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TRANSMISSION BLOCKING VACCINE STUDIES IN LEISHMANIASIS: II. EFFECT OF IMMUNISATION USING *LEISHMANIA MAJOR* DERIVED 63 KILODALTON GLYCOPROTEIN, LIPOPHOSPHOGLYCAN AND WHOLE PARASITE ANTIGENS ON THE COURSE OF *L. MAJOR* INFECTION IN BALB/C MICE

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TRANSMISSION BLOCKING VACCINE STUDIES IN LEISHMANIASIS: II. EFFECT OF IMMUNISATION USING *LEISHMANIA MAJOR* DERIVED 63 KILODALTON GLYCOPROTEIN, LIPOPHOSPHOGLYCAN AND WHOLE PARASITE ANTIGENS ON THE COURSE OF *L. MAJOR* INFECTION IN BALB/C MICE

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ABSTRACT

Background: **Safe, effective and inexpensive vaccines may be the most practical tool for control of any form of leishmaniasis. Leishmaniasis produces a state of pre-immunition which is the underlying mechanism for prolonged immunity to re-infection. Low doses of parasites has been shown to be able to induce protection in mice. It is not known, however, how immune sera from a susceptible host immunised with *Leishmania*-derived antigens when taken in by the sandfly affects the development and the subsequent transmission of the parasite to naive hosts.**

Objective: **To monitor the course of disease in BALB/c mice following challenge using *L. major* infected *P. duboscqi* which had previously fed on immunised mice.**

Methods: **BALB/c mice were immunised adequately with *Leishmania major*-derived antigens namely, crude whole parasite (WPA), recombinant 63 kilodalton glycoprotein (rgp63), lipophosphoglycan (LPG) and a cocktail composed of rgp63 plus LPG antigens. Laboratory reared *Phlebotomus duboscqi* sandflies, the natural vector for *L. major* were later allowed to feed on immunised animals, interrupted and allowed to continue feeding on infected animals for an equal amount of time until they became fully engorged. The sandflies were maintained on apples as a carbohydrate source in an insectary maintained at a temperature of 25°C and 80% relative humidity. On the seventh day these sandflies were used to infect naive BALB/c mice and the course of infection followed for a period of at least three months.**

Results: **Mice infected using sandflies which had previously fed on WPA or rgp63-immunized mice showed disease exacerbation as the infection progressed, whereas those infected using sandflies which had previously fed on LPG-immunised mice had the least lesion sizes compared to control mice infected using sandflies which had fed on saline immunised mice ($p<0.05$).**

Conclusions: **Results from this study indicate that the course of *L. major* infection in BALB/c mice was dependent on the infective dose of parasites transmitted by the sandflies. Results from this study suggests that sub-infective doses of the parasite from sandflies previously fed on animals immunised with *Leishmania-derived* antigens needs to be evaluated for their potential in vaccine development against *Leishmania* infections.**

INTRODUCTION

Zoonotic cutaneous leishmaniasis (ZCL) produced by *Leishmania major,* is an important health problem in many countries of North Africa, in Eastern Africa, Eastern Mediterranean region and South Western Asia(1). Most cases develop multiple lesions on the exposed part of the body, often on the face, that usually heal in a few months, but occasionally last for many years, causing considerable morbidity and large scars. Where treatment is inevitable, drugs commonly used are pentavalent antimonials such pentostam®, and glucocantine®. These drugs are expensive and produces side effects(2). Control of the sandfly vectors has also proven to be difficult due to inaccessibility of their breeding sites and insecticide resistance(3). With all these shortcomings, there is need to identify ways in which interference of parasite transmission can be done and thus reducing *Leishmania* infections.

Experimental and clinical attempts at immunising against *L. major* with non-viable vaccines has shown encouraging results in mice and humans(4,5). It is not known, however, how immune sera from a susceptible

host immunised with *Leishmania*-derived antigens when taken in by the sandfly affects the development and the subsequent transmission of the parasite to naive hosts. In the preceding paper in this series, it was reported that sera from BALB/c mice immunised with *L. major*-derived 63 kilodalton glycoprotein (rgp63), lipophosphoglycan (LPG), a cocktail, of rgp63 plus LPG, or heat-killed whole parasite antigens (WPA) were able to significantly reduce parasite burdens in *Phlebotomus duboscqi* sandflies(6). In the present study we sought to investigate whether sandflies which had previously fed on rgp63, LPG, rgp63 plus LPG cocktail or WPA-immunised BALB/c were able to transmit L. *major* infections to naive BALB/c mice.

MATERIALS AND METHODS

Preparation of Leishmania major -derived antigens and immunisation protocol: Preparation of rgp63, LPG or WPA antigens and immunisation protocol have already been described in detail in the preceding paper(6).

Infection of sandflies and challenge of BALB/c mice: Phlebotomus duboscqi from an established laboratory colony maintained at the Kenya Medical Research Institute (KEMRI) were used in these experiments. Maintainance of sandflies, feeding experiments have been described in detail(6). On the seventh day post-feeding, at least 25 female *P. duboscqi* sandflies were taken in a vial and used to infect four, 6-8 week old naive BALB/c mice by repeatedly transferring them from one animal to another while allowing them to feed on the left hind footpads. Every week for a period of 12 weeks, lesion sizes of all mice were measured using a direct reading vernier caliper. Sandflies used as controls were infected using sandflies which fed on saline immunised mice or infected lesions alone. The lesion areas (mm^2) was calculated by comparing the infected with the contralateral footpad(7).

RESULTS

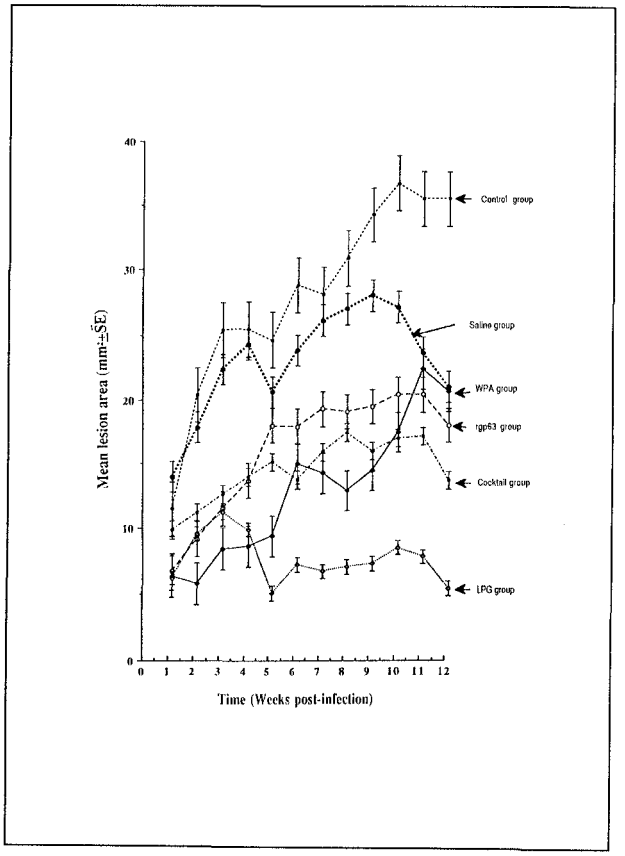
Lesion development in BALB/c mice infected with L. major using sandflies which had previously fed on immunised mice: At week 1, lesion sizes of BALB/c mice which were infected using *P. duboscqi* sandflies which had previously fed on mice immunised with *L. major*-derived antigens (rgp63, LPG or WPA groups), or controls (saline or lesion alone) had almost similar lesion sizes ($p>0.05$) (Figure 1).

At week two, mice infected using sandflies which had previously fed on WPA -immunised mice or "the WPA group" had the least lesion sizes which increased significantly as the infection progressed from week 5 ($p<0.05$). At week 10 the group lesion sizes still maintained an increase but smaller compared to that of the control groups ($p<0.05$). At week 13 when the experiment was terminated, the animals showed splenomegaly. At necropsy, cultures of spleen and infected footpads revealed the presence of parasites, an indication of visceralisation.

Mice infected using sandflies which had previously fed on rgp63 -immunised mice or "the rgp63 group" also showed significantly smaller lesion sizes in the beginning which increased as the infection progressed ($p<0.001$). At week 6 lesion sizes had increased and not significantly different from that of the control groups ($p>0.05$). From week 8 the lesion sizes started reducing and at the end of the experiment animals showed splenomegaly, and parasites were recovered from their spleen and footpad biopsies in culture (Table 1).

Figure 1

Mean lesion sizes (mm^2) of BALB/c mice infected using sandflies which had previously fed on immunized and infected mice



Mice infected using sandflies which had previously fed on LPG-immunised mice or "the LPG group" showed significantly smaller lesion sizes in the beginning ($P<0.05$) which decreased further as the infection progressed ($p<0.001$). From week 5 onwards till the end of the experiment, the group showed the least lesion sizes compared to all the groups ($p<0.001$). At the end of the experiment, the lesions had not ulcerated and animals showed splenomegaly, and parasites were recovered from their spleen and footpad biopsies in culture.

The "cocktail group" also showed significantly smaller lesion sizes in the beginning ($p< 0.001$), increasing significantly at week 4 ($p<0.01$). At week 5, their lesion sizes were intermediate in sizes compared to that of rgp63 and WPA groups but significantly smaller than that of the control groups ($p<0.05$). At week 8, the lesions began to reduce till the end of the experiment ($p<0.05$). At the end of the experiment, animals showed splenomegaly, and parasites were recovered from their spleen and footpad biopsies in culture.

DISCUSSION

BALB/c mice infected with *L. major* parasites develop a progressive infection involving large ulcerating lesions in the infected foot, loss of foot due to necrosis, metastatic spread of the parasites to secondary cutaneous sites as well as the viscera, and eventual death of the animals(8). However, mice infected using sandflies which had previously fed on LPG-immunised bloodmeals developed smaller lesion sizes mice infected using sandflies that had previously fed on rgp63, cocktail or WPA-immunised bloodmeals. It is possible that smaller lesions developed in "LPG group" mice because fewer metacyclic promastigotes were introduced by sandflies previously fed on LPG-immunised mice than sandflies that had fed on rgp63, cocktail or WPA-immunised mice(6). Such low doses of parasites have been shown to induce Thl immune responses in mice(5). While low doses of virulent parasites transmitted by an infected sandfly may be tolerated without producing a lesion, a high dose may overwhelm the immune response(9).

Mice challenged using sandflies which had previously fed on WPA-immunised mice initially showed smaller lesion sizes which increased significantly from the fifth week onwards, as the disease progressed. This observation is in agreement with previous studies which showed that heat-killed WPA in the absence of adjuvants which induced partial protection in BALB/c mice(2,10) or in humans(11). In contrast, mice infected using sandflies previously fed on mice cocktail-immunised mice developed smaller lesion sizes which reduced further in size as the disease progressed ($p<0.05$). Interestingly, however, mice infected using sandflies which had previously fed on rgp63-immunised mice showed smaller lesion sizes which exacerbated as the disease progressed.

A vaccine against any form of leishmaniasis does not necessarily have to prevent an infection. An infection may exist without an observable pathological manifestation, and disease prevention without interfering with infection may have an added advantage(2). In some cases, leishmaniasis produces a state of premunition i.e concurrent infection and immunity which may be the underlying mechanism for prolonged immunity to reinfection generally seen in recovered individuals. If this is true then a transmission blocking vaccine does not need to prevent an infection to be disease protective. Hence, a vaccine which can reduce the effective dose of the inoculum may be adequate for certain populations who develop some degree of natural immunity as a result of repeated exposure to sub-infective doses of the parasite (9).

In the present study it was observed that immunisation of animals with *L. major*-derived antigens leads to a decrease in the number of infective parasites that can develop, and hence be transmitted by the sandfly vector.

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REFERENCES

1. Desjeux, P. Cutaneous leishmaniasis: clinical aspects. In Dowlati, Y., Modabber, F., editors, Clinics in dermatology, vol. 14. Amsterdam: Elsevier Science B.V., p. 417-423, 1996.
2. Howard, J.G., Liew, F.Y., Hale, C. and Nicklin, S. Prophylactic immunization against experimental leishmaniasis .II. Further characterization of protective immunity against fatal *Leishmania tropica* infection. *J. Immun. 1982;* **132**: 450-455.
3. Kaul, S.M., Wattal, B.L., Bhatnagar, U.N. and Mathur, K.K. Preliminary observations on the susceptibility status of Phlebotomus *argentipes* and *Phlebotomus papatasi* to DDT in two districts of northern Bihar (India). *J. Commun. Dis. 1*978; **10**: 208-211.
4. Abath, F.G., Montenegro, S.M. and Gomes, Y.M. Vaccines against human parasitic diseases: an overview. *Acta tropica,* 1998; **71**:237-54.
5. Doherty, T.M. and Coffman, R.L. *Leishmania major:* Effect of infectious dose on T cell subset development in BALB/c mice. *Exp. Parasit.* 1996; **84**:124-135.
6. Tonui W.K., Mbati P.A., Anjili, C.O., Orago, A.S., Turco, S.J., Githure, J.I. and Koech, D. K. Transmission blocking vaccine studies in leishmaniasis: I. Lipopho sphoglycan (LPG) is a promising transmission blocking vaccine molecule against cutaneous leishmaniasis. *East Afr. Med. J.* 2000; **78**:84-93.
7. Nolan, T.J. and Farrell, J.P. Experimental infections of the multimammate rat *(Mastomys natalensis)* with *Leishmania donovani* and *Leishmania major. Amer. J. trop. Med. Hyg.* 1987; **36**:264-266.
8. Scott, P.A. and Farrell, J.P. Experimental cutaneous leishmaniasis: disseminated leishmaniasis in genetically susceptible and resistant mice. *Amer. J. trop. Med. Hyg. 1982*; **32**: 230-238.
9. Modabber, F. Experiences with vaccines against cutaneous leishmaniasis: of men and mice. *Parasitology,* 1989; **98**: S49-S60.
10. Scott, P.A., Pearce, E., Natvitz, P. and Sher, A. Vaccination against cutaneous leishmaniasis in a marine model 1. Induction of Protective Immunity With a Soluble Extract of Promastigotes. *J. Immunol. 1987*; **139**:221-227.
11. Convit, J., Rondon, A., Ulrich, M., Bloom, B., Castellanos, P.L., Pinardi, M.E., Castes, M and Garcia, L. Immunotherapy versus chemotherapy in localized cutaneous leishmaniasis. *Lancet,* 1987; **1**: 401-404.