
Brief Definitive Report

CHARACTERIZATION OF AN IMMUNODOMINANT *ONCHOCERCA VOLVULUS* ANTIGEN WITH PATIENT SERA AND A MONOCLONAL ANTIBODY

BY RICHARD LUCIUS,* HARTWIG SCHULZ-KEY,[‡] DIETRICH W. BÜTTNER,[§]
ANDREA KERN,* BRIGITTE KALTMANN,* JACQUES PROD'HON,^{||}
FRANK SEEBER,* ROLF D. WALTER,[§] K. C. SAXENA,[¶] AND
HANS-JOCHEN DIESFELD*

From the *Institut für Tropenhygiene der Universität Heidelberg, D-6900 Heidelberg, the
[‡]Tropenmedizinisches Institut der Universität Tübingen, D-7400 Tübingen, and the [§]Bernhard-
Nocht-Institut, D-2000 Hamburg 4, Federal Republic of Germany; the ^{||}ORSTOM, 75 Paris
Cedex 10, France; and the [¶]Central Drug Research Institute, Lucknow, India

Onchocerca volvulus is a pathogenic, tissue-dwelling nematode of man and apes, occurring mainly in sub-Saharan Africa and Latin America. The filarial worm causes chronic infections leading to inflammatory and degenerative changes of skin and eyes which can result in blindness ("river blindness") (1, 2). The narrow host spectrum of the parasite makes work on the immunobiology of the infection difficult. Yet, immunological studies are urgently needed in order to develop sensitive and specific immunodiagnostic tests and to evaluate the possibility of protective immunization. In this context, we used patient sera and mAbs to characterize a major immunodominant antigen of *O. volvulus* which we had previously identified (3, 4).

Material and Methods

Sera. Groups of onchocerciasis sera were collected in various forest and savanna regions of West Africa and in the Yemen Arab Republic, from microfilaridermic patients or individuals with localized onchocerciasis (Table I). Sera of patients infected with *Wuchereria bancrofti* were collected in the vicinity of Lucknow, India, from microfilaremic patients or persons with a history of infection. Sera of patients with *Brugia malayi* infections were obtained from microfilaremic individuals in Indonesia (courtesy of Dr. W. F. Piessens, Harvard School of Public Health, Boston, MA). 50 sera of healthy German blood donors and 9 sera of uninfected persons from the Yemen Arab Republic served as controls.

Parasites. *O. volvulus* from a savanna focus in Northern Ivory Coast and *O. lienalis* of cattle from Togo were isolated by collagenase digestion (5). Infective larvae of *O. volvulus* from a forest focus in Liberia were obtained from the heads of *Simulium* species 9 d after a blood meal on a microfilaridermic volunteer. Microfilariae were collected from skin biopsies of Liberian patients by incubation in RPMI 1640 medium. Female *O. volvulus* were cultured in medium without FCS and the supernatants were concentrated as previously described (6).

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TABLE I
Origin of *Onchocerciasis* Sera and Control Sera

Origin of sera	Patient sera	Control sera
Western Ivory Coast	20	—
Northern Ivory Coast	26	—
Northern Togo*	24	—
Southern Togo	32	—
Yemen Arab Republic	42	9
Burkina Faso 1977	14	—
Burkina Faso 1983*	13	—
Liberia	34	—
Mali	14	—
Germany	—	50
Total	219	59

* Areas subject to vector control measures by the *Onchocerciasis* Control Programme of the World Health Organization for > 5 yr.

SDS-PAGE and Immunoblotting. SDS-PAGE was performed according to Laemmli (7) using linear gradient slab gels (10–14.5% acrylamide). Minced worm material solubilized in sample buffer (1% wt/vol SDS, 5% vol/vol 2-ME, 10% vol/vol glycerol in 63 mM Tris-HCl, pH 6.8) was boiled for 2 min and sonicated. For immunoblotting, separated antigens were electrophoretically transferred onto nitrocellulose sheets and processed according to Burnette (8). Detecting antibodies were anti-human IgG or anti-mouse IgG + IgM, labeled with horseradish peroxidase (Tago, Inc., Burlingame, CA). The substrate solution consisted of 10 mM H₂O₂ and 3.3 mM 4-chloro-1-naphthol in TBS.

Monoclonal Antibodies. 6-wk-old BALB/c mice were immunized three times by subcutaneous injections of each 250 µg of female *O. volvulus* homogenized in Tris-buffered saline (TBS). The first immunization was with CFA, the subsequent ones with the incomplete adjuvant. Spleen cells were fused with cells of the myeloma line X63/Ag8 and the hybridomas cloned using established methods (9).

Immunoaffinity Chromatography. Affinity purified mAb 4A7 was coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden), and the immunosorbent was used to purify the target antigen from worm lysates in TBS. The antigen was eluted with 0.1 M glycine, pH 2.5, and the protein was concentrated by acetone precipitation using 10 mM sodium desoxicholate as a carrier. All buffers contained protease inhibitors (1 mM final concentration: ϵ -amino-*n*-caproic acid, EDTA, benzamidine, and PMSF).

ELISA. Microtiter plates were coated with 10 ng per well of immunoaffinity-purified antigen in carbonate buffer (13 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.4) overnight at 4°C. The wells were blocked with 3% BSA and sequentially reacted with serum dilutions and horseradish peroxidase conjugated anti human IgG (Tago, Inc.) with intermittent washings. Substrate (0.1% wt/vol orthophenyldiamine, 50 mM H₂O₂ in TBS) was added, the reaction was stopped with 8 N H₂SO₄, and the absorbance was read at 490 nm.

Indirect Fluorescent Antibody Test (IFAT). Cryostat sections of 4-µm thickness were prepared from adult *O. volvulus* wrapped into mouse tissue. The sections were acetone fixed on glass slides for 2 min and sequentially reacted with undiluted hybridoma supernatants and anti-mouse IgG + IgM + IgA conjugated with FITC (Nordic, Tilburg, The Netherlands) with intermittent washings.

Results

Analysis with Patient Sera. A total of 182 sera from onchocerciasis patients of seven endemic areas with ongoing *O. volvulus* transmission (Table I) were ana-

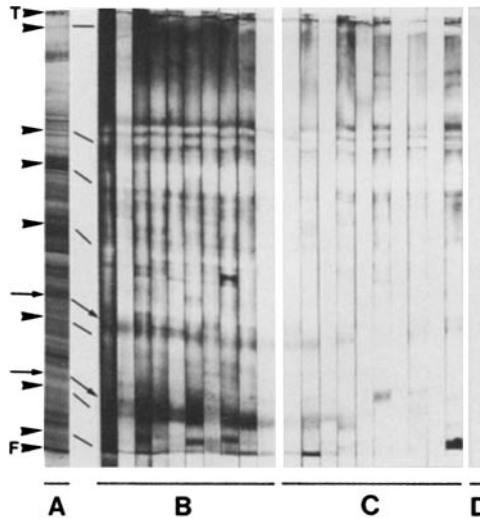


FIGURE 1. Immunoblotting analysis of the IgG response of randomly chosen patients against total antigens of female *O. volvulus*. (A) Sera from patients exposed to *O. volvulus* transmission (Western Ivory Coast, 1984). (B) Sera of patients from an area subject to vector control for >5 yr (Northern Togo, 1985). (C) Pool serum of Europeans. (Arrows) Position of the 33,000 and 21,000 M_r bands. (Arrowheads) 220, 94, 67, 43, 30, 20.1, and 14.4-kD standards. T, top; F, front.

lyzed by immunoblotting with antigens of female *O. volvulus* and two major traits emerged: First, the great majority of individuals had IgG responses against bands of 97,000, 67,000, 33,000, 21,000, 20,000, and 17,000 M_r . The bands of 33,000 and 21,000 M_r were recognized by 175 sera (96.2%), and in most cases they were prominent. Second, many other antigen bands of various M_r were recognized by only a small proportion of sera (Fig. 1 B). A comparative analysis was performed with 37 onchocerciasis sera from two regions where transmission had been interrupted by vector control measures for >5 yr (Table I). All serum donors still had microfilariae and/or nodules containing living worms in their skin. These sera had low concentrations of IgG against the 33,000 and 21,000 M_r bands and other components of lower molecular weight and it appeared that patients with old infections did not maintain their levels of antibodies against the 33,000 and 21,000 M_r bands (Fig. 1 C).

Analysis with a Monoclonal Antibody. An mAb (4A7, IgG1 with κ light chains) recognized the 33,000 and 21,000 M_r bands in immunoblots with total antigens of female *O. volvulus* and adult *O. lienalis*, but no antigens of other filarial genera (*Acanthocheilonema viteae*, *Litomosoides carinii*, *Setaria cervi*, *B. malayi*, not shown). The 21,000 M_r band appears to be a proteolytic product of the 33,000 M_r band, since it became comparatively more prominent after prolonged exposure of worm material to room temperature. The electrophoretic mobility shifted from 33,000 and 21,000 M_r under reducing conditions to 37,000 and 24,000 M_r under nonreducing conditions (not shown). In male *O. volvulus* the target epitope was present on 33,000 and 21,000 M_r bands and on a band of 39,000 M_r (Fig. 2, lane 1). In infective larvae, only a band of 133,000 M_r was recognized, whereas the target epitope was not detectable in microfilariae (Fig. 2). Antibody binding studies with ELISA revealed that the target epitope was resistant to carbohydrate digestion by 50 mM metaperiodate (4°C, 24 h). The mAb bound to the reproductive organs and muscles of both sexes in cryostat sections of adult *O. volvulus*, whereas no appreciable binding to the cuticle

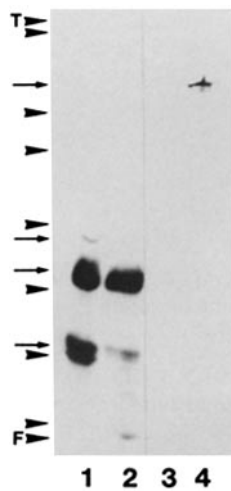


FIGURE 2. Presence of mAb 4A7 target epitope in various stages of *O. volvulus* analyzed by immunoblotting. (Lane 1) Male worms; (lane 2) female worms; (lane 3) microfilariae; (lane 4) infective larvae. (Arrows) position of 133,000, 39,000, 33,000, and 21,000 M_r bands. (Arrowheads) kilodalton standards (see Fig. 1). T, top; F, front.

was noticed (Fig. 3). The target epitope was detectable by immunoblotting on bands of 33,000, 21,000, and 20,000 M_r in concentrated culture supernatants of adult female *O. volvulus*. The amount of antigen shed was ~ 10 ng per female worm per day as compared by immunostaining of known concentrations of purified material (see below).

Studies with Purified Antigen. The target antigen of mAb 4A7 was purified by immunoaffinity chromatography in order to test its specificity in immunodiagnosis. The purification yielded a minor band of 33,000 M_r and more prominent bands of 21,000 and 20,000 M_r which were detected by mAb 4A7 in immunoblots. ELISAs using purified antigen were performed with sera of randomly chosen patients infected with *O. volvulus* (Southern Togo, $n = 20$), *W. bancrofti* ($n = 32$) or *B. malayi* ($n = 20$) and from Europeans ($n = 20$). The differences in OD reading between sera of onchocerciasis patients and the other sera were statistically significant ($p < 0.01$; Student's t test), but not clear enough to reliably differentiate between the infections. We assume that this result was due to crossreacting contaminants. Immunoblots with purified material and the same set of sera revealed that only onchocerciasis sera, but none of the other filariasis sera recognized the 33,000, 21,000, and 20,000 M_r bands (not shown). This high specificity was combined with a high sensitivity, since 18 of the 20 onchocerciasis sera reacted.

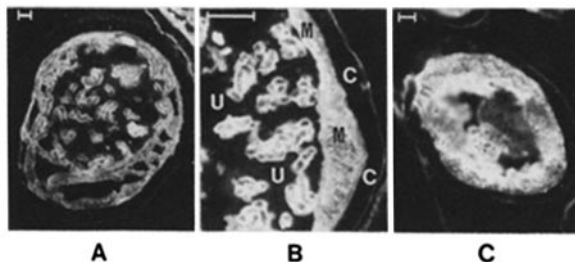


FIGURE 3. Binding of mAb 4A7 to sections of adult *O. volvulus*. (A) Female worm: fluorescence in uteri and in muscles. (B) Detail of section of a female worm: fluorescence in the uterus and the muscle layer, but not in the cuticle. U, uterus; M, muscle; C, cuticle. (C) Male worm: fluorescence in the reproductive organ and in the muscles. Bar, 100 μm .

Discussion

The immunodominant antigen of *O. volvulus* described in our study has promising properties of an immunodiagnostic component, namely specificity for the genus *Onchocerca* and a very high frequency of recognition by onchocerciasis sera. Specificity of immunodiagnostic tests is of prime importance in the context of control measures, such as the Onchocerciasis Control Programme of the World Health Organization in West Africa, in order to differentiate between onchocerciasis and other filariases occurring in the same area. In West Africa, these are infections with *W. bancrofti*, *Mansonella spp.* and *Loa loa*. Our results with sera of lymphatic filariases and initial tests with sera of the other filarial infections were encouraging. The sensitivity of the 33,000 M_r antigen is expected to be high: First, the component induces antibody responses in patients infected with various geographical strains of the parasite. Second, patients with different clinical forms of onchocerciasis were shown to have 33,000 M_r -specific antibodies in an earlier study (3). Third, the occurrence of the target epitope of mAb 4A7 in infective larvae suggests formation of 33,000 M_r -specific antibodies already in early stages of the infection. Indeed, 33,000 M_r -specific antibodies were detected in sera of experimentally infected chimpanzees already 6 mo after infection (Lucius, R., and Taylor, H. R., unpublished observation). Further studies have to clarify if the relative decrease of antibody response against the 33,000 M_r antigen of patients from vector-controlled areas could allow a serological differentiation between active and declining onchocerciasis. It remains to be established if the *Onchocerca*-specific 20,000 M_r antigen identified in several studies (10–13) is identical with a proteolytic fragment of the component characterized here.

At present, we do not have a clue to the stage-specific occurrence of the target epitope of mAb 4A7 on antigens of very different molecular weight. The antigen is encoded by a single copy gene (Lucius, et al., manuscript submitted for publication), the most likely explanation for the occurrence of a 133,000 M_r antigen in infective larvae, therefore, is the formation of a polymer. The antigen's solubility in buffers indicates that it is an intracellular component. Its high immunogenicity suggests that it is little related to host antigens. A highly immunogenic intracellular component of *Schistosoma mansoni*, paramyosin, which is phylogenetically distant from host proteins, induces protective immune responses by triggering T cells which in turn activate macrophages for parasite killing (15). It remains to be established if the 33,000 M_r antigen of *O. volvulus* also has such a protective potential.

Summary

Adult *Onchocerca volvulus* and infective larvae, but not microfilariae contain an immunodominant antigen (33,000 and 21,000 M_r in females, 39,000, 33,000, and 21,000 M_r in males, 133,000 M_r in infective larvae) which is recognized by an *Onchocerca*-specific mAb. The component is part of the reproductive organs and muscles. 96.2% of onchocerciasis sera contained antibodies detectable by immunoblotting against it. Antigen purified by immunoaffinity chromatography was specifically recognized in immunoblots by onchocerciasis sera, but not by sera from other filarial infections. The high immunogenicity, the specificity, and

the occurrence in infective larvae of this antigen indicate an immunodiagnostic potential and a possible role in the immunobiology of the parasite.

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References

1. Duke, B. O. L. 1972. Onchocerciasis. *Br. Med. Bull.* 28:66.
2. Buck, A. A. 1973. Onchocerciasis. Symptomatology, pathology, diagnosis. World Health Organization, Geneva.
3. Lucius, R., D. W. Büttner, C. Kirsten, and H. J. Diesfeld. 1986. A study on antigen recognition by onchocerciasis patients with different clinical forms of disease. *Parasitology*. 92:569.
4. Lucius, R., J. Prod'hon, A. Kern, G. Hebrard, and H. J. Diesfeld. 1987. Antibody responses in forest and savanna onchocerciasis in Ivory Coast. *Trop. Med. Parasitol.* 38:194.
5. Schulz Key, H., E. J. Albiez, and D. W. Büttner. 1977. Isolation of living adult *Onchocerca volvulus* from nodules. *Tropenmed. Parasitol.* 28:428.
6. Wittich, R. M., H. D. Kilian, and R. D. Walter. 1987. Polyamine metabolism in filarial worms. *Mol. Biochem. Parasitol.* 24:155.
7. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680.
8. Burnette, W. N. 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. *Anal. Biochem.* 112:195.
9. Galfre, G., and C. Milstein. 1981. Preparation of monoclonal antibodies: strategies and procedures. *Methods Enzymol.* 73:3.
10. Philipp, M., A. Gomez-Priego, R. M. E. Parkhouse, M. W. Davies, N. W. T. Clark, B. M. Ogilvie, and F. Beltran-Hernandez. 1984. Identification of an antigen of *Onchocerca volvulus* of possible diagnostic use. *Parasitology*. 89:295.
11. Cabrera, Z., and R. M. E. Parkhouse. 1986. Identification of antigens of *Onchocerca volvulus* and *Onchocerca gibsoni* for diagnostic use. *Mol. Biochem. Parasitol.* 20:225.
12. Cabrera, Z., and R. M. E. Parkhouse. 1987. Isolation of an antigenic fraction for diagnosis of onchocerciasis. *Parasite Immunol. (Oxf.)*. 9:39.
13. Lobos, E. and N. Weiss. 1987. Identification of non-cross-reacting antigens of *Onchocerca volvulus* with lymphatic filariasis serum pools. *Parasitology*. 93:389.
14. Lanar, D. E., E. J. Pierce, S. L. James, and A. Sher. 1986. Identification of paramyosin as schistosome antigen recognized by intradermally vaccinated mice. *Science (Wash. DC)*. 234:593.