Isolation of Antigen from *Litomosoides carinii* Macrofilariae Detecting Serum Antibodies Due to *Onchocerca volvulus* *

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Abstract. Crude aqueous *Litomosoides carinii* adult worm extract was used as antigen for the detection of antibodies in sera from African patients with proven onchocerciasis (n=45) resident in rural endemic areas of Togo and Sierra Leone. In 71% of cases this extract was found to produce 1 to 5 precipitation arcs in immunoelectrophoresis. Using a crude aqueous extract from adult *Onchocerca volvulus*, precipitation tests were positive in 75% of cases.

The complexity of the *L. carinii* crude extract was shown by PAG-disc electrophoresis, PAG-electrofocusing, immunoelectrophoresis and crossed immunoelectrophoresis with the appropriate rabbit-antiserum. An antigen detecting onchocercal antibodies was isolated by two step preparative flat bed electrofocusing in granulated gel (PEGG). The antigen (pI 6.55, molecular weight 55 to 60 kd as estimated by SDS-PAG electrophoresis) was very suitable for antibody demonstration in double diffusion test and immunoelectrophoresis. Preliminary controls for specificity were performed by diffusing the antigen against sera from human and animal helminthoses including filarial infections. In contrast to the crude *L. carinii* extract no reaction was observed with sera from helminthic infections others than filariasis.

Introduction

A wide variety of tests has been employed for the serodiagnosis of human onchocerciasis and for seroepidemiological purposes using homologous

* Supported by the Deutsche Forschungsgemeinschaft, D-5300 Bonn-Bad Godesberg, Grant Ge 167/14

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crude or in part purified (Marcoullis et al. 1978; Philipp et al. 1982 and unpublished data) adult worm antigen and in some cases microfilariae. Because the only sources of adult *Onchocerca volvulus* are the subcutaneous nodules of patients with onchocerciasis, material for the preparation of homologous antigen is limited. In contrast, *Litomosoides carinii* adult worm antigens, which have been shown to be suitable for the detection of antibodies in human onchocerciasis (Neppert 1974; Bartlett et al. 1975; Marcoullis and Gräsbeck 1976; Stappert 1979; Geyer and Zahner 1980; Ogilvie and Parkhouse unpublished data), can be obtained in sufficient quantities from experimentally infected *Mastomys natalensis* or *Sigmodon hispidus*.

Comparative immunoanalytical examination of proteins/proteins present in *O. volvulus* and *L. carinii* crude extracts prepared from adult worms revealed 12 to 13 common reactants (Geyer and Zahner 1980). Based on these results this study deals with the purification of *L. carinii* adult worm antigen suitable for the demonstration of precipitating antibodies in sera from patients infected with *O. volvulus*.

**Materials and Methods**

1. **Preparation of Crude Aqueous L. carinii Extract.** Adult male and female cotton rat filariae were removed from the pleural cavities of experimentally infected *M. natalensis* (strain "GRA-Gießen") between days 120 and 150 post-infection. The worms were washed five times in saline, once in double distilled water, frozen rapidly at -60°C, and then freeze dried.

   Lyophilized worms were homogenized in double distilled water (1 g/100 ml) using an Ultra-Turraxxy at 20,000 rpm for 3 x 30 s in N2. After overnight extraction under gentle stirring the homogenate was centrifuged at 6,000 x g for 30 min. The supernatant was saved and the sediment suspended in double distilled water (1:20 v/v), homogenized in a glass grinder, extracted and centrifuged as described above. The supernatant was stored, the sediment suspended in double distilled water (1:20 v/v) and sonicated at 20 kHz for 4 x 30 s followed by extraction and centrifugation. The supernatants were pooled and centrifuged at 78,000 x g for 1 h. The protein content was determined photometrically at 623 nm according to Lowry et al. (1951). All operations were carried out at 4-6°C. The supernatant was lyophilized, stored at -60°C and designated *L. carinii* crude antigen (LCA).

2. **Sera.** For production of antisera to crude antigen (LCA) male rabbits (*n* = 5, Alaska strain, 1 year old) were sensitized each intramuscularly with 0.2 ml LCA (2 mg protein) emulsified with 0.1 ml Freund's complete adjuvant (FCA) on days 1, 2, 3, 7, 28, 42, and 56. Booster injections (0.1 ml LCA +0.1 ml FCA) followed on days 80, 104, 128, and 152. Blood was collected from the marginal ear vein on days 66, 90, 114, 138, and 162. Sera were tested immunoelectrophoretically for antibody activity. The rabbit-antiserum to LCA for the experiments was a pool of sera showing high precipitating quality. Antiserum was stored in small quantities at -40°C. The supernatant was lyophilized, stored at -60°C and designated *L. carinii* crude antigen (LCA).

Sera from patients (*n* = 45) with proven onchocerciasis (skin snip positive) and no evidence of other parasitic infection were collected from rural populations in Togo and Sierra Leone. Controls for intestinal worms, *Schistosoma mansoni*, *S. haematobium* and *Entamoeba histolytica* were performed by repeated examinations of stool and urine samples. Serological tests were carried out for the detection of *S. mansoni* and *Echinococcus cysticus* derived antibodies (IHAT, Cellognost, Behringwerke AG, D-3550 Marburg) and for antibodies due to *S. haematobium* (Intradermal test, Schistosoma Antigen for Bilharziosis Skin Test, Behringwerke AG). The patients have also been examined for *Wuchereria bancrofti* microfilariae and plasmodia. The sera were tested for onchocercal antibodies in double diffusion test, immunoelectrophoresis