Problem set

1) Contrast positive and negative selection of developing T cells in terms of:
   a) when during development it happens — both occur at CD4+ CD8+ stage
   b) what the receptor-ligand interaction is (what molecules are involved) — TCR, CD4 or CD8 and peptide MHC complex (class I or class II).
   c) which cells are responsible for the selection and where the cells come from — thymic epithelial cells are responsible for positive selection and thymic dendritic cells and macrophage (bone marrow derived) are responsible for negative selection.
   d) which type of selection requires a greater avidity of interaction — negative selection

2) In what settings is receptor editing observed in developing T cells?
   DP T cells with surface TCR waiting to be positively selected, and anti-self DP T cells attempting to avoid self-specificity.

3) How does a developing T cell "know" whether to become a CD4+ or a CD8+ single positive T cell?
   Data favors instruction — if positively-selecting self-peptide/MHC complex is class I, then cell becomes a CD8+ SP. If selecting complex contains class II MHC, then T cell becomes a CD4+ SP.

4) Splenocytes from a H-2b mouse are mixed with irradiated macrophage from either a H-2a, H-2d, or H-2bxd mouse. In which cases would you expect the H-2b splenocytes to respond by proliferating? What type of reaction is this?
   I would expect the splenocytes to proliferate in each of the latter 3 cases. This is called a one-way MLR.

5) Bone marrow from an H-2b xd mouse is transferred into an irradiated H-2b mouse. Six weeks later, splenocytes are harvested from the transplanted mouse and mixed in vitro with irradiated macrophage from a) H-2b, b) H-2d, or c) H-2a mice. In which cases would you expect the splenocytes to proliferate?
   I would expect the splenocytes to proliferate only to H-2a macrophage since they have been negatively selected on thymic macrophage and dendritic cells of H-2b xd genotype.

6) The same bone marrow transfer experiment is performed as was described in questions 5, but then the transplanted mouse was immunized with hen egg lysozyme (HEL). T cells are harvested from after this immunization and tested for their ability to respond to HEL presented by irradiated macrophage of the following MHC types — a) H-2b, b) H-2d, and c) H-2a. In which case(s) do you expect to see antigen dependent proliferation in vitro?
   Only in the case of H-2b APC since the transplanted thymocytes were positively selected in an H-2b thymic background.

7) Why is it important to deplete bone marrow of mature T cells prior to allogeneic bone marrow transplantation?
   To avoid graft vs. host disease, an alloreaction in which donor T cells recognize host MHC-peptide complexes.

8) Two inbred mouse strains, Strain A and Strain B, have different MHC haplotypes. Bone marrow transplants are performed by first irradiating the recipient to kill ALL bone marrow derived cells, then reconstituting with bone
marrow cells from a donor mouse. Irradiated Strain A mice were reconstituted with Strain B bone marrow (so-called B into A or B>A transplant) or with bone marrow from an F1 hybrid of Strain A and Strain B expressing both sets of MHC alleles (so called AxB into A or AxB>A). What is the explanation for the observation of normal T cell dependent immunity in transplanted mouse AxB>A but impaired T cell dependent immunity in the B>A transplanted mouse? The antigen presenting cells in the B>A transplant are all of type B, but the T cells were positively selected for the ability to recognize antigenic peptides presented in the context of MHC A. Therefore, there is impaired T cell immunity. The F1 A x B donor provides APCs which can present antigen in the context of MHC A, the same MHC type as the host which positively selects the T cells, so all is well.

9) Which cells in the thymus are of epithelial origin and which are of hematopoietic origin?
Thymic epithelial cells are "epithelial" in origin, while thymic macrophage and dendritic cells are of bone marrow origin.

10) What is the difference between the two types of TCR?
Different TCR genes αβ vs. γδ. Also, γδ cells do not use CD4 or CD8 co-receptors, are generated mostly in fetal life, and are less polymorphic in TCR structure.

11) What happens to the thymus with aging, and what are the implications of this observation?
The thymus involutes with aging. This means that most memory T cells are generated in youth and survive for the rest of a lifetime. It also implies that as individuals age, they become progressively immunocompromised.

12) Draw the expected FACS profile for thymocytes stained with anti-CD4 and anti-CD8 antibodies in the setting of the following alteration:
a) wild-type
b) lck knockout
c) preT knockout
d) TCR chain knockout
e) TCR chain knockout

13) How does a cell decide whether to become an αβ or γδ cell?
Some data to favor idea that if TCR β makes in-frame rearrangement, leading to pre-TCR expression, then cell becomes an αβ. If TCR γ and δ rearrange productively before β, then cell becomes a γδ. Not certain, though.