



COMMENTARY

## Multiantigenic subunitary vaccines against tuberculosis in clinical trials: Where do we stand and where do we need to go?

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### ABSTRACT

The idea of presenting this commentary is to bring attention to the current status of clinical tests from several multiantigen vaccine candidates based on proteins produced by means of genetic engineering and molecular biology approaches and to suggest how new emerging technologies (OMICs) and bioinformatics might benefit vaccine development for better control of tuberculosis.

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Vaccination is the most important step in preventive medicine, and it has been practiced for almost 300 years, and the last reports have shown that vaccines have prevented more than 100 million cases of diseases in the United States<sup>1</sup> and that every year they prevent 2.5 million deaths worldwide.<sup>2</sup> Despite the time that vaccination has been used by humankind, the new technologies applied to vaccine development, are greatly vamping the way toward the availability of additional candidates designed with rational approaches.<sup>3</sup>

The birth of vaccines was followed by the discovery by Robert Koch and Louis Pasteur that infectious diseases are caused by microorganisms. Pasteur started drying, heating, and exposing microorganisms to oxygen or passing them in different animal hosts to attenuate them. The first bacteria to be attenuated was the one that causes chicken cholera, known as *Pasteurella multocida*<sup>4</sup> and the first human vaccine developed was against a rabies virus grown in a rabbit spinal cord and attenuated by exposure to dry air.<sup>3</sup>

Vaccines are great strategies to eradicate, control and prevent diseases, but even though generally speaking, they have go through great improvement, no vaccine has been able to control tuberculosis burden. Tuberculosis is the second disease with the highest mortality caused by a single infectious agent worldwide, with 9 million cases and 1.5 million deaths reported in 2013.<sup>5</sup>

Bacillus Calmette-Guérin (BCG) is the first and only clinically available vaccine against tuberculosis. Albert Calmette and Camille Guérin in Lyle, France, passaged a *Mycobacterium bovis* isolate, taken from a cow with tuberculous mastitis, 230 times to obtain the attenuated vaccine.<sup>6</sup> In spite of this vaccine protecting against tuberculous meningitis and other non-pulmonary tuberculosis during childhood, it does not protect against pulmonary tuberculosis, and the effect decreases gradually through the years, and it drops to almost 0 between 10 and

20 years after vaccination, it has shown a protection ranks that varies from 0 to 80%. Even though the protection varies widely, cessation of childhood BCG vaccination has been associated with a marked increase in the rate of childhood adenitis due to non-tuberculous mycobacteria.<sup>7</sup>

Over the past 15 years, funding for research has allowed the development of up to 15 new vaccine candidates, which are now being tested in preclinical and clinical trials. These trials have focused on alterations in the administration route of BCG and the evaluation of novel candidates to replace or enhance the effect of BCG. Three different types of new TB vaccines are currently in human clinical trials: live mycobacterial vaccines designed to replace BCG, subunit vaccines to boost immunity induced by BCG and therapeutic vaccines.<sup>8</sup>

Attenuated vaccines, or whole organism vaccines, can be further fractionated to reduce the number of components administrated. This can simplify the vaccine down to a single component or a mixture of a few components, which are capable of eliciting protective immunity.<sup>9</sup> This is the case of subunit vaccines, which are protein fragments that are recognized by the immune system and can produce an antigenic response, reducing the risks related to whole pathogens. Antigens are delivered usually accompanied by an adjuvant, DNA or live vector.<sup>10</sup> Adjuvants are immunostimulatory molecules that help non-living vaccines to stimulate an immunogenic response.<sup>11</sup> The first molecules to be used as adjuvants were phosphate or hydroxide salts of aluminum in the 1920s for vaccines against diphtheria and tetanus toxoids, and since then they have been successfully used to formulate most of the non-living vaccines that have been administered to billions of infants and adults. Since then, plenty other adjuvants have been developed, having different effects and helping to enhance Th1, Th2 or Th17 immune profiles.

Several highly immunogenic mycobacterial antigens have been described since the genome sequence of *M. tuberculosis* (Mtb) was published; the most studied are ESAT-6 and Ag85. Antigen 85 is a complex of proteins (Ag85A, Ag85B and Ag85C) secreted by Mtb and they are an important virulence factor. Ag85 complex proteins are crucial for cell wall synthesis playing an important role in bacteria growth and survival.<sup>12</sup> Besides, CD4+ and CD8+ T cells from individuals with active tuberculosis can recognize Ag85C.<sup>13</sup>

Vaccines that fuse several bacterial protein antigens increase the number of epitopes associated with a specific vaccine, however, this sometimes is not sufficient to enhance the response, and several molecules with potential immunogenic properties are needed, such as PAMP's and DAMP's, or interleukins, in association with fusion proteins, to increase the duration of such response.<sup>14</sup> In 2010, the safety and tolerability of the fusion vaccine candidate termed H1 (combination of ESAT-6 + Ag85B + adjuvant IC31) was demonstrated in a Phase I clinical trial.<sup>15</sup> Ag85B in combination with another antigen, TB 10.4, plus IC31, was recently shown to have an acceptable safety profile and was immunogenic in South African adults,<sup>16</sup> and testing different amounts of vaccine allowed to find that producing a more robust immune response.

Most approaches in the search for antigens for a TB vaccine have relied in culturing mycobacteria under in vitro conditions thought to resemble at least one environmental stress found during in vivo infection, often focused on oxygen<sup>17</sup> an nutrient availability. From these studies, a number of new potential antigens has been incorporated in subunitary vaccines and tested in preclinical trials, and when successful, moved toward clinical phases. Such is the case of the vaccine candidate termed H56 (comprising a fusion protein of Ag85B, ESAT-6 and Rv2660c, formulated in IC31 adjuvant) that induced antigen-specific IgG responses and Th1 cytokine-expressing CD4+T cells, where even a dose-response effect was evaluated, finding that a 15 µg dose promote more polyfunctional T-cells.<sup>18</sup> Other mycobacterial antigens, relevant at different stages of infection, have also been evaluated, such as the combination of Mtb32 (a secreted serine protease) plus Mtb39, termed M72, which in combination with adjuvant AS01<sub>E</sub> has shown an acceptable safety profile and induced a potent M72-specific CD4+ T cell response in clinical trials performed in adults, even Mtb-infected people,<sup>19-22</sup> and recently shown to induce robust T cell and antibody responses in HIV-negative adolescents living a TB-endemic setting.<sup>23</sup>

More recently, approaches to propose new vaccine candidates have been refined by finding T-cell epitopes recognized by individuals suffering from active or latent disease, with bioinformatics being fundamental in predicting new potential targets for the immune system,<sup>24</sup> either from the dominant class of antigens or those that are not as strong as the so called dominant but that still play a relevant role during some part of the interaction with the host critical for establishment of persistence of infection. OMICs technologies (genomics, transcriptomics, proteomics and metabolomics) also start to play a highly prominent role, by making possible to unravel very discrete yet quintessential molecules for *M. tuberculosis* life cycle that would result in rationally designed vaccine candidates. These will be targeting more than one key pathway that *M.*

*tuberculosis* might require during its natural history of infection, and we think this will greatly optimize research and success in dealing with TB worldwide.

## Disclosure of potential conflicts of interest

Dr. Mario Alberto Flores-Valdez and Dr. Rogelio Hernández-Pando have filed for patents before the Mexican Institute for Intellectual Property (IMPI) on live mycobacterial vaccine candidates.

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