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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].

Some procedures for control of leishmaniasis include using autoclaved *Leishmania major* vaccine[4-6], leishmanization[7], use of non pathogenic *Leishmania* strains[8], attenuated parasite[9] and vaccination by killed *Leishmania promastigote*[1] use of membrane lipophosphoglycan[10] and oral vaccine[11].

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[13] 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/Nadim 1) virulent type (at stationary phase of life cycle), at tail endings (Table 1).

Table 1: Mice groups and antigen dose injection

Protein reaction No.	Protein MW (Dalton)	Antigen dose (mg)	Route of injection	Booster dose (mg)	Challenge by *L.major*
F_1	78000	30	Sub coetaneous	30	$2x10^6$
F_2	64000	30	Sub coetaneous	30	$2x10^6$
F_3	30000	30	Sub coetaneous	30	$2x10^6$
F_4	35000	30	Sub coetaneous	30	$2x10^6$
F_5	19200	30	Sub coetaneous	30	$2x10^6$
F_6	21500	30	Sub coetaneous	30	$2x10^6$
Control	-----	PBS	Sub coetaneous	PBS	$2x10^6$

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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) till five months after the challenge with *Lieshmania 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-Witney analytical test.

DISCUSSION

Recently the numerous active strategies have lead to research on *Leishmania* vaccines[1,14,15] and TDR (Tropical Disease Research) has decided to produce an appropriate vaccine against leishmaniasis. There are two ways for designation and developing of vaccines, termed Pragmatic and Systematic. Pragmatic way is examination of crude antigenic materials together with a convenient adjuvant or without it, in human and animals. During the last years, crude materials of *Leishmania* have been sufficiently standardized but it is required to enormous accumulation of data for identification of them. Systematic way includes identification and purification of immunogenic materials[16].

There are highly genetic overlaps in the parasites between species and strains[17]. So, because of being cross-reaction between different animalian strains, we can use nonpathogenic strains as vaccine against virulent ones.

In this research, the balb/C mice have been affected. Various studies have indicated that the susceptibility to infection is controlled by genetic and vary widely between inbred stains of mice[7].

Almost, inbred strains of mice are resistant to infection with *Lieshmania major* and only a few of them are largely sensitive to it, particularly balb/C, H-2 and P/J tribes. Even injection of a few numbers of parasites to Balb/C mice, cause severe visceral leishmaniasis that it is so useful for immunologic studies.

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 Fraund`s Adjuvant) stimulates in specific cellular immunity and macrophages against antigens. In this study complete and incomplete Fraund's adjuvant was used. There may be a cross-immunity between *Leishmania* and mycobacterium[18]. After vaccination with CFA, mycobacterium causes a dermal inflammation reaction, so we decided to use incomplete Fraund's adjuvant in secondary injection.

Subcutaneously injection of 78, 64, 35, 30, 19.2 and 21.5 kDa fractions[13] 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 2×10^6 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.*[19] during purification of proteins of *Lieshmania major* by using Triton X-114, a non-anionic detergent.

Formmel *et al.*[20] caused an effective immunity in balb/C mice against *L. mexicana* and *Lieshmania major* by subcutaneously injection of 64-97 kDa proteins of *Lieshmania major* and *L. infantum* together with muramil dipeptide (MDP) adjuvant.

Cardoso *et al.*[21] 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

		Group						
Time		78 kDa	64 kDa	30 kDa	35 kDa	19.2 kDa	21.5 kDa	PBS
X±sd	1	-	-	-	-	-	1.35±0.23	1.35±0.23
	2	-	-	-	-	-	2.45±0.28	2.45±0.28
	3	-	-	-	-	-	3.46±0.32	3.46±0.32
	4	-	-	-	-	-	4.46±0.32	4.46±0.32
	5	-	-	-	-	-	5.12±0.22	5.12±0.22
	6	-	-	-	-	-	6.96±0.67	6.96±0.67
	7	-	-	-	-	-	8.24±0.57	8.24±0.57
	8	-	-	-	-	-	9.03±0.56	9.03±0.56
	9	-	-	-	-	-	10.94±0.63	10.94±0.63
	10	-	-	-	-	-	-	1.35±0.23
	11	-	-	-	-	-	-	2.45±0.28
	12	-	-	-	-		-	3.46±0.32

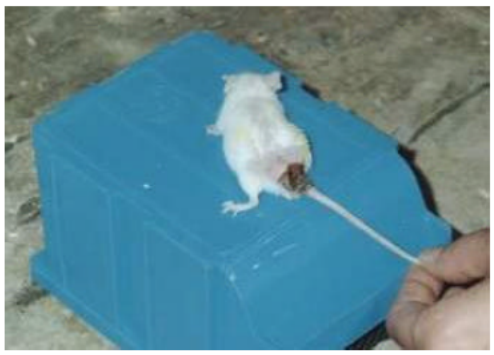

Fig. 2: Mice at group 6 manifested detectable wound

Malekzadeh *et al.*[22] isolated fractions of promastigote forms of *Lieshmania major* including: 40-60, 60-80 and 80-100 kDa proteins. Who suggested that 60-80 kDa proteins are immunogen and found it during subcutaneous vaccination of balb/C mice.

During the study on *Lieshmania major* antigenic components. Hejazi[16] 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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