CHAPTER 5.8

'MYCOBACTERIUM HABANA' AS A LIVE VACCINE CANDIDATE AGAINST TB

Iliana Valdés, Lilian Mederos, Miguel Echemendía, José Antonio Valdivia†, Ernesto Montoro

‘Many ills are derived from wealth, not from poverty: from the unjust distribution of the wealth of our world.’
Alied Bencomo Alerm

‘I guarded the light, and it extinguished itself in the darkness.’

The Ant
Dulce María Loynaz

From the series Letters from the Inxilio
The Shared Misery
Cirenaica Moreira
Photography white and black
INTRODUCTION

Five decades of TB control programs using potentially efficacious drugs have failed to reduce infection prevalence by MTB. Factors such as poverty, overcrowding, wars and breakdown of national infrastructures contribute to TB spread (1–3) showing about a third of the world’s population infected, leading to clinical active disease in approximately 9.3 million people and 1.8 million deaths every year (4).

The live attenuated BCG vaccine was developed more than 80 years ago and is still the only currently available vaccine against TB (4). Vaccine BCG in its current presentation affords variable protection which usually wanes with aging. Various reasons have been cited to explain the discrepancies in the efficacy of BCG, including generic differences in the BCG vaccine strains used in immunization program throughout the world (5).

The low efficacy of BCG vaccine has promoted the search for novel vaccines for TB using strategies aimed to the completely replacing the existing vaccine or improving the current BCG vaccine taking into account the safe history and low cost of this vaccination (6,7).

Despite previous reluctance, an expert vaccine group meeting has strongly advocated development of viable vaccines against TB. They are the most potent stimulators of protective immune response taking advantages of the large spectrum of expressed mycobacterial antigens and the ability to stimulate a combination of different T cell populations (8). New viable attenuated vaccine candidates are considered as a replacement for the existing vaccine BCG. Hence, they have to perform better than BCG regarding to protective efficacy, safety or both (8, 9).

Many alternative of mycobacterial members have been explored as putative TB vaccines. Live candidates include less virulent, naturally attenuated mycobacteria or auxotrophic mutants of MTB. Naturally attenuated *M. vaccae* and *M. microtti* have been suggested, but animal experiments showed variability in their protective
efficacy. On the other hand, recombinant *M. vaccae* and *M. smegmatis* that express MTB epitopes have also been reported. However, none of them performed better than BCG in experimental models (7).

‘*M. habana*’ was first isolated in Cuba by Valdivia et al. from human suffering from lung disease and proposed as a new species within the genus *Mycobacterium* (10, 11). However, biochemical and serological analyses (12, 13) led to it being considered synonymous with *M. simiae* serotype I, some serological differences between both microorganism remained without satisfactory explanation and the name ‘*M. habana*’ has been maintained in the literature for strains originally isolated in Cuba (14).

Different approaches had been conducted to demonstrate de vaccine potential of ‘*M. habana*’ (Figure 5.8.1). Experimental evidences suggest that vaccination with ‘*M. habana*’ TMC-5135 strain confers protection against the infection by MTB, *Mycobacterium leprae*, *Mycobacterium ulcerans* and *Leishmania donovani*, using animal models (15–19).

**Figure 5.8.1** Approaches using experimental models to study vaccine potential of ‘*M. habana*’ (Numbers in parenthesis belong to the Reference in which each strategy was used)
IMMUNOGENIC POTENTIAL OF ‘M. HABANA’ IN THE EXPERIMENTAL TUBERCULOSIS

First report concerning ‘M. habana’ protection against TB was done by Gupta et al. in 1979. This research group demonstrated the potentiality of ‘M. habana’ TMC-5135 among 19 different mycobacteria species, using murine experimental models of TB. Interestingly, they demonstrated the ability of ‘M. habana’ to protect vaccinated mice against experimental challenge with MTB H37Rv; detecting survival values 20% higher in comparison with BCG vaccination, under same experimental conditions (15).

Conversely with serologic relatedness proven by Meissner & Schroeder between ‘M. habana’ and M. simiae serotype I (12), immunogenicity experiments evidenced that the latest strain does not confer protection against experimental TB infection (15). Probably different outcomes were associated with distinctive antigenic characteristics between both strains. On this way, works carried out in the IPK by Mederos et al. (20–22), found differences in glicopeptidolipids (Table 5.8.1) and mycolic acids (Table 5.8.2) which may be related with the immunogenic ones. This hypothesis is supported by the demonstration that MTB strains with mutations at mycolic acids level show differences in pathogenic and immunogenic characteristics in comparison with the wild types (23, 24).

On the other hand Mederos et al. established structural differences in cord factor between ‘M. habana’ and M. simiae, nevertheless these variances doesn’t seems relevant on the induction of TNF-α by a macrophage cell line (25).

The only valid evidence of vaccine’s ability to induce protection is to measure disease outcome on vaccinated and no-vaccinated individuals. Potential variable results in TB vaccine trial, to assess protective efficacy include the prolonged survival, reduction in clinically-apparent disease, less pathology at the gross and/or in microscopic level, decrease bacterial burden and the anatomic restriction of bacilli at the entrance, in vaccinated animals (26).

The protective effect of vaccination with live ‘M. habana’ strains had been evaluated using multiples inoculation routes and different experimental models. Raj et al. demonstrated the restrictive capacity of ‘M. habana’ intravenously administrated on MTB burden in both immunocompetent and immunocompromised animals. Decreasing of 100-fold bacilli was observed in vaccinated animals when compared with the control group. This finding rendered lung damage limited to a small portion of the pulmonary parenchyma, absence of necrotic areas and the increase
Table 5.8.1 Polar gplipopeptidolipids from *M. habana* TMC-5135 y *M. simiae* ATCC 25275, as determined by ESI-IT-MS. Data was obtained and adapted from Mederos et al., 2008 (21)

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<td>1023</td>
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<td>2087·5</td>
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<td>1037</td>
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### Table 5.8.2 MS analysis and proposed general structure of α-mycolic acids of *M. habana* TMC-5135 y *M. simiae* ATCC 25275. Data was obtained and adapted from Mederos *et al.*, 2007 (22)

<table>
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<tr>
<th>Strain</th>
<th>Meroaldehyde chain</th>
<th>Chain length (free acid)</th>
<th>[M + Na] +</th>
<th>Monoisotopic mass</th>
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<td>m/z</td>
<td>Chain length</td>
<td>Relative proportion</td>
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<td></td>
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<td>α1(cisΔ + cisΔ) and α2(cisΔ + trans-db)</td>
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<tr>
<td></td>
<td>824.6</td>
<td>C58</td>
<td>62</td>
<td>C84</td>
<td>1258.5</td>
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<tr>
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<td>C59</td>
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<td>C85</td>
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<td></td>
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<td>C60</td>
<td>49</td>
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<td>1286.4</td>
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<tr>
<td><em>M. simiae</em> ATCC 25275</td>
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<td>866.6</td>
<td>C61</td>
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Additional note: α1(cisΔ + cisΔ) and α2(cisΔ + trans-db) for *M. habana* TMC-5135, α1(cisΔ + transΔ) for *M. simiae* ATCC 25275.
of lymphocytes in contrast with control mice. When results were expressed in terms of death caused by MTB, vaccination with ‘*M. habana*’ clearly evidenced enhances in the survival rate (survival time was enhanced by 50%), indicating that ‘*M. habana*’ administration definitely protected the animals from death because of TB (16).

Despite protective response found in those experiments, their results were slating due to the use of intravenous route during the challenge with MTB H37Rv. This method differs of the natural way used by micobacteria to cause infection in susceptible hosts. Intravenous inoculum delivery could contribute to a wider agent distribution through the mononuclear-phagocytic system, enhancing the elimination of bacilli.

Considering this situation, our research group carried out protection studies using subcutaneous vaccination with different live strains of ‘*M. habana*’ in Balb/c mice. MTB H37Rv and Beijing genotype strain were used for the intratracheal challenge. Former strain was reported hypervirulent since it induces rapid mice death with massive pneumonia and very high lung bacilli burden (27). Model of progressive pulmonary tuberculosis used is suitable to determine virulence and immune response induced by ‘*M. habana*’, since it is based on respiratory infection, which is the usual infection route in humans, and the rate of bacterial multiplication in the lungs correlates with the extend of tissue damage and mortality (28). In spite of ‘*M. habana*’ did not prevented the infection, vaccination protects against disease progression, which was significantly higher than conferred by BCG (29, 30).

Although precise correlates of protection await definition, it is generally accepted that control of mycobacterial infection is characterized by the emergence of CD4+ cells producing type I cytokine, in particular, IFN-γ (31, 32). Valdés *et al.* demonstrated that vaccination with live strains of ‘*M. habana*’ induces the expression of high quantities of protective factors, such as IFN-γ, TNF-α and iNOS and lower IL-4 amounts. Former findings added to the early granuloma formation after the infection with MTB H37Rv suggest a potent cellular immune response due to ‘*M. habana*’ vaccination (29, 30).

Several reports had been dedicated to elucidate the immunogenic role of the main antigens of ‘*M. habana*’ in order to explain protection conferred by it. Jyothi *et al.* confirmed the capacity of ‘*M. habana*’ to release extracellular proteins inducing strong proliferation of T cells with the increase of citoquines belonging to Th-1 profile. Therefore, these citoquines upregulate the activity of lysosomal enzymes (γ-glucuronidase and acid phosphates) as well as NO and H₂O₂ production. Both are potential action mechanisms of this type of vaccination (33).
Heat shock proteins (HSP) have long been considered as candidates for molecular vaccines by virtue of their strong immunogenicity and ubiquitous occurrence including mycobacteria. The most immunogenic and widely shared mycobacterium HSP is a 65 kDa antigen which bears several well defined epitopes for both B and T cells (34). Sing et al. demonstrated the protective efficacy of HSP 65 of 'M. habana' in the experimental TB. Mice vaccinated with this antigen showed low values of acid fast bacilli in lungs and spleen beside 7-8-fold higher mean survival time in comparison with unvaccinated control group (34).

Isolation and sub cellular localization of antigen carried out by Chaturvedi et al. showed significantly contribute towards the protective efficacy of 'M. habana' against TB infection. They revealed these antigens are in greater density in the membrane fraction particularly in the peripheral compartment. The observed phenomenon of protection by membrane proteins of 'M. habana' may be a result of enhancement of overall body immunity which takes care of the infection either by checking its onset and subsequent spread or by destroying the causative pathogen followed by faster clearance of the dead bacilli. The most striking results were the demonstration that separation pattern of peripheral and integral membrane proteins were the same as observed in MTB H37Rv which places 'M. habana' in relation with MTB (35).

Interestingly, Chaturvedi et al. found recognition of some secretory proteins, e.g. 15-17, 32/33, 34 and 35 kDa by TB patient’s sera. The major secretory 32, 31 and 30-kDa molecular mass proteins of 'M. habana' are also important secretory antigens of MTB with respect to their protective value seen in the guinea pig TB model (36). These findings were corroborated by Valdés et al. after the observation of a high production of IFN-γ in response to the stimulation with Ag 85 of cell suspension from lymph nodes, spleen and lungs, collected at different times after vaccination with 'M. habana'. Additionally they demonstrated that antigenic stimulation with culture filtrate antigens from MTB; induce high level of this Th-1 cytokine, reinforcing the reported antigenic relatedness between MTB and 'M. habana' (29, 30).
SAFETY OF EXPERIMENTAL VACCINATION WITH ‘M. HABANA’

Safety is the major concern for live vaccines and a critical point for "go" or "no-go" decisions. Bacilli burden in lungs and spleen, survival rate after the infection with lethal doses of live vaccine in addition to histo-pathological evaluation in target organs allow establishing safety in vaccine candidates (7, 37). On the other hand, new attenuated vaccines must exhibit greater efficacy than BCG in addition to robust safety profiles especially in immunocompromised individuals. Former issue may be assured testing the new candidate in mice and potentially other animals with severely compromised immune systems such as the SCID mouse (37-39).

A broad virulence spectrum using the endovenous route of infection had been observed in mice infected with different mycobacterial species. Collins et al demonstrated a time-slow reduction of lung bacilli burden in mice infected with ‘M. habana’ in opposition with the persistence observed in recipients of M. simiae. Additionally ‘M. habana’ infection showed scarce clues of dissemination in contrast with BCG infection (40).

Safety of endovenous vaccination with ‘M. habana’ was assessed by Raj et al. Experiments showed a total survival with reduction of bacilli load and a sterilizing tendency at the end of the study (16). Recently our research group confirmed the natural attenuation of ‘M. habana’ strains after fourth months of intratracheal infection of Balb/c mice. We observed no-changes in survival, lower colony forming units, smaller pulmonary damage and higher granuloma area in comparison with animal infected with MTB H37Rv (29, 30) (Figure 5.8.2 A-D).

Confirmation of natural attenuation was obtained after the vaccination of immunodeficient mice with different strains of ‘M. habana’ and BCG (29, 30).
Figure 5.8.2 Survival rate (A), bacilli burden (B), percentage of pneumonia (C) and granuloma formation (D) of mice infected with 'M. habana' TMC-5135 and M. tuberculosis H37Rv (control group). Asterisks represent statistical significance (p<0.05) among the indicated groups. Data was obtained and adapted from Valdés et al., 2010 (29, 30)
CONCLUSIONS

So the question remains, how will be able to control tuberculosis by vaccination in an effective way? Live mycobacterial vaccines are now considered a viable strategy for controlling the morbidity and mortality associated with TB infection worldwide. Furthermore, a live recombinant vaccine also affords the opportunity to construct strains expressing additional MTB antigens that were deleted during attenuation of BCG (41).

New attenuated vaccine strains aimed at substituting BCG, therefore, need to demonstrate robust safety profiles. Experimental results based in the virulence, immunogenicity and protection conferred by the vaccination with ‘M. habana’ fit well in the preposition of “classical vaccine” candidates should mimic natural infection as closely as possible without causing disease (42, 43). ‘M. habana’ encourage new investigations considering this mycobacteria as recipient of mycobacterial genes encoded highly immunogenic proteins.

Figure 5.8.3 Survival of nude mice vaccinated by the subcutaneous route with ‘M. habana’ TMC-5135 and BCG-Phipps (control group).
Data was obtained and adapted from Valdés et al., 2010 (29, 30)
REFERENCES


