Immunogenicity of a Polyinosinic-Polycytidylic Acid (Poly(I:C))-Adjuvanted Microparticle Vaccine for Leishmaniasis

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Statement of Purpose: There is a need for a human vaccine against leishmaniasis. Vaccines based on recombinant proteins have great potential for clinical translation; however, conventional aluminum adjuvants steer protein vaccines toward a T-helper 2 (T_H2)-type immune response, whereas protection against Leishmania parasites requires a T_H1-type response. In this study, the Leishmania donovani 36-kDa nucleoside hydrolase (NH36), a highly conserved protein among Leishmania species [1], and the adjuvant polyinosinic:polycytidylic acid (poly(I:C), low molecular weight (PICL)), a TLR3 agonist which stimulates synthesis of IFN-γ to induce a T_H1-biased immune response [2], were formulated and encapsulated in poly(lactic-co-glycolic acid) (PLGA) microparticles (MPs). We hypothesized that PLGA MP-formulated NH36 protein and PICL (MP/NH36/PICL) would elicit a stronger antigen-specific, T_H1-biased immune response in comparison to control formulations including PBS, NH36-containing MPs (MP/NH36) and non-MP (soluble) formulations (NH36, NH36/PICL).

Methods: Microparticle Formulation: MPs containing NH36 were formed using a double emulsion, solvent evaporation method, using 2 mg NH36 and 100 mg PLGA. PICL was ion-paired with DOTAP to form a complex soluble in dichloromethane, and PICL MPs were synthesized using a single emulsion method with 5 mg PICL and 100 mg PLGA. Loading of NH36 was measured by microBCA, and PICL by spectroscopic absorption at 290 nm.

In Vivo: BALB/c female mice (n = 5) were vaccinated subcutaneously with MPs containing NH36 (40 μg) +/- PICL (8, 20, or 60 μg), or with soluble NH36 (40 μg) +/- PICL (20 μg), all in 100 μL volume. Mice were boosted 3 weeks later and sacrificed after another 2 weeks. Splenocytes were analyzed for IFN-γ^+ cells (by ELISPOT), IFN-γ production (by ELISA), and proliferation. Serum IgG antibodies were analyzed by ELISA.

Results: Formulation: MPs were well-formed, and sizes ranged from 500 nm to 2 μm for NH36 and 1 to 4 μm for PICL (**Figure 1**). NH36 loading efficiency ranged from 49.5% to 108. 1% and PICL from 42.0% to 53.5%.

Serum antibody response: IgG2a responses, which are associated with T_H1 bias, were consistently higher in the mice immunized with NH36/PICL or MP/NH36/PICL vaccine formulations (**Figure 2**). A similar trend was observed in IgG2b responses (data not shown).

IFN-γ *production:* Mice immunized with NH36/PICL or MP/NH36/PICL generated a higher frequency of IFNγ-producing cells than mice immunized without PICL (**Figure 3**). The dose comparison in MP/NH36/PICL groups suggested that 20 μg is the optimal dose of MP-formulated PICL. A similar trend was observed with secreted IFN-γ ELISA (data not shown).

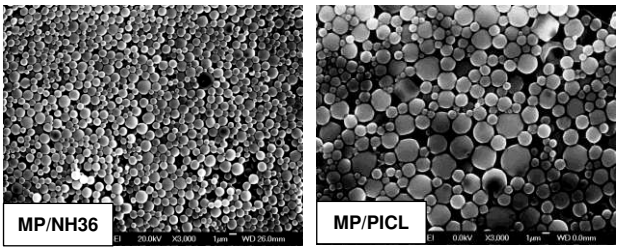

Figure 1. SEM images of PLGA microparticles (MPs).

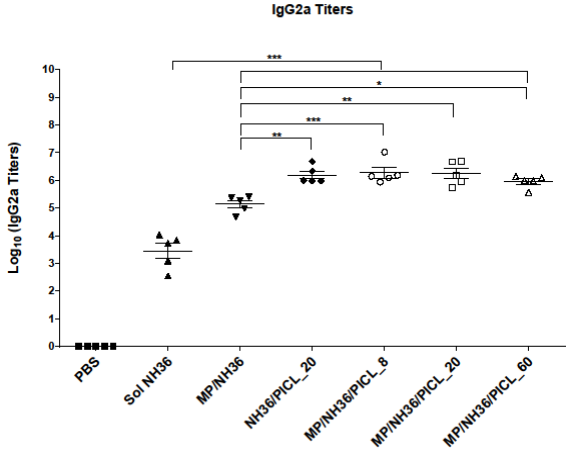

Figure 2. Serum IgG2a antibody responses.

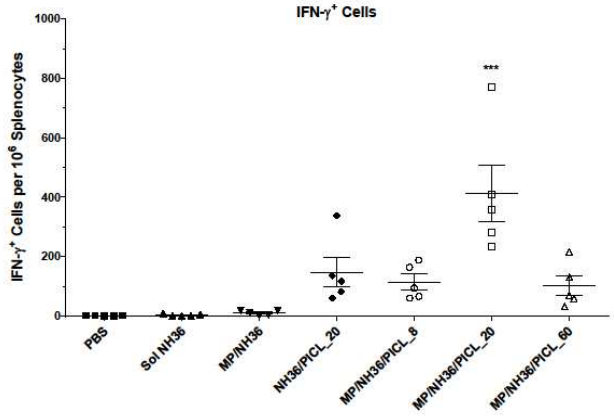

Figure 3. IFNγ-producing splenocytes (by ELISPOT).

Conclusions: Results showed that PICL enhanced the T_H1-associated IgG2a and IgG2b titers, and also increased splenocyte IFN-γ production. In comparison to the soluble NH36/PICL group, microparticle formulation significantly increased T_H1-biased cellular immune responses as evidenced by higher frequency of IFNγ-producing cells and higher total secreted IFN-γ. These results indicate a strong potential for the MP/PICL combination as an adjuvant/delivery system for a Leishmania protein vaccine.

References:

1. Dumonteil E. Infect Genet Evol. 2009; 9:1075-1082.
2. Jain, S. Expert Rev Vaccines. 2011; 10:1731-1742.