A trial Of Immunisation Against Cutaneous Leishmaniasis

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Summary:

Background: *Cutaneous leishmaniasis is an endemic protozoal disease in Iraq. Recovery from the disease confers a solid and permanent immunity. Vaccination with a living inoculum of promastigotes isolated from culture reduce the incidence of disease.*

Objective: *To show the efficacy of different types of antigens for protection of Balb/c mice against cutaneous leishmaniasis.*

Methods: *Thirty Balb Ic mice were divided in to six groups, groups 1-4 were immunized with different types of antigens (heat killed, freezing-thawing, formalin fixed and ultrasonicated). Group 5 was treated with freshly harvested viable promastigotes from liquid medium as positive control and group 6 was kept as negative control. Leishmanin test was used for estimation of hypersensitivity of skin. Results: Immunization with antigens preparation revealed that, the immunized mice became resistant to infection.*

Conclusion: *Immunization of mice against cutaneous leishmaniasis is possible by using different types of antigens. Key words: Immunisation, cutaneous leishmaniasis, mice.*

Introduction:

Cutaneous leishmaniasis is one of major protozoan disease in tropical and subtropical countries. The disease was endemic in Iraq and neighboring countries (1). Recovery from the disease confers a solid and permanent immunity, although it is usually species-specific and may be strain-specific as well. Vaccination with a living inoculum from a recently isolated culture significantly reduces the incidence of cutaneous leishmaniasis (2).

Vaccination was applied to the general practice of immunization against an infective agent. Similarly the term leishmaniasis has recently been applied to an ancient practice of deliberate infection for the purpose of inducing a long lasting immunity against old world cutaneous leishmaniasis (3). Development of vaccines against different forms of the disease is not simple and require detailed information on the pathology of the disease and risk factors (4).

The present study was planned to evaluate the immunity states of mice inoculated with different types of L. tropica antigens (formalin-fixed, heat killed, freezing-thawing and sonicated antigens), for the purpose of trial of vaccination against cutaneous leishmaniasis.

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Materials And Methods:

A thirty six Balb/C mice were used in this. experiment, mice were divided into six groups, each of six. Groups 1-4 were immunized with different types of antigens, while group 5 was considered as positive control and group 6 kept as negative control. Preparation of antigens: Viable promastigotes freshly harvested from liquid medium were suspended in 1% glucose phosphate buffer saline (PBS), pH 7.2 at 2x107 promastigotes per 0.1 ml.

Types of antigens:

Four types of antigens were used:

1-Heat killed antigens: The suspended promastigotes were exposed to 56 °C for one hour in a water bath (5).

2-Freezing-thawing antigens: The suspension of promastigotes was repeatedly frozen in liquid nitrogen and thawed (6).

3-Formalin-fixed antigens: Prepared by suspension of promastigotes in 0.5 % formalin for five minutes at room temperature (7).

4-Ultrasonicated: The suspension of promastigotes was subjected into an ultrasonic disintegrate for five period of two minutes each, with two minutes resting intervals (7).

Immunization: Mice from group 1-4 were injected intravenously in the tail vein with two weekly interval doses of prepared antigens (2X107promastigotes in a volume of 0.1 ml). Three weeks after the last immunizing injection, all groups were injected subcutaneously into foot pad with 5x 10 live promastigotes. Mice were followed up and the developed lesions were measured at weekly intervals for 16 weeks after infection (7).

Montenegro test: The promastigotes were harvested from biphasic medium, washed three times with sterile P.B.S. pH 7.2. Its concentration was adjusted to 1x10 6/ ml, killed with sterile phenol saline 0.5%, 0.1 ml of it was injected intradermaly into the back of above the base of tail. Mice were examined at 24, 48 and 72 hours by measuring the diameter of erythema and induration surrounded the site of inoculation of the antigen (8).

Results:

It is found that all mice in the protected groups (immunized mice) were resistant to a challenge with virulent L.tropica promastigotes. At day 120 after challenge, no amastigotes were detected in the smears and cultures prepared from the site of injection and also from visceral organs (spleen, liver and bone marrow). While in control group skin lesions were developed after three weeks of challenge. Follow-up for 120 days post challenge revealed that there was no detected lesion in protected groups but only small palpable nodules were detected. In contrast, the non immunized (control) group was markedly infected and the mean lesion score was reached to its last grade (grade 4).

Table 1, shows the results of delayed hypersensitivity test. It was found that, the size of erythema were 8,9,7 and 8 mm in animal group protected by formalin fixed, heat killed, sonicated and freeze-thawed antigens respectively. While the size of erythema in normal control group was less than 0.1 mm. Statistically there was significant difference between groups protected with different types of antigens. Although there was no difference between protected and infected control groups, but there was significant difference between the above groups and normal uninfected group (PO. 01).

Table. Diameter of erythema in mice after 16 weeks post immunization with different types of antigen

	Skin thickness after 16 weeks (mm) 6 mice/ rou						
Type of immunization	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**
Formalin-fixed Ag	**1.1**	**1.2**	**1.2**	**0.9**	**0.8**	**1.0**	**1.03**
Heat-killed Ag	**0.6**	**0.9**	**1.2**	**1.0**	**0.9**	**1.3**	**0.98**
Sonicated Ag	**0.9**	**1.1**	**1.2**	**1.1**	**0.8**	**0.6**	**0.95**
Freezing-thawed Ag	**1.0**	**0.9**	**1.6**	**0.7**	**1.1**	**0.9**	**1.03.**
Un immunized (infected)	**1.1**	**1.3**	**1.2**	**0.9**	**1.0**	**1.3**	**1.13**
Normal control (not infected)	**less than 0.1**						

Discussion:

The successful use of non-viable promastigotes vaccine in the absence of an adjuvants has established the feasibility of inducing substantial levels of protection against fatal L. major infection in Balb/ C mice (7).

The present study was carried under carefully standardized conditions such as experimental animals, source of the parasite and route of inoculation. All experimental animals were kept under approximately constant temperature of about 25 °C, because the high temperature (33-35 °C) and low temperature (air cooled rooms) influenced infection and inhibit multiplication of the parasite within the host (9).

For immunization, four types of killed antigens were prepared and injected intravenously in the tail vein. The intravenous (I.V.) route was used because many studies had reported that I.V. injection has given protective immunity better than subcutaneous (S.C.) or intramuscular (I.M.) injections. Lama and Cole (10) showed that irradiated L. enriettii promastigotes were totally ineffective when injected S.C. in protecting guinea pig against L. enriettii infection. Freeze-thawed L. major or live nonpathogenic L. major isolates induced protective immunity in the mice only when injected by the I.V. or I.P. routes (11&12). Liew et al. (13) reported not only that the S.C. route is wholly ineffective in protection against cutaneous leishmaniasis, but that the use of both S.C. and I.M. administration of vaccine totally suppresses induction of prophylactic immunity against infection.

It is concluded that immunization of mice against cutaneous leishmaniasis is possible by using different types of antigens.

It is recommended to carry on further studies on laboratory animals and human beings to find a suitable vaccine against the disease.

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