Liposomal SLA co-incorporated with PO CpG ODNs or PS CpG ODNs induce the same protection against the murine model of leishmaniasis

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A R T I C L E   I N F O

Article history:
Received 2 September 2011
Received in revised form 16 February 2012
Accepted 16 March 2012
Available online 29 March 2012

Keywords:
Vaccine
Liposome
SLA
CpG ODNs
Leishmaniasis

A B S T R A C T

First generation Leishmania vaccines consisting of whole killed parasites with or without adjuvants have reached phase 3 trial and failed to show enough efficacy mainly due to the lack of an appropriate adjuvant. In this study, the nuclease-resistant phosphorothioate CpG oligodeoxynucleotides (PS CpG) or nuclease-sensitive phosphodiester CpG ODNs (PO CpG) were used as adjuvants to enhance immunogenicity and rate of protection against leishmaniasis. Due to the susceptibility of PO CpG to nuclease degradation, an efficient liposomal delivery system was developed to protect them from degradation. 1, 2-dioleoyl-3-trimethylammonium-propane (DOTAP) as a cationic lipid was used because of its unique adjuvanticity and electrostatic interaction with negatively charged CpG ODNs. To evaluate the role of liposomal formulation in protection rate and enhanced immune response, BALB/c mice were immunized subcutaneously with liposomal soluble Leishmania antigens (SLA) co-incorporated with PO CpG (Lip-SLA–PO CpG), Lip-SLA–PS CpG, SLA + PO CpG, SLA + PS CpG, SLA or buffer. As criteria for protection, footpad swelling at the site of challenge, parasite loads, the levels of IFN-γ and IL-4, and the IgG subtypes were evaluated. The groups of mice receiving Lip-SLA–PO CpG or Lip-SLA–PS CpG showed a high protection rate compared with the control groups. In addition, there was no significant difference in immune response generation between mice immunized with PS CpG and the group receiving PO CpG when incorporated into the liposomes. The results suggested that liposomal form of PO CpG might be used instead of PS CpG in future vaccine formulations as an efficient adjuvant.

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1. Introduction

Leishmania species are dimorphic homoflagellate intracellular protozoan parasites of macrophage-dendritic cell lineage that cause a spectrum of diseases ranging from a self-healing cutaneous lesion to potentially fatal visceral form of disease, known as leishmaniasis [1,2]. The current control strategies are either ineffective or hard to maintain in many foci, available drugs are expensive, need multiple injections, accompany with side effects and are not always effective [3–5].

It is well known for centuries that long lasting protection induces upon recovery from cutaneous leishmaniasis (CL) or leishmanization [4,5]. Leishmania subunit vaccines has not reached phase 3 while whole killed Leishmania with or without adjuvant reached to phase 3 clinical trials. However, prophylactic studies showed a limited efficacy mainly due to the limited Th1 inducer adjuvant for use in human [4–8].

Synthetic oligodeoxynucleotides, containing unmethylated CpG motifs, are extremely efficient inducer of Th1 immune response and generation of cytotoxic T lymphocyte (CTL) which have shown to induce protection against an extensive range of viral, bacterial and some parasitic pathogens in animal models [9]. While human trials have yielded promising results [10–12], clinical use of free CpG ODNs still faces several challenges which limit their effectiveness. One of the limiting factors in the success of oligonucleotide-based immunotherapeutics is rapid degradation of unmodified ODNs (PO CpG) within the body. This problem is diminished by modifications such as the replacement of non-bridging oxygen with sulfur in phosphate linkages to prepare nuclease-resistant phosphorothioate analogs (PS CpG) [13]. Despite backbone stabilization of CpG ODNs, PS-modified ODNs are still susceptible to nuclease degradation, albeit at a lower rate [14]. More critically, phosphorothioate