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Nucleoside hydrolase DNA vaccine against canine visceral leishmaniasis

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Abstract

The Nucleoside Hydrolase (NH36) is the main marker of the FML complex of *Leishmania donovani*, antigen of the licensed Leishmune® vaccine for prophylaxis of canine visceral leishmaniasis. As a DNA vaccine in mice, it induces a TH1 immune response. We vaccinated mongrel dogs with the VR1012NH36 vaccine for prophylaxis and immunotherapy against a high dose *Leishmania chagasi* infection (7 x 10^8 infective amastigotes). The untreated controls developed more symptoms, higher parasite/lymphocyte ratio, smaller DTH reactions, lower proportions of NH36-specific CD4+ cells and sustained NH36-specific CD8+ cell counts than dogs of the prophylaxis group. In the immunotherapy treated group, enlarged DTH reactions, enhanced CD4+ and sustained CD8+ lymphocyte proportions were also detected, however, without reduction of symptoms or parasite/lymphocyte ratio, indicating that the vaccine was sufficiently potent to prevent but not to control the disease. Both treatments determined higher survival rates. Anti-FML antibodies increased in vaccinated and control dogs while anti-NH36 antibodies were only increased in vaccinees (p= 0.000). The parasite load of an untreated survivor control dog (638.05 parasites) felt outside the IC95% of that of vaccinated dogs (32.02, IC95% 9.45-64.59) suggesting that both vaccination treatments succeeded in reducing the *Leishmania* infective burden. Accordingly, an untreated control dog showed lower levels of IFN γ-β, IL-2, IL4 but not IL-10 β actin-relative quantification. We conclude that the VR1012-NH36 vaccine induces strong prophylactic protection and a milder immunotherapeutic effect against a high dose canine experimental infection with *Leishmania chagasi*

Keywords: Leishmanioses; Nucleoside hydrolase; DNA vaccines; canine visceral leishmaniasis; prophylaxis; immunotherapy

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Vaccination with the FML-saponin Leishmune® vaccine is efficient in prevention [1,2], immunotherapy [3,4] and in blocking the transmission of canine visceral leishmaniasis (CVL) between dogs and to humans [5]. NH36, a nucleoside hydrolase of 34.3 kDA and 314 amino acids, is the main antigenic marker of the FML complex [6]. It cleaves nucleosides from imported DNA to release free purine or pyrimidic bases for *Leishmania* replication and according to this very relevant function it has been recently recognized as an important and conserved phylogenetic marker of the *Leishmania* genus [7]. As a DNA vaccine (VR1012-NH36) in mice it induced 88% of protection, 91% of curative potential against visceral and 65% of protection against coetaneous leishmaniasis, developing a TH1 immune response [8].

In this work we used 19 SRD dogs (4 months old), of 4 different broods, that were vaccinated against rabies, canine distemper, Type 2 Adenovirus, Coronavirus, Parainfluenza, Parvovirus and Leptospira and treated with anti-helminthic drugs. All dogs were healthy and seronegative for *Leishmania* antibodies in the FML-ELISA assay [9]. Aiming to avoid any bias based on genetically determined susceptibility [10], each brood was randomized by draft into 3 experimental groups (saline, prophylaxis and immunotherapy). For prophylaxis, 6 dogs were vaccinated with 3 doses of 750 μg of VR1012-NH36 plasmid, through the im route on days 0, 21 and 42, while the other 13 dogs received only saline. On day 67, all the animals were challenged with 7 x 10^8 amastigotes from a *L chagasi* strain maintained for 3 passages in hamsters and originally isolated from an infected dog. On day 160, all the 13 untreated dogs were already *Leishmania*-seropositive and symptomatic. Six of them were treated for immunotherapy with 3 doses of 750 μg of theVR1012NH36 vaccine, while the other 7 remained as untreated controls. The sera of all animals was assayed for the presence of anti-NH36 and anti-FML antibodies [9] and the cellular immune response was evaluated by the assessment of the DTH response against leishmanial lysate and of the proportion of CD4+ and CD8+ specific lymphocytes after *in vitro* proliferation of PBMC with 0.5 μg NH36. Dogs were also monitored for their clinical symptoms scores and for their parasite/lymphocyte ratio in Giemsa stained smears obtained after fine needle lymph-node punctures. All cohorts were monitored until day 517. At the end of the experiment, the parasite load of the survivor dogs was evaluated in PBMC and the cytokine expression in whole blood *ex-vivo* was assayed by Real Time PCR as modified from Manna et al., (2006) using the primers and probes represented in Table 1 and the (Taq man system®) [11].

Table 1. Primers and Probes

	Primer forward	**Primer reverse**	**Probe (FAM-MGB)**
β-act.	5'CTGGCACCACACCTTCTACAA 3'	5'GCCTCGGTCAGCAGCA3'	5'GCCTCGGTCAGCAGCA3'
INFg	5'GCGGAAAAGGAGTCAGAATCT GT3'	5'GCGGAAAAGGAGTCAGA ATCTGTT3´	5'GCGGAAAAGGAGTCAGAATCTG TT3'
IL-2	5'GAAGTGCTAGGTTTACCTCAA AGC3'	5'CAGATCCCTTTAGTTTCA GAAGTGTTACA3'	5'ACACCAAGGAATTAATCAGC3'
IL-4	5'GCTCCAAAGAACACAAGCGAT AAG3'	5'CTGCCGCAGTACAGTAGC A3'	5'CTCTGCAGAAGATTTC3'
IL-10	5'CCTGGGAGAGAAGCTCAAGAC 3'	5'CACAGGGAAGAAATCGG TGACA3'	5'CTGAGACTGAGGCTGCGAC3'
L. inf.	5'GGCGTTCTGCGAAAACCG3'	5'AAAATGGCATTTTCGGGC C3'	5'TGGGTGCAGAAATCCCGTTCA3'

Furthermore, the expression of IFN gamma, IL-2, IL-4 and IL-10 were also studied by Real Time PCR after *in vitro* proliferation of PBMC of one dog of the untreated control and one dog of the immunoprophylaxis group, at day 667 and parasite load was also obtained by absolute quantification-Real Time PCR [11].

For statistical analysis means were compared by ANOVA analysis simple factorial test and by one way ANOVA- Tukey´s honestly significant difference method (SPSS for windows). To test the significance of the differences between groups we also used the 95% confidence interval of the averages. All the animals included in this investigation were treated following the guidelines for animal experimentation of the USA National Institute of Health, and experiments were done in accordance with the institutional guidelines in order to minimize animal suffering.

The results of the serological survey are summarized in **Figure 1**