Patterns of tuberculosis in the Americas - How can modern biomedicine inform the ancient past?

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Tuberculosis (TB) is an infectious disease that continues to take its toll on human lives. Paleopathological research indicates that it has been a significant cause of death among humans for at least five thousand years. Because of the devastating consequences to human health, social systems, and endangered primate species, TB has been the subject of many and varied research efforts throughout the world, efforts that are amassing an enormous amount of data concerning the causative agent Mycobacterium tuberculosis. *Despite sequencing of the* M. tuberculosis *genome and numerous molecular epidemiological studies, many questions remain regarding the origin, evolution, and future co-evolutionary trajectory of* M. tuberculosis *and humans. Indeed, the origin of pre-Columbian New World TB has been and remains hotly debated, and resolution of this controversy will likely only come with integration of data and theory from multiple disciplines. In this paper, we discuss the pre-Columbian TB controversy, and then use research from biological and biomedical sciences to help inform paleopathological and archaeological studies of this ubiquitous disease that plagued our ancient forbears.*

Key words: tuberculosis - Americas - Maya - anemia - paleopathology - iron

Tuberculosis (TB) is an infectious disease caused by species of the genus *Mycobacterium*. In mammals, TB is most often caused by members of the *Mycobacterium tuberculosis* complex: *M. tuberculosis*, *M. africanum,* and *M. canettii*, which are primarily pathogens of humans, and *M. microti* and *M. bovis*, which primarily affect other mammals. Immune-compromised humans can also be infected by and develop disease from the *M. avium* complex, usually a bird pathogen, as well as some environmental saprophytes such as *M. ulcerans* and *M. marinum.* Among mammals, the route of infection with the *M. tuberculosis* complex is typically via affected droplets coughed or sneezed from the lungs – these are then inhaled (or occasionally ingested) by close contacts (Myers & Steele 1969, Fanning 1994).

In the early and middle XX century, the prevailing belief was that TB evolved in the Old World, and was brought to other parts of the world following contact with European explorers and colonists. Medical doctors (Hrdlicka 1909, Morse 1961, Cockburn 1963) studying the New World archaeological record supported the idea with what they felt were key pieces of evidence against pre-Columbian New World TB. Hrdlicka and Morse, for example, emphasized the absence of anecdotal evidence of the disease from early explorers, colonists, and Native Americans themselves; the lack of TB lesions in pre-contact skeletons; and the extreme susceptibility of Native American groups to the disease upon European contact.

Cockburn further stressed that Native Americans exhibited characteristics of populations naïve to TB, underscoring the apparent high prevalence in all age groups and overall high mortality, and the tendency for individuals to exhibit glandular rather than pulmonary TB. Further, he argued that TB was a crowd disease, requiring large, settled populations or a domestic animal to serve as an origination point and reservoir for the disease, neither of which was believed to have existed.

In 1944, Rich had suggested that, because *M. bovis* has such a wide host range relative to *M. tuberculosis*, it was probably the ancestral form, with *M. tuberculosis* a natural mutant that then specialized in humans. Following this line of reasoning, Cockburn (1963) posited that TB originated in the Old World with cattle domestication – because the New World was colonized prior to the domestication of cattle in the Old World, it would have been impossible for *M. tuberculosis* to exist in the pre-Columbian New World.

While the above scenario seemed reasonable and was widely accepted, it did not fit with the biological and archaeological evidence from the New World. Pre-Columbian skeletal remains with lesions strongly resembling those diagnostic of TB have been recovered from many different sites in the Americas (García-Frías 1940, Requena 1945, Lichtor & Lichtor 1952, Ritchie 1952, Judd 1954, Crane & Griffin 1959). By the mid-XX century, accumulated evidence for pre-Columbian TB, including the discovery of TB-like lesions in pre-Columbian Peruvian mummies (Allison et al. 1973) led paleopathologists to hypothesize that *M. tuberculosis* was, after all, present in the Americas prior to contact (Buikstra 1976,1999). After assessing the evidence for pre-Columbian TB, Buikstra (1976) analyzed the arguments developed by Hrdlicka, Morse, and Cockburn, emphasizing several important points: the incomplete nature of early ethnohistoric and ethnographic accounts, the impact of antibiotic therapy upon contem-

porary clinical experience, the presence of large urban centers throughout the Americas, and the presence of potential animal reservoirs. It was further noted that Cockburn's "naïve" patterning didn't appear until groups were resettled onto reservations or repartimientos.

By the 1990s, the development of molecular techniques such as the polymerase chain reaction (Mullis & Faloona 1987) made possible the direct analysis of ancient pathogens when preservation permitted. A diagnostic, repetitive DNA fragment from the *M. tuberculosis* complex was obtained from ancient pre-Columbian individuals with TB-like lesions from sites in North and South America by several groups. The first research focused upon desiccated soft tissues (Salo et al. 1994), but this was soon followed by evidence recovered from bones (Arriaza et al. 1995, Braun et al. 1998).

Recent biomolecular studies on the genome of modern *M. tuberculosis* complex strains indicates that *M. bovis* arose from an *M. tuberculosis*-like precursor (Brosch et al. 2002, Baker et al. 2004, Gutierrez et al. 2005), and thus was not ancestral to *M. tuberculosis*. Further, if results of Rothschild et al. (2001) are valid, then ancient *M. tuberculosis* DNA was present in an extinct long-horned bison from Wyoming some 17,870 years ago.

Despite paleopathological and molecular evidence for ancient pre-Columbian tuberculosis, controversy still remains. Molecular evidence has not been accepted by all. Especially vocal critics thought that the recovered genetic material was likely contamination by modern organisms, or if authentic ancient DNA, it was from *M. bovis* rather than *M. tuberculosis*, following the viewpoint that *M. tuberculosis* initially arose from Old World cattle (Stead et al. 1995). Other criticisms focused upon the susceptibility of Native American groups after initial contact with European conquerors and colonists, hypothesizing that the "ancestral" condition for humans is susceptibility to TB (Stead 1997, 2001). In this scenario, Europeans were least susceptible to TB because they had encountered it longer than any other group. The high morbidity and mortality observed among Native Americans was due to immunological "naïveté" to the pathogen, and therefore pre-Columbian TB could not have existed in the New World:

"Some [paleopathologists] have erroneously deduced that TB as we know it today must have existed before {Columbus} got here. If they had been correct, the Indians would not have been decimated by TB when they were crowded into reservations" (Stead 2000).

Among the unstated assumptions in the above quote are two that are crucial for an understanding of TB among New World natives. The remainder of this paper focuses on these assumptions, and presents examples of ways in which integration of data and theory from fields in biology and biomedicine can provide a broader understanding of the ancient, and complicated, paleopathological record.

Factors affecting host responses to pathogens

A more thorough examination of the issue of Native American susceptibility to TB, and certainly the issue of pre-Columbian TB, should include work of evolutionary biologists and epidemiologists on host/pathogen co-evolution, immunological history of the host population, genetic diversity present in both the host and pathogen, and host dietary and nutritional factors. Examples of how these factors may influence our understanding of Native American susceptibility to TB and its meaning in regard to possible pre-Columbian TB follow.

Social and cultural disruption can increase susceptibility to infectious disease - The first assumption in the above quotation is that the social and cultural consequences of European contact and colonization to Native American communities were negligible. In fact, social disruption, forced relocation and/or concentration onto reservations or other inhospitable environments, extreme poverty, malnutrition, huge increases in population density, and exposure (often in heavy dosages) to many different pathogens, were all consequences of European contact and colonization that increased susceptibility to disease in general. Experimental research by Power et al. (1998), for example, showed that high doses of mycobacteria caused inefficient immune responses that precipitated disease. The authors speculated that "[i]t is natural to suppose that crowded living conditions will lead more often to the substantial infection required to establish disease". It is highly likely, then, that the high population densities resulting from widespread relocation and concentration of native groups was one of the most important factors in contact-era susceptibility to respiratory diseases.

The host-pathogen co-evolutionary trajectory depends on multiple factors - The second assumption embedded in the previously-cited quotation is that pathogens always advance toward benign forms, and/or that hosts always evolve immunity over time. However, several examples of pathogens that have not developed benign coexistence with humans come to mind: the influenza virus is documented historically from at least 15th century Europe, and probably long before (Kuszewski & Brydak 2000), and is still a significant cause of death in many parts of the world, even in developed nations (WHO 2003). This intracellular virus can modify its antigen structure to overcome host defenses, and annual flu cycles are lethal to some individuals, even with access to vaccination and excellent healthcare. Closer to the topic at hand, the evolution of drug-resistant strains of TB in many parts of the world where humans and *M. tuberculosis* have been in contact over time, and the emergence of extremely virulent strains of *M. tuberculosis* in some areas (for example, the Beijing variant; van Soolingen et al. 2005) also tend to belie the assumption that association over time always leads to benign commensalism. While there are many, and varied, reasons for the emergence of drug-resistance and virulence, the point is that a long period of interaction is only a single factor influencing the co-evolutionary trajectory a host and pathogen will take.

The immunological history of the host population - There are two basic types of acquired immunity in humans: the cell-mediated or T-helper 1 (Th1) response, and the antibody producing, or T-helper 2 (Th2) response

(Mosman et al. 1986). Although all healthy humans are capable of making both responses, the two tend to down regulate each other (Sander et al. 1995 and reviewed in D'Ambrosio et al. 1998). The Th1 response is that which is most effective against intracellular infections such as *M. tuberculosis* (Surcel et al. 1994) while the Th2 response is most effective against macroparasites.

Hurtado et al. (2003) have found that Native South Americans constantly encounter macroparasites in their environments. The Yanomamo, for example, show some of the world's highest levels of antibodies (Sousa et al. 1997, Hurtado et al. 2003) even when they are healthy, and this is thought to be due to the constant stimulation of the Th2 pathway by macroparasites. Perhaps more germane to an understanding of Native American responses to TB is the report from Hurtado et al. (2003) that among the Aché of Paraguay, a large number of individuals are incapable of making a delayed-type hypersensitivity (Th1-type) response to challenge with the tuberculin purified protein derivative test, even though there are high rates of TB in this population. In fact, individuals who either have active TB or who have a history of active (treated) TB are still unable to make a Th1 response, as are individuals who have *M. tuberculosis* DNA present in their mouths (and hence are exposed; Wilbur 2005). Thus, this exposed and infected group reacts as if it is immunologically "naïve" to the pathogen! This highlights the important role of the immune history of contact-era Native American populations in their reactions to TB, and severs any simple, direct link that might be made between susceptibility to TB and prior existence of the disease.

The genetic structure of the host population (see Anderson & May 1982, Ewald 1994) - When an infectious bacterium encounters a large population with appreciable variation at immune loci, potential hosts may vary widely in susceptibility and resistance to the bacterium. In this case, the probability of the pathogen encountering a new susceptible host quickly may be low. Here it is to the evolutionary advantage of the pathogen to become fairly benign, as virulence would lead to quick death of the host and an evolutionary dead end of the organism if a new susceptible is not encountered.

In a homozygous population, however, there is an abundance of available hosts. In this case, the bacterium that can infect a host, multiply rapidly, and infect many new hosts is at an evolutionary advantage – therefore, increased virulence is favored. Evidence thus far indicates that many Native American populations are genetically homogeneous at many loci (Salzano 2002, Mulligan et al. 2004), including those of the immune system (Wilbur 2005). In the wake of wars, poverty, starvation and waves of other diseases, heterozygosity was likely drastically reduced in contact-era Native Americans, leading to increased virulence among bacterial pathogens such as *M. tuberculosis*.

Diet and nutrition influence the course of disease - A final detailed example focuses on the pattern of presence and absence of New World archaeological evidence for TB, and synthesizes current epidemiological and molecular biological research with the archaeological record.

Many convincing cases of pre-Columbian miliary TB in South America and North America north of Mexico have been recorded by paleopathologists and documented with ancient DNA (reviewed in Buikstra 1999, Roberts & Buikstra 2003). By contrast, the ancient Maya present either none or a very few convincing cases of disseminated TB. Given that there were densely populated urban centers as well as long distance trade with areas of both North and South America (Buikstra 1999, Roberts & Buikstra 2003), complete absence of TB among the ancient Maya would be remarkable. Three broad hypotheses can be set forth to explain this apparent absence.

Hypothesis 1 - There was, indeed, no tuberculosis present among the ancient Maya. This could have been possible if either or both of the following were the case: (a) the ancient Maya were isolated from populations in which TB was prevalent – Given that disseminated TB is documented in the Greater Southwest by the XVII/XIX centuries A.D. (Buikstra 1999, Roberts & Buikstra 2003), its virtual absence from Mesoamerica prior to late pre-contact times remains enigmatic. There are two cases from West Mexico as early as the XI century AD, while three more from the Mexico City area date to late pre-Colonial times. Yet, large population aggregates and long distance trade have characterized northern Mesoamerica since pre-Classic times (Sharer 1994). While the patterns of interaction changed over time and trade routes altered, certainly an infectious disease such as TB should have spread throughout this region, as it was present in the Greater Southwest by Late Classic times. Population sizes were sufficient and while regionalized trading patterns developed during post-Classic times (Lorenzo 1995), it is difficult to believe that the region would have been sufficiently isolated to deter the spread of TB; (b) the ancient Maya were somehow resistant to infection and disease from *M. tuberculosis* – A large body of research throughout the world has examined host differences in susceptibility and resistance to *M. tuberculosis* in humans and other mammals (reviewed in Bellamy & Hill 1998, Wilbur 2005). Even among inbred laboratory animals, however, there is variation in susceptibility, and to date no population of humans has ever been found with complete resistance to *M. tuberculosis*. Further, modern Native American groups have shown high susceptibility to infection and disease from the organism (Sousa et al. 1997, Hurtado et al. 2003, Wilbur 2005). It is thus unlikely that the ancient Maya were resistant to *M. tuberculosis*.

Hypothesis 2 - Infection and even disseminated disease from M. tuberculosis were present among the ancient Maya, but these have not been recovered for various reasons: (a) poor preservation precludes recovery of individuals with skeletal TB – While Maya sites are notorious for poor bone preservation, it is hard to believe that Pott's disease, with its effusive bone formation, would not have been preserved somewhere in the Maya realm. As will be emphasized below, there is evidence for nutritional stress among the ancient Maya, whose thus compromised immune systems may have led to death relatively early in the course of the disease, before dissemination could occur. Other similarly compromised groups

from North America, however, present clear evidence of bone TB; (b) as yet undocumented mortuary customs resulted in differential disposal areas for individuals with skeletal TB – Individuals dying with obvious skeletal deformation could have been treated distinctively following death, perhaps through spatial isolation or bodily destructive mortuary treatments (Buikstra 1999, Roberts & Buikstra 2003). Dwarfs, for example, are artistically represented in many ancient Maya contexts, yet only one skeleton with possible dwarfism has been reported – Tikal Burial 24 – Structure 5D–33-1st (Coe 1990, Prager 2002). Even this example, with its short stature apparently reconstructed from either field drawings (115 cm) or in situ crown-heel length (125 cm, as accepted by Haviland 1967), is reported to have long legs of somewhat normal size (p. 322) relative to a kyphotic spine (Coe 1990) Dwarfism is clearly a possibility, though the impact of vertebral kyphosis upon stature estimates is unknown, as the remains are not presently available for study (Lori Wright, pers. commun., Nov. 19, 2005). Most dwarfs figured artistically by the ancient Maya appear to have suffered from achondroplasia, which is not the likely diagnosis in this example. Bautista Marínez and Romano Pacheco (2003) do, however, report an achondroplastic dwarf skeleton, recovered from a west Mexican context. Foot deformity in a burial from Tikal has also been described (Wright 2005), and individuals with spinal deformities not attributable to TB, including osteoarthritis, osteophytosis, and ankylosing spodylitis have been recovered from Maya archaeological sites (Tiesler & Cucina 2005). Thus, an apparent low recovery rate for individuals with deforming pathology lends modest support to theories emphasizing sampling bias based upon selective interment practices.

Hypothesis 3 - Tuberculosis was present among the ancient Maya, but for some reason dissemination from the lungs to the skeleton was extremely rare. This could potentially be attributable to either pathogen differences or to host differences: (a) differences in the strain of *M. tuberculosis* resulted in a different manifestation of disease – i.e., hematogenous dissemination did not occur. There is evidence from modern molecular studies that some strains of *M. tuberculosis* are more virulent than others (e.g., Dunn & North 1995). While it is possible that a more-virulent strain that killed individuals quickly left no bone lesions, it is uncertain how biologically plausible this is. However, even if different strains of *M. tuberculosis* were present among the ancient Maya than among North and South American natives, it is expected that with trade, the more virulent strain would have moved into any new population contacted. Thus, even if Maya had a more virulent strain that led to earlier death, and no dissemination, it is expected that upon contact with North and South Americans the more virulent strain would have been transmitted to the traders. Assuming that the traders then returned to their people and transmitted the new virulent strain, the archaeological record should a lack of TB in North and South American populations who previously showed disseminated TB.

Assuming high virulence, there would be a trend of this absence of TB as temporal and geographic distance from Maya trade routes decrease. Although temporal regional control of the archarological record is difficult, what is certain is that once introduced into the Greater Southwest, a disseminated form of TB persisted throughout the pre-Colonial period (Roberts & Buikstra 2003); (b) differences in the Maya hosts themselves resulted in a different manifestation of disease – This could have taken the form of reduced infection and disease, which were already discussed above in terms of host genetics. One might posit cultural practices that prevented infection with *M. tuberculosis* despite exposure to the organism, but such mechanisms have eluded modern public health officials for decades, despite attempts to find and implement them; (c) exposure to other pathogens in the Maya environment somehow rendered the population immune to disseminated TB – If, for example, the ancient Maya typically encountered other pathogens that encouraged a Th1 response, such would have helped them to successfully fight off TB infection and disease. However, this has not been observed at a population-wide level anywhere in the modern world to our knowledge, and in any case, at least among modern Maya, pathogens encouraging both Th1 (e.g., species of the genus *Leishmania*) and Th2 (e.g., helminths) types of immune responses are known to be endemic (Whittington 1989); (d) diet/nutrition influenced host response to exposure and infection with *M. tuberculosis* – Published sources related to diet and nutrition define two general patterns for ancient Maya. First is the widespread evidence of heavy reliance on maize consumption (Whittington 1989, Wright 1994, Chase 1997, Massey & Steele 1997, Saul & Saul 1997, Whittington & Reed 1997). The second is the apparent porotic hyperostosis and cribra orbitalia in large numbers of individuals at the sites of Corozal, Caracol, and Tayasal (Chase 1997), Cuello (Saul & Saul 1997), Colha (Massey & Steele 1997), Copán (Whittington & Reed 1997, Whittington 1989). Maya researchers have attributed the porotic hyperostosis and *cribra orbitalia* to iron-deficiency anemia secondary to an extraordinary reliance upon maize, which is iron-poor (see, for example, Whittington 1989). These two lines of evidence suggest a potential role of iron-deficiency in limiting hematogenous dissemination of TB among ancient Maya.

A review of modern experimental and epidemiological studies demonstrates the necessity of adequate serum and tissue iron for development of TB. Like most microbial pathogens, mycobacteria require iron for their existence and growth. In the case of *M. tuberculosis*, for example, limitation of iron limits growth in vitro (McReady & Ratledge 1978, Lundrigan et al. 1997). As might be expected, one line of defense that mammals have evolved against potential microbial invaders is a system to sequester iron and limit pathogen access. Host proteins such as transferrin and lactoferrin chelate extracellular iron, while intracellular iron can be stored by the protein ferratin (Finklestein et al. 1983). Upon infection, serum iron shifts to immune cells in the reticuloendothelial system (Finklestein et al. 1983, Olakanmi et al. 2002), and assimilation of dietary iron is also restricted (Ratledge 2004).

In their turn, obligate pathogens such as *M. tuberculosis* have evolved their own chelators (called side-

rophores) to compete with (or even remove iron from) the host's iron-binding proteins (Gobin & Horwitz 1996). Such competition is of great importance for both host and pathogen, as "[i]ron availability...and the bacterium's efforts to circumvent ...[its]... restriction are critical determinants of the outcome of infection with *M. tuberculosis*" (De Voss et al. 1999). Ratledge (2004) reviews experimental research on mycobacterial siderophores. In vitro, the siderophore mycobactin is produced in response to iron deficiency. Laboratory research shows that mycobactin synthesis reaches a maximum when the culture medium is iron-deficient. It is suggested that in host tissues in vivo, extracellular siderophores acquire iron from the host and transmit it to mycobactin. When the mycobacterium receives iron, it signals the repression of mycobactin synthesis, so the level of mycobactin likely remains low when iron is sufficient. The question, then, is to what extent iron deficiency in the host is necessary before synthesis of siderophores begins. Further, if hosts have very little tissue iron, there will be little iron available to the mycobacterium regardless of siderophore synthesis. Ratledge (2004) cautions against food or supplements rich in iron for TB patients: "Mild anaemia is of positive benefit to the patient particularly in the early stages of the disease as this prevents the mycobacteria gaining the iron they need for growth" (p. 124).

Several studies have documented the role of host iron stores in the outcome of infection with mycobacteria. Iron-overload in a certain strain of lab mouse was found to be "an exacerbating cofactor for tuberculosis" (Schaible et al. 2002). Multiplication of *M. tuberculosis* in mouse lungs and spleens was shown by Lounis et al. (2001) to be enhanced when iron was administered, while the number of bacilli was reduced in controls. Resistance to another pathogenic mycobacterium, *M. avium,* is also decreased in the presence of excess iron (De Voss et al. 1999). For example, mice fed iron-rich diets developed disease more rapidly than control mice, and had a higher *M. avium* bacterial load than those eating a regular diet (Dhople et al. 1996). Further, when iron-supplementation was combined with beef fat, the mice developed disseminated disease faster with smaller doses of *M. avium*. In humans, *M. avium* is a typical opportunistic pathogen of the immune-compromised, and experiments have shown that the rate of *M. avium* complex replication in human immune cells directly correlates with iron concentration (reviewed in De Voss et al. 1999).

Given the importance of iron availability to the outcome of infection with mycobacteria, it is not surprising that pulmonary TB patients are typically anemic (Morris et al. 1989), and it has been suggested that the anemia of infectious and chronic disease is an immune defense mechanism (Kent et al. 1994). In fact, Murray et al. (1978) point out that Armand Trousseau understood the importance of iron availability in TB patients as early as 1868, and in 1872 he warned against iron-supplementation for recovering patients, as this had been observed to cause a relapse of active disease.

In 1978, Murray and colleagues examined the effects of iron supplementation on the incidence of various types of infections. Iron-deficient Somali nomads were either given a course of ferrous sulfate or a placebo for 30 days. More than five times as many iron-supplemented individuals developed infections (including three cases of glandular TB or tubercular mastoiditis) compared to controls. The authors showed that infections in the iron-supplemented individuals were almost certainly due to reactivation of latent infection, and reactivation peaked as individuals neared normal iron status.

The effects of high levels of dietary iron were also examined in an autopsy series of black South Africans by Gordeuk et al. (1996). Individuals with high levels of tissue iron were found to be 17 times more likely to have died from TB than those with negligible iron, and there was a dose-response effect of iron level and risk of death by TB. When dietary iron intake was examined in another African group (Gangaidzo et al. 2001), elevated levels were found to increase the risk of active pulmonary TB, as high levels impair the ability of macrophages to suppress growth.

Macrophages are immune cells that are important first responders to infection with *M. tuberculosis*. Their role is to engulf, digest and present portions of foreign pathogens that they encounter. However, *M. tuberculosis* has developed the ability to live inside macrophages, although their growth inside and outside macrophages can be regulated by iron. Olakanmi et al. (2002) showed that *M. tuberculosis* requires iron for growth and multiplication within macrophages, and it can acquire this iron either from within the macrophage if it was preloaded prior to the infection, or from outside the cell from the host's transferrin. Further experiments (Serafin-Lopez et al. 2004) showed that iron regulates bacterial growth within macrophages as well as stimulating *M. tuberculosis* growth outside the macrophages. In mouse-macrophages, iron is crucial for *M. avium*'s survival. When infected cells were supplemented with iron, *M. avium* was able to inhibit the normal process of phagosome maturation by which macrophages digest (and thus kill) invading organisms (Kelley & Schorey 2003).

Thus it appears that, if host iron is not limited, *M. tuberculosis* can multiply in macrophages, which may eventually burst and allow bacilli into extracellular space, where they may disseminate. This led us to wonder what happens if an individual is anemic and is exposed to *M. tuberculosis*. Is it possible, given the importance of iron for growth of the organisms in the host, that infection will remain latent? Or if disease does develop, will lack of iron tend to limit dissemination due to reduced growth of bacteria? We are currently pursuing this line of questioning, also considering an additional portion of the immune system.

The human complement cascade is a component of innate immunity with multiple functions, including the lysis of foreign cells and the promotion of phagocytosis of antigens and bacterial cells. Upon encountering evidence for infection of cells, a cascade is triggered that culminates with a "Membrane Attack Complex" that creates a pore in the affected cell, penetrating the membrane and bursting the cell. While this works well on some types of infections, the consequences of bursting a cell filled with multiplying *M. tuberculosis* may be to disseminate the organism around the body.

Experiments have shown that mycobacteria can use the host's complement system to gain entry to macrophages, and Schorey et al. (1997) demonstrated a novel mechanism that only the pathogenic mycobacteria have evolved in order to use the complement system as a means of entering macrophages. We conjectured that it may be possible, as well, that such stimulation of the complement system by pathogenic mycobacteria could also stimulate cell lysis, potentially freeing mycobacteria from infected macrophages and allowing their dissemination throughout the body. The mice experimentally infected with *M. avium* (Dhople et al. 1996), for example, did indeed develop disseminated disease quickly with a very low dose of inoculum when fed extra iron. We underscore, however, that the mechanism by which dissemination occurs is unknown, and therefore our observations concerning the complement system are, at this point, merely speculation. Still, this discussion highlights areas in which future research may shed light upon the archaeological record.

The examples presented in this paper are from areas in which our current research is focusing, in addition to an ongoing ancient DNA component that explores the phylogeny of New World TB in relationship to current reinterpretations of Old World sequences. Our research program seeks to incorporate data and theories from other disciplines, focusing on acquisition of new data as well as examination of that already available in light of recent advances. In this way, we hope to understand past cycles of disease, and to help predict the forms that may be taken by future ones.

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