NOTA

SIGNIFICANT AND TRANSITORY CONTROL OF LESIONS DEVELOPMENT IN MICE IMMUNIZED WITH A GENOMIC LIBRARY OF *Leishmania amazonensis*

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ABSTRACT

DNA from a genomic library of *Leishmania amazonensis* and from pcDNA3 plasmid were used to immunize BALB/c mice. Three immunizations at two weeks intervals were done, with 50μg/0.1ml of DNA. A control group was also injected with the same volume of saline solution. Afterward, all mice were challenged with infective promastigotes of the parasite, and the development of lesions was followed during 16 weeks by dorsoventral measures of the footpad. Mice previously immunized with the genomic library were capable of controlling lesions at a significant level, with major significance between 9 and 13 weeks post challenge.

KEYWORDS: *Leishmania.* Genomic library. DNA vaccine

The novel technology of nucleic acid vaccines in infectious diseases has been recently reported (Davis et al. 1994, Ulmer et al. 1993, Hoffman et al. 1994). Defined antigens and genomic libraries from parasites have been delivered in expression vectors, and the specificity of the immune response of inoculated mice, characterized (Gurunathan et al 1997, Alberti et al 1998). In order to know if this response is able to control the *in vivo* infection, using a New World species of *Leishmania* as a model, we studied the response of mice immunized with a genomic library from *Leishmania amazonensis* to challenge with promastigotes of the same species.

The strain of *Leishmania amazonensis* MHOM/77/LTB0016, kindly provided by the Immunology Department (Fiocruz, RJ, Brazil), and the strain

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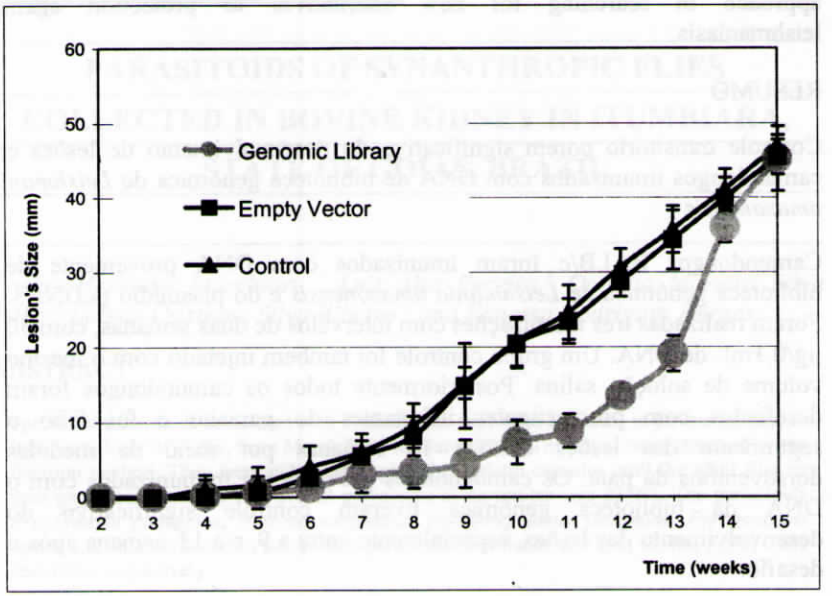
of *Escherichia coli* XL1-Blue of (New England, Biolabs) were used to construct the genomic library, and also the vector pcDNA3 (Invitrogen, USA) with a promoter of expression in eukaryotic cells. The vector was properly digested with BamHI enzyme (Boehringer Manheim). The genomic DNA from the parasite was obtained from promastigotes maintained in Schneider's medium, washed in PBS solution and resuspended in lysis buffer containing proteinase K. The DNA extraction was carried out with phenol-chlorophorm solution and precipitated in absolute ethanol. The integrity of the DNA obtained was checked by electrophoresis. The genomic DNA was digested using Sau3A enzyme from Boehringer Manheim. Ligation of DNA fragments and digested vector and posterior transformation with competent cells was carry out as recommended by Sambrook et al (1989).

The expression of the DNAs contained in the library on the muscles of inoculated mice, was then confirmed, by an immunofluorescence method, using muscle microsections from immunized mice and a pool of sera from individuals suffering from cutaneous forms of the disease, as a primary antibody. A conjugate of fluorescein and human IgG immunoglobulines (Sigma) was also used. The fluorescence was observed in slides from genomic library inoculated mice (Wild-Leitz, Heerbrug Switzerland). On the contrary, the control mice were negative to the observation.

To study the response of animals *in vivo*, two groups each of seven isogenic BALB/c mice (obtained from Centro de Producción de Animales de Laboratório, CENPALAB, La Habana) were inoculated, by intramuscular injection, with 50 µg/0.1ml DNA material from the library and empty vector, respectively; and a third group received physiological saline solution in the same volume. Three immunizations at two weeks intervals were done. All mice were challenged with $5x\ 10^6$ infective promastigotes of *Leishmania amazonensis* from third passage, by subcutaneous injection in the footpad.

The evolution of the lesions was followed by dorsoventral measures of the infected footpad once a week, during 16 weeks. The difference between the infected and noninfected footpads was calculated and the means compared. Mean differences were compared by variance analysis of double classification of repeated measures (Statistica 93 Program). The experiment was repeated twice, the figure shows the results of one of them.

In mice previously immunized with the genomic library, the lesions were evident 6 weeks post challenge. Differently, the lesions of mice in the other groups had appeared 2 weeks before (4^{th} week). In addition, the mean size of the lesions of the library immunized mice, were smaller than the lesion's mean size in the rest of mice since the 6^{th} week ($p<0.05$), difference that increased in significance between 9 and 13 weeks ($p<0.001$). After this, the lesions growth in the three groups was similar.



The differences in the appearance and mean size of lesions between controls and library-immunized mice, evidenced the immunocompetence developed in the last ones. This fact suggests that *in vivo* expressed antigens contain, at least, part of antigen determinants of native proteins.

Piedrafita et al, in 1999, reported for the first time the protection reached by immunization of genomic libraries using the murine cutaneous model of *Leishmania major*. Melby and colleagues, in 2000, reported the use of fractions of cDNA libraries for immunizing mice against *Leishmania* infection, looking for protection against visceral leishmaniasis. They described the immunocompetence reached with this approach. Interestingly, they found that greater fractions trigger the better response. In this direction, we found that the immunization with the genomic library of *Leishmania amazonensis* produced a significant and transitory control of lesions in mice challenged with promastigotes of the same species, possibly due to the combined effect of several expressed antigens, as occurs in nature.

Further studies will help to understand why this protection declines after the 15th week post challenge. Probably the injection of a lower number of parasites during the challenge will give light on this fact. On the other hand, it was impossible to continue monitoring the lesions for practical and ethical reasons, after 16 weeks. We conclude that immunization with a genomic library of *Leishmania amazonensis* in the mice model, is a good

approach in searching for new alternatives to protection against leishmaniasis.

RESUMO

Controle transitório porem significativo do desenvolvimento de lesões em camundongos imunizados com DNA de biblioteca genômica de *Leishmania amazonensis*

Camundongos BALB/c foram imunizados com DNA proveniente de biblioteca genômica de *Leishmania amazonensis* e do plasmídio pcDNA3. Foram realizadas tres imunizações com intervalos de duas semanas, com 50 µg/0,1mL de DNA. Um grupo controle foi tambem injetado com o mesmo volume de solução salina. Posteriormente todos os camundongos foram desafiados com promastigotes infectantes do parasito e foi feito o seguimento das lesões durante 16 semanas por meio de medidas dorsoventrais da pata. Os camundongos previamente immunizados com o DNA da biblioteca genômica tiveram controle significativo do desenvolvimento das lesões, especialmente entre a 9ª e a 13ª semana após o desafio.

DESCRITORES: *Leishmania*. Biblioteca genômica. Vacina de DNA

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