Immunization of Balb/C Mice by Protein Fragments of Lizard Leishmania promastigote

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Abstract: The objective of this study was the immunization of balb/C mice by protein fragments of lizard Leishmania promastigote. Mice were divided in 6 case groups and one as control. Each group received a fraction of lizard Leishmania promastigote. Then active pathogenic Leishmania major challenged them separately. We followed up all the case groups (6 groups) till five months after the challenge with Leishmania major together with control group and recorded the lesions diameter. None of the mice manifested detectable wound or nodules, except those at group 6 but the difference compared to control group was not significant according to the Mann-Whitney analytical test.

Key words: Immunization, balb/C mice, fraction of lizard Leishmania

INTRODUCTION

Differently manifested leishmaniasis is the result of infection with different types of Leishmania parasite[1]. The disease is considered as one of the important health problems in 82 countries (61 countries of the old world and 21 countries of the new world), since 12 million people are already affected and 1.5-2 million people get involved every year. In 1990, 2.1 million people lost the chance of efficient life due to infection[2]. Human and animals are infected by Leishmania, so they remain as the source of the infection. That’s the reason of complexity of leishmaniasis control efficiently[3].


The present research was planned to challenge balb/C mice with different protein fragments of lizard Leishmania promastigotes in order to detect the probability of immunization against Leishmania major.

MATERIALS AND METHODS

To assess the immunization against Leishmania major, balb/C mice at different groups (6-8 mice at each group) were inoculated subcutaneously at between ears by 30 μg of each fragment of lizard Leishmania[12] antigen fragments[12] together with Freund’s complete adjuvant and two weeks later, for the second time, together with Freund’s incomplete adjuvant (the same dosage). After 35 days, each mouse was challenged with Leishmania major (MRHO/IR/64/Nadin) virulent type (at stationary phase of life cycle), at tail endings (Table 1).

<table>
<thead>
<tr>
<th>Table 1: Mice groups and antigen dose injection</th>
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<tbody>
<tr>
<td>Protein reaction</td>
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<td>F1</td>
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<td>F4</td>
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<td>F5</td>
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<td>F6</td>
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<td>Control</td>
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</table>

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After the day 60, the appearance of nodules and wounds was noted weekly (determined as the mean of two perpendicular diameters). Nonparametric analytical test (Mann-Whitney) was used to recognize the difference between groups.

RESULTS

Since the most confident way to study the immunization against infectious agents, is to challenge the suitable laboratory animal with the antigen, clinical follow up and comparing the case and control groups, we followed up all the case groups (6 groups) fill five months after the challenge with *Leishmania major* together with control group and recorded the lesions diameter (Table 2). None of the mice at groups 1, 2, 3, 4 and 5, manifested detectable wound or nodules (Fig. 1) except those at group 6 (Fig. 2), but the difference compared to control group (group 7) was not significant according to the Mann-Whitney analytical test.

Fig. 1: Mice at groups 1, 2, 3, 4 and 5, didn’t manifest detectable wound or nodules.

Furthermore, transforming of infection to visceral form in mice is similar to human kala-azar (Peripheral blood cells and serum protein alteration).

In contrast to human, cutaneous leishmaniasis in animal models, dermal infection can progress to produce an extensive lesion without any limiting. It is supposed to protect these mice with a special antigenic component of *Leishmania* parasite in order to use it for causing immunity in human.

CFA (Complete Freund’s Adjuvant) stimulates in specific cellular immunity and macrophages against antigens. In this study complete and incomplete Friedn’s adjuvant was used. There may be a cross-immunity between *Leishmania* and mycobacterium. After vaccination with CFA, mycobacterium causes a dermal inflammation reaction, so we decided to use incomplete Friedn’s adjuvant in secondary injection.

Subcutaneously injection of 78, 64, 35, 30, 19.2 and 21.5 kDa fractions to particular groups of balb/C mice has been carried out. After immunization, we have challenged six groups of mice as test and control cases by injection of 2×10⁵ alive *Leishmania major* (MRHO/IR/64/Nadim 1).

Among the considered fractions, 21.5 kDa protein has caused efficient immunity (Mann-Whitney test, p<0.05).

There are similar studies to present research like gp63, this glycoprotein was detected by Murry et al.[9] during purification of proteins of *Leishmania major* by using Triton X-114, a non-ionic detergent.

Fornmel et al.[10] caused an effective immunity in balb/C mice against *L. mexicana* and *Leishmania major* by subcutaneously injection of 64-97 kDa proteins of *Leishmania major* and *L. infantum* together with muramyl dipeptide (MDP) adjuvant.

Cardoso et al.[11] isolated 8 fractions include of 42, 46, 63, 66, 73, 87, 97 and 160 kDa of American *Leishmania* by polyacrylamide electrophoresis gel and electro elution and tried to vaccinated the C57BL/10 mice with them.
Table 2: Mean of lesion diameter in case and control animals after exposure to active parasite

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>76 KDa</th>
<th>64 KDa</th>
<th>30 KDa</th>
<th>29 KDa</th>
<th>19.1 KDa</th>
<th>21.5 KDa</th>
<th>PBS</th>
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<tbody>
<tr>
<td>KSrd</td>
<td>1</td>
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<td>1.25±0.22</td>
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<td>2</td>
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<td>2.05±0.20</td>
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<td>3</td>
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<td>3.46±0.32</td>
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<td>4</td>
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<td>4.46±0.32</td>
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<td>5</td>
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<td>5.12±0.22</td>
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<td>6</td>
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<td>6.96±0.67</td>
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<td>7</td>
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<td>8.24±0.57</td>
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<td>8</td>
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<td>9.04±0.56</td>
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<td>9</td>
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<td>10.94±0.63</td>
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<td>10</td>
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<td>1.35±0.23</td>
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<td>11</td>
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<td>2.45±0.28</td>
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<td>12</td>
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<td>-</td>
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<td>3.46±0.32</td>
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Fig. 2: Mice at group 6 manifested detectable wound

Malekzadeh et al. [20] isolated fractions of promastigote forms of *Leishmania major* including: 40-60, 60-80, and 80-100 kDa proteins. Whose suggested that 60-80 kDa proteins are immunogenic and found it during subcutaneous vaccination of balb/C mice.

During the study on *Leishmania major* antigenic components, Hejazi [20] detected 26 bands (10-112 kDa) and found that 40 kDa protein is an active immunogen by subcutaneous injection together with BCG.

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REFERENCES