Meeting report

Mycobacteria: from genomes to disease control

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Published: 21 November 2001

Genome ***Biology*** 2001, **2(12)**:reports4032.1–4032.3

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2001/2/12/reports/4032

A report on the symposium 'Mycobacteria - new developments' at the 149th meeting of Society for General Microbiology, University of East Anglia, Norwich, UK, 10-13 September 2001.

The growing burden of mycobacterial diseases

Amongst mycobacteria are some of man's most potent microbiological adversaries. The incidence of mycobacterial diseases, such as tuberculosis, has recently increased and this, together with the completion of several mycobacterial genomes, has put mycobacteria back in the spotlight. The importance of this group of bacteria was highlighted with a special symposium on new developments in mycobacterial research at the 149th meeting of the Society for General Microbiology.

Mycobacterium tuberculosis, which causes tuberculosis in man, is a phenomenally successful pathogen, infecting a large proportion of the world's population, killing up to 3 million people a year and causing suffering in many millions more. Paul Fine and Neil Stoker (London School of Tropical Medicine and Hygiene, UK) reviewed the epidemiology of tuberculosis, emphasizing the importance of environmental and socio-economic factors. Tuberculosis acts as a social barometer: its incidence has declined steadily in the industrialized world over the last two centuries, reflecting decreased crowding, improved nutrition and hygiene, and better public health infrastructure. Such improvements have not been seen in many parts of the world, and increasing levels of tuberculosis in developing countries have been fueled by co-infection with the human immunodeficiency virus (HIV). The emergence of multi-drug-resistant strains of *M. tuberculosis*, particularly in countries where healthcare infrastructure has broken down, and variable efficacy of bacillus Calmette-Guerin (BCG), the vaccine used against tuberculosis and estimated to be the most widely used vaccine against any bacterial disease, have each contributed to the desire to explore new approaches to the vaccination, treatment and control of tuberculosis.

M. tuberculosis is not the only mycobacterium that is creating major problems. Recently identified species of mycobacteria, such as *Mycobacterium malmoense*, are increasingly being isolated, even from immunocompetent individuals, as discussed by Mike Goodfellow (University of Newcastle upon Tyne, UK). *Mycobacterium avium* and other 'opportunistic' mycobacteria are important causes of death and disease in immunocompromized patients, including those with HIV, and many millions of people still suffer the consequences of infection by the leprosy bacillus, *Mycobacterium leprae*.

Man is not the only victim of mycobacterial infection. Richard Clifton-Hadley (The Veterinary Laboratory Agency, Weybridge, UK) emphasized the importance of another close relative of the tubercle bacillus, *Mycobacterium bovis*, which has a much wider host range than *M. tuberculosis*, infecting many wildlife species in addition to cattle. Although the risk of human infection with *M. bovis* has decreased since the 1930s because of pasteurization of milk and meat inspection, there has been a dramatic increase in recent years in infection in UK cattle, and an association has been suggested between the distribution of *M. bovis* infection in cattle and in badgers, thought to be the major wildlife reservoir of *M. bovis* in the UK.

Mycobacterium paratuberculosis is another important pathogen of animals, and in man it has been linked to Crohn's disease, a chronic inflammation of the bowel, as discussed by Mike Sharp (Moredun Research Institute, Edinburgh, UK). This organism, a member of the closely related group of species referred to as the *M. avium* complex, causes chronic granulomatous enteritis in ruminants. It has now been found to infect a more extensive host range, including primates. In Scotland, rabbits have been found to be infected with *M. paratuberculosis*, raising the possibility that infection is passed on through an extensive food chain.

Mining the genomes of mycobacteria

Having established the medical and economic importance of mycobacteria, the main focus of the symposium was the remarkable advances that are being made in understanding the molecular and cellular basis of mycobacterial infection. Several mycobacterial genomes have now been sequenced, and results from comparative genomics of closely related mycobacterial species were reviewed by Stewart Cole (Institut Pasteur, Paris, France). The *M. tuberculosis* genome was the first to be sequenced and revealed an organism with broad metabolic potential: most anabolic pathways are present and it has many genes involved in lipid synthesis and metabolism. *M. tuberculosis* has complex gene regulatory pathways with 13 sigma factors, 11 two-component regulatory systems and many genes involved in transcriptional regulation. By contrast, the *M. leprae* genome is highly degenerate: many genes have been lost altogether or reduced by mutation to pseudogenes. This may provide clues as to why *M. leprae* grows so slowly and cannot be grown *in vitro;* the genome sequence suggests that *M. leprae* is likely to be recombination-deficient, less lipolytic than *M. tuberculosis*, unable to access intracellular iron, and deficient in energy production.

The almost complete *M. bovis* sequence reveals even more intriguing insights into the evolution of mycobacterial pathogenesis. It was previously thought that *M. tuberculosis* evolved recently from *M. bovis*, at the time when man began to develop an agricultural way of life. Deletions in the genome of *M. bovis* suggest, however, that *M. tuberculosis* is in fact 'older' than *M. bovis,* with the intriguing and counter-intuitive possibility that the latter has evolved by losing genes while simultaneously increasing the range of species that it can infect.

Comparisons across different *M. tuberculosis* strains were described by Dick van Soolingen (National Institute for Public Health and the Environment, Bilthoven, The Netherlands) who described the use of molecular typing techniques to analyze changes in strain distribution in recent years. Van Soolingen reported the emergence of a widely distributed genotype of *M. tuberculosis*, referred to as the 'Beijing' strain. Such changes, in which a dominant strain emerges, may reflect the selection pressures applied by the host's immune response; it is thought that the Beijing strain may be less immunogenic and hence better equipped to evade host immunity.

The challenge of the mycobacterial post-genomic era is now to understand gene regulation in a relevant biological context. Mycobacteria can survive and grow within host cells, particularly macrophages. Understanding how this is achieved is the key to understanding the pathogenesis of mycobacterial diseases. Lawrence Wayne (Veterans Administration Hospital, Long Beach, USA) has investigated *in vitro* models that might be useful in studying the ability of *M. tuberculosis* to survive inside host tissue for long periods of time without increasing in numbers, but still retaining the ability to resume replication when conditions become favorable. Oxygen depletion commonly occurs in necrotic and inflammatory tissues; an *in vitro* model of this situation, in which oxygen is slowly depleted, has been developed. The shift to hypoxic conditions results in a series of metabolic changes, such as the utilization of alternative energy sources, thickening of the cell wall, and overexpression of the α-crystallin-like protein (which is thought to contribute to the stabilization of other proteins in time of environmental stress), all of which might help long-term survival.

In order to identify genes involved in virulence, Brigitte Giguel's group (Institut Pasteur, Paris, France) have used a signature-tagged transposon mutagenesis assay. Mutants that failed to survive following inoculation into mice could be identified using this system. Several of the genes identified in this way were found to be involved in lipid biosynthesis or lipid transport, emphasizing the importance of lipids and the lipid-rich cell envelope in mycobacterial virulence. Other approaches used by this and other groups, such as Neil Stoker's, Douglas Young's and my own group, have involved specific deletion of genes by homologous recombination. Again, genes involved in virulence, such as those encoding PhoP/PhoR, a two-component system, OmpATb, a membrane protein that is involved in permeability and protection against acidic environments, and DevR, a two-component regulator, have been identified.

Large-scale analyses of proteins and genes expressed in *M. tuberculosis* bacilli isolated from infected tissues or from infected macrophage cultures were described by Philip Butcher (St. George's Hospital Medical School, London, UK). Proteomic approaches, involving two-dimensional polyacrylamide gel electrophoresis and mass spectrometry, were used to identify proteins found in bacteria isolated from infected tissues. In order to investigate gene expression at the mRNA level, a microarray has been developed that allows simultaneous assessment of the expression level of virtually all of the *M. tuberculosis* open reading frames. Although this work has largely involved establishing the techniques, genes such as *nadC*, encoding a nicotinate-nucleotide pyrophosphatase, and *sucD,* encoding succinyl-CoA synthetase, have been found to be upregulated in bacteria isolated from mouse lungs. Similar techniques are being used by Ken Duncan (GlaxoSmithKline Ltd, Stevenage, UK) to identify genes that are upregulated in *M. tuberculosis* during nutrient starvation, with a view to identifying potential new drug targets. Again, the α-crystallin-like protein was found to be upregulated along with two genes of unknown function as well as the *sigF* (stationary phase sigma factor) operon, upregulation of which is consistent with activation of the stationary phase in response to starvation.

It takes two: the response of the host cell

The interaction between mycobacteria and host cells initiates a complex series of changes in gene expression in both the bacteria and the host. I described the changes in macrophage gene expression following infection with *M. tuberculosis*. The major change following contact between bacteria and macrophages is a rapid turning on of macrophage genes involved in cell migration and recruitment. Activation of macrophages is accompanied by acidification of the bacteria-containing vacuoles. Genes involved in protecting the bacillus against this acidic environment, such as *ompATb*, appear to enable the bacteria to multiply or survive within the acidified vacuole.

Ian Orme (Colorado State University, Fort Collins, USA) described the use of animal models to investigate both innate and acquired immunity to *M. tuberculosis*. Host genes involved in the early, innate immune response, such as those encoding chemokines and bacterial receptors, may have a transient effect on early bacterial growth in the lungs, but the immune reaction is then supported by genes that are important for acquired immunity, such as those encoding nitric oxide synthase and interferon-γ, both of which are involved in antimicrobial functions of macrophages. The development of novel approaches to vaccine development involving subunits, fusion proteins and avirulent mutants was also described by Orme.

The remarkable developments in studying the molecular biology of mycobacteria that have taken place over the last decade are providing a timely opportunity now to apply post-genomic approaches to the development of new strategies for controlling diseases caused by mycobacteria, the most extensively sequenced of all groups of bacteria. Techniques for specific gene deletion, analysis of global gene expression and investigation of host-parasite interactions at the cellular and molecular level are also shedding light on the evolution of these important pathogens. The challenge for the future is to channel fundamental insights into the organism's biology towards the development of new intervention strategies for the control of disease.