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Effect of *Leishmania gerbilli* Injection on Mice Immunization Against Cutaneous Leishmaniasis (CL) Caused by *Leishmania major*

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Abstract: In the present study *Leishmania gerbilli* were used to immunize BALB/c mice against pathogenic strains of leishmania to determine whether injection of *L. gerbilli* in mice could protect them against later *L. major* inoculation. Eighty female BALB/c mice were divided by random in eight groups. Promastigotes of *L. major* and *L. gerbilli* were used. Mice were inoculated with three different doses of *L. gerbilli* (3×10^6, 2×10^7 and 5×10^7) via subcutaneous (SC) in the base of their tails or interpretoen (IP). Forty days after the first injection, all mice received the same doses as a booster. Two control groups received PBS (SC or IP) only. All BALB/c mice were inoculated subcutaneously with 2×10^6. Promastigotes of *L. major* in the base of their tails after 75 days of the first injection of *L. gerbilli*. When leishmania lesion developed (35 days after challenge), the size was measured and continued once a week for 12 weeks. Meanwhile, the liver and spleen samples of dead mice moved to culture media and examined for the parasite. Delaed Type Hypersensitivity (DTH) and immunoflurecent tests were used to determine results of immunization. Compared with the control group and the other groups that received different doses of *L. gerbilli* via IP, an evident decrease in lesion size was observed in group that received 2×10^7 promastigotes ($p<0.05$). By contrast, in those groups received *L. gerbilli* subcutaneously, no difference was observed through the different doses of inoculated parasite. Comparison of the inoculation styles showed that IP method caused smaller lesions than SC ($p<0.05$).

Key words: *Leishmania gerbilli*, immunization, BALB/c, *Leishmania major*

INTRODUCTION

Leishmania species are known as parasitic protozoan cause of many kinds of leishmaniasis include Cutaneous (CL), Visceral and muco-cutaneous Leishmaniasis. Cutaneous Leishmaniasis (CL) is an endemic disease in many parts of the world that can cause considerable dermal disease and may result in sever disfigurement. *Leishmania major* and *Leishmania tropica* are pathogenic agents of Cutaneous Leishmaniasis (CL) in man and many other animals and transmitted by the bites of infected phlebotomine sand flies in nature.

Various efforts such as Local irradiation, cryotherapy and antileishmanial drugs, are beenig made to enhance healing of the ulcer which none of them has proved satisfactory for the treatment of CL. In the search for new ways to control of leishmaniasis there has been a major emphasis on immunization methods, for example killed amastigote vaccine against fatal *L.* major (Liew *et al*., 1987), Bacilli Calmett Gurine (BCG) as a vaccine (Stephani *et al*., 1993), Recombinant BCG (Connel and Medina, 1993) and different collones of *L. major* and *L. arabica* (Peters *et al*., 1990).

Since no effective chemotherapy on cutaneous and visceral leishmaniasis is available, many efforts focused on finding effective immunization material. In the present research *Leishmania gerbilli* were used to immunize animals against pathogenic strains of Leishmania to determine whether injection of *L. gerbilli* in mice could protect them against later *L. major* inoculation. *Leishmania gerbilli*, a kind of Leishmania species causes CL in *Rhombomys opimus* (but not in *Meriones libycus*). It first reported from Kanzo and Sinkiang in China with zoonetic cycle and shows that failes to contaminated

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human (Sterelkova *et al*., 1991). It's Isoenzymatic pattern was studied in previous USSR and compared with other straines (Kelina and Passova, 1985). Because of such a close relationship between *L. gerbilli* and *L. major,* we suppose that early injection of *L. major* promastigotes may conclude further immunization in BALB/c mice.

MATERIALS AND METHODS

Animals: Eighty female Balb/C mice purchased from Pasteur Institute of Tehran and transported to laboratory of Isfahan researching center and Islamic Azad University of Falavarjan. After that, they were divided by random in eight groups following by breeding to 6-8 weeks old mice.

Parasite: Promastigotes of *L. major* (strain MRHO/IR/75/ER) which was isolated from *Rhombomys opimus* in Isfahan region (Iran) and *L. gerbilli* (strain MRHO/CN/60/GERBILLI/LON25) that was obtained from Tarbiat Modarres University (Tehran-Iran), were used. Promastigotes cultured in RPMI 1640 medium (mixed by 10% fetal calf serum, 100 μg mL^{-1} Streptomycine, 50 μg mL^{-1} Gentamycine, 100 IU mL^{-1} Penicillin) and obtained in stationary phase of culture as well as washed three times in PBS.

Immunization: Mice were inoculated with three different doses of *L. gerbilli* (3×10^6, 2×10^7 and 5×10^7) via Sub Cutaneous (SC) in the base of their tails or Inter Pretoen (IP). Forty days after the first injection, all mice received the same doses as a booster and two control groups received PBS (SC or IP) only.

Challenge: In order to study the role of immunization in Cutaneous leishmaniasis, all Balb/C mice were inoculated subcutaneously with 2×10^6 Promastigotes of *L. major* in the base of their tails after 75 days of the first injection of *L. gerbilli.* When leishmania Lesion developed (35 days after challenge), the size was measured in two dimension at right angles to each other with a caliper gauge and continued once a week for 12 weeks. Meanwhile, the liver and spleen samples of dead mice moved to culture media and examined for the parasite.

Statistical analysis: The Tukey-HSD test was used with SPSS software (Microsoft excel version 9) to analyze the data.

RESULTS

In this study, animals (*L. gerbili*-infected BALB/c mice) were injected by *L. major* at 75th day and five weeks later the nodule transformed into an ulcer which increased in size.

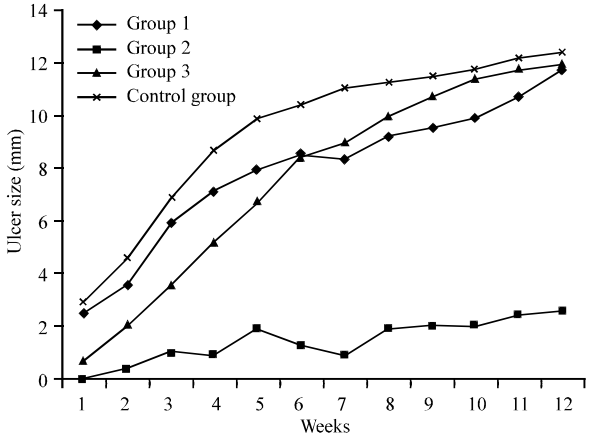

Fig. 1: Ulcer size in groups 1, 2, 3 and control

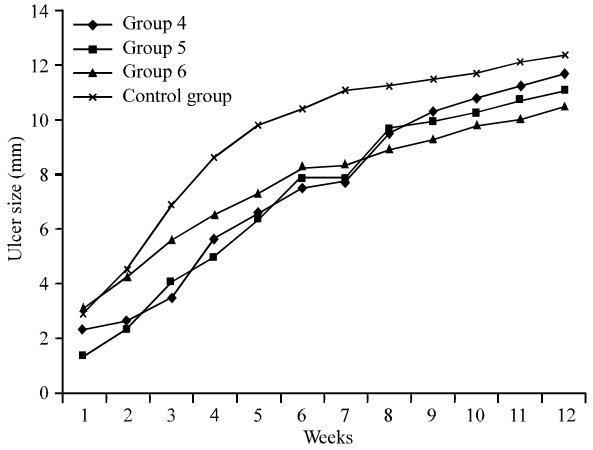

Fig. 2: Ulcer size in groups 4, 5, 6 and control

Compared with the control group and the other groups that received different doses of *L. gerbilli* via IP, an evident decrease in lesion size was observed in group that received 2×10^7 promastigotes ($p<0.05$), (Fig. 1). By contrast, in those groups received *L. gerbilli* subcutaneously, no difference was observed through the different doses of inoculated parasite (Fig. 2). Comparement of the inoculation styles, showed that IP method caused smaller lesions than SC ($p<0.05$). In other experiment, mice which received 3×10^6 or 5×10^7 *L. gerbilli* promastigotes subcutaneously or by interpretoen, no significantly difference was seen in lesion size.

DISCUSSION

In this study we used *L. gerbilli* promastigote injection as a vaccine to protect BALB/c mice against later *L. major* infection. Isoenzyme pattern of *L. gerbilli* is similar to *L. major* P-K, PEP-1 and *L. tropica* ES (Xu *et al*., 1984), so we suspected that *L. gerbilli* might presents as

a immunized agent to protect BALB/c mice. Pramastigotes of different strains of Leishmania was used before. Barrel used *L. mexicana amazonensis* Promastigotes; he used three different doses of parasite (5×10^7, 2×10^7 and 3×10^7) and showed when doses of antigen increased, it lead to more protection (Barrel *et al*., 1987). In other study they used five different doses (2×10^4, 2×10^5, 2×10^6, 2×10^7 and 2×10^8) of radiated *L. major* to protect mice against *L. major* and reported that although protection in all groups were seen; but the better results were seen in 2×10^7 promastigote derived (Liew *et al*., 1985). This results are similar to that we showed here. Liew at all reported that subcutaneously inoculation of 2×10^4, 2×10^5, 2×10^7 and 2×10^8 radiated amastigotes of *L. major* in BALB/c mice induced bigger lesions compared with control groups and doses of parasite had not been effect the size of lesions (Liew *et al*., 1985). In this study no difference were seen in lesion size of mice derived different doses of *L. gerbilli* subcuteneously. Although the mortality rate in mice was increased as the doses of parasite derived was increased.

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