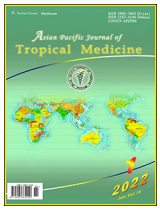

Review Article

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Liposomes as immunological adjuvants and delivery systems in the development of tuberculosis vaccine: A review

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ABSTRACT

Liposomes are phospholipid bilayer vesicles, which are biocompatible, biodegradable and nontoxic vehicles suitable for numerous drug and gene delivery applications. In this review, we discuss the prospect of using liposome technology in the development of a vaccine for tuberculosis. Tuberculosis remains an important health problem that requires the development of an effective vaccine, especially since the only approved vaccine for it continues to be the Bacille Calmette-Geurin (BCG) one developed 100 years ago. This review focuses on the different applications of liposomes toward achieving this goal. Numerous liposomal formulations showing prospect in the research stage and in clinical trials are discussed.

KEYWORDS: Liposomes; Tuberculosis vaccine; Adjuvant; Delivery system; Tuberculosis

1. Introduction

According to the World Health Organization (WHO), tuberculosis (TB) together with human immunodeficiency virus (HIV) is one of the most critical diseases and causes of mortalities in adults worldwide. TB imposes a significant economic burden on most countries around the world[1]. Although there has been a decrease in the global annual death rate due to TB during the last 15 years, this bacterium still produces an alarming number of 1.5 million deaths per year[2]. Another important aspect of the TB pandemic is the growing appearance of strains with antibiotic resistance[3]. Multidrug-resistant TB (MDR-TB) is defined as TB resistant to common drugs, such as isoniazid or rifampin. MDR-TB is estimated to currently affect 480 000 people annually, with only half of these patients receiving appropriate treatments[4]. *Mycobacterium tuberculosis* (Mtb), the causative agent of TB, may be undetectable in the lung and survive in it for prolonged periods of time in a dormant state, which makes the process of diagnosis and timely treatment difficult. Latent TB infection and the reactivation of infection can happen at any time, particularly following the immune compromise[4,5].

Vaccination is the most desirable means of preventing TB. French scientists Albert Calmette and Camille Guérin developed the Bacille Calmette-Geurin (BCG) vaccine in 1921. Exactly 100 years later, this remains the only licensed human vaccine against TB and has been used all across the world for more than 80 years[6]. BCG is a live attenuated strain of *Mycobacterium bovis* and is used in 80% of the TB endemic areas[7]. Although BCG can prevent dissemination of TB in children, its protective effect is variable and questionable in adults[8].

The advent of new technologies in vaccine development, including new adjuvant formulations, the whole-genome sequencing of Mtb, and other mycobacteria, has been used to define new TB vaccine candidates[9]. Due to the evasive nature of Mtb during the infection process, the use of the bacterial components, such as lipids, has been

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studied intensely by the researchers as one of the components for TB vaccine development[10]. Here, the use of liposomes derived from the lipid components of the bacterial cell has been considered as a special prospective vaccine candidate against the TB infection due to their capacity to induce strong humoral and immune responses[11]. In what follows, this approach to TB vaccine development will be reviewed and discussed.

2. Mechanisms of infection

TB is a major lung disease and the Mtb bacterium is the etiological agent[12]. The main port of entry for the bacterium is the respiratory tract. Upon reaching the lung, Mtb becomes engulfed by phagocytic cells such as macrophages and dendritic cells (DCs)[13]. The first-line defence against Mtb infection is the innate immune response, which is represented by macrophages, DCs, and natural killer (NK) cells among others. These cells are activated by interaction between the pathogen associated molecular patterns (PAMPs), such as glycolipids, lipoproteins, and carbohydrates on Mtb and the pattern recognition receptors (PRRs) on the host cells, which include Toll-like receptors, NOD-like receptors, and C-type lectin receptors[13,14]. This presence on the infected cells allows for the recognition of the pathogens, the reactivation of the effector cells, and the uptake of Mtb by phagocytic cells[13].

After the activation of the innate immune response, a specific immune response is produced against Mtb. This response is represented by T helper (Th) $CD4^+$ and T cytotoxic $CD8^+$ cells and accompanied by the production of specific antibodies[15]. Mtb, however, employs an effective strategy to evade both the innate and the adaptive immune responses[16,17]. With respect to the innate immunity, it inhibits apoptosis and triggers necrosis of host macrophages, which delays the initiation of adaptive immunity[18]. The manipulation of macrophage death pathways is one of the mechanisms used by Mtb to evade host defences[18]. In addition, Mtb uses various mechanisms to inhibit pathways for antigen presentation to T cells[13]. The evasion mechanisms allow Mtb to establish a persistent or latent infection in macrophages, which results in inhibition of major histocompatibility complex class Ⅱ (MHC-Ⅱ) molecule expression and antigen presentation[19]. This ability of Mtb to inhibit MHC-Ⅱ antigen presentation leads to inhibition of recognition of $CD4^+$ T cells. It is important to note that immunity to TB depends on $CD4^+$ T cells for the control of primary infection and it is essential for ongoing immune surveillance to control the infection that forms the reservoirs for reactivation of TB[19,20].

3. Liposomes

Liposomes are relatively small spherical vesicles whose membranes consist of one or more phospholipid bilayers (Figure 1). Liposomes were first reported by Bangham *et al*. in 1965 and their use has been established in several medical areas of interest, including the oral delivery of vaccines, insulin administration and cancer chemotherapy[21–23]. Liposomes have important biological and technological advantages over many other types of medication carriers and have been used with success as delivery systems for biological substances both *in vitro* and *in vivo*[24]. The efficacy of liposomes as carriers of drugs is partly due to their capacity to release the medication cargo in target cells[25]. The interest in the use of liposomes has also been tied to specificities of their composition, which is biodegradable and biocompatible[26]. They can be produced using natural or synthetic lipids and take the form of concentric bilayered vesicles in which an aqueous volume is entrapped[27]. The range of sizes of liposomes enables them to reach the targeted cells, including antigen-presenting cells such as DCs[28]. Several studies have shown that the size of liposomes depends on the preparation method[29]. The smaller sized, 50-250 nm unilamellar vesicles (ULVs) are customizable for encapsulation of hydrophilic drugs and have a longer half-life when compared to multilamellar vesicles (MLVs) with size ranging from 500-5 000 nm[27]. Another important property is their capacity to protect entrapped bioactive materials (*e.g.*, hydrophilic and hydrophobic drugs) from immediate degradation, thus increasing the efficacy of drugs and decreasing their toxicity[29,30]. Liposomes have been used to facilitate the cellular uptake of the drugs directly by the targeted cells such as macrophage and DCs[31].

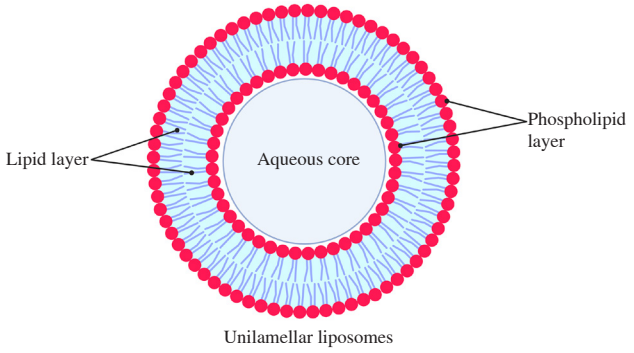

Figure 1. Basic structure of liposomes.

3.1. Synthesis of liposomes

The earliest liposome was discovered and synthesized by Bangham and colleagues using thin layer hydration (TLH) techniques, also known as Bangham techniques[22]. Due to promising advantages of liposomes in biomedical and biotechnology areas, many researchers started to produce liposome using conventional or supercritical-assisted techniques. The conventional techniques of liposome production has occurred earlier than supercritical-assisted techniques, which can performed at room temperature and high pressure system. The size of liposomes produced ranged from 1 μm up to 300 nm with poor stability, whereas the encapsulation efficiency ranged between

20% to 90%[32,33]. Supercritical-assisted techniques is a rapidly evolving modern and green technology for expanding the production of vesicles on a large scale. The size of liposomes ranged between 20 nm and 300 nm with better dimensional control as deduced from the liposomes heterogeneity measure known as polydispersity index (PDI), and the higher encapsulation efficiency of up to 90%[32-35]. Liposomes can be tailored to possess different sizes, ranging from very small (0.025 μm) to large (2.5 μm), and can be produced using different methods[29]. Different methods of synthesis of liposomes can lead to a variety of sizes and numbers of liposome bilayers[36]. Accordingly, liposomes can be classified according to their size and phospholipid bilayer number (Figure 2).

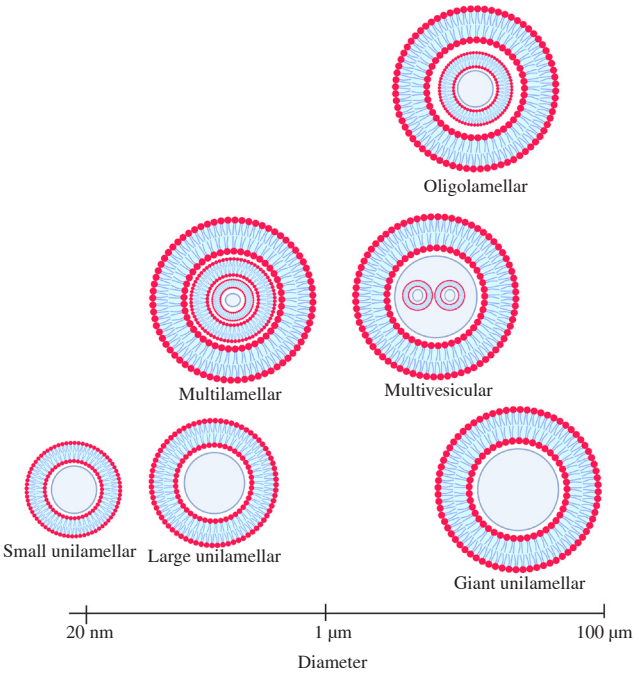

Figure 2. Classification of the liposomes based on their size and phospholipid bilayers.

3.2. Physiochemical properties of liposomes

Liposomes have been used in analytical, diagnostic and therapeutic applications owing to their unique physiochemical properties[37]. One of the important elements reported with respect to the physiochemical properties of liposomes is the phospholipid bilayer. The bilayer composition of liposomes allows the interaction with the biomolecules, such Doxil® and Depocyt® and both of these biomolecules have been used clinically so far in cancer therapy[38,39]. Different ligands such as peptides, proteins, monoclonal antibodies, and carbohydrates can be coupled to the surface of liposomes, leading to the interaction with the specific target cells, which promotes an increase in the therapeutic efficacy[38]. The addition of cholesterol to the lipid bilayer can increase their stability *in vivo* and *in vitro*[24]. Apart from that, the aqueous interiors of liposomes can be incorporated with hydrophilic and/or amphiphilic drugs, which have been used with success in targeted cancer therapy[40].

The vesicle size and the bilayer structure are the two most important factors determining the physiochemical properties of liposomes and they greatly influence the liposomal vaccine design[41]. If these two properties are appropriately tuned, liposomes can pass through the tumor vessels and concentrate in the target site. Previous studies have found that liposomes with a size smaller than 100 nm in diameter can circumvent the capture by the reticuloendothelial system (RES), have a longer half-life in blood and accumulate in the tumoral site[42]. In contrast, liposomes of larger sizes did not escape the RES uptake and got eliminated rapidly from the blood circulation[42]. Vesicle lamellarity also influences the immune response against liposome-associated antigens[41]. A previous study investigated the small unilamellar vesicles (SUVs) with no TLR agonist and showed a higher capacity to induce the spleen IFN-γ response against Ovalbumin (OVA) compared to multilamellar vesicles (MUVs)[43]. The ability of SUVs to induce a potent CD8 T cells response shows that SUV is the preferred state to potentiate innate and adaptive immune responses for an improved vaccine efficacy[43,44].

3.3. Advantages of liposomes

Liposomes have been currently approved as adjuvant/antigen delivery agents and exploited to deliver therapeutic compounds and immunomodulators for a broad range of diseases[45]. It has been reported that liposomes can incorporate antigens and targeting molecules to serve as potent vaccines[46]. In contrast to liposomes for the delivery of cancer drugs, liposomes that get rapidly cleared from the blood through elimination by phagocytic cells, hepatic Kupffer cells, and macrophages, alongside being uptaken by the target cells, may be particularly suitable for the application in vaccines[42]. One such prompt presentation to the cells of the immune system will increase the delivery efficiency and invoke less of the systemic toxicity in the organism[47]. Here it should be noted that liposomes produce less toxicological effects when they are injected in low doses[47]. Moreover, liposomes also do not cause an antigenic reaction by themselves[35], which makes them suitable for the role of carriers of antigenic loads.

There are many benefits of liposomes that stem from their structure and composition. Liposomes have the ability to facilitate the binding of the target to the liposomal surface, which make them a suitable candidate for drug delivery in general, but they are also capable to increase the therapeutic efficacy of the drug[29]. Encapsulation of drugs in liposomes improves the pharmacokinetics and also protects the drug against deactivation, which has been exploited in the delivery of unstable antibiotics[48]. For this reason, encapsulation of ciprofloxacin in SUVs significantly improved the antibacterial activity against Francisella tularensis infections[49]. With this many advantages, further studies into liposomes and their application in various fields of medicine must be delved into.

3.4. Immunostimulatory properties of liposomes

Because of their unique properties and advancements in their

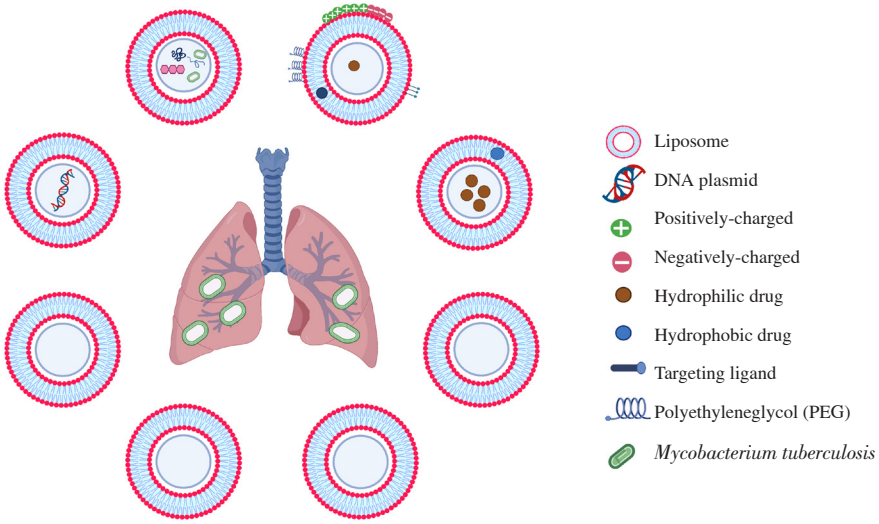

Figure 3. The use of liposomes in development of *Mycobacterium tuberculosis* vaccine.

synthesis, liposomes have been widely used to stimulate the immune response. The enhancement of the immune response is dependent on the cellular uptake of liposomes by the target cells. However, the cellular uptake of liposomes is affected by their surface charge. A previous study found that the surface charge of liposomes influences the cellular uptake through the endocytotic pathway in glioblastoma cells[50]. Meanwhile, the pharmacokinetic properties of liposomes can have a significant effect on the efficiency of the presentation of liposomes to target cells such as DCs, macrophages, and other cells[24,31]. DCs are professional antigen-presenting cells that can express cytokines, co-stimulatory molecules that regulate the primary immune response. In addition, DCs are considered phagocytic cells, too, since they have the ability to take up substances in peripheral tissues, undergo maturation and promote the T cell response[31]. DCs will process the antigens and present them on the cell surface to T lymphocytes[51,52]. Once liposomes are stably surface-modified and functionalized, they can be conjugated with several molecules such as polyethyleneglycol (PEG) and antigens to specifically target DC receptors to enhance the uptake and initiate the selective adaptive immune response[28].

Apart from DCs, liposomes also can be associated and uptaken by other target cells such as macrophages[31]. As a particulate drug carrier, liposomes will naturally target the mononuclear phagocytic system. Mononuclear phagocytic cells express a range of receptors such as scavenger receptors, mannose receptors, and integrins that can be targeted by ligands of liposomes, thus increasing the target specificity toward the macrophages. This advantage for targeting macrophages using liposomes can lead to cell activation for the treatment of chronic inflammation and cancer[31]. Besides, the addition of antigens in formulated liposomes could enhance the activation and uptake capacity of DCs, which increases the ability of DCs to induce T cell proliferation[53]. A previous study found that mannosylated liposomes cause a high expression of surface markers and stimulation of T cells[54], which suggests that they can represent a versatile delivery vehicle to enhance the immune response.

The lipid surface of liposomes can be chemically modified to increase the circulation time, accumulation time at the target site, and cellular internalization[55]. In a previous study, liposomes were modified and made multifunctional by adding functional groups to their surface. The addition of these functional groups increased the longevity of liposomes in blood, favouring the specific targeting in response to the local stimuli at the target site. Active targeting of liposomes has also been achieved by conjugation with peptides or ligands to reduce the interaction with off-target cells[55,56]. Liposomes with specific surface ligands actively targeted and interacted with cancer cell surface receptors in the tumor environment, which led to enhanced uptake and therapeutic effect at the tumor site[56]. Other studies have found that liposomes are able to target the endoplasmic reticulum (ER) and associated membranes specifically[57]. Once liposomes are inside the ER, the targeting lipid intercalates with the ER membrane and incorporates itself into ER-assembling entities such as lipid droplets and secreted proteins[57]. These ER-targeting liposomes were found to be effective for prolonged delivery of lipids and lipophilic drugs into human cells[55,57,58].

4. Liposomes in TB vaccine development

Liposomes are versatile and widely used as an efficient adjuvant and delivery system (Table 1). Although the majority of their applications as agents for the delivery of immunostimulatory molecules have been in the domain of cancer immunotherapy, they can be considered as an ideal vaccine carrier candidate, especially for TB (Figure 3). This application will be reviewed in the following section in detail.

Table 1. Summary of liposomes in tuberculosis vaccine development.

Adjuvant	References	Delivery strategy	References
CAF01	[67-76]	Passive targeting	[88-91]
AS01	[2,78,79,106-109]	Active targeting	[37,44,91,92]
DOTAP	[80-82]	Nucleic acid- Ag85A	[37,93-95]
AdHu5Ag85A	[83-86]	Live attenuated and killed whole cell vaccines-RUTI	[98-103]

4.1. Liposomes as adjuvants

The strategy to develop TB vaccine has resulted in several different liposome-based adjuvant candidates. The word 'adjuvant' comes from the Latin word 'adjuvare', meaning 'to help' or 'to enhance'[59]. The development of liposome-based adjuvants in TB vaccine formulations is indeed intended to enhance the immune responses against Mtb antigens[60]. In recent years, it has been shown that cationic liposomes in combination with other immunostimulatory factors such as TDB, MPL, and Poly Ⅰ: C can induce a solid immune response against Mtb antigens[11]. Cationic liposomes provide long-term storage for subunit TB vaccines at the injection site and have been shown to provide a potent surface charge when interacting with APCs to promote both humoral and cellular immune responses[11,44]. Cationic liposomes have been combined with dimethyldioctadecylammonium (DDA) stabilized with glycolipid immunomodulator Trehalose 6,6-dibehenate (TDB), which is a synthetic variant of the cord factor located in the mycobacterial cell wall[61]. The combination of these DDA-TDB liposomes with the mycobacterial fusion protein Ag85B-ESAT-6 is a novel TB vaccine candidate for CAF01. The stabilizing properties of CAF01 make it suitable for use in vaccine formulations and their safety was demonstrated in a Phase 1 trial. Vaccination with CAF01 resulted in highly complex immune responses with strong T cell immunity, which indicated that CAF01 might be a good candidate for future TB vaccine development[62]. Knudsen and colleagues performed a detailed comparison of five different clinical adjuvants, including CAF01, and showed a mixed Th1/Th17 profile in mice[63]. In TB, Th1 immune response is required against Mtb infection, while Th17 immunity was rapidly induced upon Mtb infection, conferring protection similar to vaccination[63,64]. The mycobacterial phospholipid from *Mycobacterium bovis* BCG lipid extract was shown to induce a potent Th1 immune response, characterized by an increase in the expression of IgG2a and IFN-α[65]. Further investigations showed that the formulation with a liposomal adjuvant is a promising system that successfully induced a prolonged uptake and activation of DCs to elicit Th1 and Th17 cells in both neonates and adults[66,67]. This adjuvanted vaccine candidate with CAF01 manages to induce a robust multifunctional memory in T cells, which is maintained for over a year post-vaccination[68]. Durable protection via T cells induced by CAF01 formulation preferentially localized to the lung site of infection[69]. Recently, incorporation of additional immunomodulatory adjuvants such as monophosphoryl A and Pam3Cys as recognition agonists into the CAF01 formulation led to new liposomal adjuvants. These new formulations effectively induced a specific immune response against the mycobacterial DNA and antigen, respectively, and they also provided an enhanced and persistent protection against the Mtb infection[2]. Other studies also found that CAF01 in combination with the anti-subunit TB vaccine, H56, results in an increased response towards polyfunctional CD4 T cells that localize to the lung parenchyma. This leads to prolonged and sustained protection to infected antigen presentation cells in mice and to date this study is still in clinical development[69,70]. These findings demonstrated liposomes not only to be a viable vaccine carrier that induces long-term protection against Mtb, but also a safe and tolerable adjuvant producing no adverse or systemic effects observed upon vaccination, notwithstanding that further more in-depth safety profiles need to be established[71].

Liposomes have been studied in comparison with alum and oil-in-water emulsions as adjuvants for TB vaccines. Preclinical studies have shown that both emulsion-based and liposome-based adjuvants provide protection against mycobacterial challenge[2]. AS01 is a liposome-based adjuvant vaccine containing two immunostimulants: 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) and saponin QS-21[72]. This liposome-based adjuvant such as AS01 caused the rapid effect after being localized to the injected muscle and draining the lymph node. Besides, AS01 also triggered a higher $CD4^+$ T cell response, as indicated by a stronger IFN-γ response, confirming that it is a suitable candidate for an adjuvant in a TB vaccine[2,72,73]. As far as the clinical trial was considered, this adjuvant system was used in the form of M72 antigen. M72-AS01E (E referring to reduced dose for pediatric use) is currently in Phase 2b clinical trial and is deemed safe in healthy adolescents and adults[74,75]. It induces immunogenicity and protection in adults with active and latent Mtb infection[76,77].

Another lipid tested as a liposomal adjuvant in an animal challenge study is 1, 2-dioleoyl 3-trimethylammonium propane (DOTAP). DOTAP is a cationic liposome-forming compound that has been used as an adjuvant in TB vaccine development. It acts as a booster of the immunogenicity of peptide and protein antigens to produce Th1 immune responses[62,78]. DOTAP in combination with a fusion protein of Mtb HspX, PPE44, and EsxV elicits a strong immune response. Mice that received this combination of DOTAP secreted more IFN-γ and IL-12, indicating a strong Th1 response[78]. Apart from that, a previous study has shown that DOTAP exhibits mucosal adjuvant effects when ovalbumin (OVA) combined with DOTAP is delivered via the intranasal route, as indicated by the strong Th2 immune response[79]. However, further development of DOTAP as a mucosal adjuvant for vaccination against TB is still needed.

The current liposomal adjuvants, in fact, are most appropriate for injection. Still, the potential for stimulating an immune response combined with the possibility of 'needleless vaccination' has provoked an interest in their use in mucosal vaccination. For example, the protection achieved by the intranasal mucosal immunization with AdHu5Ag85A was associated with the localization of antigen/adjuvant specific T cells to the lung airways[80,81]. A previous study used the rhesus macaque TB model to evaluate the safety effects of AdHu5Ag85A. The results showed that the mucosal boost immunization was safe in BCG rhesus macaque in which an enhanced antigen-specific T cells response was observed[82]. The mucosal route for TB vaccination has already reached Phase 1 clinical trials and the assessment of the levels of protection specific to this adjuvant is underway[83].

4.2. Liposomes as delivery systems

The characteristics of liposomes to entrap substances such as drugs have made liposomes an excellent tool to explore as a new drug delivery system for the development of TB vaccines[25]. Liposomal formulations are able to increase the bioavailability of drugs and reduce the treatment time[84]. Liposomes can also be targeted to specific tissues or organs by active or passive methods. Passive targeting involves the transport of liposomes to the target tissues by tailoring their surface structure to a desired systemic distribution profile. The combination of passive targeting in TB vaccine development with the inhalation route has gained interest of the researchers because liposomes owe their ability to reach alveoli macrophages to an adequate size. Once in the blood circulation, liposomes are easily taken up by phagocytic cells in the mucosal pulmonary systems[84–86]. Apart from that, the development of liposomal formulations for aerosol delivery hints at the potential for their use in intranasal TB vaccines because liposomes can thus reach the lung tissues more effectively[86]. The use of passive targeting has been employed by Gaspar *et al*., who encapsulated multilamellar vesicles of liposomes with rifabutin and thus achieved a higher concentration of the antibiotic in targeted organs compared to the treatment with free rifabutin[84,87]. Along this line, innovative inhalation therapies with liposomes may contribute to the development of TB vaccines for pulmonary administration.

Meanwhile, for the purposes of active targeting, the phospholipid bilayer of liposomes is coupled to targeting ligands, including peptides, antigens and proteins, so as to make them suitable carriers for the delivery of drugs to specific sites with improved therapeutic outcomes[25,84]. The incorporation of antigens in liposomes influences immunogenicity by inducing T cell response and indirectly increasing the availability for antibody or B-cell recognition[41,60]. Gerald and colleagues found that immunization of liposomal mycobacterial lipid antigens induced protection in guinea pigs challenged with Mtb[88]. The formulation consisting of a liposomal system with mycobacterial lipid antigens reduced the bacterial load in the spleen of inoculated animals as compared to the unvaccinated group of animals. In another study, liposomes based on phosphatidylserine (Lipo-AE) carrying a mycobacterial antigen were shown to induce the accumulation of memory T cells in the lung and reduce the bacterial load in both lung and spleen, thereby boosting BCG immunization[3]. These findings show that liposomes are convenient as delivery systems for lipid antigens *in vivo*, in part because their phospholipid bilayers are suitable for incorporation of an amphipathic antigen[88,89].

Nucleic acid vaccines have emerged as alternatives to traditional vaccines in inducing an immune response against TB. Liposomal delivery systems encapsulating DNA plasmids represent the most promising strategy to stimulate the immune responses[60,90]. For example, liposomes encapsulating a *Mycobacterium* DNA and incorporating Ag85A caused a substantial expression of DNA in the mucosal intestinal epithelium as well as in microfold cells, DCs, and Peyer's patches of the small intestine. These cellular compartments play an important role in regulating the immune response[91]. This approach has resulted in oral vaccination with liposomal-DNA Ag85A able to generate antigen-specific mucosal and systemic humoral immunity against TB[90,91]. Furthermore, liposomes incorporating the same DNA showed an enhancement in CD4 and CD8 T cell response and were capable of prolonging survival in mice infected with TB[2,92].

Finally, live attenuated and killed whole-cell vaccines have been studied as candidates for TB vaccines because of their advantages over protein-adjuvant formulations and recombinant viral-vectored ones[93]. Live whole-cell vaccines possess the ability to induce long-lasting memory immune responses by employing a broad antigen composition to stimulate the production of T cells and B cells responses[93]. Nowadays, the live attenuated vaccines have entered the preclinical and clinical developments with the recombinant BCG and attenuated Mtb[94]. One of the liposomal therapeutic vaccine candidates is a vaccine made of fragmented Mtb cells detoxified and liposomed (RUTI), a polyantigenic liposomal vaccine composed of detoxified fragmented Mtb cells. RUTI is developed to prevent active TB in subjects with latent TB infections by boosting the previous immunity through chemotherapy, which has triggered a Th1/Th2 response in infected mice[95,96]. Furthermore, RUTI has been shown to reduce the bacillary load and increase the survival rate of infected animals in short- and long-term vaccination, respectively[97]. This vaccine also facilitated a response of Th cells to a wide range of antigens, along with an increased antibody production, thanks to which it entered Phase 2 clinical trial in 2014[93,98]. The treatment with RUTI appears to be well tolerated and the immunogenicity profile in latent TB infections will be based on a single injection of a highest dose[95]. As preclinical results are similar or even enhanced compared to BCG vaccination, Phase 1 clinical trial of RUTI has indicated that it is a safe treatment option for healthy individuals, as it confidently triggers the specific T cell response against Mtb[99]. This approach of utilizing fragments of Mtb in vaccines has driven

the field to explore other species of mycobacteria. One of them is *Mycobacterium smegmatis*, a non-pathogenic *Mycobacterium* that shares several glycolipids with Mtb and that induced specific humoral immune responses against Mtb infection when it was enwrapped in liposomes[100].

5. Conclusions

Liposomes are essential drug delivery carriers characterized by a number of advantageous properties. They are generally able to safely carry therapeutic compounds to target cells, oftentimes increasing their therapeutic activity and preventing any toxic side effects. Their ability to induce specific immune responses serves as a fundamental feature of an effective delivery system and adjuvant in the development of TB vaccines. Nonetheless, further extensive research and development are required to optimize for a number of varying factors that determine the efficacy of liposomes in these applications, including the liposome sizes, surface charge, and composition of the lipid bilayers.

Conflicts of interest statement

We declare that there is no conflicts of interest.

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Authors' contributions

NEML, SA, ASNA and AN performed the literature search and drafted the manuscript. RK, VU, RM, MES, AA and MNNA supervised and revised the manuscript. All authors contributed significantly to the manuscript and approved the submitted version.

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