MOLECULAR CLONING OF AN IMMUNODOMINANT ANTIGEN OF *ONCHOCERCA VOLVULUS*

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Onchocerciasis, or "river blindness," is caused by the filarial nematode *Onchocerca volvulus*, which infects an estimated 18 million people in Africa and Latin America (1). It is one of the leading causes of blindness in sub-Saharan Africa today. However, few biochemical and immunological studies have been conducted on *O. volvulus* because humans and chimpanzees are its only known mammalian hosts (2). Since the parasite cannot be maintained in the laboratory, there is a severe shortage of parasite antigen material for the development of immunodiagnostic tests and the assessment of immunoprophylactic potential. As a first step to overcome this problem, we describe here the cloning and characterization of the gene for an immunodominant antigen of this parasite.

Materials and Methods

Immunochemistry. Female worms isolated from nodules by collagenase digestion (3) were solubilized in sample buffer (63 mM Tris-HCl, pH 6.8, 10% [vol/vol] glycerol, 3% [wt/vol] SDS, 5% [vol/vol] β-mercaptoethanol) containing 1 mM each of ε-amino-n-caproic acid, EDTA, benzamidine, and PMSF. The sample was sonicated at 500 W for 2 min and boiled for 2 min. Electrophoresis of the supernatants was in 10–14.5% gradient polyacrylamide gels followed by transfer to nitrocellulose (4). The filters were saturated with 3% BSA, serially reacted with the primary antibodies and horseradish peroxidase–conjugated antibodies against mouse IgG or human IgG, and developed using 4-chloro-1-naphthol as substrate. Frozen sections of female worms of 4 μm thickness were fixed with acetone on glass slides for 2 min and serially reacted with mAb 4A7 and anti–mouse IgG antibodies conjugated with FITC.

DNA Manipulations. DNA sequence determinations (5) and Northern and Southern blots (6) were conducted as described.

Results and Discussion

In previous studies we identified and characterized an immunodominant antigen of *O. volvulus* that is potentially an important diagnostic reagent (7, 8). Immunoblots demonstrated that this antigen is specific for Onchocerca since it is recognized by sera of 96% of onchocerciasis patients but not by sera from patients infected with other filarial parasites. mAb 4A7 directed against this antigen binds to components
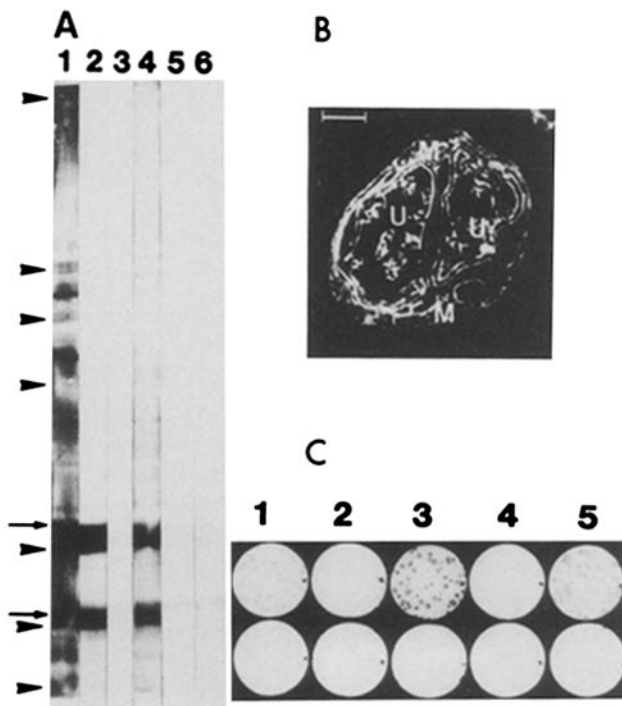
FIGURE 1.   Onchocerciasis patient serum, mAb 4A7, and a monospecific mouse serum recognize the M_r 33,000 antigen of female *O. volvulus* and its M_r 21,000 fragment. (*A*) Immunoblot of total antigens from adult female parasites probed with patient serum (lane *1*); mAb 4A7 (lane *2*); an unrelated mAb (lane *3*); polyclonal monospecific mouse serum directed against affinity-purified M_r 33,000 antigen (lane *4*); mouse preimmune serum (lane *5*); conjugate only (lane *6*). Arrowheads are M_r markers of 220, 94, 67, 43, 30, 20, and 14.4 × 10^3, respectively; arrows mark the positions of the M_r 33,000 and 21,000 bands. (*B*) Binding of mAb 4A7 to antigens in the uteri (*U*) and muscle (*M*) of a female *O. volvulus* in a frozen section. Bar represents 25 µm. (*C*) Recombinant phage Ov33-3 (*top row*) or λgt11 phage (*bottom row*) probed with mAb 4A7 (lane *1*); an unrelated mAb (lane *2*); monospecific mouse serum directed against mAb 4A7-purified M_r 33,000 antigen (lane *3*); preimmune mouse serum (lane *4*); affinity-purified human anti-*O. volvulus* antibodies (lane *5*).

of M_r 33,000 and 21,000 in adult parasite extracts (Fig. 1 *A*, lane *2*). The 21,000 M_r component is probably a proteolytic fragment of the 33,000 M_r component since it is much more prominent in extracts of dead worms than live worms (not shown). A polyvalent mouse serum generated by immunization with mAb 4A7-purified antigen also binds to these two bands on immunoblots (Fig. 1 *A*, lane *4*). In extracts of infective larvae obtained from the black fly vector, the target epitope of mAb 4A7 is located on a M_r 133,000 band that has not been characterized further because of a shortage of larvae material (8). The epitope recognized by the mAb is not present in extracts of microfilariae isolated from skin snips. Examination of frozen sections of adult worms with mAb 4A7 by immunofluorescence assay reveals that the antigen is present in the reproductive organs and muscles but not in the cuticle (Fig. 1 *B*). The lack of this antigen on the surface of adult worms and its absence from microfilariae in the skin suggests that the host immune response to it is induced by release of the antigen from the adult.

The polyvalent serum directed against the 33,000 M_r antigen (Fig 1 *A*, lane *4*) was used to immunoscreen 650,000 phage of an unamplified λgt 11 cDNA library prepared from poly(A)⁺ RNA of adult *O. volvulus* collected from patients in Kumba,

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      R  T  T  T  M  K  I  L  F  C  L  L  L  L  A  I  T  A  L  E  A  G  V  V  K  R  Y  N  K  R  F  A  G  F  N  V  A  G  I     39
CAAGAACAACAACAATGAAAATTCTTTTCTGTTTGTTATTGCTCGCGATAACAGCATTGGAAGCAGGTGTAGTAAAAAGGTACAATAAACGTTTTGCTGGATTTAATGTTGCCGGAATTG    120

   G  G  N  A  G  C  V  V  V  D  N  K  L  F  A  N  S  F  F  L  R  E  L  T  T  E  E  Q  R  E  L  A  Q  Y  I  E  D  S  N  R     79
GTGGAAATGCTGGATGTGTCGTTGTGGATAATAAACTGTTTGCAAACAGTTTCTTTCTTCGTGAATTGACAACTGAAGAACAACGAGAACTTGCCCAATACATTGAAGATTCAAATCGTT   240

   Y  K  E  E  V  K  E  S  L  E  E  R  R  K  G  W  Q  L  A  R  D  G  K  E  D  S  K  V  L  S  A  L  A  E  K  K  L  P  K  P    119
ACAAGGAGGAAGTAAAAGAATCATTGGAAGAACGACGTAAAGGATGGCAATTAGCACGAGATGGCAAGGAAGATTCAAAAGTTTTATCGGCTCTTGCAGAAAAGAAACTTCCGAAACCAC   360

   P  K  K  P  S  F  C  S  A  G  D  T  T  Q  Y  Y  F  D  G  C  M  V  Q  N  D  K  I  Y  V  G  R  A  Y  V  R  D  L  T  P  D    159
CGAAAAAACCATCCTTCTGCTCTGCTGGTGATACAACTCAGTACTACTTTGATGGTTGCATGGTTCAGAATGATAAAATCTATGTTGGACGAGCATATGTACGAGATTTGACACCAGATG   480

   E  V  T  Q  L  K  T  F  D  A  K  M  T  A  Y  Q  K  Y  L  S  S  T  I  Q  K  Q  V  D  S  L  F  G  E  K  S  N  L  F  N  L    199
AAGTGACTCAATTGAAAACATTTGATGCCAAAATGACAGCTTATCAGAAATATTTATCTTCTACCATCCAAAAACAAGTGGATAGCTTGTTCGGTGAAAAATCAAATCTGTTCAATTTAT   600

   F  A  D  T  R  T  E  A  T  S  Q  A  S  D  D  A  T  A  G  A  T  T  T  Q  A  P  V  E  A  P  E  P  P  H  F  C  V  A  I  Y    239
TCGCTGATACACGTACTGAGGCCACGTCGCAAGCATCCGATGATGCTACGGCTGGTGCGACAACAACGCAAGCTCCAGTTGAAGCACCAGAACCACCACATTTCTGCGTCGCAATCTATT   720

  *
GAAAACAAACTTGGACGTGAAAATGCCGAATAAAAATTTCAATTTATTCTTTTGCATTTTCATTTTTTTTTGTATATGCATATGATATTTGTGCATTATTTTTTAATATGAAATCATAATT   840

ATCATGTTTTTTTTTTTTTATCATTCAGGTTTTATTTCAATTTATTCAAGTGAAAAATGGAAAAAAAAAAGTTACTTTTAAATGCTGATTTTTGTATCAATTTAGCTATTTTTCTCTTCGT   960

TTTGCTAACCCTGATTACTGTTTATTA                                                                                                   987
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FIGURE 2. The nucleotide sequence of the Ov33-3 cDNA and deduced amino acid sequence. Restriction fragment termini labeled for the sequence determination were generated by Hpa II, Nde I, Sca I, and Eco RI. The indicated reading frame is in phase with the β-galactosidase gene of λgt11.

Cameroon, West Africa (9). Four recombinant phage clones gave a positive signal. Their different-sized cDNAs crosshybridized under high stringency conditions, indicating that they all encode the same sequence. Clone Ov33-3, which contained the largest Eco RI cDNA insert, produces a recombinant fusion protein that is also recognized by both mAb 4A7 and human patient sera (Fig. 1 C). This 1.0-kb cDNA was subcloned into pUC12 and its sequence was determined (Fig. 2). An open reading frame encoding 239 amino acids is in phase with the reading frame of the β-galactosidase gene of λgt11, followed by a 3' nontranslated region of 254 nucleotides that is missing the 3' poly(A) tail. Comparison of the sequence with the nucleotide and amino acid sequences in GenBank did not reveal any significant similarities.

To estimate the abundance of the corresponding mRNA in adult parasites, 16 ×

FIGURE 3. Radioactive Ov33-3 cDNA was used to probe (A) a Northern blot of 2 µg of total RNA from O. volvulus collected from patients in the villages of Kumba (K) and Touboro (T) in Cameroon, and from patients in Mali (M) and Guatemala (G), and (B) a Southern blot containing Hind III digests of 5 µg of genomic DNA from the same organisms. Hind III fragments of λDNA were used as standards (st). The probe hybridizes to a 1.2-kb RNA and a 14-kb Hind III restriction fragment in organisms from all four locations.
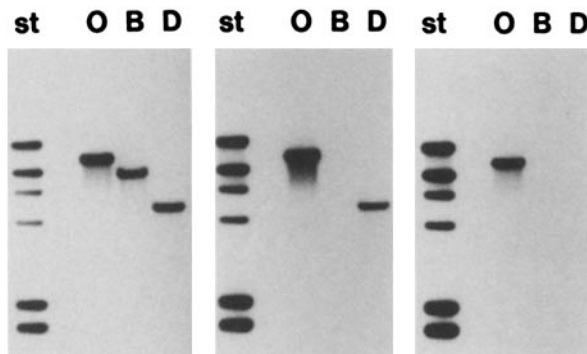
FIGURE 4.   Hind III digests of 5 μg of genomic DNAs from *O. volvulus* (*O*), *B. malayi* (*B*), and *D. immitis* (*D*) were probed with Ov33-3 cDNA and the single filter washed successively under low (*left*), moderate (*middle*), and high (*right*) stringency. The washings were two 1-h incubations in 0.1% SDS, 0.1% sodium pyrophosphate, 1× SET at 50°C (low) or 65°C (moderate), and the same SDS and sodium pyrophosphate concentrations in 0.1× SET at 65°C (high). Standard length fragments (*st*) were the same as in Fig. 3. The probe hybridizes to Hind III fragments of 14 kb in *O. volvulus*, 9.5 kb in *B. malayi*, and 5.5 kb in *D. immitis*.

10^3 recombinants of the unamplified cDNA library were screened with Ov33-3 cDNA. It hybridized to the phage DNA in 48 plaques, indicating that its mRNA constitutes 0.3% of the total poly(A)$^+$ RNA in the adult worms. The cDNA was also used to probe blots of RNA and genomic DNA from adult *O. volvulus* collected at four widely spaced locales, two savanna regions in Africa (near Touboro, Cameroon and Bamako, Mali), a forest region in Africa (Kumba, Cameroon), and in Central America (near Guatemala City, Guatemala). *O. volvulus* causes blindness much more frequently in savanna regions than in the forest, an observation that has been attributed to different genetic forms of the parasite in the two habitats (10). The Northern blot (Fig. 3 *A*) indicates that a 1.2-kb mRNA for this antigen is present in parasites from all four places and suggests that the Ov33-3 cDNA lacks only ~200 nucleotides of the mature message. In a Southern blot (Fig. 3 *B*), the cDNA hybridizes to the same 14-kb Hind III fragment in all four genomes. This result, as well as those obtained with other restriction enzymes, indicates that there is little or no heterogeneity in this genomic region among *O. volvulus* from the four different locations.

To determine whether the Ov33-3 gene is also present in filarial parasites of other genera, we examined the extent of its crosshybridization with other DNAs. Fig. 4 (*left*) shows that in a Southern blot washed under low stringency conditions, the cDNA hybridizes to a single Hind III fragment of a different size in the DNAs of *O. volvulus*, *Brugia malayi* (a human lymphatic filarial parasite) and *Dirofilaria immitis* (the dog heartworm). However, with moderate stringency it does not hybridize to *B. malayi* DNA (Fig. 4, *middle*) while in high stringency it does not hybridize to either *B. malayi* or *D. immitis* DNA (*right*). Thus, the gene is different in the three parasites although the corresponding *D. immitis* sequence is more similar to the *O. volvulus* gene than is the *B. malayi* sequence. These nucleotide differences most likely cause amino acid changes in the corresponding protein that form the basis for the specificity of mAb 4A7 for Onchocerca. The similarities in the gene, on the other hand, are confined to parasitic nematodes since, even under very low stringency, the cDNA probe does not bind to DNA from *Caenorhabditis elegans*, a free living nematode (not shown).

This paper presents the first characterization of a gene in *O. volvulus*. The three

previous reports on the DNA of this important parasite described the identification of noncoding repetitive DNA segments that may be useful for epidemiologic and diagnostic studies (10–12). Here we describe a protein and its gene that may also contribute to these kinds of studies. Furthermore, it could have major immunological significance. Studies on murine schistosomiasis show that, in addition to surface components such as the M_r 28,000 antigen of *Schistosoma mansoni* (13), proteins like paramyosin and glutathione-*S*-transferase also induce partial protective immunity against challenge infections (14, 15). An assessment of the immunoprophylactic potential of the 33,000 M_r antigen, and all other *O. volvulus* antigens, will be difficult because of the high degree of host specificity of the parasite. However, further characterization of the Ov33-3 protein product should provide more information about its immunological significance and its potential as an immunodiagnostic reagent and vaccine component.

Summary

We have cloned and characterized the gene for an immunodominant antigen of *O. volvulus* that is recognized by the sera of 96% of patients with onchocerciasis. Its 1.2-kb mRNA constitutes 0.3% of adult worm poly(A)$^+$ RNA and its cDNA sequence reveals that it is not a highly conserved structural protein such as actin or tubulin. Similar but not identical genes occur in the genomes of related filarie, *Brugia malayi* and *Dirofilaria immitis*. The recombinant antigen has both immunodiagnostic and immunoprophylactic significance.

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