1) What are the danger signals that mediate dendritic cell activation?
- Inflammatory cytokines
- Toll like receptors

2) What are the substrates of TLR3, 7, 9?
- dsRNA, ssRNA, unmethylated CpG DNA

3) Why is TLR9 localized in the endosomes/lysosomes?
- No really clear. A hypothesis is that the intracellular localization of TLR9 may be critical to its ability to discriminate self from viral nucleic acids (self DNA present in the serum would not be endocytosed contrarily to viruses/bacteria)

4) How can different carbohydrates recognized by the different C-type lectins?
- C-type lectin receptors (CLR) are proteins that contain carbohydrate recognition domains (CRDs) and bind carbohydrate. Depending on the amino acid sequence, the CRD has specificity for mannose, galactose or fucose structures. Moreover, interaction of these carbohydrate structures with the different CLRs is dependent on carbohydrate branching, spacing and multivalency.

5) What are the major differences between an immature DC and a activated DC?
- Immature: Highly endocytic, low level of MHC molecules, low level of costimulatory molecules
- Activated: poorly endocytic, high MHC levels, high level of costimulatory molecules

6) How would you use DC to vaccinate?
- Multiple possibilities. For example: Purify immature DC from blood (or differentiate them in vitro using appropriate growth factors), incubate them with antigen in presence of TLR ligands, inject.

7) Which cell types in the immune system are capable of killing virally infected cells? How do each of these cell types recognize infected cells?
- Cytotoxic T cells and NK (Natural Killer) cells.
- CTL are TCR+ CD8+ and recognize MHC class I / foreign peptide complexes to kill target cells. Activation of CTLs require signal 1, signal 2, plus IL-2
- NK cells have an array of activating and inhibitory receptors. They kill cells which have lost class I MHC expression.

8) What mechanisms are used the CTL to kill target cells? Why don't neighboring cells get killed via a "bystander" effect?
- CTL secrete perforin and granzymes. Perforin makes pores in target cells and granzyme diffuses in to target cell through pores and activates apoptosis cascade. The Fas / Fas-L system provides a second killing mechanism.
Bystander effects are avoided because the CTL polarizes its secretory machinery so that granules are secreted towards target cell. Also, CTLs express cathepsin B which cleaves perforin.

9) Would expression of an anti-apoptotic gene product protect virally infected cells from CTL killing? How/why?

Yes. Killing mechanisms depend in large part on the activation of apoptosis in target cells.

10) CD8⁺ T cells require co-stimulation in order to become armed effector T cells (CTLs). How do CTLs receive this co-stimulation?

Costimulation comes from B7 on the APC or infected cell binding to CD28 on the CTL. Naïve CD8 cells also require an exogenous source of IL-2, frequently provided by TH1 cells.

11) Most all tumor cells express mutant proteins. Why don't CTL recognize and kill tumor cells based on expression of mutant (altered) self-proteins? Suggest one or two strategies for activating anti-tumor immunity based on what you know about the requirements for CTL activation.

CTL don't always recognize tumor cells because most tumor cells do not express co-stimulatory molecules. One might overcome this problem by transfecting tumor cells with an expression vector encoding the B7 gene for example. Another strategy is to transfect tumor cells with cytokines which attract and activate dendritic cells (GM-CSF is often used).

12) What are the different types of inhibitory receptors on NK cells? What is there structure? How are they thought to signal?

They all have ITIMs in their cytoplasmic domains, or associate with proteins which contain ITIMs. Presumably, these ITIMs associate with PTPs which counteract PTK activity.

13) Why don't alloreactive T cells recognize and kill fetuses?

The answer is not completely known. One factor involved is that placental trophoblasts express very little class I MHC on their surface, diminishing the potential for attach by alloreactive T cells. They avoid NK cell killing by expressing the non-classical class I molecule HLA-G.
14) What types of immune deficiencies would you expect in a β2-microglobulin knockout mouse?
   a) Sketch a FACS plot of splenocytes from WT and mutant spleen labeled with anti-CD4 and anti-CD8 antibodies.

   ![FACS Plot]

   b) Would you expect the mutant mice to be unusually sensitive to infection with viruses, intracellular bacteria, or extracellular bacteria? Why? What other defense mechanisms might serve to protect the mutants?

   They should be sensitive to both viruses and intracellular bacteria (to a lesser extent). NK cells would help kill virally infected cells. TH1 cells (CD4+) would still be available to help activate killing mechanisms within macrophage, etc.

   c) How would NK cells be affected by this mutation? (a paradoxical answer).

   NK cells would NOT kill cells based only on absent MHC class I expression. But recall that the decision to kill is a balance of inhibitory and stimulatory influences, so the NK cells might still be functional.

15) Outline how engagement of FAS activates the apoptosis pathway in cells.

   FAS-L binding trimerizes FAS on the target cell surface. This brings together multiple copies of FADD which is bound to the death domain on the cytoplasmic tail of FAS. FADD then recruits caspase 8 which auto-activates by self-proteolysis. Active caspase 8 then activates subsequent caspases and cleaves their downstream targets.

16) Contrast the functions of the complement system and cytotoxic T lymphocytes in immunity. Point out similarities and differences between these two effector systems.
Both systems protect the host by inserting a pore into a lipid bilayer. In the case of complement, the target is bacteria; in the case of CTL the target is an infected self-cell. The structures of the pores are similar. Complement does not engage the apoptosis machinery the way CTLs do, however. Complement also plays a significant role in inflammation which CTL do not. Complement avoids self reactivity by the activity of inhibitors expressed on self-cells; CTLs avoid self-specificity by tolerance mechanisms and cell polarization towards targets.