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Protection against cutaneous leishmaniasis by intranasal vaccination with lipophosphoglycan

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Abstract

We previously showed the opposing effect of systemic and mucosal vaccination with whole *Leishmania amazonensis* antigen (LaAg). Here, the role played by lipophosphoglycan (LPG) as the key disease-promoting component of intramuscular (i.m.) LaAg and its usefulness as a defined intranasal vaccine was investigated in murine cutaneous leishmaniasis. BALB/c mice were twice vaccinated by the i.m. route with 25 μg of intact LaAg or with LaAg that was pretreated with anti-LPG 3A1-La monoclonal antibody, prior to infection with *L. amazonensis*. LPG neutralization rendered the otherwise disease-promoting LaAg antigen protective, as observed by the smaller lesion sizes and reduced parasite burden. The increased resistance was accompanied by a markedly lower antigen-driven TGF-β and IL-10 responses in the lesion-draining lymph nodes, concomitant with significantly higher IFN-γ production. To test for intranasal efficacy, 10 μg of affinity-purified LPG and its parental LaAg were twice instilled in the nostrils prior to *L. amazonensis* infection. In both cases, similarly slower lesion growth and lower parasite burden were found that was associated with increased IFN-γ and IL-10 responses in the lesion-draining lymph nodes. These results support a role for LPG in the dual route-related effect of LaAg and shows its strong potential as a defined needle-free and adjuvant-free vaccine for cutaneous leishmaniasis.

Keywords: *Leishmania*; Lipophosphoglycan; Vaccine

1. Introduction

Leishmaniasis is a complex of diseases caused by the intracellular protozoan parasite *Leishmania* that affects over 12 million people worldwide especially in developing countries [1]. Depending on the parasite species and the immunological status of the host, the disease manifestations may range from chronic cutaneous lesions to lethal visceral infection [2].

Vector control is particularly difficult and other disease control measures currently include toxic chemotherapy and dog culling for visceral leishmaniasis as in Brazil [3,4]. So far, no human vaccine has been approved to prevent any form of the disease despite considerable effort [5]. The most reportedly tested clinical vaccine against cutaneous leishmaniasis is one comprised of a cocktail of heat-killed promastigotes of five *Leishmania* stocks that was substituted in the last decade for antigens of a single strain of *Leishmania amazonensis* (LaAg, or Leishvacin®) for standardization reasons [6,7]. However, despite the immunogenicity to healthy volunteers, the efficacy of the injected cocktail vaccine or LaAg has not been confirmed [8,9]. Indeed, subcutaneous LaAg was shown to promote disease aggravation in Rhesus monkeys despite the accompanying expansion of TH1-type immune responses such as high IFN-γ production

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and delayed-type hypersensitivity reaction [10]. Recently, we showed in mice that the disease-promoting effect of intramuscular LaAg is due to parasite-specific activation of TGF-β production in the lesion-draining lymph nodes regardless of the concomitantly upregulated IFN-γ [11]. These findings are not unexpected, since contrary to murine *L. major* infection where enhanced Th1 responses are associated with protection, in *L. amazonensis* infection Th1 cells may promote non-healing disease by recruiting macrophages to the site of infection and increasing intracellular amastigote replication [12].

Mucosal administration of disease-promoting antigens has been used as a feasible strategy to induce immunotolerance and protection against autoimmune and allergic diseases [13]. We demonstrated that orally administered LaAg confers protection to different strains of mice against cutaneous leishmaniasis caused by either *L. amazonensis* or *L. major* [14]. The intranasal administration of LaAg proved to be even more effective than the oral route, with the convenience that it is easier to administer and requires lower antigen doses [15]. The nature of the disease-promoting components in LaAg is unknown but the leishmanial homologue of receptors for activated C kinase (LACK) protein that activates strong TH2 responses in BALB/c mice may play an important role since intranasal vaccination with plasmidial DNA codifying LACK also induced strong protection in BALB/c mice against *L. amazonensis* infection [15]. However, in that study, recombinant LACK protein failed to induce protective immunity most likely due to its soluble nature that hinders uptake by the M cells of the nasal mucosa. This is a problem that will probably be encountered by other disease-promoting soluble proteins such as cystein proteases [16] and the *L. amazonensis* glycoprotein gp 10/20 [17].

Another leishmanial antigen that has been reportedly demonstrated to promote disease enhancement when administered by the s.c. route to BALB/c mice is the *L. major* glycoconjugate derived from lipophosphoglycan (LPG) [18]. LPG is naturally particulated, a condition that may favor its uptake by M cells and increase protection against enzymic digestion in the nasal mucosa. However, significant molecular differences exist between LPG from different *Leishmania* species, specially as concerns the sugar composition and sequence of branching sugars attached to the conserved repeat unit Gal (β1,4) Man (α1)-PO_4 backbone and in the cap structure [19,20]. The LPG of *L. mexicana* and *L. amazonensis* has a distinctive 3 position of this Gal residue partially substituted by β (1,3) glucose [21]. Thus, before the disease-promoting activity of *L. major* LPG is generalized and extended to LaAg, it has to be certified in *L. amazonensis*.

Aiming at developing a needle-free and adjuvant-free vaccine against leishmaniasis, we evaluated here whether the disease-promoting effect of intramuscular LaAg can be attributed to LPG and whether purified *L. amazonensis* LPG can be used as a more defined mucosal/intranasal vaccine against cutaneous leishmaniasis.

2. Materials and methods

2.1. Animals

BALB/c mice were originally obtained from Jackson Laboratory (Bar Harbor, Maine). They were bred and maintained at our own facilities using sterilized bedding, filtered water and pelleted food. Animals were used at 6–8 weeks of age in all experiments.

2.2. Parasites

For infection, *L. amazonensis* (designation MHOM/BR/75/Josefa) promastigotes rendered fluorescent by transfection with green fluorescent protein were used throughout, as described previously [11,22]. Parasites were routinely isolated from mouse lesions and maintained at 26 °C as promastigotes in Dulbecco-modified Minimum Essential Medium (D-MEM, Sigma Chemical Co., USA) containing 10% heat inactivated fetal calf serum (HIFCS, Cultilab, Brazil) and antibiotics (50 U/ml penicillin + 50 μg/ml streptomycin and occasionally with 1000 μg/ml geneticin).

2.3. Vaccines

2.3.1. LaAg (*L. amazonensis* antigen)

This was prepared from the PH8 strain of *L. amazonensis* (designation IFLA/BR/67/PH8), as previously described [11,14,15]. Briefly, infective promastigotes were cultured in M199 medium (Cultilab, Brazil) containing 10% HIFCS to reach stationary-growth phase, then washed three times in phosphate buffered saline (PBS) and subjected to three cycles of freezing and thawing. The whole antigen was lyophilized, stored at −20 °C and reconstituted with PBS immediately before use. Alternatively, LaAg was incubated for 30 min at 4 °C with anti-*L. amazonensis* LPG monoclonal antibody (3A1-La MAb) diluted at 1:500 in PBS. 3A1-La MAb was obtained and purified as described previously [23,24]. Irrelevant rat IgG2b (R&D Systems, Minneapolis, USA) was used as isotype antibody control.

2.3.2. LPG

This was also extracted from stationary-growth phase-promastigotes of PH8 strain of *L. amazonensis*, as described previously [25]. Briefly, saline-washed promastigotes were extracted at 80 °C with 45% aqueous phenol for 10 min. After dialysis and freeze-drying, the aqueous layer was applied to a column (2 cm × 100 cm) of Bio Gel P-60. The eluted material was freeze-dried and the LPG was recovered by double extraction with chloroform/methanol/water (10:10:3), releasing a soluble fraction and the residue containing LPG. The presence of protein contaminants was evaluated by silver staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Also, the presence of lipophosphoglycan-associated kinetoplastid membrane protein-11 (KMP-11), a key protein contaminant