NAME (printed): _____

7.23
Spring 2022
Exam #1

- Please answer questions **completely** (including descriptions where asked for) yet **succinctly** (essentially all question sections can be answered with just 1-2 sentences!).
- You may use any paper or electronic notes for this exam, so long as downloaded to a device – however, any outside communication (messaging, internet, or communication within the class – even to access notes – will be considered academic dishonesty).
- This exam contains **13 pages**, including the cover sheet
- You have 80 minutes to answer the exam.

Problem 1: / 20 pts

Problem 2: / 30 pts

Problem 3: / 22 pts

Problem 4: / 28 pts

TOTAL: /100 pts

NAME (printed): _____

Problem 1 (20 points)
Even though hematopoietic stem cells (HSCs) were first identified over 30 years ago, there are still controversies about aspects of HSC lineage and function. One such controversy is whether every HSC can produce each blood cell type with equal probability, or if some HSCs are biased to create certain cell lineages more than others.

You discover a new cell surface marker, CD 9000, which you propose can identify lymphoid-biased HSCs when used to sort bone marrow in conjunction with traditional HSC cell surface markers.

a) (5 points) What are the traditional cell markers used to sort mouse HSCs?

Lin^-, $Sca1^+$, $cKit^+$ (LSK cells)

b) (5 points) Given you are proposing lymphoid bias, list two cell types that you would hypothesize would be generated in greater proportion by CD 9000+ HSCs than by normally sorted, "bulk" HSCs?

T, B, NK cells

c) (5 points) To test your hypothesis, you take CD 45.1+ (a congenic marker) HSCs sorted via traditional cell surface proteins, and mix them 1:1 with CD 45.2+ CD 9000+ HSCs. You then inject this mixture into a cohort of lethally irradiated mice. If you examine the blood of the irradiated animals, briefly (no more than 2-3 sentences) describe what you would observe in (i) two weeks and (ii) six months if your hypothesis is correct.

i. Two weeks after, a $CD45.1^+$ cells will reconstitute the entire immune system, while the $CD45.2^+$ cells will be over-represented in the lymphoid cells ($CD3^+$, $CD19^+$, or $NK1.1^+$ cells)
ii. No change

d) (5 points) Suppose your hypothesis is incorrect, and CD 9000+ "HSCs" are actually lymphoid-biased progenitors rather than true HSCs. How does this change your answer to part c?

Answer would be unchanged at 2 weeks – at six months, proportion of $CD45.2^+$ lymphoid cells will be decreased/gone as compared to the $CD45.1^+$ cells.

NAME (printed): _____

Problem 2 (30 points)

After completing the second problem set in 7.23, you and a few of your classmates decided to start a company based upon the Wittrup lab Lumican technology. As a reminder, Lumican binds to collagen (a common extracellular matrix component) with high affinity, effectively localizing the Lumican and anything linked to it to the injection site. Your new company wants to create Lumican fusions as treatments for MRSA (methicillin-resistant Staphylococcus aureus), which is the cause of ~20,000 deaths in America a year. These infections typically start in the skin, but then can spread systemically. Your goal is to stop the infection at the skin.

Your company proposes two possible Lumican fusions. For each, list **one** additional molecule that will interact with the fusion protein (other than collagen). Also list **one** immune effect that this molecule causes, and **one** immune cell that may be involved in this response. **Note: each of these may have multiple correct answers! Please provide only one – listing multiple answers may result in deduction of points.**

A) (10 points) A stabilized version of C3(H_2O)

i. Other interacting molecule

Complement component B

ii. Immune effect caused by the molecule fused to lumican (no more than 1 sentence!)

Initiation of the alternative pathway of complement activation

iii. Possible immune cell involved

Phagocyte, neutrophil

B) (10 points) Interferon beta

i. Other interacting molecule

IFNAR1/2

ii. Immune effect caused by the molecule fused to lumican (no more than 1 sentence!)

Induction of pSTAT1 signaling; antiviral immunity

iii. Possible immune cell involved

DC, NK cell, etc - many answers possible

NAME (printed): _____

c) (6 points) At a quarterly company review, you realize that your team contains a saboteur! (it's a real Willy Wonka/Slugworth situation). One of the suggested fusion proteins they suggested is highly unlikely to be effective against MRSA. Which of the Lumican fusions is **less** likely to have anti-bacterial effect? Justify your reasoning in no more than 1-2 sentences.

Type I interferons are primarily thought of as anti-viral immune effectors – therefore you would expect this to be less effective for an antibacterial response. Curse that Slugworth!

d) (4 points) Your team is tasked with increasing MRSA T cell responses. Keeping in mind MRSA is an extracellular pathogen, would you focus on increasing MHC-I or MHC-II antigen presentation?

MHC-II

NAME (printed): _____

Problem 3 (22 points)
A key role of PRRs is to not only distinguish between self and non-self, but to prime the immune response to the *type* of pathogen encountered, as different pathogens may require different immune effector programs.

a) (2 points per table entry) As an example, please compare and contrast the following TLRs by filling out this table:

	TLR3	TLR5
Cellular localization	Endosome/lysosome	Cell surface
Typical ligand	dsRNA	Bacterial flagellin
Primary signaling adapter(s)	TRIF/TRAF3	MyD88/IRAK1/4
One anti-microbial function caused by ligand recognition	Activation of type I interferons	Cytokine secretion

b) (6 points) In no more than 2 sentences, explain how aspects of TLR3 recognition, localization, and signaling primes an anti-viral immune response as compared to TLR5.

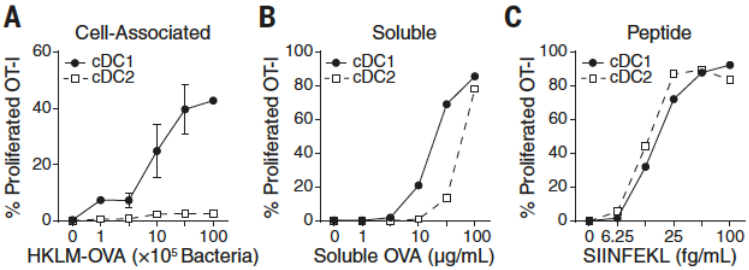
Endosomal localization and recognition of virus-like RNAs are poised to recognize viruses as they infect a cell. Activation of type I interferons is the most important early anti-viral immune effector program.

NAME (printed): _____

Problem 4 (28 points)

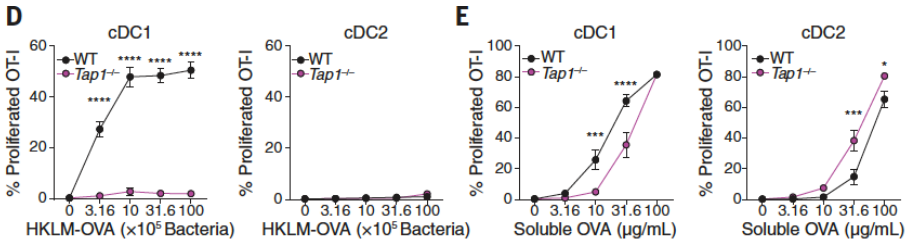
You heard from a co-worker that the source of antigen tremendously affects the induced immune response. Looking for interesting research publications, you come across this work which analyzes antigens derived from whole bacteria or soluble proteins/peptides.

a) (8 points) The first data you look at shows percent of activated $CD8^+$ T cells following co-culture with dendritic cells (DC). Prior to the co-culture, DC were exposed to heat-killed bacteria expressing ovalbumin (HKLM-OVA, labeled as "Cell-Associated"), soluble OVA (labeled as "Soluble") or SIINFEKL, an immunogenic peptide of OVA (labeled as "Peptide"). Based upon the data below, state which DC subset is specialized to present cell-associated antigens (heat-killed bacteria expressing OVA) and justify your answer in one sentence.



cDC1 – presentation of soluble antigen as well as peptide are comparable between cDC1 and cDC2, excluding stimulatory differences but when the antigen source is cell associated antigens when only cDC1 can activate T cells.

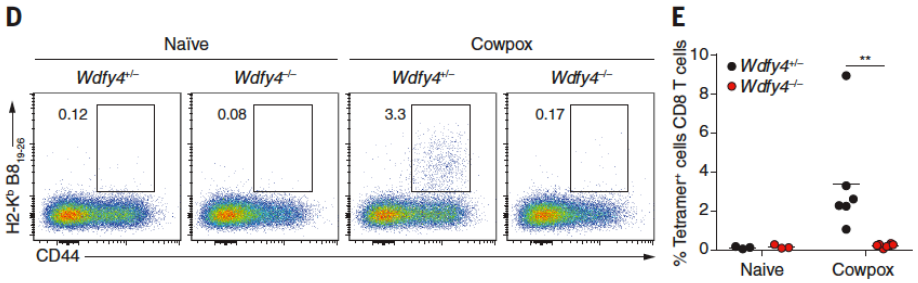
b) (8 points) By using the data shown below, please explain which presentation pathway is required for the presentation of cell-associated antigens and soluble antigens on cDC1.



Based on the data shown, cross-presentation of cell-associated antigens requires the cytosolic pathway of cross-presentation, as Tap1 is required for effective presentation of cell associated antigens.

NAME (printed): _____

The last piece of data shows the $CD8^+$ T cell response induced by DC following exposure to Cowpox infected cells (resulting in H2-KbB8 positive T cells, with H2-KbB8 being a tetramer of H2-Kb displaying a peptide derived from a cowpox protein). Please note that mouse DCs themselves cannot directly get infected by cowpox. You also note that Wdfy4 proficient (+/-) and deficient (-/-) DCs were used.



c) (6 points) Please explain what information can be gained by looking at H2-KbB8+ T cells, and what the observed difference between Wdfy4 proficient (+/-) and deficient (-/-) DCs implies (1-2 sentences).

H2KbB8 are T cell responding to a Cowpox virus-derived peptide presented on MHCI, thus induction of this cell population implies effective cross-presentation. The fact that only +/- mice show the induction of this T cell population implies that Wdfy4 is involved in cross-presentation.

d) (6 points) Please speculate in which presentation pathway Wdfy4 is involved, and explain your reasoning (1 sentence).

Based on the fact that Tap1 is required for cross-presentation of cell associated antigens and that Wdfy4 shows a similar deficiency the data suggest Wfdy4 is involved in the cytosolic pathways of cross-presentation.