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A new approach for development of vaccine against visceral leishmaniasis: Lipophosphoglycan and polyacrylic acid conjugates

Adil M. Allahverdiyev[1]✉, Rabia Cakir Koc[1], Melahat Bagirova[1], Serhat Elcicek[2], Serap Yesilkir Baydar[1], Olga Nehir Oztel[1], Emrah Sefik Abamor[1], Sezen Canim Ates[3], Murat Topuzogullari[1], Sevil Isoglu Dincer[4], Zeynep Akdeste[1]

[1]*Yildiz Technical University, Department of Bioengineering, Istanbul, Turkey*

[2]*Firat University, Department of Bioengineering, Elazig, Turkey*

[3]*Yeni Yuzyil University, Biomedical Engineering, Istanbul, Turkey*

[4]*Abdullah Gul University, Material Science and Nanotechnology Department, Istanbul, Turkey*

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ABSTRACT

Objective: To determine the antileishmanial vaccine effectiveness of lipophosphoglycan (LPG) and polyacrylic acids (PAA) conjugates on *in vivo* mice models.

Methods: LPG molecule was isolated and purified from large-scale *Leishmania donovani* parasite culture. Protection efficacies of LPG alone, in combination with Freund's adjuvant, in a physical mixture and in conjugate (consisting of various LPG concentrations) with PAA, were comparatively determined by various techniques, such as cultivation with the micro-culture method, assessment of *in vitro* infection rates of peritoneal macrophages, determination of parasite load in liver with Leishman-Donovan Units, and detection of cytokine responses.

Results: Obtained results demonstrated that the highest vaccine-mediated immune protection was provided by LPG-PAA conjugate due to all parameters investigated. According to the Leishman-Donovan Units results, the sharpest decline in parasite load was seen with a ratio of 81.17% when 35 μg LPG containing conjugate was applied. This value was 44.93% for the control group immunized only with LPG. Moreover, decreases in parasite load were 53.37%, 55.2% and 65.8% for the groups immunized with 10 μg LPG containing LPG-PAA conjugate, a physical mixture of the LPG–PAA, and a mixture of LPG + Freund's adjuvant, respectively. Furthermore, cytokine results supported that Th1 mediated protection occurred when mice were immunized with LPG-PAA conjugate.

Conclusions: It has been demonstrated in this study that conjugate of LPG and PAA has an antileishmanial vaccine effect against visceral leishmaniasis. In this respect, the present study may lead to new vaccine approaches based on high immunogenic LPG molecule and adjuvant polymers in fighting against *Leishmania* infection.

1. Introduction

Leishmaniasis, which is caused by the *Leishmania* species, are intracellular parasites of mammals, and is one of the largest public health problems in 98 countries and territories around the world, including Turkey. It is known that nearly 350 million people are at risk for this infection. Every year, approximately 1–1.5 million cases of cutaneous leishmaniasis and 500 000 cases of visceral leishmaniasis occur worldwide [1,2]. Visceral leishmaniasis (VL), or kala-azar, is known as the most serious form of leishmaniasis and is the second most deadly parasitic infection, following malaria. Treatment with chemotherapy is prolonged, costly, and toxic and requires frequent monitoring and infrastructure that may be beyond the capacity of health systems where VL is endemic [3–7]. There is currently no vaccine available for any form of leishmaniasis [8]. Therefore, development of a vaccine against leishmaniasis has been

✉First and corresponding author: Adil M. Allahverdiyev, Yildiz Techical University, Department of Bioengineering, Istanbul, Turkey.
Tel: +90 0212 383 46 39
E-mail: adilmoglu@gmail.com

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insistently advocated by the World Health Organization. One of the basic reasons for this suggestion is the recognition of vaccination as the only prevention strategy because of the unquestionable role of the immune system in controlling *Leishmania* infection. Hence, various vaccine approaches have been pursued for many years [9].

To date, developed vaccines were investigated in three groups: first-, second- and third-generation vaccines. Vaccinations with live virulent parasites (termed leishmanization) or with killed parasites are considered first-generation vaccines; vaccinations with subunits, purified fractions, recombinant vaccines in heterologous microbial vectors, and genetically or otherwise attenuated live parasites are considered second-generation vaccines; and DNA-based vaccines are considered third-generation vaccines [10]. However, all of these vaccine trials had serious disadvantages despite their advantages. Among first-generation vaccines, the use of killed parasites is considered to be confident, but their efficacies were demonstrated to be low. The use of attenuated live *Leishmania* parasites (called leishmanization) was suspended by reason of the fact that parasites can achieve virulence again, even many years later [11,12]. In recent times, genetically modified *Leishmania* parasites have been used as live-attenuated vaccine candidates. In general, genetically modified live *Leishmania* parasites are obtained by the elimination of virulence genes such as dyhydrofolate reductase, biopterin reductase, cystein proteases or heat shock proteins and vaccination with these antigens achieved promising results in animal studies. However, safety concerns are still an obstacle that prevents their use in clinical trials [13–15]. Second-generation vaccines using antigen fragments of parasites provide efficient protection against leishmaniasis compared to other groups of vaccines. These vaccine candidates are obtained in two different ways: one is isolation and purification of native antigens from a parasite culture, the other one is producing antigens by using recombinant DNA technology [11]. The success of subunit vaccines based on recombinant proteins or peptides which are found in second-generation vaccines, has been demonstrated, but was also variable to poor [16]. Despite the fact that the immunogenicities of several recombinant antigens were investigated on animal models, only a few of them achieved progression to clinical trials that were performed on primates, dogs and humans (preclinical studies) [17,18]. In one of these efforts, recombinant A2 protein of *Leishmania chagasi*, which was used in combination with saponin adjuvant, provided partial (40%) protection against canine leishmaniasis and this protection rate was found to be sufficient to develop a licensed canine vaccine that is named Leish-tec® [19]. Clinical trials of other recombinant proteins such as Leish-111F/MPL-SE have been done on humans, however, there are currently no licensed human vaccines based on recombinant antigens [20,21].

Recently, second generation-vaccine development studies have also focused on the native surface glycoconjugates of parasites. One of the important surface antigens of *Leishmania*, which is called the Fucose-Mannose Ligand (FML) possess high immunogenic features. By considering the antigenicity of FML, researchers prepared a vaccine formulation including FML and a saponin adjuvant that was isolated from Quillaja saponaria. This vaccine candidate underwent Phase I-III clinical trials and has been licensed as Leishmune® [22]. In different endemic regions of the world, this vaccine is being used in humans with success indicating that antigenic molecules isolated from Leishmanial parasite surfaces have great potential to be formulated as vaccine candidates and provide strong protection.

Like FML, lipophosphoglycan (LPG) is another important surface glycoprotein of *Leishmania* parasites. LPG covers all surfaces of parasites including flagella and plays an important role in the survival of parasites, both in humans and in vector organisms. The basic LPG structure in all *Leishmania* species consists of a 1-O-alkyl-2-lyso-phosphatidyl inositol lipid anchor, a heptasaccharide glycan core, a long phosphoglycan (PG) polymer composed of (Galb1-4Mana1-PO4) n repeat units (n = 10–40), and a small oligosaccharide cap [23].

In one study, it was shown that intranasal vaccination with *Leishmania amazonensis* LPG was an important immunomodulatory molecule [24]. Other experiments have shown that LPG provided protection to *Leishmania major* (*L. major*) infections in BALB/c mice [25–27]. However, protection was demonstrated to be dependent upon the use of adjuvants such as liposomes or killed *Corynebacterium parvum* and the integrity of the molecule. Therefore, LPG may be a good vaccine candidate only when it is used with appropriate adjuvants.

As compared with whole-cell or virus-based vaccines, subunit vaccines are poorly immunogenic and require the presence of adjuvants to stimulate protective immunity [28,29]. However, the most effective adjuvants generally cause significant inflammation. This may be essential for adjuvanticity, but their use in humans may be precluded because of unacceptable side effects. For approximately the past two decades, vaccine research has been focused on the alternation of the alum type of an adjuvant in order to increase immunogenicity. Biodegradable polymers are being used as adjuvants and drug carriers, because of their biocompatible, nontoxic nature and their biodegradable properties. Polymers that are chosen as excipients (adjuvants) for parenterally administered vaccines should meet some requirements, including being biodegradable, safe, antigen compatible and permeable, stable *in vitro*, easy to process and, ideally, inexpensive [30].

Polyacrylic acids (PAA) that are strongly negatively charged compounds with high molecular weight demonstrate adjuvant effects for both humoral and cell-mediated immunity [31,32]. Previously, synthetic polymers of PAA and more hydrophobic derivatives containing alkyl-esters significantly enhanced the antibody response against numerous inactivated model protein antigens [33,34]. These fully synthetic constructs are potentially safe in that they have not induced adverse effects in animal models and display potentially low cytotoxicity [35]. There are only a limited number of vaccine studies in the extant literature demonstrating the adjuvant properties of PAA against infectious diseases. In one of these studies, PAA conjugate was reported as a sufficient adjuvant for protection against haemorrhagic nephritis enteritis, a disease caused by a polyomavirus, since it increased the antibody response significantly in gooses [36]. However, to date, its efficacy as an adjuvant has not been investigated against leishmaniasis.

Considering the high immunogenic properties of the LPG molecule, adjuvant features of PAA and the convenience in chemical structures of each molecule to compose a conjugation, we suggest that a formulation that includes LPG-PAA conjugates could be an important vaccine candidate against Visceral Leishmaniasis. However, to the best of our knowledge, there are no antileishmanial vaccine studies based on LPG-PAA conjugates in the literature. Therefore, for the first time, this study aimed to implement conjugation between PAA and the highly immunogenic LPG molecule found on the surface of *Leishmania* parasites, to investigate the effectiveness of LPG-PAA conjugates as a vaccine candidate on animal models and to reveal their role in the development of new vaccines against leishmaniasis.

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