

## *Onchocerca volvulus* Glycolytic Enzyme Fructose-1,6-Bisphosphate Aldolase as a Target for a Protective Immune Response in Humans

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**To identify potential vaccine candidates for the prevention of infection with the filarial nematode *Onchocerca volvulus*, we screened an *O. volvulus* L3 stage cDNA library with sera from putatively immune (PI) subjects, and a prominent immunogenic clone of 1,184 nucleotides was identified. It contained an open reading frame of 363 amino acids encoding the glycolytic enzyme fructose 1,6 bisphosphate aldolase (*Ov-fba-1*). Immunolocalization experiments demonstrated that the protein was most abundantly expressed in metabolically active tissues, including body wall muscle and the reproductive tract of adult female worms. Immunoelectron microscopy of L3 demonstrated binding in the region where the cuticle separates during molting, in the channels connecting the esophagus to the cuticle, and in the basal lamina surrounding the esophagus and the body cavity. Among subjects from areas where this organism is endemic specific humoral and cellular immune responses to recombinant protein were observed in both PI and infected subjects, whereas responses were not observed among subjects who had not been exposed to *O. volvulus*. Despite the absence of differential responsiveness in parasite-exposed human populations, when the recombinant was tested for protective efficacy in a mouse chamber model, a reduction in survival of larvae by ca. 50% was seen. This observation provides support for the further study of this parasite enzyme as a vaccine candidate in larger animal models.**

Despite vector control and the widespread use of ivermectin in areas where *Onchocerca volvulus* is endemic, onchocerciasis continues to occur in significant parts of Africa and small pockets in Latin America. Recent epidemiologic studies indicate that, while chemotherapy with ivermectin results in relief of symptoms, community-based ivermectin distribution and vector control may not result in the eradication of infection (22).

In order to identify novel larval-stage antigens as potential vaccine candidates, an *O. volvulus* L3 stage cDNA library was screened with pooled sera from a well-defined human population residing in region of Ecuador where onchocerciasis is endemic (8). Prominent among the multiple reactive recombinants identified by immunologic screening were isolates encoding fructose 1,6 bisphosphate aldolase (*Ov-fba-1*). We therefore undertook detailed characterization of this filarial enzyme and tested it for protective efficacy in an animal model of onchocerciasis. In so doing, we demonstrated its potential as a target for the induction of protective immunity to onchocerciasis.

### MATERIALS AND METHODS

**Human sera.** Sera were obtained from a group of *O. volvulus*-infected and putatively immune (PI) subjects from a region of Ecuador where the organism is

endemic (8). Infection status was documented based on clinical history, nodule palpation, ophthalmologic evaluation for ocular microfilariae, and parasitologic evaluation through skin snip examination supplemented by PCR of the skin for an *O. volvulus*-specific repeat DNA sequence (28). Exposure to infection was documented in all subjects from the area based on long-standing residence in the region. The infection status of the subjects was documented with four clinical and parasitologic evaluations made over a period of 13 years. Sera from 10 PI subjects identified were used in a pool for initial library screening. These 10 sera were selected from the available 34 subjects on the basis of the longest duration of documented negative skin snips. Sera from 42 *O. volvulus*-infected adults were used for testing for differential recognition, while sera from 22 unexposed North American subjects who had never visited a region where filarial infections are endemic were used as negative controls.

**cDNA expression library screening.** The *O. volvulus* L3 library (SAW94WL-OvL3) (20), kindly provided by Steven A. Williams (Department of Biological Sciences, Clark Science Center, Smith College, Northampton, Mass.), was screened by using standard techniques. The immunoscreening was done with pooled human PI sera diluted to 1:500. Secondary incubation was with peroxidase-conjugated polyclonal affinity-purified Fc-specific goat anti-human immunoglobulin G antibody (Jackson ImmunoResearch) diluted 1:500,000. Positive plaques were detected by incubation of the filters with the ECL chemiluminescence detection reagent (Amersham).

**DNA sequence analysis.** Complete automated double-stranded sequence was obtained after *in vivo* excision and rescue into the pBluescript plasmid of positive  $\lambda$  phage plaques (Rapid Excision Kit; Strategene). Sequence chromatographs were verified manually; the derived sequence was then compared with the public protein and nucleotide database (GenBank) by using the BLASTn and BLASTx algorithms. The DNA sequence of *Ov-fba-1* was deposited in GenBank (accession no. AF155220). Multiple sequence alignments were performed by using the CLUSTAL IV algorithm. Amino acid sequence differences between the human aldolase and *Ov-FBA-1* were correlated with the three-dimensional structure of human aldolase A (PDB accession number 1ALD [9]) by using a molecular modeling package (Look; Molecular Applications Group).

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**Dot blot hybridization.** One microgram of genomic DNA was used for dot blot hybridization. A gene-specific DNA probe was generated by PCR incorporation of digoxigenin-conjugated dUTP (Roche Molecular Biochemicals) into the PCR product with gene-specific primers. After overnight hybridization with the DNA probe at a concentration of 5 ng/ml at 65°C, stringency washes were undertaken twice at 65°C for 15 min with 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate. Positive signal was detected by chemiluminescent assay according to the manufacturer's instructions (Roche Molecular Biochemicals).

**Northern blot analysis.** RNA was extracted from onchocercal nodules that had been surgically excised from *O. volvulus*-infected subjects in Ecuador and then stored in Triazol (Life Technologies) in liquid nitrogen until RNA extraction. RNA was simultaneously extracted from 10 ml of fresh human blood. RNA (5 µg) obtained from the nodules or from human blood was subjected to Northern blot analysis using the gene-specific DNA probe generated by PCR amplification (above) at a concentration of 100 ng/ml. Hybridization was conducted overnight at 45°C. Two stringency washes were carried out at 65°C with 0.5× SSC–0.1% sodium dodecyl sulfate, and detection was carried out by using the Genius detection system (Roche Molecular Biochemicals).

**Production of recombinant *Ov-FBA-1*.** The *Ov-fba-1* full-length sequence was subcloned by PCR into the prokaryotic expression plasmid pRSET (Invitrogen), generating the recombinant plasmid pOVFBA. The plasmid was transformed into the *Escherichia coli* expression host BL21(DE3)-pLysS (Novagen) and protein expression induced with IPTG (isopropyl-β-D-thiogalactopyranoside). Analysis of different cellular fractions revealed that the recombinant protein was predominantly present in an insoluble form within inclusion bodies (data not shown). Attempts to produce protein in soluble form by alteration of growth conditions were unsuccessful. Purification of the recombinant protein was therefore undertaken by nickel-agarose affinity chromatography (Qiagen) under denaturing conditions. In addition, a glutathione *S*-transferase (GST) fusion polypeptide was prepared, and soluble recombinant protein was purified on glutathione beads.

**Generation of polyclonal antisera.** Polyclonal antisera was produced in both mice and rabbits. Female CAF1 mice (five per group) were immunized by intraperitoneal injection with 7 µg of recombinant protein emulsified in 200 µl of Freund's complete adjuvant (Sigma) or with adjuvant alone. Animals were boosted at day 40 by with the same quantity of recombinant in incomplete Freund's adjuvant. Mice were bled at day 100. Rabbits were immunized with 70 µg of the GST fusion protein with Freund's complete adjuvant for the first immunization and then boosted three times with recombinant protein in Freund's incomplete adjuvant.

**Immunolocalization.** Immunohistochemistry of adult *O. volvulus* female worms was undertaken with murine antisera (26), while immunoelectron microscopy of *O. volvulus* L3 was undertaken with rabbit antisera (15). Control experiments were conducted with murine sera from adjuvant-immunized mice and with rabbit anti-GST and preimmune sera.

**Immunoblot analysis of human and murine antibody responses.** Immunoblot analysis was done by standard techniques. Individual strips were incubated with human or mouse sera diluted 1/500. Strips were incubated with goat anti-human or anti-mouse polyclonal antibody (Fc specific; Jackson Laboratories) diluted to 1/50,000 and developed by using the ECL chemiluminescent substrate (Amersham).

**Human cellular immune response to *Ov-FBA-1*.** Analysis of T-cell proliferation and cytokine production was conducted exactly as described previously (4) by using freshly isolated peripheral blood mononuclear cells (PBMC) from PI, *O. volvulus*-infected, and "endemic controls" (i.e., ethnically identical individuals living in a nearby area where no *O. volvulus* transmission has occurred). Responses to pokeweed mitogen, to *O. volvulus* adult, larval, and microfilarial antigen, and to *Ov-FBA-1* were tested in concentrations of 5 and 1 µg/ml. The recombinant antigen was added to the cell culture wells from a diluted stock of recombinant antigen solubilized in 8 M urea at a concentration of 840 µg/ml. In control experiments conducted simultaneously, an equal volume of the buffer was added to control wells, with no observed cytotoxicity or nonspecific stimulation (data not shown). T-cell proliferation levels were expressed as stimulation indices (SI).

**Vaccination studies.** Protective immunity was assessed in a murine model by challenge infection using diffusion chambers as previously described (2). Male BALB/cByJ mice, 6 to 8 weeks old, were immunized by subcutaneous injection into the nape of the neck with 25 µg of urea-solubilized antigen in 0.1 ml of phosphate-buffered saline emulsified with 0.1 ml of Freund complete adjuvant (Sigma). A booster immunization was given 14 days later with the same quantity of antigen in Freund incomplete adjuvant. A control group was immunized in an identical fashion with adjuvant alone. A single diffusion chamber containing 25

live *O. volvulus* L3 larvae was surgically inserted into a subcutaneous pocket created in each mouse. Diffusion chambers were recovered 21 days postchallenge, and their contents analyzed to assess larval survival. Larvae recovered from diffusion chambers were considered live if they exhibited motility. Differences in survival of L3 between groups was evaluated by using the multifactorial analysis of variance test.

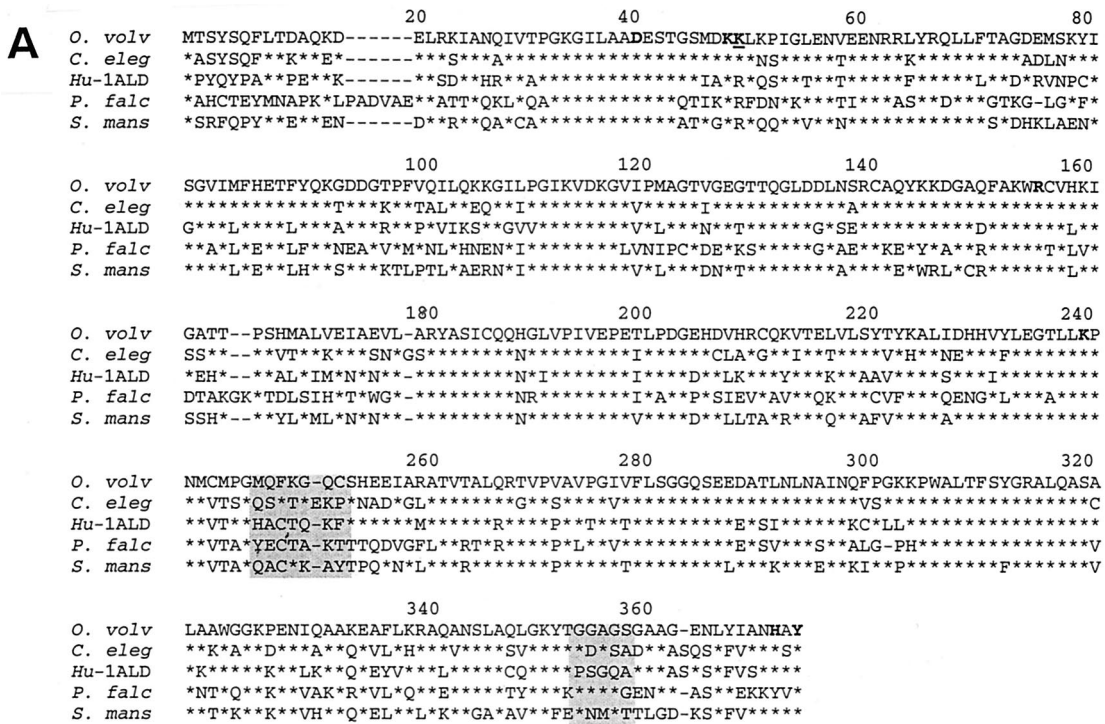
## RESULTS

**Cloning and sequence analysis of *Ov-FBA-1*.** When serum pools from the *O. volvulus*-infected and immune subjects were used to screen the *O. volvulus* L3 cDNA library, 65 positive recombinants were identified from  $9 \times 10^5$  phage. DNA sequence analysis indicated that 13 of the 65 were identical isolates encoding fructose 1,6 bisphosphate aldolase (*Ov-fba-1*). One isolate was subjected to complete double-stranded DNA sequence analysis. The full-length sequence (NCBI accession no. AF155220) was 1,184 nucleotides long and contained an open reading frame encoding a polypeptide of 363 amino acids (Fig. 1A). Sequence comparison of the deduced amino acid revealed a high level of identity with other eukaryotic aldolase sequences (70% human aldolase A [M11560], 63% *Drosophila* aldolase [pirADFF], 81% *Caenorhabditis elegans* [CE-1, P54216], 63% *Schistosoma mansoni* [P53442], and 54% *Plasmodium falciparum* [M28881]). Multiple sequence alignment (Fig. 1A) with other eukaryotic aldolase sequences revealed high homology, with absolute conservation of all but one residue associated with enzymatic activity (Asp<sup>33</sup>, Lys<sup>41</sup>, Lys<sup>146</sup>, Arg<sup>148</sup>, Lys<sup>229</sup>, His<sup>361</sup>, and the terminal Tyr). At residue 42, the sequence of *Ov-fba-1* and the *C. elegans* aldolase diverged from other eukaryotes in that a lysine residue replaced the highly conserved arginine residue found in other eukaryotes (Fig. 1A).

When the *Ov-FBA-1* sequence was compared with a three-dimensional representation of human aldolase derived by X-ray crystallography, two regions of significant divergence were identified on the external surface of the human molecule, at residues 238 to 244 and residues 345 to 350 (Fig. 1B). In contrast, other sites of divergence in amino acid sequence between human and *O. volvulus* aldolase were generally located within the internal structure of the enzyme (data not shown).

**Related sequences in other species.** To verify that the cloned gene was derived from *O. volvulus* and not from another source (such as the *Simulium* vector from which the parasite material for library construction had been harvested), as well as to investigate for similar sequences in other filarial parasites, an *Ov-fba-1* probe was used in a genomic dot blot (Fig. 2A). Hybridization was observed with genomic DNA from other filarial parasites (*Dirofilaria immitis*, *Wuchereria bancrofti*, and *Brugia malayi*) but not from the free-living nematode (*C. elegans*), the arthropod vector of onchocerciasis (*Simulium damnosum*), or humans.

**Northern blot analysis.** To verify the transcription of aldolase mRNA and to determine the size of the native transcript, Northern blot analysis was undertaken by using the full-length cDNA sequence to probe a blot containing total RNA from an onchocercal nodule and from uninfected human blood. A band was observed at 1.6 kb in the lane containing *O. volvulus* nodule RNA but not in the lane containing only human RNA (Fig. 2B).



**B**

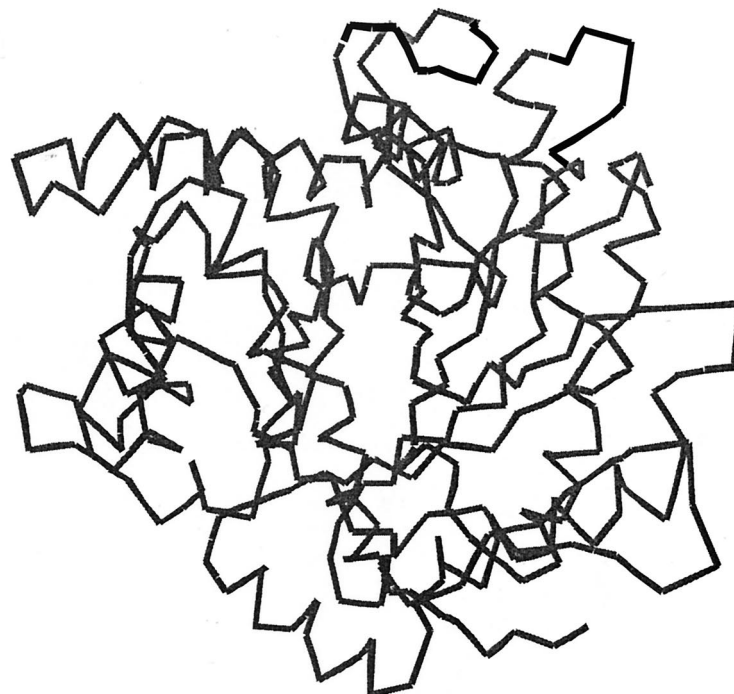


FIG. 1. (A) CLUSTAL IV multiple sequence alignment of deduced amino acid sequence of aldolase from *O. volvulus*, human aldolase A (pdb1ALD), *Drosophila* (pirADFF), *C. elegans* (spP54216), *S. mansoni* (spP53442), and *P. falciparum* (gbM28881). The amino acid residues associated with enzymatic activity (Asp<sup>33</sup>, Lys<sup>41</sup>, Lys<sup>42</sup>, Lys<sup>146</sup>, Arg<sup>148</sup>, Lys<sup>229</sup>, His<sup>361</sup>, and the terminal Tyr) are highlighted. The sequence divergence at residue 42 from other eukaryotes (where Lys residue replaced the highly conserved Arg) is underlined. Amino acid residues where significant sequence diversity exists between human and *O. volvulus* and where the structural study indicates that these residues are located on the external surface of the protein are shaded. (B) Projection of three-dimensional structure of human aldolase. The amino acid residues at positions 238 to 244 and positions 345 to 350, where there is significant sequence divergence between the *O. volvulus* and human sequences, are highlighted.

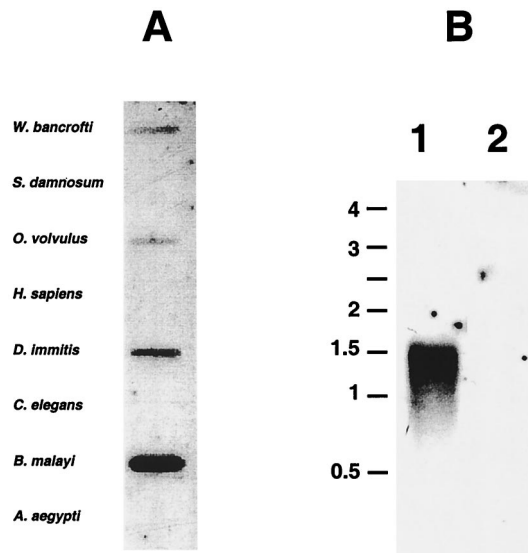


FIG. 2. (A) Genomic DNA from filarial parasites, the free-living nematode *C. elegans*, arthropod vectors of filariasis, and humans was subjected to dot blot analysis with a labeled *O. volvulus* *Ov-fba-1* DNA probe. (B) Northern blot analysis of the total RNA from *O. volvulus* (lane 1) and human leukocytes (lane 2) with a labeled *Ov-fba-1* DNA probe.

**Localization of *Ov-FBA-1*.** Recombinant protein was over-expressed in an *E. coli* expression system, purified in denatured form by nickel-agarose affinity chromatography. Polyclonal antisera was raised in mice and used in Western blot analysis of protein extract from adult worms. A band of ca. 41 kDa was observed by using pooled sera from animals immunized with recombinant aldolase (Fig. 3A, lane 1), but not pooled sera from mice immunized with adjuvant alone (Fig. 3A, lane 3). A band of identical molecular mass was observed in an immunoblot of *O. volvulus* L3 antigen with the aldolase-specific anti-

sera (data not shown). Additional identical bands of lower molecular mass were observed in the blot with the aldolase-specific and control antisera. The identity of these bands was not determined.

Immunohistochemistry of sections of adult female worms with pooled murine hyperimmune antisera demonstrated specific staining in the musculature of the body wall, the ovarian-uterine wall, the esophageal wall and, in the morula-stage, developing microfilariae (Fig. 4). The sites corresponded to metabolically active tissues and rapidly dividing cells. Immunoelectron microscopy with antisera raised against soluble GST-*Ov-Fba-1* demonstrated binding in L3 in the region where the cuticle separates during molting from L3 to L4 (Fig. 5A), in the channels connecting the esophagus to the cuticles (21) (Fig. 5B), and in the basal lamina surrounding the esophagus and the body cavity (Fig. 5C). Immunoelectron microscopy results with control, preimmune, and anti-GST sera were negative (data not shown).

**Humoral and cellular immune responses to *Ov-FBA-1* in human populations.** The presence of a specific antibody response to *O. volvulus* aldolase among individuals exposed to *O. volvulus* infection was tested by immunoblotting. A band at the expected molecular mass of ~42 kDa was observed in 37 of 42 (88%) *O. volvulus*-infected subjects, while 23 of 34 (68%) PI subjects recognized the recombinant (see Fig. 3B, where immunoblots of four representative subjects from each group are shown). Additional specific bands of higher molecular masses were observed among *O. volvulus*-exposed subjects. None of the sera from the 22 "nonendemic" subjects recognized the recombinant aldolase upon Western blotting (Fig. 3B).

Because T-cell responses to *O. volvulus*-specific antigens have been shown to be associated with protection in human populations (7, 8), the cellular immune response to *Ov-FBA-1* was assessed by using freshly isolated PBMC from individuals whose immune status had been previously characterized (7, 8). In none of the 19 "endemic" (unexposed) control subjects (i.e., subjects from endemic areas) did PBMC culture with *Ov-FBA-1* result in significant T-cell proliferation ( $SI > 2$ ), while 2 of 15 *O. volvulus*-infected and 1 of 19 PI subjects demonstrated T-cell responsiveness to the recombinant antigen. Assay of gamma interferon (IFN- $\gamma$ ) production in cell culture supernatants resulted in similar findings, with measurable cytokine levels from 1 of 20 "endemic" control subjects, 4 of 17 PI subjects, and 4 of 12 *O. volvulus*-infected subjects. Five of eight *O. volvulus*-infected subjects produced interleukin-5 (IL-5) in response to *Ov-FBA-1*. Although there was no clear distinction between the cellular immune responses between the PI and infected subjects, it is clear that the *Ov-FBA-1* contains at least one (and perhaps more) T-cell epitope in that it was able to induce both IL-5 and IFN- $\gamma$  production, as well as a proliferative response in a few individuals.

**Protection experiments.** Recombinant *Ov-FBA-1* was tested for its ability to mediate protection against challenge infection in a murine model. Immunized mice were challenged with *O. volvulus* L3 larvae enclosed in a diffusion chamber. A reduction in survival of larvae by ca. 50% was seen in two sets of studies (Fig. 6; experiment 1,  $18 \pm 18$  versus  $39 \pm 22$  [ $P = 0.039$ ]; experiment 2,  $22 \pm 19$  versus  $45 \pm 23$  [ $P = 0.034$ ]; *Ov-FBA-1* versus control, mean  $\pm$  the standard deviation).

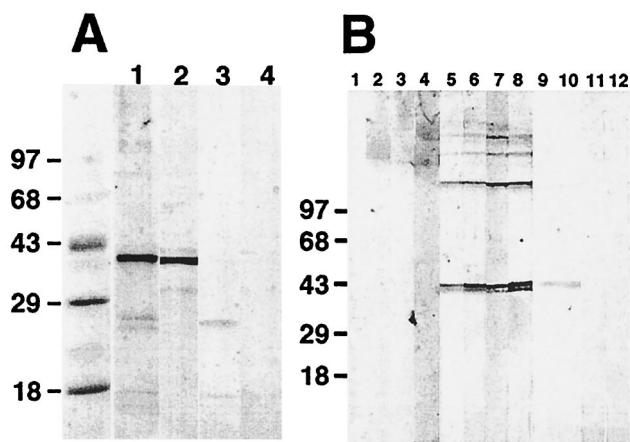


FIG. 3. (A) Immunoblot analysis using polyclonal murine antisera to purified recombinant (lanes 1 and 2) *Ov-FBA-1* or control antisera from adjuvant-immunized mice (lanes 3 and 4). Lanes 1 and 3, *O. volvulus* adult antigen; lanes 2 and 4, purified recombinant aldolase. (B) Immunoblot analysis of antibody response to *Ov-FBA-1* among human subjects. Lanes 1 to 4, control unexposed subjects; lanes 5 to 8, *O. volvulus*-infected subjects; lanes 9 to 12, PI subjects.

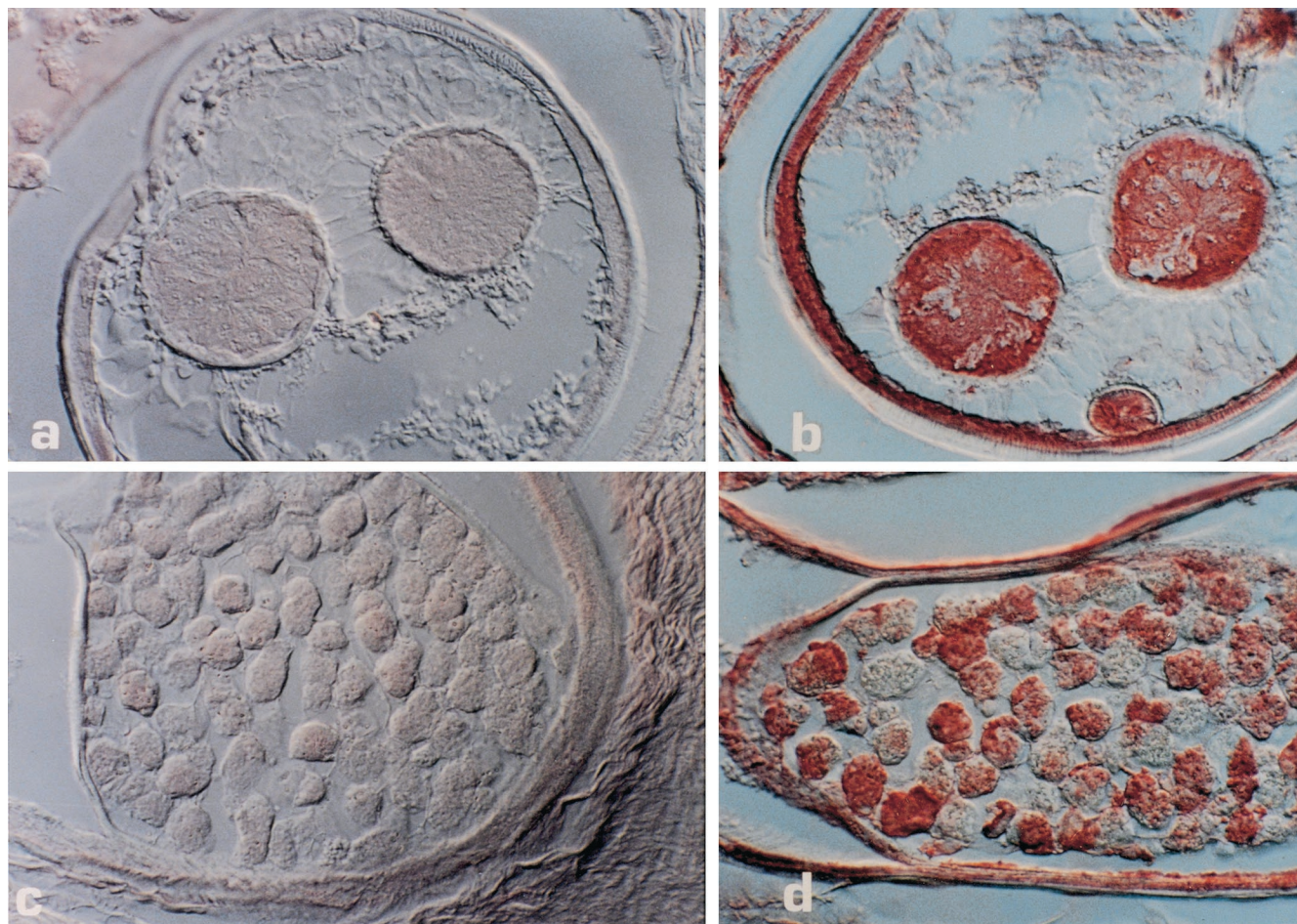


FIG. 4. (A) Immunohistochemical localization of *Ov*-FBA-1. Paraffin sections of an adult female *O. volvulus* were incubated with antibodies prepared against *Ov*-FBA-1 and binding detected with horseradish peroxidase-conjugated secondary antibodies. (a and b) Transverse section showing the presence of early morula-stage microfilariae inside the uterus of the female worm. (c and d) Similar transverse sections showing late morula-stage microfilariae. Positive staining was localized to the musculature of the body wall, the ovarian/uterine wall, the esophageal wall and in the late-morula-stage, developing microfilariae. Panels a and c are adjacent serial sections of panels b and d and represent controls with sera from adjuvant-immunized animals.

## DISCUSSION

Using a strategy to identify immunogenic recombinant molecules expressed by the larval stages of *O. volvulus* and to characterize the immune response to these antigens, we identified a potentially important vaccine candidate for the prevention of *O. volvulus* infection. This antigen, *Ov*-FBA-1, not only has recognition sites for human T and B cell responses but also can mediate protection in the best available small-animal model for *O. volvulus* infection. Moreover, by targeting this molecule, one that is critical for the glycolytic pathway in the parasite, a vaccine approach to target other molecules in related pathways (now identified through the Filarial/Onchocerca Genome Project) may provide a generic means to generate additional components for a multivalent vaccine. Indeed, such multivalent vaccines are envisaged for many parasitic infections, the most notable being malaria (24).

The induction of an immune response by vaccination with an enzyme that is so closely related to the human enzyme raises the theoretical concern that such a response may result in autoimmune disease. Autoantibodies to human aldolase have

been described in two settings. Brown et al. (3) reported that an antibody response to rabbit muscle aldolase could be detected in >50% of sera of patients with a mixture of acute and chronic viral hepatitis. In a study of autoantibodies to proteins present in an osteoclast cell line in patients with a variety of arthritic diseases (rheumatoid arthritis, systemic lupus erythematosus, osteoarthritis, and gout) (27), an antibody response to purified rabbit aldolase A was observed in 6 of 62 patients with rheumatoid arthritis. Of note, seroresponsiveness was seen at a dilution of 1/11, and the antibody response was greater to denatured rabbit aldolase than to the enzyme in native form. This is of interest, given the fact that the recombinant *Ov*-FBA-1 used in this study had been purified under denaturing conditions. We have been able to easily raise monoclonal antibodies that bind to the parasite aldolase but fail to recognize the human or rabbit homolog (data not shown). This finding suggests that the immunogenic component of the filarial aldolase is quite distinct from its mammalian counterparts. Other filarial orthologs of mammalian proteins with very similar protein sequences have shown promise as vaccine candidates.

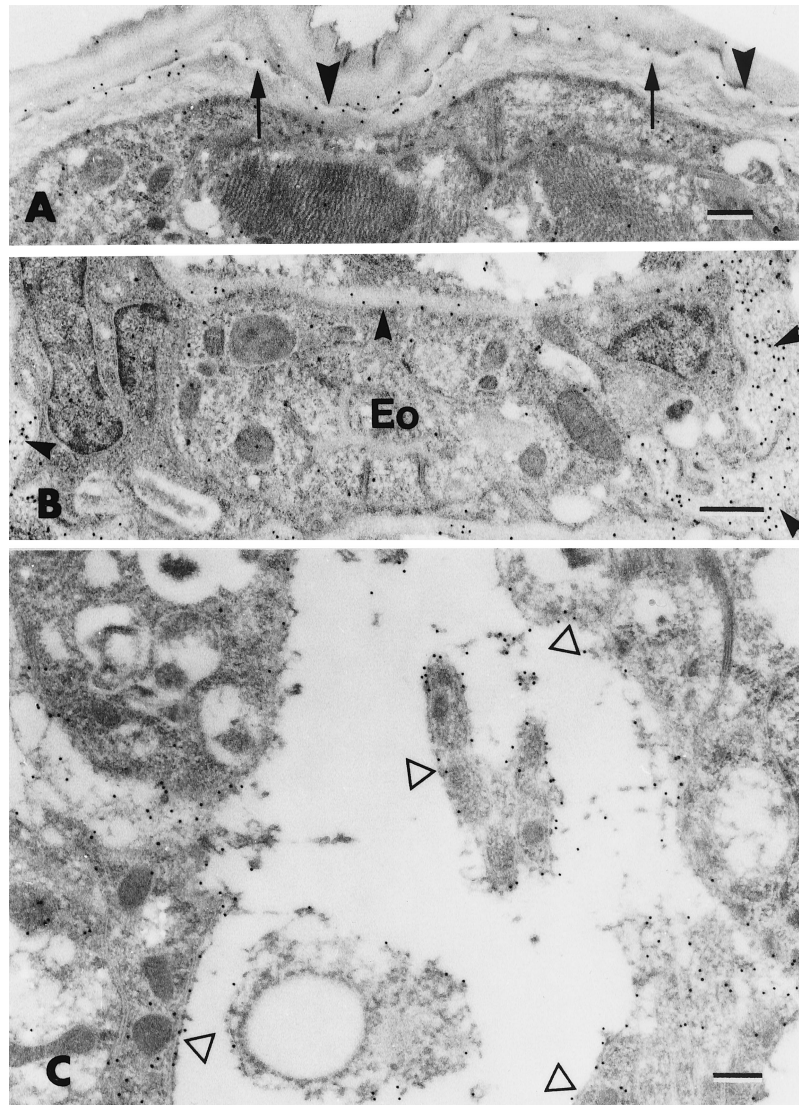


FIG. 5. Ultrastructural localization by immunoelectron microscopy of the parasite protein recognized by rabbit antibodies against recombinant *Ov*-FBA-1. Thin sections of *O. volvulus* larvae during molting of L3 to L4 in vitro were incubated first with antibodies raised against recombinant aldolase and then with protein A coupled to 15-nm gold particles for indirect antigen localization (bar = 0.5  $\mu$ m). Note the regions where the cuticles of L3 (arrowheads) and L4 (arrows) separate (A), the channels (small arrowheads) connecting the esophagus (Eo) to the cuticle (B), and the basal lamina (open arrowheads) surrounding the esophagus and the body cavity (C).

These include paramyosin (18), tropomyosin (16), and calponin (15).

Further, the molecular modeling undertaken here has led to the identification of potentially important immunogenic portions of the parasite molecule with significant sequence divergence from the human enzyme. Confirmation of the hypothesis that these regions of the parasite protein are the major epitopes for the immune response will require further work in mapping T- and B-cell epitopes of the parasite protein. It will also be important to investigate for autoimmunity when the vaccine candidate is tested in other animal models, including the bovine *Onchocerca ochengi* (12) and primate models (11).

Fructose 1,6 biphosphate aldolase (EC 4.1.2.13) is a glycolytic enzyme that catalyzes the cleavage of fructose 1,6 biphosphate into two triose sugars, glyceraldehyde 3-phosphate and

dihydroacetone phosphate. As would be predicted from the phylogenic data, the present study confirms that the enzyme in *O. volvulus* is a member of the class I aldolase family (14). In its native form, class I aldolase exists as a tetramer of  $\sim$ 40-kDa subunits. While the identity of the multiple bands at higher molecular weights observed on immunoblots with reactive human sera was not determined here, these bands may represent reactivity to polymerized enzyme. The catalytic cycle of aldolase is well studied, and the amino acid residues critical to substrate binding have been defined. Of interest, an arginine residue at position 42 in all other eukaryotes has been localized adjacent to the phosphate group on the C<sub>6</sub> of the fructose and therefore is considered to be important in the catalytic cycle (10). In *O. volvulus*, this residue has been replaced by a lysine residue. Study of available expressed sequence tag (EST) data

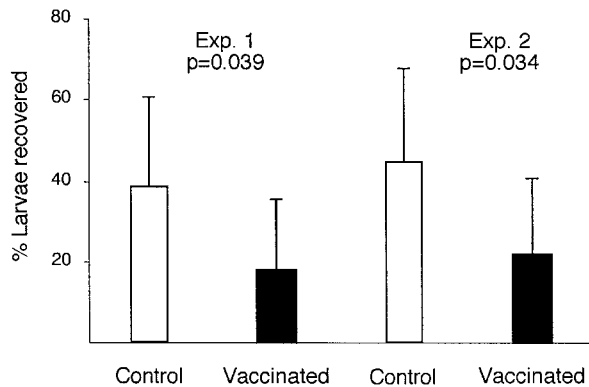


FIG. 6. *Ov*-FBA-1 vaccination studies. Male mice (3 to 6 weeks old; six animals per group) were vaccinated with *Ov*-FBA-1 or with adjuvant alone and implanted with diffusion chambers containing 25 *O. volvulus* L3. Chambers were recovered 21 days later, and larval survival was assessed.

indicates that this transition is also present in other filarial parasites (*B. malayi* and *W. bancrofti*) and in *C. elegans* but not in *Strongyloides stercoralis* (data not shown). The implication of this sequence divergence on enzymatic activity was not explored here, but we were able to demonstrate that the recombinant enzyme shows enzymatic activity (data not shown).

It is of interest to note that fructose biphosphate aldolase was also cloned by immunoscreening with hyperimmune sera from *S. mansoni*, another helminth parasite of major medical importance (6). Also, as we have observed, *S. mansoni* aldolase is abundantly expressed (13, 23), induces a strong antibody and cell-mediated immune response (13), and shows promise as a vaccine candidate (5). The great phylogenetic distance of these two parasites suggests that the enzyme may perform some common function important for tissue-dwelling helminths and, therefore, be subject to concerted evolutionary pressure.

A possible explanation for the lack of segregation in immune response among PI and infected subjects is the role of concomitant immunity in protection, whereby older individuals, although infected, may have developed a protective response to new infection. In this light, the significant protection observed in our rodent model of filariasis where immunity is directed to the L3 larvae is of particular interest.

The observation that the greatest concentration of the enzyme, as determined by light microscopy, is found in the highly metabolically active tissues of the adult worm, such as the musculature of the uterus and the body wall, as well as in rapidly dividing cells of the morula-stage microfilariae is not unexpected given the role of this enzyme in glycolysis. In contrast, an unexpected finding was the localization of the protein by immunoelectron microscopy in sections of the larval stages of the parasite where glycolysis may not be expected to be so active but where essential events occur during the transformation from L3 to L4, namely, the cuticles, channels connecting the esophagus to the cuticle, and the body cavity (Fig. 5). Of interest, these experiments demonstrated binding of aldolase-specific antibodies in the same regions to those identified by the antibodies from the mice immunized with irradiated L3 and partially protected against L3 challenge in diffusion chambers (2). Given that the larvae undergo an L3-L4 molt while in

the chamber (1), this observation may provide some clue to the mechanism of protection. The 5- $\mu$ m pore size of the membranes allows all components of the immune response to enter including cells. However, it is not possible to ascertain from these experiments whether the immune response is protective through antibody or cell-mediated mechanisms or both. While a large standard deviation was observed in the vaccination experiments, the results obtained in this study are comparable to those obtained with other antigens (2).

Circumstantial evidence supporting the hypothesis that aldolase may assume some particular importance for parasite development in the host is provided by analysis of the EST data set. A total of 74 EST entries in the *B. malayi* and *O. volvulus* filarial genome projects datasets appear to represent aldolase transcripts (30 of 22,392 for *B. malayi* versus 44 of 3,694 for *O. volvulus*) (19; data not shown). While enumerating the total number of isolates in each EST data set may not accurately reflect the relative transcriptional activity, it appears that transcription of *O. volvulus* aldolase may be upregulated in L3 (27 of 3265 EST), while it was represented in only 7 of 3,694 molting L3 EST. Small numbers of EST were also present in the microfilariae, L2, and female adult datasets (6, 2, and 2, respectively). However, it will be necessary to further investigate possible stage-specific transcriptional activation by formal quantitation.

It is possible that the protection observed in the rodent model may not predict the outcome of vaccination in human populations. This underlines the importance of undertaking protection studies in larger animals, including nonhuman primates, where immune mechanisms can be dissected by using human reagents. This is of particular importance in light of our growing ability to modulate the quality of the immune response with different cytokines, adjuvants, routes of administrations, immunostimulatory sequences, etc. These studies will also provide further data to answer the important question of whether vaccination with a protein with significant amino acid sequence similarity to the mammalian enzyme results in autoimmune disease.

Our hypothesis that parasite antigens identified by screening of stage-specific cDNA libraries by using sera from putatively immune subjects would lead to the identification of antigens preferentially recognized by immune subjects was not borne out here. However, this highlights the potential importance of concomitant immunity, with antigens recognized by both PI and infected subjects as potential vaccine candidates. Selection of vaccine targets based on our knowledge of likely targets for protection is an appealing notion and has led to the selection of promising vaccine candidates for onchocerciasis (2, 25) and other helminth parasites (17). It is clear, however, that other vaccine candidates with less appeal as "rational vaccine targets" for onchocerciasis (2) have shown promise in small animal models and are being tested in cattle naturally exposed to bovine onchocerca.

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