how can the acquired response supplement, or aid, the innate response?

An innate response is required for a substantial acquired response. Macrophages and DC, can kill pathogens, but more importantly, they are effective at presenting antigens, via MHC II (and MHC I, DC cells), to T-cells to activate them.

Several examples of the acquired response feeding back to enhance the innate:
i) antibodies can activate the classical pathway of complement.
ii) Th cells can release cytokines that attract macrophages to the site of infection.

10. (12 pts) Differences in the diversity and specificity of the B-cell receptor (BCR), MHC, and the T-cell receptor (TCR) are important in the normal function of the immune system.
i) Briefly describe how diversity is generated for BCR or TCR and MHC (i.e. pick B or T, and do MHC). Comment on whether the diversity is at the level of a single cell, within a single organism, or the population.
ii) Briefly describe the specificity of these proteins and how the specificity (or lack of) is an essential property of the immune system.

i) Diversity of the B/T cells are at the level of the individual. These receptors are homogeneous on any given cell, but there are $10^{10}$ different receptors. This diversity is obtained by segmental joining of DNA sequences to generate variable exons, imprecision joining, N and P base addition.

Diversity of the MHC is at the cellular level. Any given cell expresses a large number of different MHCs due to the co-expression of multiple MHC genes. All cells in an individual are the same. There are significant differences between each individual due to the large number of alleles in the population.

ii) Antibodies and the TCR are very specific in their recognition. This is to ensure: i) the production of antibodies that are specific for their target, showing little (no) crossreactivity to self antigens. ii) only a small number of T-cells are activated and that T-cells will not recognize self-antigens due to cross reactivity.

MHC are not very specific in the presentation of their peptides. This is to ensure a wide sampling of peptides from a pathogen are displayed, to enhance the possibility that a T-cell can be found to activate an immune response.
11. (4 pts) Discuss **one** genetic deficiency that could cause a severe combined immunodeficiency (SCID).

The most severe form of SCID (T-, B-) can be caused by a loss of Rag1/Rag2 - so that no B or T cell receptors can be generated, leading to death of the cells. In addition, enzymes involved in nucleotide metabolism, adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiencies can also lead to death of the B and T cell due to the high demand for nucleotides during proliferation.

Selective B or T deficiencies can be due to i) loss of signaling kinases (Bruton's, B-cells), (Zap kinase, T-Cells) or loss of IL2R function (T cells)

12. (6 pts) What enzymes in the HIV lifecycle are currently inhibited by anti-HIV drugs. What are the roles of these enzymes in the HIV lifecycle?

**Reverse transcriptase** - copies viral RNA to dsDNA, prior to integration.
**HIV protease** - responsible for maturation of immature proteins to mature proteins that assemble to form the virus.

13. (6 pts) Please do **one** of the following choices:
   **Choice A:** Briefly describe how you might make an HIV vaccine using measles virus.
   **Choice B:** Certain HIV resistant individuals are missing a protein. What is this protein, and why are they resistant to HIV?
   **Choice C:** How did biochemical studies confirm the major features of the TCR-MHC interaction as observed in the crystal structure?
   **Choice D:** How do class I and class II MHCs differ in their ability to present peptides.
   **Choice E:** Discuss one application of “engineered” antibodies in disease treatment.

   **Choice A:** Clone the gene for HIV glycoproteins into the measles viral genome, infect the person with the recombinant virus and the may produce a B or Tc cell response. This is generally unsuccessful because of the high mutation rate of the HIV virus.

   **Choice B:** Certain HIV resistant individuals are missing a protein. What is this protein, and why are they resistant to HIV? They are missing (or have a deletion) in CCR5, a cytokine receptor that is used by M-tropic HIV viruses to infect macrophages.

   **Choice C:** Direct binding of mutant peptides to MHC was measured to confirm which residues on the peptide contacted the MHC. Transgenetic mice were used to produce homogenous T-cell receptors, and then mutant peptides were tested for the ability to activate T-cells bearing this receptors.

   **Choice D:** Only significant difference is length - class I are limited to 8-9 residues, no limit for class II due to the open binding pocket.

   **Choice E:** Bite antibodies - contain anti-CD3 and anti-tumor antigen. Target Tc cells to tumor cells, leading to death of the tumor cell. Antibodies used to treat cancers would cause serum sickness, unless they are humanized or chimeric with human constant regions.
14. (14 pts) The Complement System. Please answer all of the following.

i. What functions does the complement system play in innate immunity? Briefly describe each of these functions (precisely mention the effector, how it is generated? how it mediates its function?) 6pts

ii. How does the complement system directly participate in eliminating viral particles and virally infected cells? 4pts

iii. Describe the effects of a genetic disorder that perturbs the expression of the C3 complement subunit 4pts

i. The complement system has 3 functions in innate immunity: 1) Recruiting and activating immune cells through the release of anaphylotoxines C3a & C5a generated by the proteolysis of C3 and C5 complement subunits (by C3 convertase), respectively. C3a and C5a attract and activate immune cells. 2) Opsonization of pathogens through C3b (opsin) coating of pathogen surface which facilitates their phagocytosis by CR1 on phagocytes. C3b is the result of C3 subunit proteolysis by C3 convertase. 3) Killing pathogens by punching holes on the pathogen's membrane through formation of MAC (membrane attacking complex). Proteolysis of C5 subunit generates C5b-membrane bound fragment, which recruits C6, C7, C8 and C9 subunits to form the MAC.

ii. The complement can kill directly the parasite through formation of the MAC on its surface

iii. C3 complement subunit deficiency leads to sever primary immunodeficiency symptoms:
   - No production of C3a -> no activation of mast cells, no recruitment of immune cells
   - No production of C3b -> no opsinization -> inefficient phagocytosis
   -> no formation of C5 convertase -> no MAC

15. (5 pts) What are the differences between active and passive immunization? Give an example for each.

<table>
<thead>
<tr>
<th>Passive</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provides transient immunity (few week)</td>
<td>Provides long-lasting immunity</td>
</tr>
<tr>
<td>Relies on Ig transfer</td>
<td>Relies on Bmemory and Tmemory cells</td>
</tr>
<tr>
<td>Does not solicit the immune system of the</td>
<td>Solicits the immune system of the immunized</td>
</tr>
<tr>
<td>immunized person</td>
<td>person</td>
</tr>
<tr>
<td>e.g. placental transfer (IgG)…</td>
<td>e.g. vaccination…</td>
</tr>
</tbody>
</table>

16. (6 pts) The Gut-Associated Lymphoid Tissue (GALT) is the digestive tract immune system. Describe how does the GALT protect the body against ingested pathogens?

The GALT contains B cells, T cells, Plasma cells and APC (DC, Macrophages)

Continuously, M cells (microfold cells) inserted into the endothelium membrane would sample microorganisms from the lumen environment and present them to the Dendritic cells and B cells that are nestled within the M cell invaginations. Dendritic cells and B cells can migrate to the GALT and present processed peptides from these microorganisms to TH cells. In case of activation of a B cell by a pathogen, the activated B cell differentiates into a plasmocyte that secretes IgA against the recognized pathogen. IgA is then transported via the endothelial membrane and released into the lumen were they bind, neutralize and opsonize the pathogen preventing it from affecting the organism.
17. (10 pts) The flu virus evades the immune system by undergoing antigenic shift and antigenic drift. Describe each of these immune evasion mechanisms and clearly state the cause of each one. 5pts each

The antigenic shift is the small and gradual change in surface antigens that allows the virus to evade immunity by loss of antigen recognition. It is caused by point mutations affecting its genome. The lack of proofreading activity of the viral RNA polymerase allows random point mutations to occur which accounts to the antigenic shift.

The antigenic drift is rather a large and abrupt change in the antigens resulting from a change in the genomic composition of the virus. It is driven by genome recombination of different flu strains (e.g. human flu virus and bird flu virus). The recombination occurs when a third organism called host (e.g. pig) gets infected by both strains, then the viral genomic materials get mixed (in the pigs infected epithelial cells) and new viral particles bearing a recombined genome are made.

18. (10 pts) Answer two questions of your choice from the following questions. 5pts for each

Choice A: What causes histamine release by Mast cells? (detail your answer)
Choice B: What is histamine’s role in hypersensitivity reaction type I?
Choice C: What is an adjuvant? What is its role in a vaccine?
Choice D: Give an example of a vaccination strategy and highlight its pros and cons

Choice A: Histamine is stocked into granules in the cytoplasm of Mast cells. The crosslinking of IgE-bound FcR at the surface of Mast cells (e.g. after a second exposure to an allergen) generates an intracellular signal (causes Ca+ cations release) which induces the fusion of the cytoplasmic granules with the plasma membrane (i.e. degranulation) and the release of histamine outside of the cell.

Choice B: In HS type I, histamine released from Mast cells induces
- Vasodilation of blood vessel -> increase in vascular permeability -> recruitment of immune cells
- Bronchoconstriction due to contraction of smooth muscle cells enwrapping the lung airways and stimulation of mucus secretion
- Intestinal hypermotility (involuntary muscle contractions) due to the effect on the gut’s smooth muscle

Choice C: Compound added to a vaccine that elicits inflammatory response to accelerate, prolong, or enhance the adaptive immune response against the vaccine antigen. Adjuvants also prolong the release of antigens.

Choice D: Check lecture material (Immunization lecture)

19. (14 pts) Trypanosoma brucei is a protozoan parasite that infects humans. The ensuing humoral immune response is mainly raised against the parasite surface protein Variant Surface Glycoprotein (VSG). Despite the efficiency of the secreted anti-VSG antibodies in clearing out the parasite, the infection persists. Please do all of the following:

i) Describe how the anti-VSG antibodies kill the parasite? 3pts

By binding to the Variant Surface Glycoprotein the Abs
- opsonize the parasite -> facilitate phagocytosis by phagocytes
- activate the classical pathway of the complement system -> lysis by MAC and opsonization by C3b

No hypersensitivity reaction is involved in this process, because the parasite is circulating while Mast cells are tissue residents

ii) What evasion strategy used by the trypanosome allows the infection to persist? Briefly explain the mechanism. 3pts

The trypanosome uses an antigenic shift strategy to evade the Abs immune response. The locus of the VSG protein contains one transcriptionally active gene located at an expression site and few hundreds of inactive VSG encoding genes that precede it. Each of these genes has a different primary sequence and is composed of 6 cassettes. To evade the immune
response the trypanosome duplicates inactive cassettes and transposes them into the expression site to replace the active gene. Hence, the trypanosome starts expressing a new VSG protein variant different in its primary sequence from the precedent variant → the circulating Abs won’t recognize the new VSG variant and cannot eliminate the parasite. (You get full credit even if you do not mention the cassettes and you talk about the gene as a whole)

iii) Complete the following graph, by representing the variations of the trypanosome blood concentration after several weeks of infection (Left Y axis) 2pts

iv) Describe the presented pattern and explain its causes: 3pts Periodically, trypanosomes that underwent an antigenic shift will evade the immune response and quickly multiply and thrive (raise of blood titer). However, the immune system reacts to the new parasite and produces effective Abs that kill the new (escaping) variant (decline of blood titer). Only remain the parasites that were able to undergo a new antigenic shift, which resist the immune response and multiply quickly to start a new cycle.

v) Overlay on the same graph the levels of circulating anti-VSG antibodies (Right Y axis) 3pts

Generated anti-VSG antibodies are VSG variant specific and their secretion decreases overtime (but not within a week)

20. (6 pts) How is gene therapy used as a treatment for patients with severe combined immunodeficiency (SCID)? 4pts What could be the risks of using such therapeutic strategy? 2pts

In gene therapy, the patient’s immune stem cells are extracted from his bone marrow, and then infected with a virus to supplement the copy of the non-working gene. Cells that have integrated the gene and express the missing protein are sorted and injected back into the patient body. The genetically modified stem cells repopulate the bone marrow, and produce healthy immune cells → this experimental technique allows a replacement of the patient deficient immune system by his fixed immune stem cells.

The risks incurred could be:
- The uncontrolled insertion of the gene of interest in the genome can perturb the expression of important genes. Also, it can induce the expression of oncogenes.
- The virus carrying the gene has to be safe and not cause genome instability or interfere with cell function.
21. (16 pts) Fill out the following comparative table. 1pt/cell

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
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<tbody>
<tr>
<td><strong>Antibody</strong></td>
<td>IgE</td>
<td>IgM/IgG</td>
<td>IgG</td>
<td>No Abs (TH1 immune response)</td>
</tr>
<tr>
<td><strong>Antigen</strong></td>
<td>- Parasite</td>
<td>- Cell surface proteins</td>
<td>- Soluble antigen</td>
<td>- Modified proteins (Hapten\carrier)</td>
</tr>
<tr>
<td></td>
<td>- Soluble antigen</td>
<td>- Modified proteins</td>
<td></td>
<td>- Graft</td>
</tr>
<tr>
<td><strong>Effector</strong> (The agent that causes the immune response)</td>
<td>- Mast cells</td>
<td>- NK cells (ADCC)</td>
<td>- Neutrophils</td>
<td>- Macrophages</td>
</tr>
<tr>
<td></td>
<td>- Eosinophils</td>
<td>- Complement (MAC)</td>
<td>- Complement (C3a, C5a)</td>
<td></td>
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<tr>
<td><strong>Mediator</strong> (cytokines)</td>
<td>Histamine TNF/ECF/IL8</td>
<td>C3a, C5a</td>
<td>C3a, C5a</td>
<td>INFγ</td>
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<td>MIF</td>
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<td>MCP-1</td>
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<td>TNFa</td>
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22. (16 pts) The following graph depicts the variation of the HIV viral load (Right Y axis-brown curve-) in an HIV seropositive patient. Please answer all of the following questions.

i) Describe the viral load variations labeled 1 to 4 in the figure and the precise cause of each of them. 4pts

ii) Complete the graph by representing the variations of the \( T_{H}^{CD4^+} \) blood cell count (Left Y axis) knowing that prior to infection the patient had 800 cell/\( \mu \)l. Explain the variations shown by your curve 4pts

iii) On the same graph Delineate and label the 3 stages of HIV infection leading to the patient death (you can draw lines parallel to the Y axis) 3pts

iv) Overlay, on the same graph, the curve (in dotted line) representing the variation of circulating anti-HIV antibodies since infection occurred. Then, explain the variations shown by your curve 5pts

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i) 1- The viral load increases rapidly because HIV virus multiplies quickly in \( T_{H} \) cells
   2- The viral load decreases significantly due to the anti-HIV immune response that takes place
   3- The HIV virus persists in the blood at a steady level due to the pressure of the immune system
   4- The viral load increases rapidly because the failing immune system can no longer keep it under control + immune evasion + drug resistance

ii) The \( T_{H}^{CD4^+} \) blood cell count decreases initially because the rapid multiplication of the virus kills them. After few weeks the antiviral immune response fights back and brings the virus level down. The \( T_{H}^{CD4^+} \) level recovers (partially or totally). However, because of the chronic infection, the spread of the infection and the damage caused to the immune system, the \( T_{H}^{CD4^+} \) level continues to decrease \( \rightarrow \) collapse of the immune system

iii) The anti-HIV antibodies start being produced few weeks after infection. The circulating level increases quickly to bring the viral load down. Antibodies continue to be produced to keep the virus on check. The virus mutates and evades the immune system (through antigenic drift) while actively multiplying \( \rightarrow \) The Abs level decreases as a result of the immune evasion and the collapse of the immune system