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Norma Andrews' Lecture Part 2: *Leishmania spp* and Leishmaniasis

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1. Keywords and Terms

Leishmania species, Leishmaniasis, protozoa, macrophage, procyclic promastigote, amastigote, metacyclic promastigote, blood born disease, Lipophosphoglycan (LPG), sand fly, insect vector

2. Lecture Notes

Leishmania spp (species) are protozoans that cause Leishmaniasis in humans. They belong to the same family as the *Trypanosoma* species and are closely related. They are intracellular parasites. The incidence of disease is more wide-spread than that of *T. cruzi* and is present in Africa, the Middle East, regions around the Mediterranean Sea, and Central and South America. This is also a disease of poverty and is present in underdeveloped regions of the world, but in contrast to *T. cruzi*, development of these countries is not really stopping the transmission of *Leishmania* species. Recently the

disease is moving from rural areas to more urban areas, and as it moves it comes into contact with susceptible individuals (there are ~300 million susceptible people in the world) and the incidence may increase. In 2007 there were approximately 12 million people in the world that were infected.

Leishmaniasis

There are many different species of *Leishmania* and the disease that they cause is directly linked to the species of *Leishmania* with which a person is infected. The cutaneous form of Leishmaniasis is caused by *L. major*, *L. tropica*, or *L. mexicana*. The cutaneous form is characterized by a skin lesion that is normally self-healing. The mucocutaneous form is caused by *L. braziliensis* and this is characterized by degradation of the mucosal membranes and can cause severe disfigurement. The most severe form of Leishmaniasis is the visceral form that is caused by *L. infantum* (also called *L. chagasi*) and *L. donovani*. This form is characterized by severe enlargement of the liver and spleen, fever, weight loss, and anemia. The mortality rate is highest with the visceral form of Leishmaniasis.

History

Even though the disease has been around for a long time, it wasn't until 1900 – 1903 when the *Leishmania* organisms were first characterized. William Leishman, a British medical doctor that was analyzing samples from patients in India, and Charles Donovan, an Irish investigator, noticed that the macrophages in the spleen of infected people are heavily infected with organisms that looked similar to trypanosomes; they were initially called Leishman-donovan bodies. In 1904, Leonard Rogers and in 1908, Charles Nicolle took spleen tissue from patients and put it in culture and they were able to show that a second extracellular form of the organism appeared in these cultures. This indirectly indicated that the organisms had a life cycle similar to *Trypanosoma* and possibly an insect vector. It wasn't until 1924 that John Sinton discovered the first clues about the vector. He noticed that the incidence of visceral Leishmaniasis was associated with the distribution of a specific species of sand fly. In 1928, Knowles was able to detect *Leishmania* in sand flies yet it wasn't until 1942 that Swaminath formally demonstrated that sand flies could transmit *Leishmaniato* humans. Around this time (1940s) the life cycle of *Leishmania* was beginning to be understood; the parasite replicates prolifically in the sand fly gut and is then transmitted to humans/dogs/rodents

after a blood meal via regurgitation. The parasite then invades and replicates within the macrophages of the host.

In 1984-1985 David Sacks at the National Institute of Health (NIH) identified the infective stage of *Leishmania*. He showed how the different forms of the parasite are resistant or susceptible to the complement system. He showed that the procyclic promastigote form differentiates to the complement resistant metacyclic promastigote form in the sand fly mid gut. In 1990, Malcolm McConville and David Sacks showed that differentiation into metacyclic promastigote forms involves the surface molecule Lipophosphoglycan (LPG), which is a major component of the surface coat of the parasite. In the procyclic promastigote form, LPG is a shorter molecule with sugar side chains terminating in galactose. In the metacyclic promastigote form, LPG is an elongated molecule with sugar side chains that terminate in arabinose. This modification of LPG promotes detachment of the infective form of the parasite from the mid-gut of the sand fly. He later went on to show in 1994, that vectoral competence for different species of *Leishmania* is associated with LPG polymorphisms. This means that the ability of specific species of sand flies to transmit a specific species of *Leishmania* is based on different polymorphisms of the LPG molecule. This showed the molecular properties of the parasites that caused specific associations with vectors.

Since the portion of the life cycle that occurs in the insect has been studied extensively, the next frontier is to understand replication in the human host, especially because the parasite replicates inside macrophages, which is normally the cell that would kill the parasite. We do not completely understand how the parasite is able to survive within macrophages. It is now known that the entry of the parasite into the macrophage occurs via receptor mediated phagocytosis using complement receptors CR1 and CR3, which bind to the complement proteins, which have opsonized the parasite. Jean Claude Antoine at the Pasteur Institute has shown that *Leishmania* survives in intracellular compartments that share properties with lysosomes – they are acidic, contain a full set of lysosomal enzymes (hydrolases), and the membranes contain lysosomal membrane proteins (i.e. MHC II). These parasites can replicate quite well, despite the harsh conditions.

Life Cycle

When the sand fly bites a human, dog, or rodent, it regurgitates the organism into the bite wound. The organism then infects macrophages and replicates intracellularly. In the sand fly the parasite is in the promastigote form that changes from procyclic to

metacyclic, and the metacyclic promastigote form is the infectious form that is regurgitated into the bite. This form invades macrophages and then transforms into the amastigote form, which replicates within the macrophage. The doubling time is ~12 hours and the life cycle takes several days. Eventually the macrophage is lysed and the amastigote form is released into the blood.

Control of Human Transmission

The control of transmission is complicated because it is difficult to limit exposure of humans with sand flies because they are very small insects. The simple measures that have proved very efficacious in control of the spread of malaria (i.e. bed nets), have not worked in this specific case because the sand flies are so small they can pass through the bed nets. There is a strong need for new, less toxic drugs to treat the different forms of Leishmaniasis. The drugs that are currently being used throughout the world to treat Leishmaniasis are very toxic, but there are some new drugs that could prove useful to treat the disease.

3. Review Questions

1. True/False *Leishmania* species are extracellular pathogens?
2. Where are *Leishmania* species, and consequently the disease, endemic? Circle all that apply.
 - a. Australia
 - b. Middle East
 - c. Regions around the Mediterranean Sea
 - d. North America
 - e. Central and South America
 - f. Asia
 - g. Africa
 - h. Northern Europe
3. What is the name of the human disease caused by *Leishmania* species?
4. Name the different types of Leishmaniasis. Which is the most severe? Which is the most mild?

5. True/False *Leishmania* only undergoes human-to-human transmission.
6. When a human gets infected with a *Leishmania* species, it replicates in this cell type.
7. What is the vector for disease transmission of Leishmaniasis?
8. True/False Each specific species of sand fly is associated with a specific species of *Leishmania*.
9. Circle the forms that have been observed for *Leishmania*.
 - a. Epimastigote
 - b. Deltamastigote
 - c. Metacyclic promastigote
 - d. Metacyclic amastigote
 - e. Amastigote
 - f. Procyclic promastigote
 - g. Picyclic amastigote
10. Changes in the molecule lipoposphoglycan (LPG) are involved in the transition from what form to what form?
11. How do *Leishmania* species invade macrophages?
12. Why is it difficult to control Leishmaniasis disease spread?

4. Answers to Review Questions

1. False – they are intracellular pathogens
2. Middle East, Regions around the Mediterranean Sea, Central and South America, Africa
3. Leishmaniasis
4. cutaneous –most mild; mucocutaneous, visceral – most severe
5. False

6. Macrophages
7. Sand flies
8. True
9. Epimastigote, metacyclic promastigote, amastigote, procyclic promastigote
10. Procyclic promastigote to metacyclic promastigote
11. Receptor mediated endocytosis
12. The sand fly is VERY small and so it is very difficult to limit human contact with the disease vector. It can pass through mosquito nets – which is normally what is used to limit human contact with insect disease vectors (i.e. malaria). Flying insects are more difficult to control because spraying homes is not as effective.

5. Discussion Questions

1. *Leishmania's* infectious form – metacyclic promastigotes – is resistant to complement lysis but not opsonization. How is this efficacious for the parasite? We know that changes in LPG accompany the transition from procyclic promastigote to metacyclic promastigote. Do you think that the LPG molecule is involved in the resistance to complement lysis? What other factors might govern complement resistance?
2. Since Leishmaniasis is a disease of poverty, would eliminating poverty eliminate the disease? What else might need to be considered when trying to eliminate the spread of *Leishmania* spp? How would you accomplish the elimination of Leishmaniasis? What characteristics of the disease vector need to be considered in order to effectively eliminate disease transmission? What factors might be involved in the recent move of the disease to more urban areas?
3. Vaccination has proven effective as a means of disease prevention. Do you think a vaccine could be developed for *Leishmania*? What are some barriers to the development of a vaccine?

4. MHC Class II is expressed on the surface of the compartments in which *Leishmania* replicates within the macrophage. This protein is usually only expressed by activated macrophages. How might the parasite be interfering with the normal function of MHC II? Why would this be good strategy?
5. Why are there so few drugs available to treat Leishmaniasis? Why is it hard to find and develop a drug for treatment?

6. Answers to Discussion Questions

1. When the parasite enters the human host, its goal is to replicate itself. In order to do this, it needs to have some sort of mechanism for evading the host's immune system. This pathogen is quite clever in the fact that it is resistant to host complement lysis, but uses the complement system in order to invade macrophages via opsonization and CR1 and CR3. It is possible that the modification to LPG is responsible for resistance to complement lysis, but there is still a lot that is unknown about this mechanism. The modifications of LPG are one thing that might be involved, but the transition from procyclic promastigote to metacyclic promastigote probably involved many changes – not only to surface molecules but also in gene expression. In order to have a good understanding of what is happening, we need more detailed studies of the changes that occur during the transition from procyclic promastigote to metacyclic promastigote.
2. In recent years, Leishmaniasis has begun to spread from rural areas to more urban areas. This is a clue that the elimination of poverty might not lead to elimination of the disease. Even though poverty is one factor, there seems to be some other factor(s) that are governing disease spread. One important aspect of limiting disease spread is control of the insect vector. The measures taken thus far have not proved efficacious in blocking human contact with the sand fly. Theoretically, if contact with the sand fly could be greatly decreased or eliminated, then disease spread would also be decreased or eliminated. New strategies for controlling the insect vector are needed – this could come in many forms (smaller pores in bed nets, insecticide spraying, elimination of breeding habitat, etc.). Some of the factors that might influence the spread of the disease to more urban areas could be human population migrations, changes in local habitats that favor insect breeding and survival,

increased levels of poverty in urban areas that cause large numbers of people to gather in small spaces with little sanitation, etc.

3. Development of a vaccine for *Leishmania* has proven to be difficult, probably because the parasite has a very efficient strategy for evading the host immune system. There are some possibilities for vaccines that are currently being tested, but we still don't know enough about the biology of the parasite to ensure that a vaccine will be feasible in the near future. Also, there are other barriers to vaccine development. It is a very expensive process, and if there is not a demand great enough to allow vaccine developers to recoup their costs, it can only happen with the help of effective public-private partnerships. This is a critical aspect for moving forward, considering that the countries where disease transmission is endemic are very poor.
4. Expression of MHC II in macrophages that have *Leishmania* growing in them suggests that the macrophages are being activated. However, there is also evidence that there is something in the pathway leading to antigen presentation that is being disrupted. There are many different steps that have to occur in order to effectively present *Leishmania* antigens to the other parts of the immune system. If any one of these steps is disrupted, then the antigens will not be presented and the rest of the immune system probably won't get activated to assist in clearance of the parasite. This is a very good strategy for the the parasite to prevent the rest of the immune system from becoming activated, which allows it to replicate unchecked within the host. Since the "goal" of the parasite is to replicate itself, this is an effective way to avoid being killed by the host.
5. Since *Leishmania* is a eukaryote similar to human cells, it is difficult to find metabolic pathways that are used only by *Leishmania*. If there were drugs that interfere with a metabolic pathway shared between the parasite and humans it would probably be too toxic to be used as a treatment. There is still a lot that is unknown about the biology of *Leishmania*, and this is a major barrier for drug development. Also, drug development is expensive and time consuming, so public-private partnerships are also very important to finance the process.

7. Explain or Teach These Concepts to a Friend

1. Explain the life cycle of a *Leishmania* spp. Make sure to include the host and insect forms of the parasite. Be as detailed as possible. Also include why *Leishmania* transmission is very difficult to control in human populations.
2. Explain the difference between cutaneous, mucocutaneous, and visceral Leishmaniasis. What is one of the major factors that influences which type of Leishmaniasis that develops? Make sure to include which type is most likely to end in death of the host.
3. What has been done to control the spread of *Leishmania* spp? How effective has it been and what else might need to be done to effectively stop human infections?

8. Research the Literature on Your Own

1. What is the mortality rate for *Leishmania* infections? What characteristics of the parasite could influence this?
2. What drugs are available to treat *Leishmania* infections? How good are they? Is it feasible to treat all infected people in the world?
3. There are many different clinical forms of Leishmaniasis that vary greatly in severity. Even though all the species belong to the same genus, the diseases that they cause are quite different. What are the differences between the many different species of *Leishmania* that governs the type of clinical disease that develops? How are they similar?
4. In what countries or regions is *Leishmania* transmission still endemic? How many people are currently infected with the disease? Has there been a decrease or increase in the number of people infected worldwide (lecture given in April 2007)? Why?
5. Why have some countries been more successful than others at controlling disease spread? What factors might have influenced this?
6. What more has been discovered about *Leishmania* in the past couple of years? What proteins are important for pathogenesis? Is there a known population of

humans that is resistant to *Leishmania* infection? If so, why are they resistant to infection?