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Evolutionary changes in the genome of *Mycobacterium tuberculosis* and the human genome from 9000 years BP until modern times

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SUMMARY

The demonstration of *Mycobacterium tuberculosis* DNA in ancient skeletons gives researchers an insight into its evolution. Findings of the last two decades sketched the biological relationships between the various species of tubercle bacilli, the time scale involved, their possible origin and dispersal. This paper includes the available evidence and on-going research. In the submerged Eastern Mediterranean Neolithic village of Atlit Yam (9000 BP), a human lineage of *M. tuberculosis*, defined by the TbD1 deletion in its genome, was demonstrated. An infected infant at the site provides an example of active tuberculosis in a human with a naïve immune system. Over 4000 years later tuberculosis was found in Jericho. Urbanization increases population density encouraging *M. tuberculosis*/human co-evolution. As susceptible humans die of tuberculosis, survivors develop genetic resistance to disease. Thus in 18th century Hungarian mummies from Vác, 65% were positive for tuberculosis yet a 95-year-old woman had clearly survived a childhood Ghon lesion.

Whole genome studies are in progress, to detect changes over the millennia both in bacterial virulence and also host susceptibility/resistance genes that determine the NRAMP protein and Killer Cell Immunoglobulin-like Receptors (KIRs). This paper surveys present evidence and includes initial findings.

1. Introduction

Microbial infections played a key role in shaping life on earth and have been a major selector for the evolution of all present species. Evidence exists that demonstrate infectious diseases were already present in our remote ancestors [1,2]. Considering the impact of *Mycobacterium tuberculosis* (MTB), in all probability it has had a greater influence on the genetic selection of the *Homo sapiens* population than any other infectious agent.

The molecular identification of human pathogens in ancient human remains has recently opened new scientific fields that provide considerable insight into the history and evolution of host, pathogen and their interaction. This allows us to track changes in the ancestral tubercle bacillus as it became more and more exposed to the internal environment and immune system of its human host. Conversely, it is possible to track changes in the genes of the human population that confer resistance or susceptibility to disease over time.

TB is related to population density [3], transmitted from human to human living in close contact. However, the origin of the disease,

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the earliest hosts of MTB and its evolution remain unclear. The evolution of the bacteria cannot be considered in isolation. It is important to realise how TB has influenced the human development over the millennia, particularly our resistance/susceptibility genes Figure 1. MTB experienced an evolutionary bottleneck when it became an obligate pathogen and has a clonal relationship with different human lineages [4]. Subsequent co-evolution has resulted in the majority of TB infections being latent. In past eras of low human population density, MTB adapted over time in response to host-adaptive changes and *vice versa*. This process, which can be defined as mutualism, is a biological interaction between individuals of two different species where both individuals derive a fitness benefit. As the host becomes more resistant, strains better able to colonise the resistant host will predominate, thus starting off another cycle. More virulent MTB strains will attack their human host, killing the most susceptible and leaving the more resistant as survivors. However, when human populations were sparse, this could break the chain of transmission of the pathogen. The development of antibiotics has shortened the mutualistic cycle significantly, but the combination of HIV co-infection, antimicrobial therapy and increased global human population density is leading to the emergence of some MTB strains that are both more transmissible but also more virulent [5].

2. The impact of palaeomicrobiological investigations of archaeological human material

2.1. Questions to be addressed

Archaeologists should seek to correlate research questions with historical events. For example, did past invasions introduce new pathogens, or more virulent strains of pathogens into susceptible populations? Thousands of indigenous peoples in the Americas died from exposure to European strains of MTB, measles and smallpox [6]. Another possible scenario is that invaders may have brought new pathogens with them on return to their place of origin. A good example of this is the introduction by European colonialists of venereal syphilis from South America.

A further question one has to ask is what was the genetic status of *Homo erectus* or predecessor species regarding the underlying genetic basis of host resistance and susceptibility to tuberculosis. Did ancestral hominids have the precursors of modern host susceptibility/resistance genes or were these acquired late? Is the 'Out of Africa' theory of the origin of human TB proposed by Gutierrez et al. [7] capable of being verified by a study of human remains, or will these show that TB developed in several areas and that this is the explanation for the variability of the organism in different geographical areas?

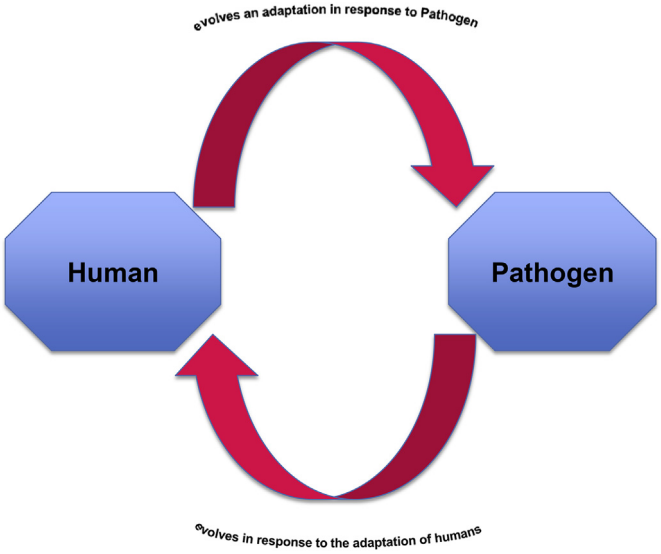

Figure 1. Co-evolution between human and pathogens. Evolution of one species in response to characteristics of another.

The majority of TB patients in the world today never progress to active disease. The World Health Organisation estimates that approximately one-third of the global population is infected but only 10% of immunocompetent people progress to active disease during their lifetime [8]. Our current immunity may be the result of Darwinian selection only, or may depend upon whether particular genes are switched on or off – a mechanism that can result in rapid adaptation. It must be remembered that other non-genetic factors influence human susceptibility to infection such as dietary deficiencies, stress and trauma [9]. Long-term climatic changes have an impact on vegetation and agriculture [10] whereas local variations in climate may influence transmission of MTB by infectious aerosols. Temperature changes will determine whether humans spend more time in the open air or enclosed spaces, for example.

2.2. Significant findings

With the first reported finding of MTB DNA in ancient skeletons based on amplification of a small (123 bp) DNA target that was specific for the MTB-complex [11] a new era of research into microbial pathogen evolution became possible. In addition to skeletal remains, calcified and mummified tissues also proved to be good sources of MTB ancient DNA (aDNA)[Mic] [12]. Our knowledge was enhanced with the finding of MTB in a 17000-year-old Pleistocene bison from Natural Trap Cave, Wyoming [13]. Spoligotyping revealed that the Pleistocene bison lesions contained aDNA from the *M. tuberculosis* complex, possibly MTB *or Mycobacterium africanum*, but distinct from *Mycobacterium bovis*. The consensus bison spoligotyping pattern was compared with the combined database collated by the National Institute of Public Health and Environment (RIVM), Utrecht, The Netherlands and the Veterinary Science Division, Department of Agriculture and Rural Development, Belfast, N. Ireland. No exact matches were found on the database. However, in a computer analysis comparing a library of defined species, the highest similarity was from *M. africanum* (82.3%), then *M. tuberculosis* – MTB (76.6%), with *M. bovis* having only 72.7% similarity.

The original aDNA findings in the Pleistocene bison were confirmed ten years later by finding species-specific MTB cell wall lipid biomarkers [14]. We have used this method of independent confirmation of our MTB aDNA findings since 1998 [15] because lipid analysis uses methods based on the direct detection of femtogram quantities of target molecules, with no need for any amplification. This is a more rigorous method of independent confirmation than sending part of the specimen to another laboratory for analysis.

The Pleistocene bison contained MTB-complex aDNA but the particular lineage has not yet been identified. The earliest known human MTB was detected and characterised in samples from the submerged Neolithic site of Atlit Yam, a 9000-year-old settlement submerged in the sea off the coast of Haifa in Israel [16]. The findings were confirmed by lipid analysis and the preservation was sufficiently good that it was possible to confirm that the MTB had experienced the TbD1 deletion, found only in human lineages. This is of particular significance as this was a Pre-Pottery site with the earliest evidence of animal domestication in the Levant.