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Norma Andrews' Lecture Part 3: Current Research: Strategies for Cell Invasion and Intracellular Survival

Teaching Tools were prepared by Norma Andrews and Beki Renberg, a graduate student in the Andrew's lab.

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1. Review Questions

1. *T. cruzi* enter a host cell by a process that is:

    a. dependent on host cell actin polymerization
    b. dependent on host cell microtubules

2. How did studying the entry mechanism of *T. cruzi* lead to a better understanding of normal cellular function(s)?

3. Synaptotagmin (Syt) is:

    a. an enzyme that cleaves disulfide bonds
    b. a protein produced by *Leishmania* that causes membrane fusion
    c. a protein that couples calcium concentrations with membrane fusion

d. a glycoprotein that in involved in nuclear transport

4. True/False If you mutate or change parts of Syt VII, *T. cruzi* is still able to invade host cells and replicate intracellularly.

5. When *T. cruzi* invades the host cell, it acquires a membrane that is derived from the host cell. Where does it come from?

6. Lysosomal fusion with the plasma membrane is also important in the retention of the parasite inside the host cell. How do we know this?

7. The normal function of lysosomes is to degrade proteins, but in the case of *T. cruzi*, this doesn't seem to happen because the parasite is not degraded even though it is living in a lysosomal like compartment (acidic with lysosomal proteins). What other parasite(s) do/does this same thing?

8. Where does *Leishmania* replicate within the cell?

9. Nramp1 and Nramp2 are host proteins that are responsible for depleting iron from within endocytic compartments. Why are these proteins important?

10. What is LIT 1?

11. If the host is depleting the iron that is in the lysosomal compartments by using Nramp1 and Nramp 2, how does *Leishmania* acquire iron?

a. by using the Na+/K+ pump
b. by using LIT1
c. by diffusion
d. by degrading Nramp1 and Nramp2

12. Do LIT1 -/- mutants cause lesions in mice? Can parasites be isolated from host tissues?

13. Do we know in which cell types *Leishmania* persists in host tissue?

2. Answers to Review Questions

1. B

2. It leads to the discovery that the behavior of conventional lysosomes in mammalian cells is controlled by cytosolic calcium concentrations and that there is a population of peripheral lysosomes that is near the plasma membrane that function more as calcium-regulated secretory vesicles rather terminal compartments of the endocytic pathway, which was thought previously.

3. C

4. False

5. lysosome membranes

6. When you inhibit lysosomal fusion, the entry of the parasite is reversible. By using the lysosomal membranes to surround itself, the parasite can anchor itself to the microtubules within the cell (because lysosomes have proteins on their surfaces that interact with microtubules and anchor them).

7. *Leishmania* spp

8. Lysosomal compartments

9. The most important function is to transport iron to the cytosol for the cell to use it in normal cellular processes. Secondarily, this is a defense mechanism of the host cell. Since almost all known pathogens require iron to grow well, if the lysosome is deficient in iron, then there is very little iron available for the parasite to use to grow and theoretically it would die, thus preventing an infection.

10. Leishmania Iron Transport protein

11. B

12. No; Yes

13. It is still unknown in which cell types *Leishmania* persists within the host tissues.

3. Discussion Questions

1. What types of experiments were done to investigate the entry of T. cruzi into cells? What were the conclusions?
2. What are the effects of increases in cytosolic calcium concentrations on lysosomes? What other cellular processes can increases in cytosolic calcium affect?
3. What did the Andrews lab show using Total Internal Reflection Microscopy (TIRFM)?
4. What is the molecular machinery involved in controlling lysosomal exocytosis? How was this shown?
5. The Andrews lab used Leishmania to study intracellular pathogen survival. How can this work be related to the study of T. cruzi?
6. What are the functions of Nramp1 and Nramp2? Why are they important?
7. How does Leishmania acquire iron in lysosomal compartments? How did Dr. Chau Huynh show this?
8. What are some major questions that need to be addressed next?

4. Answers to Discussion Questions

1. Immunofluorescence microscopy was used to visualize actin, T. cruzi, and lysosomes localization within the cell. When T. cruzi and actin were visualized it was noted that actin did not surround the phagocytosed particle as is normally observed during phagocytois. When drugs that disrupt actin or microtubules were added, it was observed that the outcome of invasion was only affected when

microtubules were disrupted. It was also observed that lysosomes co-localize with T. cruzi as the parasite is invading the host. The following model was proposed to explain these observations: Lysosomes move along microtubules to the sight of parasite entry. T. cruzi makes an agonist that binds to a receptor on the host surface. Receptor binding activates phospholipase C which in turn makes IP3. IP3 mediates the release of intracellular calcium stores increasing the concentration of cytosolic calcium. Increased calcium promotes the fusion of lysosomes with the plasma membrane thus providing the membrane that surround the parasite as it invades the cell.

2. An increase in cytosolic calcium concentrations promotes the fusion of lysosomes with the plasma membrane. These changes in calcium also affect phagocytosis and membrane repair mechanisms. It was also learned that lysosomal compartments are not necessarily dead ends but can be transformed into secretory organelles and used when needed. During phagocytosis, this lysosomal fusion is the mechanism by which more membrane is added to the site where a particle is being phagocytosed.

3. They used TIRFM to directly visualize lysosomal exocytosis. The lysosomes were labeled with a fluorescence marker and it was possible to see the lysosomes getting closer to the cell surface and eventually fusing to plasma membrane (this is seen as a flash of fluorescence). This observation led to the conclusion that there is a population of peripheral lysosomes that reside near the plasma membrane and can be induced to exocytose by increases in intracellular calcium. It also helped to show that the distribution of lysosomes within the cell can be membrane proximal and not exclusively perinuclear as was previously thought.

4. Syt VII, v-SNARE, and t-SNARE are the proteins that are involved in the fusion of lysosomal compartments with the plasma membrane. Syt VII couples calcium concentrations to membrane fusion events by binding calcium in its two calcium-binding domains and interacting with the SNAREs. This facilitates formation of the SNARE complex that promotes membrane fusion. This was shown by using immunofluorescence microscopy to confirm that Syt VII is on the lysosome (co-localizes with Lamp1), and when Syt VII is depleted from the cell one can see that lysosomal exocytosis is inhibited.

5. Members of the Andrews lab study how Leishmania grow in lysosomal-like compartments that contain degradative enzymes. The information gained from these studies can be applied to T. cruzi because Trypanosoma and Leishmania are members of the same family and therefore are closely related. They share common

mechanisms for intracellular growth; Leishmania replicates in acidic, lysosomal-like vacuoles while T. cruzi enters cells via a mechanism that involves lysosomes.

6. They are iron transporters that deplete iron from the endocytic pathway. Nramp 1 is known to be a susceptibility gene for some intracellular pathogens. Nramp 1 and 2 function as defense mechanisms by depleting iron from lysosomal compartments, thus restricting the pathogens access to iron which they need for growth.

7. Dr. Huynh used a homology search within the genome to find genes that could be involved in iron acquisition. He found a gene that encoded a protein that he called LIT1 (Leishmania Iron Transporter 1) that looked like an iron transporter that was well characterized in Arabidopsis. It had eight trans-membrane domains and the same highly conserved residues previously shown to be critical for iron transport. He hypothesized that this protein was probably a member of the ZIP family of metal transporters. He made an antibody to LIT1 and found that it was only expressed during the intracellular stage of growth – about 24 hours post infection in cell culture. He also isolated the LIT1 gene and put it into strains of yeast that were defective in iron transport. He was able to show that LIT1 transports iron because it can rescue the growth of yeast defective in iron transport. Using homologous recombination, he made strains of Leishmania that did not have the gene for LIT1. These mutant Leishmania could not take up iron and could not replicate in host cells. Dr. Huynh confirmed that LIT1 was necessary for Leishmania infection by showing that the mutant could not cause lesions when injected into the foot pads of mice.

8. How (and in what cell type) does Leishmania persist in host tissues? How does Leishmania evade the host immune system? What are the requirements for Leishmania survival within the host?

5. Explain or Teach These Concepts to a Friend

1. Explain the signaling cascade that is involved in T. cruzi invasion. Include all known proteins, signaling molecules, and enzymes involved.

2. Explain how Nramp1 and Nramp2 are a host defense mechanism and how Leishmania circumvents them.

3. Explain what happens in LIT1 -/- mutants of Leishmania in cell invasion and replication.

6. Papers for Journal Club

Burleigh, B.A. and Andrews, N.W. The mechanism of Trypanosoma cruzi Invasion of Mammalian Cells. Annu Rev Microbiol. 1995. 49:175-200.

This review specifically address the invasion mechanism used by T. cruzi to get inside host cells. They discuss some of the different molecules that are involved in the cell invasion process.

Reddy, A., Caler, E.V., and Andrews, N.W. Plasma membrane repair is mediated by Ca(2+)-regulated exocytosis of lysosomes. Cell. 2001. 106(2):157-169.

This paper talks about plasma membrane repair and the involvement of Ca(2+). It also talks about synaptotagmin VII and its role in membrane repair. It also discusses the role of lysosomes in plasma membrane repair.

Andrade, L.O., and Andrews, N.W. Lysosomal fusion is essential for the retention of Trypanosoma cruzi inside host cells. J Exp Med. 2004. 200(9):1135-1143.

This is the paper where the Andrews lab shows that in order for T. cruzi to be retained within the cell, lysosomal fusion is necessary. They discuss some aspects of intracellular survival of pathogens.

Huynh, C., Sacks, D.L., and Andrews, N.W. A Leishmania amazonensis ZIP family iron transporter is essential for parasite replication within macrophage phagolysosomes. J Exp Med. 2006. 203(10):2363-2375.

This is the paper where the Andrews lab showed the existence of LIT1 and its involvement in parasite survival in macrophages. LIT1 is characterized and they show that some Leishmania species can persist in mice even when they lack the ability to grow within macrophages. They talk of the role of iron acquisition in relation to virulence.