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A COMBINATION DNA VACCINE ENCODING NUCLEOSIDE HYDROLASE 36 AND GLYCOPROTEINE 63 PROTECTS FEMALE BUT NOT MALE HAMSTERS AGAINST *LEISHMANIA MEXICANA*

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Summary:

Leishmaniasis is a group of diseases caused by protozoan parasites of the *Leishmania* genus. Previous studies have shown that a DNA vaccine encoding *Leishmania donovani* antigen nucleoside hydrolase 36 and *L. mexicana* glycoprotein 63 is protective in mice. We investigated here the efficacy of this DNA vaccine to induce protection in golden hamsters. Male hamsters were more susceptible to infection by *Leishmania mexicana* than females. Following immunization with two doses of the DNA vaccine, only females resulted protected while males developed normal lesions.

KEY WORDS : leishmaniasis, vaccine, hamster.

Résumé : UNE COMBINAISON DE VACCINS D'ADN CODANT POUR L'HYDROLASE DE NUCLÉOSIDE 36 ET LA GLYCOPROTÉINE 63 PROTÈGE LES HAMSTERS FEMELLES MAIS PAS LES MÂLES CONTRE *LEISHMANIA MEXICANA*

La leishmaniose est un groupe de maladies causé par des parasites protozoaires du genre Leishmania. Des études antérieures ont montré qu'un vaccin d'ADN codant pour les antigènes hydrolase de nucleoside 36 de Leishmania donovani et la glycoprotéine 63 de L. mexicana est protecteur chez la souris. Nous avons étudié ici l'efficacité de ce vaccin pour induire une protection chez le hamster. Les hamsters mâles ont montré une plus grande susceptibilité à l'infection que les femelles. Après la vaccination avec deux doses de vaccin d'ADN, seules les hamsters femelles furent protégées, alors que les mâles développèrent des lésions de taille normale.

MOTS CLÉS : *leishmaniose, vaccin, hamster.*

Leishmaniasis is a group of diseases caused by protozoan parasites of the *Leishmania* genus. They are obligate intracellular parasites of host macrophages and cause different forms of disease, depending on the *Leishmania* species. Because of their major disease burden, intensive efforts have been devoted to vaccine development against this parasite (Dumonteil *et al*., 2001; Palatnik-de-Sousa, 2008). *L. mexicana* causes localized cutaneous leishmaniasis, and is one of the most frequent species found in Mexico (Andrade-Narvaez *et al*., 1990; Garcia-Miss *et al*., 1990). In some cases, it may also lead to diffuse as well as visceral leishmaniasis (Ramos-Santos *et al*., 2000; Velasco *et al*., 1989).

DNA vaccines have been shown to induce a preferentially Th1 immume response, which is necessary for the elimination of intracellular parasites and are thus a promising strategy to control *Leishmania* (Dumonteil, 2007). Previously we found that a DNA vaccine encoding *L. donovani* antigen nucleoside hydrolase (NH)36 induced protection against both *L. chagasi* (visceral leishmaniasis) and *L. mexicana* (cutaneous leishmniasis) in mice (Aguilar-Be *et al*., 2005). Further studies indicated that a recombinant NH vaccine was also protective against *L. major* (Al-Wabel *et al*., 2007) and that the NH36 DNA vaccine was usefull for the therapy of *L. chagasi* murine infection (Gamboa-León *et al*., 2006). We further optimized this vaccine by combining it with a plasmid encoding *L. mexicana* glycopreotein (GP)63 and aluminium phosphate as an adjuvant, which improved its protective efficacy against *L. mexicana* in BALB/c mice (Rosado-Vallado *et al*., 2005). While these results in mice are encouraging, it is necessary to further evaluate this DNA vaccine in other animal models. Hamsters are considered a highly susceptible host (Garg and Dube, 2006) for a variety of *Leishmania* species, including *L. major*, *L. mexicana, L. guyamensis, L. panamensis, L. infantum* and *L. chagasi* (Arruda *et al*., 2002; Melby *et al*., 2001; Oliveira and Cecchini, 2000; Requena *et al*., 2000; Soliman, 2006; Travi *et al*., 2002). We thus investigated here the efficacy of a DNA vaccine encoding NH36 and GP63 to induce protection in male and female golden hamster.

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MATERIALS AND METHODS

IMMUNIZATION

Plasmid DNA vaccines encoding *L. donovani* NH36 and *L. mexicana* GP63 were purified from a fresh culture of transformed DH5a *Escherichia coli* bacteria as described before (Rosado-Vallado *et al*., 2005). Purity and quality of the plasmids was assessed by agarose gel electrophoresis, restriction digestion profiles and spectroscopic analysis. Groups of 6-12 syrian golden hamsters (*Mesocricetus auratus)* were immunized *via* intramuscular with two doses of 100 µg of DNA encoding NH36 and GP63 antigens and 45 µg of $AlPO_4$ as adjuvant two weeks apart, as used before in mice (Rosado-Vallado *et al*., 2005). Control groups received saline solution or an identical dose of the empty plasmid vector with adjuvant. The protocol was approved by the institutional Bioethics Committee and all animal handling was performed according to established guidelines.

L. MEXICANA INFECTION

Three weeks after the second immunization dose, hamsters were infected in the left hind foot pad with 500 cultured *L. mexicana* metacyclic promastigotes (strain MNY/BZ/62/M379). Disease development was monitored for up to 17 weeks after infection by weekly measurement of footpad size with a vernier caliper, and lesion size was expressed as the difference in size between the infected and the contralateral uninfected footpad as described before (Saravia *et al*., 2005). Footpad swelling is indeed well correlated with parasite load during murine infection with *L. mexicana* (Aguilar Torrentera *et al*., 2002), as well as in golden hamsters infected with *L. brasiliensis* or *L. amazonensis* (Sinagra *et al*., 2007). It is thus a reliable indicator of disease progression.

RESULTS

Measurement of footpad lesion size showed that male golden hamsters were significantly more susceptible to *L. mexicana* infection than females, as their lesions grew larger and faster than females (Fig. 1). After 17 weeks of infection, control males presented lesion twice as large as control female hamsters. DNA vaccinated females developed significantly smaller lesions than vector or saline control females, indicating that they were significantly protected by the DNA vaccine (Fig. 1A). By the end of the experiment, vaccinate females hamsters presented a reduction in lesion size of 63 % compared to control animals. On the other hand, vaccinated males presented lesions similar to non-vaccinated controls, suggesting that the vaccine had no effect (Fig. 1B). Also, there was a slight and transient exacerbation of the lesions in males immunized with the empty plasmid vector between weeks 4-11 weeks of infection, but this did not reach statistical significance.

DISCUSSION

We evaluated here the efficacy of a DNA vaccine encoding NH36 and GP63 in a hamster model for the first time. The higher susceptibility of male golden hamsters to *L. mexicana* infec-

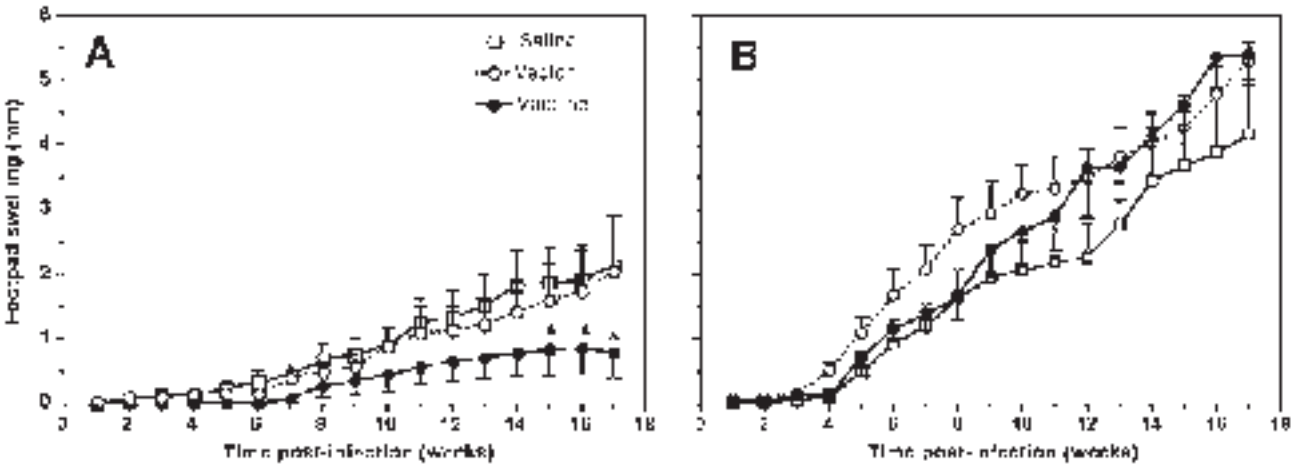

Fig. 1. – Lesion development in immunized hamsters infected with *L. mexicana.* Groups of 6-12 hamsters were immunized with a combination DNA vaccine encoding NH36 and GP63, the empty vector or saline solution. Following s.c. infection with 500 *L. mexicana* metacyclic promastigotes, lesion size was evaluated by weekly measurement of footpad thickness. Females (A) developed significantly smaller lesions than males (B). The DNA vaccine was able to significantly reduce lesion size in females (A) but not male hamsters (B). * indicates a significant difference with the saline control group (Tukey post-hoc test, $p < 0.05$).

tion is in agreement with a previous report (Saravia *et al*., 2005). Male DBA/2 mice have also been reported to be more susceptible to *L. mexicana* infection (Satoskar & Alexander, 1995) and male hamsters to *L. panamensis* and *L. guyanensis* (Travi *et al*., 2002). In both mice and hamsters, this difference in susceptibility was attributed to differences in the pattern of Th1/Th2 cytokines expressed, with susceptible males having higher levels of IL-4, IL-10 and TGF-β (Travi *et al*., 2002) or lower levels of IFNγ (Satoskar *et al*., 1998). Pregnancy (Osorio *et al*., 2008) and lactation (Gomez-Ochoa *et al*., 2003) have both been found to reduce susceptibility to *Leishmania* infection in female hamsters. Gender differences in the incidence of visceral leishmaniasis has also been reported in human and dog populations, with males being usually more susceptible (Roberts *et al*., 2001; Snider *et al*., 2009). Sexual hormones are thought to contribute to the difference in cytokine production (Ahmadi & McCruden, 2006; Lezama-Davila *et al*., 2007; Roberts *et al*., 2001; Travi *et al*., 2002). Indeed, gonadectomy and hormone therapy in mice suggest that estrogens are particularly associated with the ability of females but not males to produce IFNγ and a Th1 immune response (Roberts *et al*., 2001). Sex-differences in susceptibility to infection have also been observed for other parasites such as Plasmodium (Klein *et al*., 2008; Snider *et al*., 2009). We further found that protection against *L. mexicana* was gender-specific, with female hamsters being significantly protected by the DNA vaccine, while males failed to show protection. Very variable protection has actually been obtained in the hamster model of *L. mexicana* infection. Indeed, a *pfr*-2 DNA vaccine provided transient protection in males hamsters, but exacerbated disease in females (Saravia *et al*., 2005). On the other hand, rPFR-2 protein immunization provided no significant protection in either male or female hamsters against *L. mexicana*, but induced protection in female hamsters against *L. panamensis*, while a prime-boost immunization with both *pfr*-2 DNA and protein induced protection against *L. mexicana* in females (Saravia *et al*., 2005). Taken together, these results are difficult to extrapolate to other species, including humans. A clinical trial of an autoclaved *L. major* vaccine plus BCG in iranian childrens (6-15 years old) resulted in a higher protection in boys compared to girls, but the reasons for this difference were unclear (Sharifi *et al*., 1998). Women are thought to have a greater ability to mount a Th1 immune response and would thus be expected to be more successfuly vaccinated (Snider *et al*., 2009). In any case, these data indicate that vaccination against *Leishmania* in humans is likely to be affected by gender and should be taken into account in vaccine trials and future vaccination campains.

Nonetheless, the 63 % reduction in footpad lesion we observed in female hamsters is noteworthy, and this study expands the range of hosts in which the DNA vaccine encoding NH36 and GP63 can induce protection. It thus strengthens the use of this DNA vaccine approach, alone or in combination with additional antigens such as sand-fly salivary antigens (Gomes *et al*., 2008), for further evaluation in additional animal models.

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