

Questions on Johnson *et al.* (2005) & Stinear *et al.* (2007)

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1. Which mechanisms use the *M. ulcerans*' toxin mycolactone in order to suppress the immune system of its host?
2. Recently, the entire genome of *M. ulcerans* has been mapped by scientists at Monash University. Have treatments against this pathogen arisen from this work?
3. PCR using the IS2404 target it has been used to detect *M. ulcerans*; however, the method is not specific for the pathogen and is only 40-80% sensitive. Which other reliable methods are used for a more accurate diagnosis?
4. You mention that research into Buruli Ulcer is limited because the potential to generate profits from treating this condition is minimal. What do you recommend, or what would you like to see happen, to change this problem? Is the current profit-driven pharmaceutical market best, or should we attempt to transition politically toward government-controlled, government-funded pharmaceutical research (would this do any good for Africans with Buruli Ulcer)?
5. How is a vaccine created? What steps would it take to create an effective vaccine

against *M. ulcerans*? (I'm unclear as to how exactly you use knowledge on the *M. ulcerans* genome to prevent the condition).

Answer: Vaccines are interesting because there are so many ways to approach making them. The most rudimentary way is to concentrate the disease organism and heat kill it. Then you give the dead organism to the subject and hope that you get an immune response that will protect it from future infections. Another way is to systematically determine which proteins of the disease organism cause an immune response. The protein can be given to the subject in concentrated form. The advantage to this is you know exactly what is being injected and there is no worry about any of the disease organism surviving the heat treatment. A more sophisticated approach is to develop a live attenuated vaccine. This is done by determining what genes make the organism virulent, then those genes are targeted for deletion. The idea is that the live organism will target the body just like the unaltered form and expose the subject to all the proteins it normally does but dies before it causes the full blown disease. Another clever vaccine method is a DNA vaccine. In this method DNA, that codes for select antigens, is delivered directly to cells. This can be done using an attenuated disease organism as a kind of Trojan horse. Once the DNA is delivered the organism dies and the cell begins to produce antigens that the subject will be exposed to.

In almost every case it is necessary to know what genes are important, where to find them in the genome so they can be manipulated and how the proteins they code for interact with one another in order to develop an effective vaccine.

Additional steps in the U.S. are getting the vaccine approved by extensive testing in animal models, then humans and then getting the government to approve its distribution. This sound simple, but often takes millions of dollars and many

years to accomplish.

From *Jason Hansen*

6. If we use the BCG vaccine to control *M. ulcerans*, what is the potential for *M. ulcerans* to evolve resistance to this somewhat effective vaccine? Could *M. ulcerans* and *M. bovis* potentially hybridize, thereby rendering the BCG vaccine totally ineffective? Or is this not a concern at all due to host isolation?
7. Is it likely that other arboviral infections like Lyme disease and West Nile evolved in the same way as *Mycobacterium ulcerans* (evolving to aerobic respiration, and protection against sunlight)?
8. Are the symptoms from *M. ulcerans* and *M. marinum* similar? What has been used to treat these infections?
9. What could be the possible reasons for the focal distribution of Buruli ulcer, even within endemic regions?
10. In context of the disease progression, it's mentioned in the paper that "in the course of the natural history of the disease, the immunosuppressive effect of the toxin is somehow overcome by the host, immunity develops and healing commences". Could you elaborate on that?
11. What do you mean by the statement that, *M. ulcerans* is passing through an 'evolutionary bottleneck' as it adapts to life in a specialized niche environment?
12. Have you considered the implications if *Mycobacterium ulcerans* were to acquire resistance genes to the antibiotics used for treatment (rifampin and streptomycin)? Were any resistance genes detected when sequencing the genome, and were plasmids other than the virulence plasmid detected and also sequenced?

13. Due to the 98% sequence identity between *M. ulcerans* and *M. marinum*, is it possible that a vaccine developed for *M. marinum* would be effective in preventing lesions by *M. ulcerans*? Would there be less ethical conflict in developing a vaccine for the milder form of disease which seldomly affects humans, so it might be easier to get approval for clinical trials, etc?
14. If aerosol droplets are potentially able to be inhaled and infect the respiratory tract, do you think this infection could become systemic? and if so, what damage could be caused to the body, and how long would it take to be detected if the symptoms are very mild and no outward lesions were identified? Do you think the body's immune system would respond and clear before extensive damage was done?
15. Why is *M. ulcerans* maintaining so many pseudogenes?
16. Is there evidence that pseudogenes, although not complete, may still code for active proteins?
17. Is larval debridement therapy considered for treatment of *M. ulcerans* in advanced cases?
18. It was noted that, lacking nitrate and fumerate reductase systems, *M. ulcerans* is not capable of growth under anaerobic conditions. How does this characteristic relate to the pathogen's ability to cause the typical extensive necrosis in the SQ space? I am under the impression that this is directly related to the Mycolactone toxin, but is this one of the factors that limits infection?
19. With regards to reductive evolution and *M. ulcerans*: I think I understand the concept of how it is possible in obligate intracellular bacteria for genes to be rendered inessential due to homologous host genes and the ability of the bacteria to import protein/

metabolic products from the host. With loss of the genes due to the intracellular environment acting as a bottleneck preventing recovery from mutations of these inessential genes, I am pretty sure that this is representative of reductive evolution. Is this concept safe to apply to *M. ulcerans* as an extracellular bacterium or are there different mechanisms that allow this to happen?

20. How does the mycolactone concentration change throughout infection? Is eventual recovery via host immune response allowed only when the toxin's concentration subsides?
21. In the article 'Buruli ulcer (*M. ulcerans* infection): New insights, New Hope for disease control' the development of ulcerative *M. ulcerans* disease is associated with a shift from the Th-1 to the Th-2 phenotype. Could you please explain why there is a shift from Th-1 to the Th-2 immune response.
22. According to the article 'Reductive evolution and niche adaptation inferred from the genome of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer, *Mycobacterium ulcerans*, is found predominantly in an extracellular form in infected human tissue. Is there any reason for this extracellular stage.
23. Why there is no effective vaccination against *M. ulcerans*?
24. Results from Stinear et al. (2007) supported a specific mechanism of divergence between the two *Mycobacterium* species. Can "genome-related" related research such as this also provide a timeline of divergence?
25. Stinear et al. (2007) also stated that *M. ulcerans* doesn't produce the same carotenoids that protect *M. marinum* from sunlight exposure. Thus, *M. ulcerans* must inhabit areas with less sunlight. What selection pressures could have driven this adaptation?
26. The paper by Johnson et al. (2005) basically concluded that the ultimate goal in controlling the future spread of Buruli ulcer is via development of a safe and effective

vaccine. In class discussions, we have talked about controlling several diseases through other mechanisms (e.g., providing purified drinking water, using screens on houses, etc.). Once more is learned about *M. ulcerans* and its biology, is there a potential for controlling the spread of Buruli ulcer by way other than a vaccine (e.g., possibly managing surrounding waterways to reduce the persistence of host species preferred by *M. ulcerans*, etc.)?

27. Why are the lesions generally painless? They look terribly painful. Is there any instance in which sterile fly larvae have been used to clean out the dead tissue from the ulcer?
28. If left untreated, how large would a person expect the lesion to get? And how long would it be likely to last until it is healed?
29. How long does it take for immunity to develop?
30. Is this disease transferrable from human to human? How do most infections occur?..human to human, environment to human, insect to human?
31. Where is *Mycobacterium ulcerans* surviving in the environment? It wasn't clear if it was in insects, water, plants, soil, etc.
32. In the Johnson et al paper, it is stated that 'aerosols arising from contaminated water may disseminate *M. ulcerans* and infect humans via the respiratory tract', is there any evidence of lesions or ulcers appearing in the lungs?
33. In both papers it is stated that living close to stagnant or slow moving water is a risk factor for the disease, has any effort been made to provide these communities with fresh water sources to better evaluate this hypothesis?
34. Are other mammals afflicted by this disease, like with the water mold pythium insidiosum that causes similar necrotic ulcers?

35. Can spread occur through human to human contact? Can children in the same household get infected from each other, especially if sharing a bed?
36. It was proposed that the bacteria are spread by the bites of water bugs, how common is it for these bugs to bite humans, or other mammals?
37. Since semi-aquatic hemiptera are thought to be involved in transmission of *M. ulcerans* have any 'land-based' hemiptera such as bed-bugs, kissing bugs (or other parasites-fleas, ticks, lice) been implicated in transmission?
38. What ultimate benefits do *M. ulcerans* derive from production of mycolactone other than increased virulence on a dead-end host? The cost of mycolactone appears high, ie. longer replication time, increased sensitivity to UV, diminished metabolic capacity, and would seemingly disadvantage the bacteria in it's aquatic environment.
39. Since the organism has adaptive properties for regional invasion and evasion of the host immune response, have therapeutic options such as local vasodilators, lipophilic topicals, anti-toxin compounds or antibody therapy been tested or developed?
40. Please explain the concept of reductive evolution and genome downsizing (for those of us with minimal background in this field). Can you recommend texts or other references which might clearly elaborate on these and other topics such as "genomic signatures".
41. Based on our previous readings of Ewald (1996), it would stand to reason that if *Naucoris cimicoides* is serving as a vector or intermediate host for *M. ulcerans*, then it would be selected to have a minimum impact on the direct survivorship of the vector. Conversely, if *N. cimicoides* is the primary host then we might expect to see significant virulent effects. Is there any information on how infection by *M. ulcerans* may affect the mortality rate of *Naucoris cimicoides*?

42. Along the same lines, if *N. cimicoides* acts as a vector for *M. ulcerans*, we'd expect selection to favor it to modify its behavior to, perhaps, increase the rate at which it bites the primary host. Conversely, if *N. cimicoides* is an intermediate host and *M. ulcerans* requires it to be predated upon by the primary host, we might expect the *M. ulcerans* to modify *N. cimicoides*' behavior to increase its susceptibility to predation. Is there any information on how infection by *M. ulcerans* may affect the behavior of *Naucoris cimicoides*?