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Short Communication

Evaluation of recombinant gp63, the major *Leishmania* surface glycoprotein, as a diagnostic molecule for leishmaniasis in vervet monkeys

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The diagnosis of visceral leishmaniasis (VL) has mainly depended on the demonstration of parasites in the spleen, bone marrow or lymph nodes through smears, culture or animal inoculation. Neither of these diagnostic methods is entirely suitable for routine epidemiological studies and present obvious disadvantages in clinical practice. Moreover, parasites are scanty, irrespective of the degree of severity of the clinico-pathological picture so that repeated microscopic examination of aspirated material may fail to demonstrate the amastigotes (Mohammed et al., 1986). Visceral leishmaniasis is characterized by high titres of both specific and non-specific antibodies (Bray, 1976). Shreffler et al. (1993) reported high levels of anti-gp63 antibodies in VL patients. Capitalising on the elevated antibody levels, investigators have developed serological tests such as indirect immunofluorescence, agglutination tests and enzyme-linked immunosorbent assay (ELISA) (Bray et al., 1976; Harith et al., 1986; Ho et al., 1983; Okong'o-Odera et al., 1993). These tests are slowly gaining importance as complementary procedures to existing techniques for routine diagnosis of VL. However, widespread use of serodiagnostic assays of VL has been restricted by cross-reaction between the crude parasite antigens and sera primarily from patients with malaria, trypanosomiasis, cutaneous leishmaniasis, lepromatous leprosy and mucocutaneous leishmaniasis (Harith et al., 1986; Jaffe et al., 1988).

Serodiagnosis of leishmaniasis using purified parasite antigens would be of value for the diagnosis of individual cases, epidemiological studies (Jaffe et al., 1988) and possibly for monitoring response to therapy and for detecting relapses (De Cock et al., 1985). Recently a major surface antigen gp63, to *Leishmania* species was cloned (Button et al., 1988). The roles of this molecule in the binding of promastigotes to macrophages has been extensively studied (Russel et al., 1986; Russel, 1987). The use of recombinant gp63 as a vaccine molecule has been examined in

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mice with promising results (Yang et al., 1990). Studies are also ongoing in our laboratory to examine the molecule as a vaccine candidate in the vervet monkey model of the disease. Recombinant gp63 molecules from *L. chagasi* was reported to have some potential as serodiagnostic antigen (Reed et al., 1990). Work of Shreffler et al. (1993) suggested that recombinant gp63 may be useful in serology-based diagnostic assay for VL. The current study examines the diagnostic potential of recombinant gp63 molecule from *L. major* for serodiagnosis of cutaneous and visceral leishmaniasis in vervet monkey model.

Sera from eleven vervet monkeys with *Leishmania* infections were identified and used for this study. Five samples were from animals with subclinical *L. donovani* infection, four samples from animals self-cured from *L. major*, two samples from animals with either clinical *L. major* or *L. donovani* infection and three samples from naive vervet monkeys (negative controls). Monoclonal antibody 235 against gp63 (courtesy of Dr. McMaster, University of British Columbia, Canada), serum from gp63 vaccinated vervet monkey and serum raised in rabbit against killed *L. major* promastigotes served as positive controls. ELISA as described by Ho et al. (1983) was conducted. Recombinant gp63 synthesized in *Escherichia coli* was used at a concentration of 10 μg/ml to coat ELISA plates. Rabbit anti-monkey IgG whole molecule conjugated to horse radish peroxidase served as secondary antibody (Sigma Immunochemicals, UK). Orthophenyl diamine (OPD; Sigma Immunochemicals, UK) was the chromogenic substrate. Positive values in the test were defined as those >2SD above the mean of the control sera at a dilution of 1/625 (absorbance $=0.246$).

High levels of anti-gp63 antibodies were demonstrated in serum from gp63 vaccinated animals. The optical density achieved in the ELISA assay using such antiserum was comparable to those obtained with monoclonal antibody 235 and polyvalent rabbit anti-promastigotes serum. Animals with clinical *L. donovani* and *L. major* disease had higher antibody levels when compared to either self cured *L. major* or *L. donovani* asymptomatic cases. Animals with clinical leishmaniasis could be easily differentiated from the negative controls even at a dilution of 1/3125. Self cured *L. major* and *L. donovani* asymptomatic animals had low antibody levels but could still be distinguished from the negative controls at a serum dilution of 1/625, except animal no. 1008. Sensitivity and specificity based on the results from subclinical and self cured leishmaniasis was estimated as 89% and 100% respectively. The results are illustrated in Fig. 1 and Table 1. They go on to demonstrate the serodiagnostic potential of recombinant gp63 molecule for both cutaneous and visceral leishmaniasis during clinical and asymptomatic infections in the vervet monkeys. The results are also consistent with the reports by Okong'o-Odera et al. (1993) and Shreffler et al. (1993) on the applicability of the molecule for the serodiagnosis of the disease in humans. More so, this antigen could serve as a tool for monitoring response during therapy.

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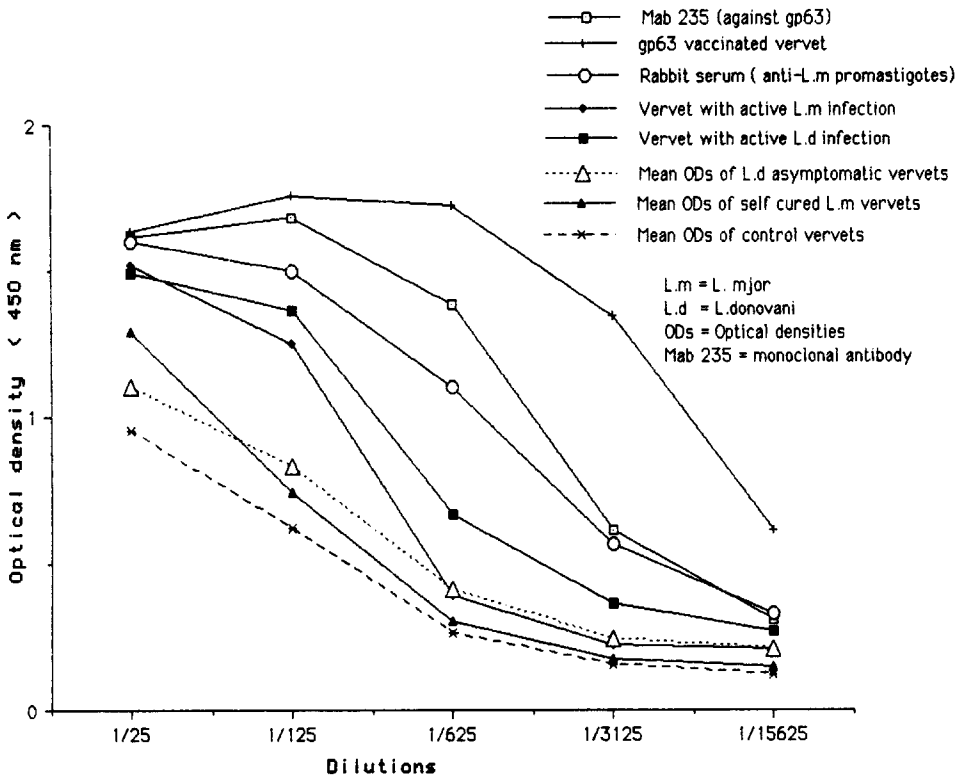

Fig. 1. Reactivity of sera at different dilutions to gp63 molecules.

TABLE 1

Anti-gp63 ELISA optical density results obtained with MAb, rabbit serum and sera from vervet monkeys with different forms and stages of leishmaniasis

Animal/Mab.	Optical density (OD), 450 nm. serum dilution		Serum description
	1/625	1/3125	
Mab 235	1.382	0.615	Monoclonal abs against gp63
Rabbit serum	1.102	0.566	Vaccinated with *L.m.* promastigotes
Ver. 1474	1.725	1.344	Vaccinated with gp63 antigen (positive control)
Ver. 1241	0.669	0.366	Clinical *L. donovani* infection
Ver. 1453	0.394	0.223	Clinical *L. major* infection
Vers. 1011, 1071, 693, 396	0.316	0.181	Self cured *L. major* animals (mean OD)
Vers. 1008, 1078, 1381, 1317, 1296	0.400	0.245	Cryptic *L. donovani* infections (mean OD)
Vers. 1429, 1396, 1573	0.241	0.145	Naive animals (negative control) (mean OD)

MA, monoclonal antibody; Ver, vervet monkey; Vers, vervet monkeys; abs, antibodies.

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