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Veterinary Microbiology 112 (2006) 127–139
veterinary microbiology
www.elsevier.com/locate/vetmic

Recent advances in our knowledge of *Mycobacterium bovis*: A feeling for the organism☆

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Abstract

Significant and rapid progress has been made in our knowledge and understanding of *Mycobacterium bovis* since the last international *M. bovis* conference 5 years ago. Much of this progress has been underpinned by the completion of the genome sequence. This important milestone has catalysed research into the development of a number of improved tools with which to combat bovine tuberculosis. In this article we will review recent progress made in the development of these tools and in our understanding of the organism, its evolution and spread. Comparison of the genome sequence with those of other members of the *Mycobacterium tuberculosis* complex has enabled insights into the evolution of *M. bovis*. This analysis also indicates that the *M. tuberculosis* complex have the propensity to adapt to new host species. The use of high throughput molecular typing methods has revealed that the recent bovine tuberculosis epidemic in Great Britain is being driven by a number of clonal expansions, which cannot be explained by random mutation and drift alone. Completion of a number of mycobacterial genome sequences has allowed the development of antigen mining techniques that rapidly identify *M. bovis*-specific genes. These can then be used as reagents in the gamma interferon assay to increase the specificity of the assay and also to discriminate between Bacillus of Calmette and Guérin (BCG) vaccinated animals and those infected with *M. bovis*. In the longer term, comparisons between the genomes of *M. bovis* and BCG will allow insight into how BCG became attenuated following serial passage on artificial growth media and reveal clues into how to improve the vaccine efficacy of BCG.

Keywords: *Mycobacterium bovis*; Bovine tuberculosis; BCG; Genome; Evolution; Clonal expansion; Vaccination; Diagnosis; Antigen mining; Tuberculin test

1. Introduction

Considerable advances have been made in our knowledge of *Mycobacterium bovis* since the last International Conference on *M. bovis* held in Cambridge, UK at the turn of the millennium. The aim of this article is to review progress made over the past 5 years and to focus on work that has been

☆ "In the future attention undoubtedly will be centered on the genome, and with greater appreciation of its significance as a highly sensitive organ of the cell". Barbara McClintock

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0378-1135/$ – see front matter.
doi:10.1016/j.vetmic.2005.11.050

underpinned by the completion of the *M. bovis* genome sequence.

2. The genome sequence

One of the key advances over the last 5 years in our understanding of *M. bovis* has been the elucidation of the complete genome sequence of the pathogen. The genome contains ~4000 genes, revealing every potential virulence factor and antigen; the goal now is to identify which of the 4000 genes encodes these properties! The availability of the genome sequence of *Mycobacterium tuberculosis* allows us to perform comparative analyses that are providing insight into some of the key differences between the human and bovine bacillus (Cole et al., 1998; Garnier et al., 2003). Added to this, the genome sequence of *M. bovis* BCG Pasteur has been recently completed (http://www.sanger.ac.uk), a data set that will help focus research on both improving the Bacillus of Calmette and Guérin (BCG) vaccine and also in determining the genetic basis for its attenuation.

The genome publication has already described in depth our initial analyses of the *M. bovis* sequence, and it is not our intention to go over this again in this paper. Instead we will focus on some of the key phenotypes of *M. bovis* and how the genome sequence is allowing us to determine the genetic basis for these features.

2.1. Antigenic variation

The cell wall is the interface between the bacillus and host; hence in many pathogens genes encoding cell wall structures show the greatest degree of variation due to selection. Therefore, it is not surprising that the greatest degree of sequence variation between the human and bovine bacilli is in genes encoding cell wall and secreted proteins.

The most striking variation in the expression of antigens between the human and bovine bacilli is the elevated expression of two serodominant antigens, MPB70 and MPB83, in the bovine bacillus. MPB83 is a glycosylated cell wall-associated protein, while MPB70 is a secreted protein that can account for 10% of *M. bovis* culture filtrate proteins (Hewinson et al., 1996). As a route to determine its function, the structure of MPB70 has been determined by nuclear magnetic resonance (NMR) spectroscopy. These studies revealed that MPB70 shows structural similarity to eukaryotic intracellular matrix proteins that are involved in interactions between the cell membrane and the extracellular *milieu* (Carr et al., 2003). The solvent-exposed surfaces are the regions where MPB70 and MPB83 show greatest variation, suggesting that they have evolved to play functionally distinct roles. Hence, while the precise function of these proteins is still unknown, the striking difference in expression and putative function in interaction with the host suggest that they might play a role in determining host preference. It is also noteworthy that substrains of BCG show variation in expression of MPB83 and MPB70, with for example, BCG Russia producing high levels of both antigens whereas BCG Pasteur is a low producer (Wiker et al., 1996). The genetic basis for this difference in expression has recently been shown to be due to a mutation in some BCG strains in the positive regulator of *mpb83/mpb70* (Charlet et al., 2005). However, it is unclear whether the difference in expression of such key antigens has any impact on the protective efficacy of different BCG strains against *M. bovis* challenge.

A group of known antigens affected by deletions from *M. bovis* is the ESAT-6 family. The ESAT-6 protein was originally described as a potent T-cell antigen secreted by *M. tuberculosis*, and belongs to a >20-membered family that contains other T-cell antigens such as CFP-10 and CFP-7 (Brodin et al., 2004). The hallmark member of the family, ESAT-6, is part of the RD1 deletion that led to the attenuation of *M. bovis* BCG, a *locus* that also encodes CFP10, another ESAT-6 family member and T-cell antigen. Six ESAT-6 proteins, encoded by Rv2346c, Rv2347c, Rv3619c, Rv3620c, Rv3890c (Mb3919c) and Rv3905c (Mb3935c) in *M. tuberculosis*, are missing or altered in *M. bovis*. The consequences of their loss are difficult to predict, although they may impact on antigen load either singly or in combination.

Lipid differences are also seen between *M. tuberculosis* and *M. bovis*, with one of the key variations being the production of a phenolic glycolipid (PGL) in *M. bovis*. It has been demonstrated that *pks1* codes for the biosynthesis of PGL in *M. bovis*, while in many *M. tuberculosis* strains the gene is disrupted so no PGL is produced (Constant et al., 2002). Some