Microbiology 204
Discussion Session #1 October12, 2018
1-2:30PM CL220/223
Faculty leader: Cliff Lowell

Keszei et al., Constitutive activation of WASp in X-linked neutropenia renders neutrophils hyperactive. JCI, *in press*, 2018

Everyone should read this paper before coming to class. You do not need to read the supplemental materials and supplemental figures, although the Discussion Leader may choose to have the class discuss one or a few supplemental figures.

See below if you have an assignment, in which case you have some additional preparation for class. Assignments rotate among the students.

REMEMBER: there are no "correct" answers to most of these questions. They are suppose to stimulate conversation.

LEARNING OBJECTIVES:
To learn about the various genetic causes of neutropenia and neutrophil dysfunction. To re-learn about how WASp controls actin dynamics (taught in Cell Biology!), and how actin regulation affects neutrophil migration and adhesion.

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

2. Review the various causes of congenital neutropenia. How do these mutations affect neutrophil development? Remind the group of the major genetic causes of chronic granulomatous disease and various forms of leukocyte adhesion deficiency disorders.

3. Review the leukocyte adhesion cascade (rolling, firm adhesion, migration). How do neutrophils migrate in tissues? Remind the group how neutrophil polarization is maintained during tissue migration. What other cells migrate this way (amoeboid-like) in tissues?

4. Review Wiskott Aldrich Syndrome. This is caused mainly by loss of function mutations in the WASp gene. What are the clinical manifestations? Review how WASp works to regulate Arp2/3 in the actin dynamics pathway. Find a schematic picture of WASp to show the regulatory domains. Review the mutations that cause X-linked neutropenia. What is the interesting clinical conundrum about these XLN patients?

5. Review Figures 1 and 2. For figure 1, focus mainly on panels C, D, E and H. In particular, what does panel H show? For figure 2, focus mainly on panels B and D (just the numbers in the saliva). How are neutrophils in the saliva different than cells in the blood?

6. Review Figure 3, mentioning the supplemental data in figure 3S. What is the major finding of the mouse models of human XLN? (Answer = they don't have XLN!! We can come back to

discuss this later). Data in figure 3D is most convincing. What is the cortical actin cytoskeleton in neutrophils?

7. Review Fig. 4, focusing on panels C and D. This is a nifty mixed chimera approach which sets up a competitive situation between the WT versus WASp L272P and I296T neutrophils. Describe why you think phenotypes may come out in mixed cell competitive situations which may not be apparent in 100% knockout situations.

8. Review Fig. 5, focusing on panels B, C then E-H. I wished they had done the phosphorylation experiments by western blot. What is the advantage of doing these experiments by intracellular staining/FACs?

9. Review Fig. 6. This figure is very dense. Stick to panels A-C, panel H and panel M. You can mention that PI3K signaling may be downstream of Btk, but the obvious phenotype is seen with relative sensitivity to Btk inhibitors, shown in panel M. Maybe make up a signaling stick diagram to explain these integrin signaling pathways.

10. Review Fig 7, focus on panels G-J. Why is there such a big difference in extracellular ROS production between WT and the WASp mutants with fMLP stimulation, but not so big with PMA and none at all with *s. aureus* infection? The answer is in the Y-axis. What limits the release of extracellular ROS? (answer is the cortical actin cytoskeleton → but how do you test this?).

11. Review Fig 8, focus on panel B, C and I. These data are pretty subtle, but what are the authors trying to argue?

12. Coming back to the beginning, why do you think human XLN patients have so little problem with infections? The biggest problem with this paper is that the murine XLN mutations did not produce neutropenia (but did produce more activated neutrophils that accumulated in the tissues). There is one important experiment they did in the humans but not in the mice, which may have helped – which was that (answer = figure 1H). Why do you think there is such a difference between human and mouse neutrophils? What is the normal ratio of neutrophils/lymphocytes in the blood of experimental mice versus humans? Why is that? This simple observation illustrates a caveat we always have to remember in all the mouse experiments we will talk about in Micro204!

Student assignment #s

1. Cody Mowery
2. Nick Mroz
3. Jennifer Umhoefer
4. Adam Wade-Vallance
5. Benjamin Wheeler
6. Brian J Woo
7. Lowis Zhu
8. Marissa Chou
9. Julie Cole
10. Rachel DeBarge
11. Ki Hyun Kim
12. Darwin Kwok
13. Suraj Makhija
14.
15.

(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).