CHAPTER 5.9

TB VACCINES BASED ON PROTEOLIPOSOMES AND LIPOSOMES FROM NON PATHOGENIC MYCOBACTERIA

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Although it is estimated that TB accompanied humanity from as early as 5000 BC, even now, on many occasions, the first reaction of the patient to the disease is denial.

‘Tis some visitor, I muttered, tapping at my chamber door; Only this, and nothing more’.

_The Raven_
Edgar Allan Poe

_The Trace_
Niuris Madrazo Castro
Materic art. (concrete and asphalt) wood; 150 × 105 cm
INTRODUCTION

Liposomes and proteoliposomes have been used as delivery systems and vaccines against infectious diseases (1, 2, 3). In the specific case of TB vaccine development, liposomes have been used in different variants of vaccine formulation which demonstrated their ability to stimulate specific humoral and cellular immune response and provide protection against MTB in different animal models (4). Recently, the use of proteoliposomes obtained from non-pathogenic mycobacteria as an alternative for the development of new TB vaccines has been reported (5). In this chapter we will present some of the main results obtained with these two technology platforms for the development of new vaccines against TB.

LIPOSOMES

Different vaccine formulations using liposomes have been evaluated as experimental vaccines against TB. Of particular interest is the cationic adjuvant formulation CAF01 composed of DDA as a delivery vehicle and synthetic mycobacterial cord factor as the immunomodulator (4, 6, 7, 8, 9, 10). This formulation induced superior specific cellular and humoral immune response against ovalbumin compared to other currently used adjuvants (4). The induced response is independent of the stimulation of toll like receptors (TLR) 2, 3, 4 and 7 (4). The use of this adjuvant with MTB antigens induces strong protection against challenge with MTB in experimental animals (4, 6, 11). Other combinations of DDA with lipidic fractions of BCG demonstrated high adjuvanticity and the induction of strong protection when combined with MTB antigens (12, 13). The combination of BCG with a TB vaccine in cationic liposomes produces a synergistic effect with a positive impact on immunogenicity and protection (14). MTB antigens encapsulated in liposomes and adjuvanted with alum have also been shown to be immunogenic and induced protective responses against MTB but with lower efficacy to those immunized with the same antigens in IFA (15). Another strategy used is the encapsulation of DNA vaccines into liposomes (16, 17, 18). DNA vaccines coding for the hsp65 antigen of *Mycobacterium leprae* formulated in cationic liposomes have been
evaluated (16). This formulation resulted in better protective effect after intranasal administration compared to the intramuscular route in mice (16). In another experiment, DNA vaccines expressing mycobacterial hsp65 and IL-12 delivered by the hemagglutinating virus of Japan (HVJ) formulated in liposomes showed protection against MTB challenge in mice, guinea pigs and monkeys (17). A DNA vaccine expressing Ag85A of MTB encapsulated in liposomes and administrated by the oral route induced specific cellular and antibody responses at the intestinal mucosa (18). The encapsulation of mycobacterial mannophosphoinositides (PIMs) with lipid A in liposomes demonstrated protection in mice after challenge with MTB (19). In another study, the formulation of an extract of MTB lipids in liposomes with adjuvants induced protective responses in guinea pigs (20).

The experimental vaccine, RUTI, which comprises MTB fragments formulated in liposomes demonstrated prophylactic and therapeutic effects against MTB in animal models (21, 22) and is now in clinical trials (23).

Our group has been working with liposomes comprising total lipid extracts of *M. smegmatis* formulated with diestearoylphosphatidyl choline and cholesterol (24) or those using only the lipid extract (25). Animals immunized with liposomes obtained by the first approach elicited an IgG response which was cross-reactive with lipids from MTB (24). In animals immunised with the liposomes containing only the lipid extract of *M. smegmatis* a cross reactive IgG response against the cell wall fractions of MTB was observed (25). Interestingly, the addition of adjuvants such as montanide or alum abrogated these responses (25). These liposomes were recognised by the sera of active pulmonary TB patients, suggesting the expression of cross-reactive MTB lipids, during the active infection in humans (25). Another strategy with the use of liposomes has been the encapsulation of BCG strains. Lipid formulations containing BCG administered by the oral route to different species induced good immunogenicity and protection against *M. bovis* (26–33). A mmaA4 gene deletion mutant of *M. bovis* BCG formulated with cationic liposomes produced superior protection than BCG against MTB challenge in mice (34).

**PROTEOLIPOSOMES**

Proteoliposomes (PLs) comprise detergent extracts of the outer membrane components of bacteria (1, 35). To date, there have only been a few examples of licensed and available PL-based vaccines. One of them, the Cuban anti-meningococcal vaccine, VA-MENGOC-BC™ (1, 36, 37), is a classic example of a vaccine consisting of PLs obtained from the outer membrane of *Neisseria meningitidis* serogroup B. This *N. meningitidis* PL has also been shown to have potent adjuvant activity when
used with other vaccine antigens either in its native vesicle form, or as a cochleate form (41). PLs has the advantage of stability allowing heterologous antigens to be incorporated into them to be used as delivery system (42). In contrast to liposomes, PLs contains LPS, proteins and other molecules in their structure known as pathogen associated molecular patterns (PAMPs) with immune potentiator and modulator effects (43). Protollin™, a non-covalent complex consisting of PL from Neisserial species and Shigella flexneri 2a LPS (1:1), is an example of a PL construct with good adjuvanticity for administration of various antigens. In animal studies and clinical trials, Protollin™ has been shown to have strong adjuvant properties for bacterial and viral glycoprotein antigens (44, 45).

The mycobacterial cell wall consists of a variety of antigenic compounds which is organized as a central covalent axis integrated by peptidoglycan (PG)-arabinogalactan (AG)-mycolic acids, and in the upper segment by free lipids, proteins, phosphatidylinositolmanosides (PIMs), tioecerol, lipomanann (LM) and lipoarabinomann (LAM) (38). These components, which are important molecular effectors involved in the infection process, have been reported to induce protective response in mice against TB. Thus the use of cell wall components as potential vaccine candidates is an attractive approach for TB vaccine development (39, 40).

*M. smegmatis* (Ms) and BCG are non-pathogenic and have high levels of genomic and antigenic homology with MTB (46, 47). These characteristics provide important advantages for these strains to be potentially useful for the development of new vaccine candidates against TB. Our group is currently evaluating the use of PLs from *M. smegmatis* and BCG as new vaccine candidates against TB. Using bioinformatics we determined the potential presence of T and B cell epitopes form MTB in the cell wall, external membrane and secreted proteins of *M. smegmatis* and BCG, and found that PLs from *M. smegmatis* and BCG potentially include multiple T and B cell epitopes from MTB (48, 49). We found that these PLs elicited the production of cross-reactive immune responses against MTB antigens at the cellular and humoral levels in mice, corroborating the earlier bioinformatics studies (5, 48, 49). Recent experiments demonstrated the protective capability of these PLs in intratracheal challenge experiments with MTB in mice (unpublished results).

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Development of New Vaccines Against TB


