Annals of Parasitology 2021, 67(3), 367–386
doi: 10.17420/ap6703.352
Copyright© 2021 Polish Parasitological Society

Review article

An overview on liposomal delivery and adjuvant development for leishmaniosis vaccines

Masoud SOOSARAEI[1], Hajar Ziaei HEZARJARIBI[2], Mahdi FAKHAR[3], Javad AKHTARI[4], Reza Zolfaghari EMAMEH[5]

[1]Student Research Committee, Department of Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Farah Abad, Sari 48471-91971, Iran
[2]Department of Parasitology, Toxoplasmosis Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Farah Abad, Sari 48471-91971, Iran
[3]Toxoplasmosis Research Center, Iranian National Registry Center for Lophomoniasis and Toxoplasmosis, School of Medicine, Mazandaran University of Medical Sciences, Farah Abad, Sari 48471-91971, Iran
[4]Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Mazandaran University of Medical Sciences, Farah Abad, Sari 48471-91971, Iran
[5]National Institute of Genetic Engineering and Biotechnology Shahrak-e Pajoohesh, km 15, Tehran - Karaj Highway, P.O. Box: 14965/161, Tehran, Iran

Corresponding Author: Mahdi Fakhar; e-mail: mahdif53@yahoo.com

ABSTRACT. Leishmaniosis is caused by different species of *Leishmania* parasites. The available treatments for this disease have not provided strong consistent results yet. The weak response of current chemotherapeutics can be attributed to their deficient effects on stealth parasites inside macrophages, rapid clearance from the site of action, and systemic side effects in high doses. To enhance leishmaniosis vaccine efficacy, it is a valuable strategy to use liposomes as vaccine delivery systems due to combined increase in technological advances and understanding of the immune system. Liposomes that contain and deliver immunostimulators and antigens are now being developed to target diseases that require stimulation of both humoral and cell-mediated immune responses. Hence, using particulate adjuvants, like liposomes for effective delivery to the antigen presenting cells (APCs) is important for improving leishmaniosis vaccine efficacy. This study aimed at reviewing liposomal adjuvants in vaccine development with specific accentuation on their adjuvant mechanism and surface charge. It also examined how specific physicochemical qualities of liposomes and the particle size during formulation design can affect the immune response

Keywords: adjuvants, leishmaniosis, liposome, physicochemical characteristics, vaccine

Introduction

Leishmaniosis is caused by various strains of *Leishmania* parasites. These parasites which grow and survive in macrophages of mammalian host are transmitted by gnawing of contaminated sand fly species. Four primary kinds of this disease include: cutaneous leishmaniosis (CL), mucocutaneous leishmaniosis (MCL), visceral leishmaniosis (VL), and post-kala-azar dermal leishmaniosis (PKDL). The most well-known form of this infection is CL, which causes cutaneous lesions. Meanwhile, the most severe form is VL, which causes a fatal systemic disease. The majority of available drugs for treating leishmaniosis are toxic, need multiple injections, and have limited efficacy. Additionally, in some cases, drug resistance is inevitable [1,2]. Immunity to *Leishmania* spp. in animal models generally relies upon development of T-helper 1 (Th1)-mediated immune response characterized by high production of interleukin-12 (IL-12) and interferon-γ (IFN-γ). In mice, CD4+ Th1 cells mediate resistance in *Leishmania major* (*L. major*)-infected mice whereas CD4+ Th2 cells promote susceptibility [3].

Contrary to the relationship between Th1-mediated immune response and protection from infection, genuine assurance against the malady

needs the association of both Th1- and Th2-mediated immune responses from vaccination. In addition, some recent observations support that complex cell-mediated immune responses (CMIs) can have an impact on the result of leishmaniosis, especially CL [4].

The leishmaniosis vaccine applicants are usually originated from *Leishmania* spp. and can be classified into three types including: (a) live *Leishmania* spp., containing new genetically modified constructs; (b) first-generation vaccines consisting of killed or fractions of the parasite; and (c) second generation vaccines which are very much characterized *Leishmania* molecules such as recombinant proteins or DNA vaccines [5].

Although many live *Leishmania* preparations for liposomal adjuvant development were recommended as vaccine candidates against leishmaniosis, only few ones proceeded to clinical trials. The immunization with inoculation of live *L. major* promastigotes is recognized as leishmanization (LZ), which has already been utilized in countries like Iran. Although LZ was regarded as the best vaccination technique against CL, there were wide confinements in its institutionalization along with some security worries about the use of LZ. Moreover, advancement of *Leishmania* vaccine utilizing whole killed parasites has been tested.

Among second generation vaccines (DNA molecules or recombinant proteins), only Leish-F3, Leish-F2, and Leish-111f achieved clinical trials. These vaccines included recombinant poly proteins and were formulized with monophosphoryl lipid A (MPL) in a stable oil-in-water emulsion utilizing squalene. Since nearly the use of any *Leishmania* antigen with IL-12 impelled the guarding in animal models, the absence of a proper adjuvant is the major significant disadvantage of using recombinant antigens as vaccines [6,7].

DNA vaccination against leishmaniosis is considered as a promising technology from second-generation vaccination category, yet no improvement has been achieved for application in humans up to now [8]. Similarly, there are immunizations of non-*Leishmania* origin from sandfly salivary glands, which contain immunologically active molecules able to interfere with the host immune responses and markedly promote *Leishmania* infectivity [9]. Development of an effective vaccine against CL is promising since long-term protection is induced upon recovery from CL caused by LZ or natural and regular infection [10,11].

Thus, decades of studies to develop *Leishmania* vaccine have resulted in just few original vaccines being evaluated in phase III clinical trials [11]. Restricted efficacy in leishmaniosis vaccines is somewhat identified with the absence of an appropriate adjuvant.

Accordingly, this review article is based on previous experimental studies and attempts to outline some of the liposomal delivery and adjuvants that have been tested in *Leishmania* vaccines. Additionally, it attempts to briefly describe a portion of the safe reactions incited by these antigen-adjuvant vaccines.

Adjuvants in leishmaniosis vaccines

The capability to enhance the immune response of vaccines by specific compounds was first illustrated with aluminium salts, termed ‘‘adjuvants‘‘, added to constricted or killed pathogens. A systematic methodology for successful development of an effective vaccine needs exact data about the physicochemical properties of a characterized antigen and adjuvant along with the knowledge on formulization of antigen and adjuvant together to produce a safe, steady, and immunogenic vaccine.

The capacity of leishmaniosis vaccines is identified with the capacity to frame a station for prolongation of antigen presentation to APCs. Through direct interaction with APCs, the effective adjuvants animate the human immune system. Vaccine adjuvants have a large and heterogeneous nature and are divided into immune stimulants and delivery systems. The immune stimulants interact with special receptors, for example, Toll-like Receptors (TLRs) and others, while delivery systems animate the immune responses by various systems, depending on their specific characteristics [12,13].

Therefore, current vaccines include adjuvants such as pathogen-derived subcellular components, nucleic acid sequences, peptides, and recombinant proteins [14–17].

Numerous adjuvants with various properties and methods of activity have been utilized in vaccination against leishmaniosis. Some of these properties are clustered as immunostimulatory adjuvants, for example, cytokines (GM-CSF, IL-12, IL-2), monophosphoryl lipid A (MPL), saponins (QS-21, QuilA), CpG oligonucleotides or muramyl dipeptides or tripeptides and derivatives

(MDP/MTP-PE). They are generally pathogen-associated molecular patterns (PAMPs) and are monitored carefully in an expansive scope of pathogens [18]. PPAMPs are the basis of numerous immunostimulatory adjuvants, explicitly those which bind to nucleotide-binding oligomerization domain-like receptors (NLRs) or toll-like receptors (TLRs) [19]. In this classification, IL-12 is the best adjuvant for leishmaniosis, at any rate in mice. But, it is not prescribed to be used in humans because of the risk of side effects, failure to induce long-term immunity, short *in vivo* half-life, and the overall cost [20–22].

Another classification comprises of particulate adjuvants including lipid-, mineral-, or polymer-based delivery systems [23]. The particulate adjuvants, specifically target antigens to the site of activity, improve and regulate the sort of invulnerable reactions, and even upgrade the possibility to cross-present antigens in APCs. Additionally, they upgrade and enhance the ingestion and take-up of antigens by APCs. According to formulation data, particulate adjuvants serve as a warehouse for controlled arrival of antigens, decrease vaccine dose, and increase the steadiness of antigens.

Besides, they might be utilized to enhance the solvability of antigens in aqueous medium to make them proper for parenteral injection [24]. The particulate adjuvants, for example, liposomes, polymeric microspheres, and emulsions have been utilized effectively to deliver *Leishmania* antigens in preclinical models of leishmaniosis, as well as other preclinical forms of infectious diseases [25]. A novel and highly attractive technique for the discerning plan of powerful adjuvants is the blend of immunostimulatory and particulate adjuvants, which has just reached clinical trials. A liposomal vaccine adjuvant system contains two immuno-stimulants such as QS-21 and a MPL called AS01, which are instances of some ongoing procedures. These adjuvants have been utilized for the clinical trials of tuberculosis, herpes zoster, and malaria vaccines [24,26].

Liposomes as vaccine antigens and adjuvants

Liposomes are recognized as spherical vesicles made out of amphipathic phospholipids. They are grouped based on the number of lipids in the bilayer, as large unilamellar vesicles (LUVs), small unilamellar vesicles (SUVs), or multilamellar vesicles (MLVs) with ranging in size between 0.02 to 10 µm in diameter. The size and morphology of liposomes are coordinated by their arrangement and preparation technique [27]. In comparison with other particulate systems, liposomal adjuvants have a couple of positive key conditions for immunization improvement, for example, like safety and biodegradability [28]. Liposomes are routinely made out of lipids, for example, phosphatidylcholine (PC) and cholesterol, which are consolidated typically in cell layers, and as a result, the biodegradable liposomes are created. The liposomal watery central stage is a compartment for representation of hydrophilic particles, while the lipid bilayer stage can be used for hydrophobic mixes [29]. Therefore, a wide range carbohydrate, protein, nucleic acid, and small molecule antigens can be utilized in liposomes.

Liposomes can incorporate a wide variety of antigens, such as malaria, hepatitis A, tetanus toxoid, diphtheria, influenza, and leishmanial proteins; they can be produced with various compositions, bilayer fluidities, sizes, and charges, and have been considered as the antigen delivery systems [30]. In the vaccine related applications, the most vital elements of liposomes are the protection against antigens, antigen clearance from the body, and the conveyance of such antigens to the APCs. Liposomes can cause the antigen incorporation and transfer into the APCs cytosol to incite both cell-mediated immune (CMI) and humoral responses under the antigen presentation pathways by MHC classes I and II. It has been proposed that the liposome uptake occurs through phagocytosis, pinocytosis, or endocytosis procedures [31].

In addition, liposomes are ready to declare different types of chemokine, such as using dendritic cells for declaration of CCL2 (chemokine (C-C motif) ligand 2), CCL3, and CCL4. Moreover, liposomes shield the antigens through a quick debasement inside the APC, and henceforth drag out to T-cells [32]. Utilization of liposomes as the antigen delivery systems is related to a few favourable circumstances, including their low toxicity, biodegradability, and easy preparation. In any case, the efficiency of liposomes is influenced by different physical factors, like their surface charge, phospholipid organization, size, and vesicle steadiness [33]. In a previous reports on the measurement of liposome, the harmony between Th1/Th2 was demonstrated, which can be modified

through changing the span of vesicle. In this study, lipid vesicles were designed with three sizes of 100, 400, or 1000 nm and co-encapsulated with *leishmanial* rGP63 antigen to measure the impact of vesicles on the leishmaniosis cases in BALB/c mice. As indicated by the outcomes, utilization of bigger liposomes (400 and 1000 nm) created about less footpad injuries and parasite troubles in the spleen of BALB/c mice post-contamination with *L. major*. Also, the production of IFN-γ by spleen cells was just expanded in the mice inoculated with bigger liposomes (400 and 1000 nm sizes). As it was observed, in the murine model, bigger liposomes (with sizes equivalent or more noteworthy than 400 nm) instigate CMI response, while smaller liposomes (100 nm) induce humoral response against leishmaniosis [34]. The surface charge of a vesicle has been considered as the adjuvant impact of a liposome. Indeed, the net surface charge of liposomes can be changed through joining positively charged (e.g. dimethyldioctadecylammonium bromide (DDAB)), stearylamine, dioleoyl trimethyl ammonium propane) or negatively charged lipids (phosphatidic acid, phosphatidyl glycerol, phosphatidyl serine (PS), or diphenylcyclopropenone (DCP)). For example, Afrin et al. [35] applied adversely and decidedly charged liposomes containing phosphatidic corrosive and stearylamine, respectively. As the result, they found a correlation with the contrarily charged liposome (comprising of egg lecithin/phosphatidic corrosive/cholesterol; 7:2:2) or the impartial one (comprising of egg lecithin/cholesterol; 7:2). In addition, in the decidedly charged liposome (comprising of egg lecithin/stearylamine/cholesterol; 7:2:2), the layer antigens (LAgs) of *L. donovani* promastigote showed an impressive response against VL, with delayed-type hypersensitivity (DTH) and antibody (Ab) response.

In a previous study by Badiee et al. [36], after preparation of liposomal rGP63 antigen associated with different phospholipids (EPC, DPPC, or DSPC), the corresponding adjuvanticity of such liposomes were compared in infected BALB/c mice with *L. major*. They found that the liposome bilayer composition affects the resultant immune response in mice similar to immunization with liposomes associated with DPPC or DSPC promoted Th1-type immune response, while the ones with EPC led to a Th2-type immune response [36]. Furthermore, in another study, LAg encapsulated in lipid vesicles, which were produced utilizing cholesterol and DSPC (Tc=54°C) and used for intraperitoneally (i.p.) immunization. According to the results, the challenge of protection against *L. donovani* was associated with differentiation of IFN-γ producing Th1 cells and DTH responses [37]. These results showed that liposomes which are prepared with higher-Tc phospholipids seem to be more efficient in promoting the Th1-type immune response and protection, and hence can be further investigated to produce an optimized vaccine against leishmaniosis.

Badiee et al. [38] performed a study on BALB/c mice to assess the effect of liposome charge on stimulating a Th1-type immune response and protection against leishmaniosis and found that negatively and positively-charged liposomes were produced by addition of DCP or DDAB to the neutral liposome, respectively. Their results showed that in comparison with positively-charged liposomes (consisting of dipalmitoyl phosphatidylcholine (DPPC)/DDAB/cholesterol; 2:1:1), incorporation of recombinant GP63 antigen in neutral liposomes (consisting of DPPC/cholesterol; 2:1) is more effective in promoting Th1-type immune response, while using rGP63 antigen in association with negatively charged liposomes (consisting of DPPC/DCP/cholesterol; 2:1:1) resulted in a Th2-type immune response [38]. Hence, such studies demonstrate the essential role of surface charge on the extent and type of the resulting immune response.

In another study, the adjuvanticity and also the protective efficacy of positively-charged liposomes (consisting of egg lecithin/stearylamine/cholesterol; 7:2:2) against *L. donovani* in BALB/c mice were found to be higher than those of the negatively charged liposomes (consisting of egg lecithin/phosphatidic acid/cholesterol; 7:2:2) or the neutral ones (consisting of egg lecithin/cholesterol; 7:2) [38]. The immunization using soluble *Leishmania* antigen (SLA) alone and in positive, neutral, and negative liposomes resulted in different protection levels, correlated with IL-4 and IFN-γ production, in which for maximum protection, there was a skewing toward IFN-γ-producing Th1-type immune response. In addition, it was also demonstrated that SLA in positively-charged liposome vaccine could be applied for immunotherapy in murine against established visceral infection. The results showed that desirable resistance was associated with stimulation of Th1-type immune responses and

inhibition of IL-4 and IL-10 production [39].

In the research studies carried out by Shimizu et al. [40,41] on BALB/c mice, SLA was encapsulated in liposomes, which were coated with neoglycolipids containing oligomannose residues (Man3 or Man5). These liposomes were i.p. administrated and the results were indicative of strong induction of an antigen-specific Th1-type immune response. In such studies, induction of the protective response in BALB/c mice against *L. major* infection may be due to the intense induction of a Th1-type immune response specific to the encapsulated antigen, post-immunization by i.p. administration of SLA-oligomannose-liposome, and its stimulation is also triggered by peritoneal CD11b cells, i.e. macrophages.

Regarding to phospholipid composition of the liposome, Bhowmick et al. [42] assessed a potential vaccine corresponding to LAg, uses reverse-phase evaporation vesicles (REVs), dehydration-rehydration vesicles (DRVs), or multilamellar vesicles (MLVs) to protect BALB/c mice against experimental VL. While a partial resistance was observed in the case of LAg alone or incorporated in REV, nearly complete protection was obtained using cationic liposomes prepared by both MLV and DRV methods. Such protection was mainly of Th1-type immune responses, indicated by the increase in DTH and IgG2a isotype Abs as well as IFN-γ production. The encapsulation of LAg in MLV was associated with durable CMI, and it was observed that mice challenged for ten weeks following vaccination were also able to strongly resist the experimental challenge [42]. In order to improve the vesicle stability, it would be possible to replace vitalized phosphatidylcholine (PC, Tc, -10°C) using phospholipids associated with high transition temperatures (Tc) [43] like distearoyl phosphatidylcholine (DSPC, Tc 54°C), DPPC (Tc 41°C), and dimyristoyl phosphatidylcholine (DMPC, Tc 23°C). In the case of membranes containing antigens with high molecular mass, a correlation between the Tc of phospholipids and the resulting immune response (cellular or humoral) has been reported [44]. It seems that an optimal fluidity of liposomes is required for them to be captured by APCs.

Chavoshian et al. [45] examined the effect of sphingomyelin (SM) liposomes associated with SLA on the promoted immune response against leishmaniosis. Their results indicated that SM-liposome-SLA promoted strong Th2-type immune responses; hence, it cannot be considered as a suitable strategy to promote Th1-type immune response and protect the BALB/c mice against *Leishmania* infection. Furthermore, Sharma et al. [46] produced non-PC liposomes, i.e. escheriosomes from lipids of *Escherichia coli*. They observed that soluble *Leishmania* antigen (sLAg) of *L. donovani* promastigote entrapped in egg PC/cholesterol liposome (EPC-sLAg) or sLAg with incomplete Freund's adjuvant, immunized BALB/c mice through i.p. administration of escheriosome-induced, which led to more intense CD8+ cytotoxic T lymphocyte (CTL) response. In another set of experiments, hamsters immunized with ELsLAg were better protected in comparison with the EPC-sLAg-immunized hamsters. They suggested that escheriosomes can be used to deliver the desired antigen to cytosol of APCs.

In another part of liposomal delivery and adjuvant studies in the immunization against leishmaniosis, different routes of immunization were applied to evaluate the protection impacts of liposomes of *Leishmania* antigens for inducing the immune system against leishmaniosis. In a number of studies, different liposome-encapsulated *Leishmania* antigens such as rGP63 [47–49], sLAg [50], and recombinant *L. major* stress-inducible protein 1 (rLmSTI1) [51,52] were applied for injection via subcutaneous (SC) administration. Other studies focused on the IP administration of liposome-entrapped antigens such as LAgs; [53–56], *L. major* lipophosphoglycan, LPG [39], sLAg [57], GP63 derived from *L. donovani* promastigotes [58], and some polypeptides isolated from *L. donovani* [59].

To identify the immunological correlation between the protective and non-protective routes, Bhowmick et al. [60] made a comparison between the protective efficacy corresponding to LAg, either free or incorporated in positively-charged liposomes through four different administration methods, including i.v., i.p., subcutaneous (s.c.), and intramuscular (i.m.) against *L. donovani* infection in BALB/c mice. They observed that compared to the mice immunized via the s.c. and i.m. routes, which were not protected, the mice immunized via the i.v. and i.p. routes using LAg, either alone or entrapped in liposomes, were protected against the infection with *L. donovani*. In another study conducted by Bhowmick et al. [55], the protection efficacy in the BALB/c mice model was enhanced using LAg in association with the

toll-like receptor (TLR) against monophosphoryl lipid A-trehalose dicorynomycolate (MPLTDM) or cationic liposomes against experimental VL using the i.p. administration method. Due to the inadequacy of i.p. method for human immunization, in another research, they utilized vaccines that were a combination of cationic liposomes, MPL-TDM, and rGP63 as the protein antigen through the s.c. route. Such injections caused enhanced immune responses, which induced higher protection level against VL in the studied mouse model [61]. They also assessed the immune response and protection promoted by the liposome of SLA in association, with MPL-TDM via s.c. administration and found that such BALB/c mice immunization using SLA encapsulated in liposomes or in association with MPL-TDM induced partial protective levels against experimental VL leishmaniosis. However, liposomal SLA containing MPL-TDM adjuvant resulted in much higher protective levels in the spleen and liver of mice infected with *L. donovani* [62].

The liposomal combination is also a suitable vaccine delivery system, and MPL-TDM as an immunopotentiator has been validated by some studies. In 2014, Das and Ali [62] also demonstrated the higher efficacy of an antigenic cocktail of type I, II, and III cysteine proteases (CPs) encapsulated in cationic liposomes in association with MPL-TDM in a hamster model against *L. donovani*. The CPs of *Leishmania* were encapsulated in cationic liposomes associated with MPL-TDM to provide a desirable class of vaccines in human VL. In a previous study, the ability of a 78 kDa antigen from *L. donovani* was investigated. This antigen was in association with various adjuvants, namely autoclaved *L. donovani* antigen (ALD), liposomal encapsulation, MPL-A, recombinant IL-12, and Freund's adjuvant (FCA) against VL leishmaniosis in murine model. According to the results, the vaccine consisting of the 78 kDa antigen and recombinant IL-12 exhibited the highest level of protective efficacy, after which the vaccines containing liposome-incorporated 78 kDa and 78 kDa +MPL-A were observed to elicit Th1-type immune response indicated by the elevated IL-2, IFN-γ, and IgG2a production [63].

The most common *Leishmania* spp. in the liposomal delivery and adjuvant development studies was *L. major*: 21 (53.76%). MRHO/IR/75 /ER 19 (46.17%) and MHOM/IN/83/AG83 11 (26.73%) had the most frequency, respectively. Iran 19 (48.64%) and India 15 (38.4%) had the highest rate of leishmaniosis prevalence (Table 1). SLA 18 (43.74%) had the highest frequency among the antigens used. Moreover, BALB/c 36 (85.68%) was the host/model with the highest frequency. Most of the injections were s.c. inoculation for *L. major*. The highest frequency of molar ratio were related to 7,2,2 and 2,1 with 13 (24.96%) (Tables 2–4).

Effect on immunogenicity by altering the fluidity of liposomal bilayers

Liposomal membrane fluidity is governed by the level of saturation of hydrocarbon chains, and consequently the strength of van der Waals forces that hold adjacent chains [4]. The required energy to disrupt these chains and alter their packing effectiveness, and consequently their level of fluidity/rigidity is defined by the main phase transition temperature (Tm, of the lipid). At the Tm, the lipid bilayers change from a rigid and ordered structure to a loosely disordered and fluid one. The decreasing of hydrocarbon chain length and introducing C=C bonds lead to a reduction in the Tm of the liposome and an increase in bilayer fluidity and permeability. Several studies have described how modification of bilayer fluidity can alter the immunogenicity of liposomes. For example, Schwendener [5] showed that neutral phosphatidylcholine liposomes, in their solid phase at physiological temperatures, induce high cell-mediated and humoral immune responses, whereas liposomes in their fluid-phase give rise only to humoral immune responses, suggesting that the overall humoral immune response is not significantly affected by changes in fluidity. A recent study which investigated adjuvanted influenza vaccines with liposomes composed of a new type of polycationic lipid described no significant changes in immunogenicity (hemagglutination inhibition titers) on substitution of the saturated lipid chains with monounsaturated analogues [82]. However, the transition temperatures of these liposomes or their membrane state have not been mentioned *in vivo*. There are several possible explanations for the alteration of the immune response obtained by changing the liposome fluidity. One explanation could be the uptake mechanism by APCs, which varies depending on the fluidity of the liposomes, resulting in different pathways of antigen presentation and immunostimulation of APCs. Another explanation relates to the depot effect, whereby lengthening antigen or adjuvant retention

Table 1. Characteristics of studies included in this study

Leishmania species	Strain	Country	Antigen	Lipid	Molar ratio	Size (nm)	Host/model	Injection	References
L. major	NR	United Kingdom	Colloidal mixed	DPPC: Chol	1,1	NR	BALB/c	SC	[64]
				DSPC: Chol	1,1				
				PC: Chol	1,1				
L. major	MHOM/IL/67/ Jericho 2	United Kingdom	rgp63	DSPC: Chol	NR	100-2000	BALB/c	SC	[65]
L.major	MRHO/IR/75/ER	Iran	LmSTI1	DSPC: Chol	2,1	1110	BALB/c	SC	[51]
L. major	MRHO/IR/75/ER	Iran	LmSTI1	DSPC: Chol	2,1	1070	BALB/c	SC	[52]
L. major	NR	Iran	rgp63	DPPC: Chol	2,1	NR	BALB/c	SC	[37]
				DDAB: DPPC: Chol	2,1,1				
				DCP: DPPC: Chol	2,1,1				
L. major	MRHO/IR/75/ER	Iran	rgp63	DPPC: Chol	2,1	100	BALB/c	SC	[34]
				DPPC: Chol	2,1	400			
				DPPC: Chol	2,1	1000			
L. donovani	MHOM/IN/83/ AG83	India	SLA	Egg lecithin: Chol	7,2	178	BALB/c	IP	[36]
				Egg lecithin: Chol: SA	7,2,2	181			
				Egg lecithin: Chol: PA	7,2,2	170			
L. donovani	MHOM/IN/83/ AG83	India	rgp63	DSPC: Chol: SA	7,2,2	NR	BALB/c	IP	[58]
L. donovani	MHOM/IN/83/ AG83	India	LAg	DSPC: Chol: SA	7,2,2	405	BALB/c	IP	[38]
L. major	MRHO/IR/75/ER	Iran	Cysteine proteinas	DOTAP: cetyl Palmitat: Chol	3.2:1	NR	BALB/c	SC	[66]
L. major	MRHO/IR/75/ER	Iran	SLA	SM: Chol	1,1	155	BALB/c	SC	[45]
L. donovani	MHOM/IN/83/ AG83	India	Cysteine proteinas	DSPC: Chol: SA	7,2,2		hamster	IP	[62]
L. donovani	MHOM/IN/83/ AG83	India	LAg	DSPC: Chol	7,2,2	NR	BALB/c	SC	[42]
L. donovani	MHOM/IN/1983 /AG83	India	LAg	DSPC: Chol	7,2	NR	hamster	footpad	[54]
				DPPC: Chol	7,2				
				DMPC: Chol	7,2				
L. donovani	MHOM/IN/1983/ AG83	India	SLA	Egg lecithin: Chol: SA	7,2,2	NR	BALB/c	IP	[57]
L. donovani	MHOM/IN/83/ AG83	India	rgp63	DSPC: Chol: SA	7,2,2	NR	BALB/c	SC	[61]
L. donovani	MHOM/IN/80/ Dd8	Iran	SLA	EPC: Chol	7,3	NR	hamster and BALB/c	IP	[46]
L. donovani	MHOM/IN/83/ AG83	India	SLA	Egg lecithin: Chol: SA	7,2,2	NR	hamster and BALB/c	IV and IC	[53]
L. donovani	MHOM/IN/83/ AG83	India	SLA	Egg lecithin: Chol: PA	7,2,1	NR	BALB/c	IP	[56]
L. major	MRHO/IR/75/ER	Iran	SLA	DSPC: Chol			BALB/c	SC	[67]
L. donovani	MHOM/IN/83/ AG83	India	SLA	Lecithin: Chol: SA	7,2,2	337	BALB/c	IP	[55]
L. donovani	MHOM/IN/83/ AG83	India	SLA	Lecithin: Chol: SA	7,2,2	332	BALB/c	SC and IP	[68]

Table 1. Characteristics of studies included in this study

Leishmania species	Strain	Country	Antigen	Lipid	Molar ratio	Size (nm)	Host/model	Injection	References
L. major	MRHO/IR/75/ER	Iran	SLA	DOTAP: Chol	1,1	86	BALB/c	SC	[69]
L. major	MRHO/IR/75/ER	Iran	SLA	DOTAP: Chol	2,1	360	BALB/c	SC	[70]
				DOTAP: Chol	2,1	351			
L. major	MRHO/IR/75/ER	Iran	SLA	DOTAP: Chol	1,1	278	BALB/c	SC	[50]
L. major	MRHO/IR/75/ER	Iran	rgp63	Egg lecithin: Chol	1,1	NR	BALB/c	SC	[47]
L. major	MRHO/IR/75/ER	Iran	rgp63	DPPC: DDAB: Chol	2,1,1	1010	BALB/c	SC	[48]
L. major	MHOM/SU73/ 5KSKH	Japan	SLA	DPPC: Chol: Man5-DPPE	1, 0.48, 0.005	NR	BALB/c	SC	[44]
L. major	MRHO/IR/75/ER	Iran	ALM	PC: Chol: DDAB	7,2,1	1600	C57BL/6	SC	[71]
L. donovani	NR	India	gp63	Egg lecithin: Chol: PA	7,2,2	NR	BALB/c	IP	[72]
L. major	MRHO/IR/75/ER	Iran	SLA	DOTAP: Chol	2,1	400	BALB/c	SC	[73]
L. infantum	MHOM/BR/1970/ BH46	Brazil	LiHyR	DPPC: Chol	6,4	400	BALB/c	SC	[74]
L. major	MRHO/IR/75/ER	Iran	SLA	DSPC: Chol: Mal-PEG2000-IgG	2,1,1	219	BALB/c	SC	[75]
L. donovani	MHOM/IN/80/ Dd8	India	FTP	PC: SA: Chol	7,2,1	NR	BALB/c	SC and IV	[76]
L. donovani	MHOM/IN/83/ AG83	India	LD	DSPC: Chol: SA	7,2,2	NR	BALB/c	IP	[77]
L. major	MRHO/IR/75/ER	Iran	SLA	DDA: TDB: Chol	4,5,3	762	BALB/c	SC	[78]
				DDA: TDB	4,5	1395			
				DDA	4	1200			
				DDA: Chol	4,3	736			
L. amazonensis	NR	Brazil	GPI-anchored proteins	DPPC	1,2,1	220	BALB/c	IP	[79]
L. major	MRHO/IR/75/ER	Iran	SLA	EPC: Chol	2,1	200-400	BALB/c	SC	[80]
L. major	MRHO/IR/75/ER	Iran	SLA	DSPC: Chol	2,1	400	BALB/c	SC	[81]

Explanations: SC=subcutaneous, IP=intraperitoneal, IV=intravenous, IC=intracardiac

at SOI leads to increased exposure of APCs to antigen or immunostimulators, respectively. In support of this, some recent studies have shown that membrane rigidity has a significant effect on the ability of these liposomal vaccines to be retained at the SOI (paper in preparation). The liposomes which composed of more rigid bilayers were able to form a long-term depot of vaccine components (protein antigen and liposome) at the SOI and drained slowly to the local lymph node. In addition, their fluid counterparts were not as strongly retained at the SOI and showed faster draining of liposomal components to the local lymph node. Liposome rigidity correspondence to improved immunogenicity, even though both liposomal formulations expressed comparable size and surface charges and showed similar antigen biodistribution and kinetic profiles. The great number of such studies investigating how the modification of bilayer fluidity alters the immunogenicity of liposomes incorporates cholesterol to stabilize lipid bilayers. However, this adds a level of complexity to the interpretation when comparing, for example, lipids with different chain lengths or degrees of saturation [83–86]. In addition to altering the gel-to-fluid phase transition that is obtained by changing acyl-chain length or degree of saturation of the membrane lipids, cholesterol eliminates the solid-ordered and fluid-disordered phases by reorganization of the membrane lipids [87].

In a study by Nakano et al. [88], the humoral immune response mediated by neutral phosphati-

Table 2. Description of characteristic of *Leishmania* sp.

Variable	Category	n (%)
Leishmania species	*L. major*	21(53.76)
	L. donovani	16(40.96)
	L. infantum	1(2.56)
	L. amazonensis	1(2.56)
Strain	MHOM/IL/67/Jericho 2	1(2.43)
	MHOM/IN/1983/AG83	2(4.86)
	MHOM/IN/80/Dd8	2(4.86)
	MHOM/IN/83/AG83	11(26.73)
	MHOM/SU73/5KSKH	1(2.43)
	MRHO/IR/75/ER	19(46.17)
	MHOM/BR/1970/BH46	1(2.43)
	NR	4(9.76)
Country	Brazil	2(5.12)
	India	15(38.4)
	Iran	19(48.64)
	Japan	1(2.56)
	United Kingdom	2(5.12)
Antigen	ALM	2(4.86)
	Colloidal mixed	1(2.43)
	Cysteine proteinas	2(4.86)
	FTP	1(4.86)
	GPI-anchored proteins	1(2.43)
	LAg	3(7.29)
	LD	1(2.43)
	LmSTI1	2(4.86)
	rgp63	9(21.87)
	SLA	18(43.74)
	LiHyR	1(2.43)
Host/model	BALB/c	36(85.68)
	C57BL/6	2(4.76)
	hamster	2(4.76)
	hamster and BALB/c	2(4.76)

dylcholine-derived liposomes was shown to be reduced after incorporation of cholesterol. By contrast, Bakouche and Gerlier [86] found that incorporation of cholesterol increased the immunogenicity of the liposomes, suggesting that it is not so much associated to solid packing of the lipid membrane but rather related to ordered conformation of the lipid involved in the immunogenicity of the liposomes. Van Houte et al. [89] observed the incorporation of cholesterol into fluid as well as solid haptenated liposomes increased their immunogenicity, whereas liposomes with an intermediate Tm (~ 40°C) were already highly immunogenic and thus were briefly affected by the incorporation of cholesterol. Based on these results, they concluded that the induction of humoral immune responses by liposomes required an 'intermediate' fluidity of the lipid bilayer. The overall effect of incorporation of cholesterol is therefore not consistent, suggesting that the effect might also be related to other unknown factors.

Role of active targeting to APCs

Liposomes deliver their substance into cells by both passive and active targeting mechanism. The

Table 3. Description of characteristic injection type, size of liposome and molar ratio

Variable	Category	n (%)
Injection	Footpad	1(2.56)
	Intraperitoneal	10(25.6)
	Intravenous and intracardiac	1(2.56)
	Peritoneal	1(2.56)
	Subcutaneous	24(61.44)
	Subcutaneous and intravenous	1(2.56)
	Subcutaneously and intraperitoneally	1(2.56)
Molar ratio	1,1	7(13.44)
	1,2,1	1(1.92)
	2,1	13(24.96)
	2,1,1	4(7.68)
	3,2,1	1(1.92)
	7,2	4(7.68)
	7,2,1	3(5.76)
	7,2,2	13(24.96)
	7,3	1(1.92)
	4,5,3	1(1.92)
	4,3	1(1.92)
	4,5	1(1.92)
	6,4	1(1.92)
	1, 0.48, 0.005	1(1.92)

Table 4. Description of molar proportion of lipids

Lipids	n (%)
Chol: DPPC	1(1.69)
DCP: DPPC: Chol	1(1.69)
DDA	1(1.69)
DDA: Chol	1(1.69)
DDA: TDB	1(1.69)
DDA: TDB: Chol	1(1.69)
DDAB: DPPC: Chol	1(1.69)
DDAB: PC: CHOL	1(1.69)
DMPC: Chol	1(1.69)
DOTAP: Chol	5(8.45)
DOTAP: cetyl Palmitat: Chol	1(1.69)
DPPC	1(1.69)
DPPC: Chol	7(11.83)
DPPC: Chol: Man5-DPPE	1(1.69)
DPPC: DDAB: Chol	1(1.69)
DSPC	1(1.69)
DSPC: Chol	8(13.52)
DSPC: Chol: Mal-PEG2000-lgG	1(1.69)
DSPC: Chol: SA	5(8.54)
DSPC: Chol	2(3.38)
Egg lecithin: Cho: SA	1(1.69)
Egg lecithin: Chol	2(3.38)
Egg lecithin: Chol: PA	2(3.38)
Egg lecithin: Chol: SA	3(5.07)
EPC: Chol	2(3.38)
Lecithin: Chol: SA	2(3.38)
PC: Chol: DDAB	1(1.69)
PC: SA: Chol	1(1.69)
PC: Chol	1(1.69)
SM: Chol	1(1.69)
EPC: Chol	1(1. 69)

physicochemical properties of liposomes, for example, surface charge, size, and significant role in passive targeting to APCs, and active targeting intended to increase the delivery of content-specific interplay with a target cell [90], DCs make a basic connection between inborn and versatile resistance. Among various sorts of APCs, the powerful antigen exhibiting properties of DCs make them significant targets for the delivery of vaccine immunogenic fixings. Presentation of ligands or monoclonal antibodies like anti-ClecA, anti-DC-SIGN Abs, mannose, or anti-DEC for DC surface receptors enhanced the productivity of targeted delivery of liposomes to DCs [91].

A liposomal SLA formulation comprising of cholesterol, mannopentaose, dipalmitoyl phosphatidyl ethanol amine (Man5-DPPE), and DPPC was accumulated to consider its defensive response in BALB/c mice against leishmaniosis [92]. The Man5-DPPE is a blended neoglycolipid which can interface with mannose receptors of APCs. The liposomes coated with Man5-DPPE prompted insurance against leishmanial infection compared with non-covered liposomes. Both footpad swelling and parasite stack at nearby lymph nodes diminished considerably in the mice vaccinated with Man5-DPPE-coated liposomes. Some authors expressed that actively targeted liposomes are a more appropriate adjuvant than charged liposomes as their adjuvanticity is due to an effective carbohydrate. The protein interaction through the mannose receptor is a lot more grounded and more particular than interplay by charge. The coupling of target-special Abs to the liposomal surface to make immunoliposomes has been identified as a promising method to accomplish dynamic targeting of liposomes. In an examination, immunoliposomes were set up by uniting non-immune mice IgG onto the liposomal surface [93]. The outcomes showed that in spite of the fact that liposomes are impressive adjuvants to induce defence against *L. major* challenge in mice, more grounded CMI was actuated when immunoliposomes were exploited. They reasoned that DCs that endocytosed immunoliposomes by means of their FcγRs most likely exhibited antigen on MHC I and MHC II atoms all the more proficiently, prompting viable incitement of CD8 just as CD4 T cells [94]. Altogether, it ought to be noticed that active targeting to DCs utilizing liposomes is an appealing way to create potent defensive cellular immunity (CMI) against leishmaniosis, yet at the same time few examinations have assessed this impact. Today, it is broadly acknowledged that focusing on antigens to DCs through some particular receptors inspires noteworthy antigen-special immune responses contrasted with nonreceptor-interceded antigen take-up by micropinocytosis or different types of endocytosis. Therefore, ongoing information recommends that combinatorial focusing of various DC subsets may essentially

upgrade the adequacy of DC targeting. The improvement of such combinatorial methodologies is possible in liposomal formulization and would enable the researchers to attain a potent Th1 immune response against leishmaniosis.

Role of surface charge

The combination of lipids in liposome bilayers determines the surface charge. Except for a few special cases, positively charged liposomes are taken by APCs to a much higher degree than a negatively-charged or neutral liposomes. Since cationic substances communicate with negatively charged molecules on the surface of APCs, they target antigens for endocytosis more proficiently than other kinds. Also, positively charged liposomes made out of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), a cationic lipid with fusogenic properties, induce the generation of CD8 T cell responses, which requires antigen presentation in the context of the MHC I [95]. The vast majority of studies in the leishmaniosis field have utilized DOTAP, dimethyl dioctadecyl ammonium bromide (DDAB), or stearylamine (SA) for liposomal formulation. Notwithstanding, other ordinarily utilized cationic lipids, for example, N-[1-(2,3-dioleoyloxy) propel]-N,N,N-trimethylammonium (DOTMA), 3-β-[N-(N,N'-dimethylaminoethane) carbamoyl] cholesterol (DC-Chol), can be utilized as well [96]. Afrin et al. [53] assessed the immunogenicity of LAg alone or in relationship with positively-charged liposomes. The liposomes essentially ensured the hamsters and BALB/c mice, in which the splenic parasite load was reduced. Additionally, a successful incitement of all immunoglobulin G (IgG) isotypes, especially IgG2a, with liposomes happened. Another study compared negatively charged liposomes of upgrade security against LAg with control groups. The results showed that the level of protection by liposomal LAg was not notably different from that induced by free LAg, and interestingly, anionic liposomes elicited strong antibody responses, particularly IgG1. They summarized that the Th2 response was prevailing with anionic formulation [56]. Bhowmick et al. [58] demonstrated that gp63 encapsulated in cationic liposomes comprising of DSPC initiated noteworthy conservation against advanced VL in BALB/c mice. The defensive viability after vaccination against liposomal gp63 was additionally dose-dependent. The control of infection progression and parasitic weight in mice was related with upgrade of IL-4 and decrease of IFN-γ. Ravindran et al. [8] showed the adequacy of three adjuvants, cationic liposomes, MPL, trehalose dicorynomycolate (TDM), and BCG to confer protection against murine VL. Although each of the three formulations obtained considerable conservation against *L. donovani*, the most abnormal level of protection was exhibited by liposomal LAg.

Thakur et al. [76] determined the defensive viability of freeze-thawed *L. donovani* (FTP) antigen blended with various adjuvants including cationic liposomes, saponin, MPL or alum. Cationic liposomes were made out of SA, PC, and cholesterol. The highest protection was accomplished with liposome-encapsulated FTP antigen. Higher delayed-type hypersensitivity (DTH) response and the most extreme decrease in parasite load were induced by liposomal FTP over different adjuvants. Also, Bhowmick et al. [77] compared the efficacy of different antigens as antibody competitors including polypeptides 51 (LD51), 31 (LD31), 91 (LD91), and 72 (LD72)-kDa encapsulated in cationic liposomes made out of SA, DSPC, and cholesterol in BALB/c mice. Their results exhibited that liposomal LD51 and LD31 inoculation decreased parasite burden to the best degree over different formulations. The investigation of cytokine responses in immunized mice demonstrated that all the immunized groups produced IL-4, IFN-γ and IL-12. In addition, Bhowmick et al. [38] assessed the defensive efficacy of soluble *Leishmania* antigen in positive, negative, and neutral liposomes and assessed the immune response for protection against *L. donovani* in a BALB/c model. The liposomes were set up with cholesterol and egg lecithin in the presence of either phosphatidic corrosive (PA) or SA. The best formulation for vaccine contained positively charged liposomes which were then utilized for immunotherapy. This vaccine induced >90% deletion of parasites in both spleen and liver. These findings recommended that an initiated immune balance by positively charged liposomes towards Th1 is powerful for both effective immunization and immunotherapy. It appears that the kind of cationic lipids and their physicochemical properties blended with antigen have a key role in the produced immune response. DDAB is normally utilized in liposome formulation to prompt a Th1 response dependent on two primary systems. The first is to plan positively charged liposomes, and the

second one to utilize the inherent adjuvanticity of engineered quaternary ammonium compounds. But, they usually prompt blended Th1/Th2 reactions as anti-*Leishmania* antibodies. The capacity of rgp63-containing liposomes made out of positively-charged lipid (DDAB), negatively charged lipid (DCP) and neutral lipid (DPPC) to assess protection against leishmaniosis was compared in mice [97].

In another study, a cationic liposomal SLA vaccine was additionally created utilizing cholesterol and DDAB in the presence or absence of trehalose-6,6-dibehenate (TDB). The findings demonstrated that vaccination with liposomal SLA composed of TDB or DDAB induces a blended Th1/Th2 invulnerable reaction and is not a fitting procedure for enlistment of a Th1 response and protection against leishmaniosis. Interestingly, DOTAP as a synthetic ammonium compound induced a strong Th1 response and protection in BALB/c mice against *L. major*. Liposomal SLA demonstrated the lowest footpad swelling and the most reduced spleen and footpad parasite load after the challenge. This group additionally demonstrated the higher IgG2a titer, highest IFN-γ production rate, and lower IL-4 levels. The protective adequacy of liposomal SLA vaccination was also dose-dependent, with 50 µg of protein indicating optimal protection. Overall, the surface charge of liposomes has a critical role in the type and extent of produced immune responses, and the cationic lipids toned to be custom fitted to accomplish an immune response in formulation with each antigen [47].

In a different study, egg sphingomyelin (SM) containing saturated acyl chains was utilized to create a stable negatively charged liposome containing SLA. The results showed that mice getting these liposomes demonstrated fundamentally huge footpad swelling, higher parasite trouble in the foot, and higher IL-4 levels compared with the group vaccinated with buffer, which were not protected against leishmaniosis. The immune response not only relies upon the surface charge of liposomes, but also in relationship with liposome planning strategy. Resistant reactions were affected by comparative LAg-containing cationic liposomes arranged by three distinct techniques, including reversed-phase evaporation vesicles (REV), dehydration-rehydration vesicles (DRV) and conventional MLV. LAg in DRV or MLV, but not in REV, induced practically total protection. The examination of immune response in DRV and MLV bunches demonstrated elicitation of humoral immune response as well as CMI as confirmed by IFN-γ, LAg-specific antibodies, and DTH responses. Upregulation of IgG2a and low expression of IgG1 were seen in sera from BALB/c mice inoculated with LAg either in DRV or MLV, proposing that the cell immune response was essentially Th1 in these animals. Additionally, cationic MLV induced sustained Th1 immunity after utilizing as adjuvant with protein antigens.

In conclusion, positively-charged liposomes initiate more grounded immune responses compared with equivalent negatively-charged or neutral formulations. It is also important to note that extracellular matrix in the injection site which has a negative charge, can maintain liposomes at the injection site for a longer period of time. This may bring about skewing the evoked immune response toward Th1; however this is not valid in any case, at any rate on account of DDAB liposomes, as mentioned in the previous lines. Impact of liposome size and vesicle lamellarity are of the most noteworthy factors in liposomal vaccine formulation. Particle size may impact the depleting energy of liposomes; as little liposomes are cleared faster from infusion locales than large ones [96,97]. Little particles, not exactly a couple of nanometers, are generally transported to the blood, while bigger particles, < 150 nm, are openly transported into the lymphatic vessels. The liposomes over a couple of hundred nanometers in size will be trapped in the interstitial space for a longer time period or transported by dendritic cells (DCs) [98].

Badiee et al. [37] investigated the efficacy of liposome size on protection rate and type of generated response against leishmaniosis. Liposomal rgp63 with various sizes (100, 400, 1000 nm) were arranged and vaccinated into BALB/c mice. The findings of this study demonstrated that immunization with small size (100 nm) liposomes actuated a Th2 response, while the large size (400 nm,) liposomes actuated a Th1 response and protection in mice. There was no significant difference in the kind of induced immune response between 1000, 400 nm, or extruded liposomes.

The response against liposomal antigens might be affected by liposome lamellarity. Bhowmick et al. [41] compared the immune response in mice with LAg encapsulated in REV (unilamellar, 897nm), DRV (multilamellar, 882 nm), and MLV (multilamellar, 405 nm). REV evoked higher IgG1, while MLV and DRV elicited higher titer of IFN-γ and IgG2a. Accordingly, cationic MLV and DRV

were considered as appropriate adjuvants and antigen delivery systems for designing subunit vaccines.

To the best of our knowledge, there are no similar investigations demonstrating the impact of liposome lamellarity and size on immune responses against leishmaniosis. However, the results of studies in other models of the disease propose that liposomes with a size scope of 250–700 nm in diameter move the T-helper profile of immune response toward Th1 through expanding both determinations at the infusion site and transit to depleting lymph nodes. It should also be considered that this is typically appropriate on account of negatively-charged or neutral liposomes and not for cationic liposomes. The cationic liposomes of any size range are in part immobilized at the infusion site in view of their electrostatic collaboration with extracellular matrix substance.

Role of phospholipid composition and bilayer fluidity

The lipid bilayer of liposomes can be in various physical stages relying upon the particular lipid structures. Lipids with a primary stage change temperature (Tm) below 37ºC will be in a fluid crystalline state in the body, while they will be in a gel state if the Tm is above 37ºC. The physical condition of the bilayer can influence handling of the vaccine components, intracellular trafficking, and endocytosis which may impact immune responses [99]. In general, there is a correlation between the Tm of phospholipids and created immune response for membrane-related antigens [100]. Nonetheless, a phospholipid creation that prompts powerful immune responses to a particular antigen may not really induce a similar immunity to others. Thus, designing a liposomal adjuvant tailored for each antigen independently is needed [101,102].

Although the mechanisms of bilayer fluidity on liposomal adjuvanticity have not been clearly known yet, it is suggested that two mechanisms be included in the meantime: (a) the rate of antigen discharge from the bilayer vesicles at the site of infusion and (b) the method of liposomal interplay with APCs. In the first case, solid liposomes become unsteady *in vivo* at a slower rate than liquid ones; thus they present antigens more proficiently to APCs. Also, fluidity has an impact on endocytosis, extent of liposomal fusion, and processing by APCs; regarding endocytosis and fusion, solid liposomes would not be appropriate choices. Thus, it appears that liposomes need an optimal fluidity for an antigen to initiate an ideal response [28]. The liposomal rgp63 composed of distearoylphosphatidylcholine (DSPC, Tm 54°C), egg phosphatidylcholine (EPC, Tm <0°C), or dipalmitoylphosphatidylcholine (DPPC, Tm 41°C) was prepared in an investigation to create anti-*Leishmania* vaccine in BALB/c mice [41]. The liposomes composed of DSPC and DPPC elicit a Th1 immune response against *L. major* with no considerable difference between them; but, liposomes prepared with EPC induced a Th2 safe response.

BALB/c mice immunized i.p. with proteoliposomes showed a total protection (90%). This investigation proposed that liposomes advance the slow arrival of antigens at the site of infusion and trigger the immune system. Broadly speaking, liposomes with higher Tm phospholipids are appropriate formulations to induce the Th1 response and to secure mice against leishmaniosis. High Tm phospholipids or expanding the amount of cholesterol in the formulation increase the consistency of packing of phospholipids or the stability of vesicle bilayers at the body's temperature, and therefore oppose phospholipid loss to the extracellular matrix. Accordingly, liposomal integrity is protected and entangled antigens stay with the transporter for longer time periods leading to greater adjuvanticity. Along with the role of fluidity on the adjuvanticity, lipid combination of the liposome importantly affects immune responses, especially when these lipids have inherent immunostimulatory properties. Non-phosphatidylcholine (non-PC) liposomes (escheriosomes) prepared from *E. coli* lipids were utilized to deliver LAg to APCs [49]. The vaccine leads to powerful humoral immunity, just as CMI, both in BALB/c mice and hamsters. Immunization of BALB/c mice with escheriosomes elicited stronger cytotoxic T-lymphocyte (CTL) response compared with LAg entrapped in LAg or egg PC/cholesterol liposomes managed with inadequate Freund's adjuvant. Also, the delivery of LAg by means of escheriosomes upgraded the expression of costimulatory signals (CD86 and CD80). The escheriosome-immunized hamsters were found to be better protected than those immunized with liposomal LAg. The escheriosome-vaccinated hamsters were observed to be better secured over those vaccinated with liposomal LAg. They deduced that escheriosomes

can possibly deliver antigens to cytosol of APCs. In another study, gp63 and *Leishmania* promastigote lipophosphogly were reconstituted into liposomes composed of segregated phospholipids from the trypanosomatid flagellate crithidia fasciculate, and vaccinated intravenous (IV), subcotaneus (s.c.) or intraperitoneal injection (i.p.) into laboratory inbred (CBA/ca) or BALB/c mice [49]. Perfect protection was obtained just in CBA/ca mice by SC inoculation of liposomal antigens. It was interesting that antigen-containing liposomes did not cause infection intensification compared with non-liposomal unrefined parasite extracts. Moreover, Samiei et al. [103] examined a strategy to develop an artificial liposome-based cell that contained sections of *L. major*. They focused on developing another strategy for engrafting plasma membrane vesicles of parasites into liposomes and did not assess immune responses. They recommended that the existence of whole-cell membrane constituents of parasites (glycol phospholipids, proteins and glycoproteins) could be fundamental for getting a defensive immune response. In addition, adding a Th1-inducer adjuvant in the structure of engrafted liposomes could enhance the ability of prepared vaccines to protect against intracellular parasites that are dependent on Th1 responses. Finally, McConville et al. [39] reported that the dominating cell surface glycoconjugated antigen of *L. major* contained a GPI-like membrane anchor. At the point when this antigen was captured in multilamellar liposomes and infused into mice, the animals were protected from CL.

Future perspective

The advancement of effective vaccines with high safety profiles has brought about more research studies dependent on peptide vaccines or subunit protein. Liposomes offer a very intriguing framework to dominate these problems as they are able for giving two essential characteristics: adjuvant properties and antigen delivery. Intensive development efforts have resulted in a number of different liposomal vaccines on the market against several pathogens such as influenza and hepatitis A virus, but not for leishmaniosis. But it should also be taken into account that liposomal vaccines against leishmaniosis elicit different perspectives beyond common vaccines as a result of the quickly expanding interdisciplinary field of liposome technology. Since an impressive prophylactic vaccine for leishmaniosis should have a powerful therapeutic efficacy, some recent studies have demonstrated that liposomes can be utilized in this regard. As a matter of fact, numerous vaccines formulations designed for prophylaxis should also have been utilized for immunotherapy against leishmaniosis. Furthermore, immunotherapy alone or blended with accessible chemotherapies is a choice for patients with the non-healing type of CL lesions.

In a nutshell, there are still numerous barriers to defeat before broad clinical utilization of liposomal vaccines in composition with anti-*Leishmania* medicates in human. It appears that the technique for association of antigen with liposome essentially influences the magnitude and type of produced immune response. Encapsulation, surface adsorption, covalent surface phospholipid conjugation and noncovalent surface connection are the most widely recognized methods of antigen relationship with liposomes [28]. Several studies demonstrated that highest CMI is produced when the antigen is conjugated to the liposome surface rather than encapsulation. Regarding humoral response, the difference is substantially more noteworthy for antigens conjugated to the liposome surface. Conversely, it was observed that the surface conjugation and encapsulation for antigens induced similar CMI [28].

From the pharmaceutical perspective, the main difficulties in creating liposomal vaccine formulations are scalability, stability, optimization and standardization of products. Since every physicochemical property of liposomes (surface modification, colloidal stability, size, structure, and composition) add to the clinical result, any little batch-to-batch variety in liposome portrayal can cause serious changes in product efficiency and performance. Thus, the adaptability procedure of liposome preparation must be trustworthy and predictable. Another specialized test is to produce reliable liposomes at industrial scale, which typically kills numerous lab-scale planning techniques. Most studies introduced in this survey demonstrated some promising findings, however none of the arranged liposomes have reached clinical trial yet. The reason may be the formulation difficulties, for example, consistency and versatility issues. It should also be noted that a close collaboration between parasitologists, vaccinologists, pharmaceuticals, and immunologists is necessary to build up an impressive, steady, safe, and affordable

liposomal vaccine. The flexibility of liposomal adjuvants allows for the delivery of different active factors (for example, targeting ligands antigens and focusing on ligands) with the capability to enter into different kinds of APCs, just as focusing on their receptors. In addition, pattern recognition receptor (PRR) agonists, like CpG ODN, can be effectively incorporated into liposomal formulations to balance the extent and inclination of T-helper in the immune response. This leads to improved efficacy and development of liposomal adjuvants. These unique properties make liposomes a challenging topic to study, but fascinating to researchers who are eager to improve anti-*Leishmania* vaccine in patients. It is good to specify that due to the inborn properties of the antigens themselves or the relationship of the numerous variables engaged with liposomal formulation, the formulation parameters must be constantly improved cautiously for each new liposomal vaccine candidate. One of the issues confronting vaccines is the potential unspecific and unanticipated immunostimulatory activities of adjuvants when combined with various antigens.

Therefore, adjuvants are currently just used combined with explicit antigens as a vaccine and not as individual substances. Subsequently, the present adjuvant-based approach depends on testing the adjuvant with every conceivable antigen and portion mixed through chosen administration routes. Finally, since the function of adjuvants has not been completely assessed, their results cannot be generalized when combined with specific vaccine antigens. This requires more data on adjuvant mechanisms and the immune system itself. Exogenous pathogenic upgrades, such as lipopeptides, peptidoglycans, lipopolysaccharide (LPS), ssRNA and flagelin have all been recognized as agonists of adjuvant development, and TLRs has normally leant towards improvement of engineered (thus mass-producible and less lethal) analogs of such molecules. It is progressively evident that creation of little modifications in the administration route, size of liposomes, or kind of lipid monomers effectively affect the viability of the vaccine, just as which arm of the immune response is specially focused on. Liposomes offer a significant favourable superiority over aluminium-based mineral salt and o/w vaccine adjuvants that are authorized at present, since they are profoundly flexible and equipped for animating both CD4 (TH1 and additionally TH2) and CD8 T cells, contingent upon the joining of TLR-stimulators, bacterially determined particles, or immunogenic lipids.

References

[1] Palumbo E. 2009. Current treatment for cutaneous leishmaniasis: a review. *American Journal of Therapeutics* 16: 178-182. doi:10.1097/MJT.0b013e3181822e90

[2] Vandome F.P., McBrewster A.F., Miller J. 2009. Leishmaniasis. 1st ed. Alphascript Publishing, Mauritius.

[3] Biedermann T., Zimmermann S., Himmelrich H., Gumy A., Egeter O., Sakrauski A.K., Seegmüller I., Voigt H., Launois P., Levine A.D., Wagner H. 2001. IL-4 instructs T_H1 responses and resistance to *Leishmania major* in susceptible BALB/c mice. *Nature Immunology* 2: 1054-1060. doi:10.1038/ni725

[4] Scott P., Novais F.O. 2016. Cutaneous leishmaniasis: immune responses in protection and pathogenesis. *Nature Reviews Immunology* 16: 581-592. doi:10.1038/nri.2016.72

[5] Khamesipour A., Rafati S., Davoudi N., Maboudi F., Modabber F. 2006. Leishmaniasis vaccine candidates for development: a global overview. *Indian Journal of Medical Research* 123: 423.

[6] Coler R.N., Reed S.G. 2005. Second-generation vaccines against leishmaniasis. *Trends in Parasitology* 21: 244-249. doi:10.1016/j.pt.2005.03.006

[7] Gillespie P.M., Beaumier C.M., Strych U., Hayward T., Hotez P.J., Bottazzi M.E. 2016. Status of vaccine research and development of vaccines for leishmaniasis. *Vaccine* 34: 2992-2995. doi:10.1016/j.vaccine.2015.12.071

[8] Okwor I., Uzonna J. 2009. Vaccines and vaccination strategies against human cutaneous leishmaniasis. *Human Vaccines* 5: 291-301. doi:10.4161/hv.5.5.7607

[9] Andrade B.D., De Oliveira C.I., Brodskyn C.I., Barral A., Barral-Netto M. 2007. Role of sand fly saliva in human and experimental leishmaniasis: current insights. *Scandinavian Journal of Immunology* 66: 122-127. doi:10.1111/j.1365-3083.2007.01964.x

[10] Khamesipour A., Dowlati Y., Asilian A., Hashemi-Fesharki R., Javadi A., Noazin S., Modabber F. 2005. Leishmanization: use of an old method for evaluation of candidate vaccines against leishmaniasis. *Vaccine* 23: 3642-3648. doi:10.1016/j.vaccine.2005.02.015

[11] Khanjani N., González U., Leonardi-Bee J., Mohebali M., Saffari M., Khamesipour A. Vaccines for preventing cutaneous leishmaniasis. *Cochrane Database of Systematic Reviews* 2009,1: cd007634. doi:10.1002/14651858.CD007634

[12] Leroux-Roels G. 2010. Unmet needs in modern vaccinology: adjuvants to improve the immune response. *Vaccine* 28 (Suppl. 3): C25-C36. doi:10.1016/j.vaccine.2010.07.021

[13] Alving C.R., Peachman K.K., Rao M., Reed S.G. 2012. Adjuvants for human vaccines. *Current Opinion in Immunology* 24: 310-315. doi:10.1016/j.coi.2012.03.008

[14] Pérez O., Romeu, B., Cabrera O., González E., Batista-Duharte A., Labrada A., Pérez R., Reyes L., Ramírez, W., Sifontes S., Fernández N., Lastre M. 2013. Adjuvants are key factors for the development of future vaccines: lessons from the Finlay adjuvant platform. *Frontiers in Immunology* 4: 407. doi:10.3389/fimmu.2013.00407

[15] Reed S.G., Orr M.T., Fox C.B. 2013. Key roles of adjuvants in modern vaccines. *Nature Medicine* 19: 1597.

[16] Zepp F. 2010. Principles of vaccine design-lessons from nature. *Vaccine* 28: 14-24. doi:10.1016/j.vaccine.2010.07.020

[17] Joshi M.D., Unger W.J., Storm G., van Kooyk Y., Mastrobattista E. 2012. Targeting tumor antigens to dendritic cells using particulate carriers. *Journal of Controlled Release* 161: 25-37. doi:10.1016/j.jconrel.2012.05.010

[18] Bhowmick S., Ali N. 2008. Recent developments in leishmaniasis vaccine delivery systems. *Expert Opinion on Drug Delivery* 5: 789-803. doi:10.1517/17425247.5.7.789

[19] Higgins S.C., Mills K.H. 2010. TLR, NLR agonists, and other immune modulators as infectious disease vaccine adjuvants. *Current Infectious Disease Reports* 12: 4-12. doi:10.1007/s11908-009-0080-9

[20] Campos-Neto A. 2002. Anti-*Leishmania* vaccine. In: Leishmania: world class parasites. (Ed. J.P. Farrell). Springer, Boston, MA 4: 169-190.

[21] Vajdy M., Srivastava I., Polo J., Donnelly J., O'hagan D., Singh M. 2004. Mucosal adjuvants and delivery systems for protein, DNA and RNA based vaccines. *Immunology and Cell Biology* 82: 617-627. doi:10.1111/j.1440-1711.2004.01288.x

[22] Gurunathan S., Prussin C., Sacks D.L., Seder R.A. 1998. Vaccine requirements for sustained cellular immunity to an intracellular parasitic infection. *Nature Medicine* 4: 1409-1415. doi.org/10.1038/4000

[23] Palatnik-de-Sousa C.B. 2008. Vaccines for leishmaniasis in the fore coming 25 years. *Vaccine* 26: 1709-1724. doi:10.1016/j.vaccine.2008.01.023

[24] Reed S.G., Bertholet S., Coler R.N., Friede M. 2009. New horizons in adjuvants for vaccine development. *Trends in Immunology* 30: 23-32. doi:10.1016/j.it.2008.09.006

[25] Badiee A., Shargh V.H., Khamesipour A., Jaafari M.R. 2013. Micro/nanoparticle adjuvants for antileishmanial vaccines: present and future trends. *Vaccine* 31: 735-749. doi:10.1016/j.vaccine.2012.11.068

[26] Garçon N., Chomez P., Van Mechelen M. 2007. GlaxoSmithKline adjuvant systems in vaccines: concepts, achievements and perspectives. *Expert Review of Vaccines* 6: 723-739. doi:10.1586/14760584.6.5.723

[27] Kshirsagar N.A., Pandya S.K., Kirodian B.G., Sanath S. 2005. Liposomal drug delivery system from laboratory to clinic. *Journal of Postgraduate Medicine* 51: 5-15.

[28] Watson D.S., Endsley A.N., Huang L. 2012. Design considerations for liposomal vaccines: influence of formulation parameters on antibody and cell-mediated immune responses to liposome associated antigens. *Vaccine* 30: 2256-2272. doi:10.1016/j.vaccine.2012.01.070

[29] Sharma A., Sharma U.S. 1997. Liposomes in drug delivery: progress and limitations. *International Journal of Pharmaceutics* 154: 123-140.

[30] Schwendener R.A. 2014. Liposomes as vaccine delivery systems: a review of the recent advances. *Therapeutic Advances in Vaccines* 2: 159-182. doi:10.1177/2051013614541440

[31] Rao M., Alving C.R. 2000. Delivery of lipids and liposomal proteins to the cytoplasm and Golgi of antigen-presenting cells. *Advanced Drug Delivery Reviews* 41: 171-188. doi:10.1016/S0169-409X(99)00064-2

[32] Portuondo D.L., Ferreira L.S., Urbaczek A.C., Batista-Duharte A., Carlos I.Z. 2015. Adjuvants and delivery systems for antifungal vaccines: current state and future developments. *Medical Mycology* 53: 69-89.

[33] Akbarzadeh A., Rezaei-Sadabady R., Davaran S., Joo S.W., Zarghami N., Hanifehpour Y., Samiei M., Kouhi M., Nejati-Koshki K. 2013. Liposome: classification, preparation, and applications. *Nanoscale Research Letters* 8: 102. doi:10.1186/1556-276X-8-102

[34] Badiee A., Khamesipour A., Samiei A., Soroush D., Shargh V.H., Kheiri M.T., Barkhordari F., Mc Master W.R., Mahboudi F. Jaafari M.R. 2012. The role of liposome size on the type of immune response induced in BALB/c mice against leishmaniasis: rgp63 as a model antigen. *Experimental Parasitology* 132: 403-409. doi:10.1016/j.exppara.2012.09.001

[35] Afrin F., Rajesh R., Anam K., Gopinath M., Pal S., Ali N. 2002. Characterization of *Leishmania donovani* antigens encapsulated in liposomes that induce protective immunity in BALB/c mice. *Infection and Immunity* 70: 6697-6706. doi:10.1128/IAI.70.12.6697-6706.2002

[36] Badiee A., Jaafari M.R., Khamesipour A., Samiei A., Soroush D., Kheiri M.T., Barkhordari F, McMaster W.R., Mahboudi F. 2009. Enhancement of immune response and protection in BALB/c mice immunized with liposomal recombinant major surface glycoprotein of *Leishmania* (rgp63): the role of bilayer composition. *Colloids and Surfaces B: Biointerfaces* 2009 74: 37-44. doi:10.1016/j.colsurfb.2009.06.025

[37] Mazumdar T., Anam K., Ali N. 2004. A mixed Th1/Th2 response elicited by a liposomal formulation of *Leishmania* vaccine instructs Th1 responses and resistance to *Leishmania donovani* in susceptible BALB/c mice. *Vaccine* 22: 1162-1171. doi:10.1016/j.vaccine.2003.09.030

[38] Badiee A., Jaafari M.R., Khamesipour A., Samiei A., Soroush D., Kheiri M.T., Barkhordari F., McMaster W.R., Mahboudi F. 2009. The role of liposome charge on immune response generated in BALB/c mice immunized with recombinant major surface glycoprotein of *Leishmania* (rgp63). *Experimental Parasitology* 121: 362-369. doi:10.1016/j.exppara.2008.12.015

[39] Bhowmick S., Ravindran R., Ali N. 2007. Leishmanial antigens in liposomes promote protective immunity and provide immunotherapy against visceral leishmaniasis via polarized Th1 response. *Vaccine* 25: 6544-6556. doi:10.1016/j.vaccine.2007.05.042

[40] Shimizu Y., Yamakami K., Gomi T., Nakata M., Asanuma H., Tadakuma T., Kojima N. 2003. Protection against *Leishmania major* infection by oligomannose-coated liposomes. *Bioorganic and Medicinal Chemistry* 11: 1191-1195. doi:10.1016/S0968-0896(02)00644-2

[41] Shimizu Y., Takagi H., Nakayama T., Yamakami K., Tadakuma T., Yokoyama N., Yokoyama, N., Kojima N. 2007. Intraperitoneal immunization with oligomannose-coated liposome-entrapped soluble leishmanial antigen induces antigen-specific T-helper type immune response in BALB/c mice through uptake by peritoneal macrophages. *Parasite Immunology* 29: 229-239. doi:10.1111/j.1365-3024.2007.00937.x

[42] Bhowmick S., Mazumdar T., Sinha R., Ali N. 2010. Comparison of liposome based antigen delivery systems for protection against *Leishmania donovani*. *Journal of Controlled Release* 141: 199-207. doi:10.1016/j.jconrel.2009.09.018

[43] McConville M.J., Bacic A., Mitchell G.F., Handman E. 1987. Lipophosphoglycan of *Leishmania major* that vaccinates against cutaneous leishmaniasis contains an alkylglycerophosphoinositol lipid anchor. *Proceedings of the National Academy of Sciences* 84: 8941-8945. doi:10.1073/pnas.84.24.8941

[44] Gregory G. 2006. Liposome technology. New York (NY), CRC Press.

[45] Chavoshian O., Biari N., Badiee A., Khamesipour A., Abbasi A., Saberi Z., Jalali S.A., Jaafari M.R. 2013. Sphingomyelin liposomes containing soluble *Leishmania major* antigens induced strong Th2 immune response in BALB/c mice. *Iranian Journal of Basic Medical Sciences* 16: 965.

[46] Sharma S.K., Dube A., Nadeem A., Khan S., Saleem I., Garg R., Mohammad O. 2006. Non PC liposome entrapped promastigote antigens elicit parasite specific CD8+ and CD4+ T-cell immune response and protect hamsters against visceral leishmaniasis. *Vaccine* 24: 1800-1810.

[47] Jaafari M.R., Ghafarian A., Farrokh-Gisour A., Samiei A., Kheiri M.T., Mahboudi F., Barkhordari F., Khamesipour A., McMaster W.R. 2006. Immune response and protection assay of recombinant major surface glycoprotein of *Leishmania* (rgp63) reconstituted with liposomes in BALB/c mice. *Vaccine* 24: 5708-5717. doi:10.1016/j.vaccine.2006.04.062

[48] Jaafari M.R., Badiee A., Khamesipour A., Samiei A., Soroush D., Kheiri M.T., Barkhordari F., McMaster W.R., Mahboudi F. 2007. The role of CpG ODN in enhancement of immune response and protection in BALB/c mice immunized with recombinant major surface glycoprotein of *Leishmania* (rgp63) encapsulated in cationic liposome. *Vaccine* 2007 25: 6107-6117. doi:10.1016/j.vaccine.2007.05.009

[49] Russell D.G., Alexander J. 1988. Effective immunization against cutaneous leishmaniasis with defined membrane antigens reconstituted into liposomes. *The Journal of Immunology* 140: 1274-1279.

[50] Shargh V.H., Jaafari M.R., Khamesipour A., Jalali S.A., Firouzmand H., Abbasi A., Badiee A. 2012. Cationic liposomes containing soluble *Leishmania* antigens (SLA) plus CpG ODNs induce protection against murine model of leishmaniasis. *Parasitology Research* 111: 105-114. doi:10.1007/s00436-011-2806-5

[51] Badiee A., Jaafari M.R., Khamesipour A. 2007. *Leishmania major*: immune response in BALB/c mice immunized with stress-inducible protein 1 encapsulated in liposomes. *Experimental Parasitology* 115: 127-134. doi:10.1016/j.exppara.2006.07.002

[52] Badiee A., Jaafari M.R., Samiei A., Soroush D., Khamesipour A. 2008. Coencapsulation of CpG oligodeoxynucleotides with recombinant *Leishmania major* stressinducible protein 1 in liposome enhances immune response and protection against leishmaniasis in immunized BALB/c mice. *Clinical and Vaccine Immunology* 15: 668-674. doi:10.1128/CVI.00413-07

[53] Afrin F., Ali N. 1997. Adjuvanticity and protective immunity elicited by *Leishmania donovani* antigens encapsulated in positively charged liposomes. *Infection and Immunity* 65: 2371-2377.

[54] Mazumdar T., Anam K., Ali N. 2005. Influence of phospholipid composition on the adjuvanticity and protective efficacy of liposome-encapsulated *Leishmania donovani* antigens. *Journal of Parasitology* 91: 269-274. doi:10.1645/GE-356R1

[55] Ravindran R., Bhowmick S., Das A., Ali N. 2010. Comparison of BCG, MPL and cationic liposome adjuvant systems in leishmanial antigen vaccine formulations against murine visceral leishmaniasis.

BMC Microbiology 10: 181.

[56] Afrin F., Anam K., Ali N. 2000. Induction of partial protection against *Leishmania donovani* by promastigote antigens in negatively charged liposomes. *Journal of Parasitology* 86: 730-735. doi:10.1645/0022-3395(2000)086[0730:IOPPAL]2.0.CO;2

[57] Mazumder S., Ravindran R., Banerjee A., Ali N. 2007. Non-coding pDNA bearing immunostimulatory sequences co-entrapped with leishmanial antigens in cationic liposomes elicits almost complete protection against experimental visceral leishmaniasis in BALB/c mice. *Vaccine* 25: 8771-8781. doi:10.1016/j.vaccine.2007.10.028

[58] Bhowmick S., Ravindran R., Ali N. 2008. gp63 in stable cationic liposomes confers sustained vaccine immunity to susceptible BALB/c mice infected with *Leishmania donovani*. *Infection and Immunity* 76: 1003-1015. doi:10.1128/IAI.00611-07

[59] McCall L.I., Zhang W.W., Ranasinghe S., Matlashewski G. 2013. Leishmanization revisited: immunization with a naturally attenuated cutaneous *Leishmania donovani* isolate from Sri Lanka protects against visceral leishmaniasis. *Vaccine* 310: 1420-1425. doi:10.1016/j.vaccine.2012.11.065

[60] Bhowmick S., Mazumdar T., Ali N. 2009. Vaccination route that induces transforming growth factor β production fails to elicit protective immunity against *Leishmania donovani* infection. *Infection and Immunity* 77: 1514-1523. doi:10.1128/IAI.01739-07

[61] Mazumder S., Maji M., Ali N. 2011. Potentiating effects of MPL on DSPC bearing cationic liposomes promote recombinant GP63 vaccine efficacy: high immunogenicity and protection. *PLOS Neglected Tropical Diseases* 5: e1429. doi:10.1371/journal.pntd.0001429

[62] Das A., Ali N. 2014. Combining cationic liposomal delivery with MPL-TDM for cysteine protease cocktail vaccination against *Leishmania donovani*: evidence for antigen synergy and protection. *PLOS Neglected Tropical Diseases* 8: e3091. doi:10.1371/journal.pntd.0003091

[63] Nagill R., Kaur S. 2010. Enhanced efficacy and immunogenicity of 78kDa antigen formulated in various adjuvants against murine visceral leishmaniasis. *Vaccine* 28: 4002-4012. doi:10.1016/j.vaccine.2010.01.015

[64] Kahl L.P., Scott C.A., Lelchuk R., Gregoriadis G, Liew F.Y. 1989. Vaccination against murine cutaneous leishmaniasis by using *Leishmania major* antigen/liposomes. Optimization and assessment of the requirement for intravenous immunization. *The Journal of Immunology* 142: 4441-4449.

[65] Kahl L.P., Lelchuk R., Scott C.A., Beesley J. 1990. Characterization of *Leishmania major* antigen-liposomes that protect BALB/c mice against cutaneous leishmaniasis. *Infection and Immunity* 58: 3233-3241.

[66] Doroud D., Zahedifard F., Vatanara A., Najafabadi A.R., Taslimi Y., Vahabpour R., Torkashvand F., Vaziri, B., Rafati S. 2011. Delivery of a cocktail DNA vaccine encoding cysteine proteinases type I, II and III with solid lipid nanoparticles potentiate protective immunity against *Leishmania major* infection. *Journal of Controlled Release* 153: 154-162. doi:10.1016/j.jconrel.2011.04.011

[67] Golali E., Jaafari M.R., Khamesipour A., Abbasi A., Saberi Z., Badiee A. 2012. Comparison of *in vivo* adjuvanticity of liposomal PO CpG odn with liposomal PS CpG ODN: Soluble *Leishmania* Antigens. Antigens as a model. *Iranian Journal of Basic Medical Sciences* 15: 1032-1045.

[68] Ravindran R., Maji M., Ali N. 2011. Vaccination with liposomal leishmanial antigens adjuvanted with monophosphoryl lipid-trehalose dicorynomycolate (MPL-TDM) confers long-term protection against visceral leishmaniasis through a human administrable route. *Molecular Pharmaceutics* 9: 59-70.

[69] Firouzmand H., Badiee A., Khamesipour A., Shargh V.H., Alavizadeh S.H., Abbasi A. 2013. Induction of protection against leishmaniasis in susceptible BALB/c mice using simple DOTAP cationic nanoliposomes containing soluble *Leishmania* antigen (SLA). *Acta Tropica* 128: 528-535. doi:10.1016/j.actatropica.2013.07.021

[70] Shargh V.H., Jaafari M.R., Khamesipour A., Jaafari I., Jalali S.A., Abbasi A., Badiee A. 2012. Liposomal SLA co-incorporated with PO CpG ODNs or PS CpG ODNs induce the same protection against the murine model of leishmaniasis. *Vaccine* 30: 3957-3964. doi:10.1016/j.vaccine.2012.03.040

[71] Sohrabi Y.A., Jaafari M.R., Mohammadi A.M., Eskandari S.E., Khamesipour A. 2005. Evaluation of immune response against leishmaniasis in resistance C57 BL/6 mice immunized with liposomes containing autoclaved *Leishmania major* with BCG. *Cellular and Molecular Biology Letters* 10: 98.

[72] Afrin F., Rajesh R., Anam K., Gopinath M., Pal S., Ali N. 2002. Characterization of *Leishmania donovani* antigens encapsulated in liposomes that induce protective immunity in BALB/c mice. *Infection and Immunity* 70: 6697-6706. doi:10.1128/IAI.70.12.6697-6706.2002

[73] Mehravaran A., Rezaei Nasab M., Mirahmadi H., Sharifi I., Alijani E., Nikpoor A.R., Akhtari J., Hojatizade M. 2019. Immunogenicity and protection effects of cationic liposome containing imiquimod adjuvant on leishmaniasis in BALB/c mice. *Iranian Journal of Basic Medical Sciences* 22: 922-931.

[74] Ribeiro P.A., Dias D.S., Novais M.V., Lage D.P., Tavares G.S., Mendonça D.V., Oliveira J.S., Chávez-Fumagalli M.A., Roatt B.M., Duarte M.C., Menezes-Souza D. 2018. A *Leishmania* hypothetical protein-

containing liposome-based formulation is highly immunogenic and induces protection against visceral leishmaniasis. *Cytokine* 111: 131-139. doi:10.1016/j.cyto.2018.08.019

[75] Eskandari F., Talesh G.A., Parooie M., Jaafari M.R., Khamesipour A., Saberi Z. 2014. Immunoliposomes containing Soluble *Leishmania* Antigens (SLA) as a novel antigen delivery system in murine model of leishmaniasis. *Experimental Parasitology* 146: 78-86. doi:10.1016/j.exppara.2014.08.016

[76] Thakur A., Kaur H., Kaur S. 2015. Studies on the protective efficacy of freeze thawed promastigote antigen of *Leishmania donovani* along with various adjuvants against visceral leishmaniasis infection in mice. *Immunobiology* 220: 1031-1038. doi:10.1016/j.imbio.2015.05.014

[77] Bhowmick S., Ali N. 2009. Identification of novel *Leishmania donovani* antigens that help define correlates of vaccine-mediated protection in visceral leishmaniasis. *PloS One* 4: 5820. doi:10.1371/journal.pone.0005820

[78] Hojatizade M., Badiee A., Khamesipour A., Mirshafiey A., Akhtari J., Mehravaran A., Alavizadeh S.H., Abbasi A., Saberi Z., Nikpoor A.R. Jaafari M.R. 2017. DDA/TDB liposomes containing soluble *Leishmania major* antigens induced a mixed Th1/Th2 immune response in BALB/c mice. *Nanomedicine Journal* 4: 71-82.

[79] Colhone M.C., Silva-Jardim I., Stabeli R.G., Ciancaglini P. 2015. Nanobiotechnologic approach to a promising vaccine prototype for immunisation against leishmaniasis: a fast and effective method to incorporate GPI-anchored proteins of *Leishmania amazonensis* into liposomes. *Journal of Microencapsulation* 32: 143-150. doi:10.3109/02652048.2014.958203

[80] Mehravaran A., Mirahmadi H., Akhtari J. 2019. Liposomes containing the imiquimod adjuvant as a vaccine in the cutaneous leishmaniasis model. *Nanomedicine Journal* 7: 29-39.

[81] Mehravaran A., Nasab M.R., Mirahmadi H., Sharifi I., Alijani E., Nikpoor A.R., Akhtari J. 2019. Protection induced by *Leishmania major* antigens and the imiquimod adjuvant encapsulated on liposomes in experimental cutaneous leishmaniasis. *Infection, Genetics and Evolution* 70: 27-35. doi:10.1016/j.meegid.2019.01.005

[82] Even-Or O., Samira S., Rochlin E., Balasingam S., Mann A.J., Lambkin-Williams R., Spira J., Goldwaser I., Ellis R., Barenholz Y. 2010. Immunogenicity, protective efficacy and mechanism of novel CCS adjuvanted influenza vaccine. *Vaccine* 28: 6527-6541. doi:10.1016/j.vaccine.2010.04.011

[83] Nakano Y., Mori M., Nishinohara S., Takita Y., Naito S., Kato H., Taneichi M., Komuro K., Uchida T. 2001. Surface-linked liposomal antigen induces IgE-selective unresponsiveness regardless of the lipid components of liposomes. *Bioconjugate Chemistry* 12: 391-395. doi.org/10.1021/bc0001185

[84] Dancey G.F., Yasuda T., Kinsky S.C. 1978. Effect of liposomal model membrane composition on immunogenicity. *The Journal of Immunology* 120: 1109-1113.

[85] Yasuda T., Dancey G.F., Kinsky S.C. 1977. Immunogenicity of liposomal model membranes in mice: dependence on phospholipid composition. *Proceedings of the National Academy of Sciences* 74: 1234-1236. doi:10.1073/pnas.74.3.1234

[86] Bakouche O., Gerlier D. 1986. Enhancement of immunogenicity of tumour virus antigen by liposomes: the effect of lipid composition. *Immunology* 58: 507-513.

[87] Mannock D.A., Lee M.Y., Lewis R.N., McElhaney R.N. 2008. Comparative calorimetric and spectroscopic studies of the effects of cholesterol and epicholesterol on the thermotropic phase behaviour of dipalmitoylphosphatidylcholine bilayer membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1778: 2191-2202. doi:10.1016/j.bbamem.2008.05.004

[88] Nakano Y., Mori M., Yamamura H., Naito S., Kato H., Taneichi M., Tanaka Y., Komuro K., Uchida T. 2002. Cholesterol inclusion in liposomes affects induction of antigen-specific IgG and IgE antibody production in mice by a surface-linked liposomal antigen. *Bioconjugate Chemistry* 13: 744-749. doi.org/10.1021/bc0155667

[89] Van Houte A.J., Snippe H., Schmitz M.G., Willers J.M. 1981. Characterization of immunogenic properties of haptenated liposomal model membranes in mice. V. Effect of membrane composition on humoral and cellular immunogenicity. *Immunology* 44: 561-568.

[90] Bachmann M.F., Jennings G.T. 2010. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nature Reviews Immunology* 10: 787-796. doi:10.1038/nri2868

[91] Badiee A., Davies N., McDonald K., Radford K., Michiue H., Hart D., Kato M. 2007. Enhanced delivery of immunoliposomes to human dendritic cells by targeting the multilectin receptor DEC-205. *Vaccine* 25: 4757-4766. doi:10.1016/j.vaccine.2007.04.029

[92] Takagi H., Furuya N., Kojima N. 2007. Preferential production of IL-12 by peritoneal macrophages activated by liposomes prepared from neoglycolipids containing oligomannose residues. *Cytokine* 40: 241-250. doi:10.1016/j.cyto.2007.10.005

[93] Nakanishi T., Hayashi A., Kunisawa J., Tsutsumi Y., Tanaka K., Yashiro-Ohtani Y., Nakanishi M., Fujiwara H., Hamaoka T., Mayumi T. 2000. Fusogenic liposomes efficiently deliver exogenous antigen through the cytoplasm into the MHC class I processing pathway. *European Journal of Immunology* 30: 1740-1747.

doi:10.1002/1521-4141(200006)30:6<1740::AID-IMMU1740>3.0.CO;2-U

[94] Bhowmick S., Ali N. 2008. Recent developments in leishmaniasis vaccine delivery systems. *Expert Opinion on Drug Delivery* 5: 789-803. doi:10.1517/17425247.5.7.789

[95] Gregory A.E., Williamson D., Titball R. 2013. Vaccine delivery using nanoparticles. *Frontiers in Cellular and Infection Microbiology* 3: 13. doi:10.3389/fcimb.2013.00013

[96] Carstens M.G., Camps M.G., Henriksen-Lacey M., Franken K., Ottenhoff T.H., Perrie Y., Bouwstra J.A., Ossendorp F., Jiskoot W. 2011. Effect of vesicle size on tissue localization and immunogenicity of liposomal DNA vaccines. *Vaccine* 29: 4761-4770. doi:10.1016/j.vaccine.2011.04.081

[97] Oussoren C., Zuidema J., Crommelin D.J., Storm G. 1997. Lymphatic uptake and biodistribution of liposomes after subcutaneous injection.: II. Influence of liposomal size, lipid composition and lipid dose. *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1328: 261-272. doi:10.1016/S0005-2736(97)00122-3

[98] McLennan D.N., Porter C.J., Charman S.A. 2005. Subcutaneous drug delivery and the role of the lymphatics. *Drug Discovery Today: Technologies* 2: 89-96. doi:10.1016/j.ddtec.2005.05.006

[99] Tandrup Schmidt S., Foged C., Smith Korsholm K., Rades T., Christensen D. 2016. Liposome-based adjuvants for subunit vaccines: formulation strategies for subunit antigens and immunostimulators. *Pharmaceutics* 8: 7. doi:10.3390/pharmaceutics8010007

[100] Kersten G.F., Crommelin D.J. 1995. Liposomes and ISCOMS as vaccine formulations. *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes* 1241: 117-138. doi:10.1016/0304-4157(95)00002-9

[101] Davis D., Gregoriadis G. 1987. Liposomes as adjuvants with immunopurified tetanus toxoid: influence of liposomal characteristics. *Immunology* 61: 229-234.

[102] Gregoriadis G., Panagiotidi C. 1989. Immuno-adjuvant action of liposomes: comparison with other adjuvants. *Immunology Letters* 20: 237-240. doi:10.1016/0165-2478(89)90086-2

[103] Samiei A., Tamadon A.M., Samani S.M., Manolios N., Sarvestani E.K. 2014. Engraftment of plasma membrane vesicles into liposomes: a new method for designing of liposome-based vaccines. *Iranian Journal of Basic Medical Sciences* 17: 772.

Received 24 February 2020
Accepted 18 June 2021