Microbiology 204
Discussion Session #5 November 9, 2018
1-2:30PM CL220
Faculty leader: Adrian Erlebacher

Haymaker et al. Grail in CD8+ T cell cytotoxicity. Nat Commun 8:239, 2017

Everyone should read this paper before coming to class. In this paper, the supplemental data file has some important information that we should discuss.

LEARNING OBJECTIVES:
To understand mechanisms of CD8 T-cell regulation and how maninpulation of these mechanisms could foster anti-tumor immunity.

STUDENT ASSIGNMENTS
1. Discussion leader (see instruction page for advice on leading the discussion).

2. Describe what a ubiquitin ligase is and what the major ubiquintin ligases are in T-cells. Not mentioned in the paper is the de-ubiquitinas A20 (a major research topic in the Ma lab at UCSF), whose gene (TNFAIP3) is highly associated with autoimmune diseases and lymphoma. Describe its role in T-cells.

3. Describe the results in Fig. 1a-d. How are antigen specific TIL cells detected in the tumors.

4. Describe the results in Fig 4s. These results suggest both CD8 T-cell intrinsic defects as well as Treg functional defects in GRAIL-KO mice.

5. Describe the results in Fig. 1f, which show that GRAIL-KO CD8+ T-cells from TIL populations respond better to the Ova peptide. But what cell types are presenting peptide in this experiment? Do you think this response would have been seen in EL-4 tumor injected mice? If the CD8+ T cells in the GRAIL KO are controlling that tumor (Fig. S3), then what antigen are they seeing?

6. Describe the results in Fig. 2a and b. These data (Fig. 2b) suggest that the effect of GRAIL is intrinsic to CD8 T cells, since adoptive transfer of GRAIL-KO/OT-1 specific CD8 cells lead to tumor loss in the EG-7 model. But why did the authors have to use OT-1 transgenic T-cells? Shouldn't just regular GRAIL-KO CD8 cells work? And why don't the WT OT-1 transgenic CD8 cells recognize the Ova expressing EG-7 tumor cells? The data in the rest of this Fig can be only briefly mentioned.

5. Describe the results in Fig. 3f, which is the most important panel in the figure. (the other panels show that GRAIL KO CD8 T-cells are hyperactive in vitro. Panel F is a complicated in vivo CTL cell killing assay. Explain this experiment.

6. Describe the results in Fig. 4a-e. Pretty straightforward, although it is unclear how the authors came to check IL-21R levels. Describe some of the know functions of IL-21 (Wikipedia is fine).

7. Describe the results in Fig. 4f. Compare this result with Fig. S10, which shows that normally, IL-21 has no effect on CD8 T-cell anti-tumor immunity. Does this seem sort of self-conflicting?

8. Describe the results in Fig. 5a and 5d. Why did the authors do this experiment by 293T cell transfection and not directly in T-cells (similar to the experiment in Fig. S8b).

9. Describe the results in Fig. 6. This is very straightforward and makes good sense.

10. Describe the results in Fig. 7. It seems these days that you have to have some patient data in the paper to get to a higher journal. While these data make general sense, they contrast the mouse model, which shows that only TIL cells (those in the tumor bed) and not in the spleen, upregulate GRAIL (Fig. S1). So it may be surprising that peripheral blood CD8+ cells in lymphoma patients upregulate GRAIL.

Student assignment #s

1. Iowis Zhu
2. Marissa Chou
3. Cody Mowery
4. Jennifer Umhoefer
5. Adam Wade-Vallance
6. Benjamin Wheeler
7. Darwin Kwok
8. Suraj Makhija
9. Nick Mroz
10. Rachel DeBarge
11. Julie Cole
12. Ki Hyun Kim
13. Brian J Woo
14.

(NOTE : if you will miss a discussion session, inform Dr. Lowell in advance; if assignments have already been made, you should additionally make a trade with one of your classmates who does not have an assignment that week so that your assignment is covered).