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Cloning and Characterization of a Potentially Protective Chitinase-Like Recombinant Antigen from *Wuchereria bancrofti*

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While there is no direct evidence demonstrating the existence of protective immunity to *Wuchereria bancrofti* infection in humans, the presence of individuals, in populations in areas where infection is endemic, with no clinical evidence of past or current infection despite appreciable exposure to the infective larvae, suggests that protective immunity to filarial parasites may occur naturally. Earlier work indicated that such putatively immune individuals generated antibodies to a 43-kDa antigen from larval extracts of the related filarial parasite *Brugia malayi* that was recognized by only 8% of the infected population. With rabbit antiserum raised against this 43-kDa antigen, this current study identified a recombinant clone, WbN43, with an insert size of 2.3 kb, from a *W. bancrofti* genomic expression library. The recombinant fusion protein was differentially recognized by the putatively immune individuals but not by the infected patients. The coding sequence (684 bp) from the 5′ end had significant sequence similarity to chitinases from *Serratia marcescens*, *Bacillus circulans*, *Streptomyces plicatus*, and *B. malayi*. Peptide sequencing of the expressed product also defined a chitinase-like sequence. Molecular characterization indicated WbN43 to be a low-copy-number gene, with expression predominantly in infective larvae and microfilariae but not in adult parasites.

The presence, in areas where lymphatic filariasis is endemic, of a variable proportion of the adult population without any clinical manifestations of infection despite life-long exposure to the infective third-stage (L3) larvae, has long been an observation suggestive of the existence of protective immunity. Since it is impossible to determine resistance to reinfection directly, the study of such immunity in human populations has been particularly difficult because of the inability to distinguish between truly immune individuals and those considered concomitantly immune (infected but resistant to reinfection). Taking a more indirect approach, we have previously reported a group of individuals living in an area where *W. bancrofti* infection is endemic (Mauke, Cook Islands) whom we defined as putatively immune (PI) on the basis of rigorous clinical, parasitological, and serological criteria (13). These individuals were infection free by all available clinical and laboratory standards but demonstrated significantly greater humoral and cellular responsiveness to parasite antigens than did their compatriots with active filarial infection (30, 32, 33, 36, 49). When immunoblot analysis was used to compare the qualitative antigen recognition patterns between these two groups of individuals, no distinct differences were seen with either the adult or microfilarial antigen extracts of *Brugia malayi*, a closely related filarial species. However, when the responses to L3 antigens were assessed, 100% of the PI individuals and only 8% of the infected, microfilaremic subjects recognized a 43-kDa larval-stage antigen (13). It was thus of great interest to characterize this 43-kDa L3 antigen because of its potential as a protective immunogen.

We report here the cloning and characterization of a *W. bancrofti* recombinant (WbN43), obtained by screening a *W. bancrofti* genomic expression library (38), using rabbit antibodies raised against the isolated *B. malayi* 43-kDa antigen. Sequence analysis of WbN43 has shown the recombinant antigen to have significant similarity to chitinase. Since mammalian hosts of filarial parasites do not contain chitin, this molecule, as well as the enzymes involved in chitin metabolism, are potential candidates for vaccine development.

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MATERIALS AND METHODS

Parasites. Microfilariae of *W. bancrofti* were obtained from blood of patients with high levels of microfilaremia living in Madras, India, and purified as described previously (38). Adult parasites and microfilariae of *B. malayi* were obtained from the peritoneal cavities of jirds. Infective larvae (L3) of *B. malayi* were obtained from The University of Georgia (J. McCall, Athens, Ga.).

Patients. As previously described (13), 7 adults (1 man and 6 women) living in Mauke, Cook islands, an area where subperiodic bancroftian filariasis is hyperendemic, were defined as PI (variously referred to as infection free or endemic normal); 12 others (5 men and 7 women) were identified as clearly infected, microfilaremic. All PI individuals were amicrofilaremic on eight membrane filtration evaluations of their blood, had no historical or physical indication of filarial infection, had none of the reactions to administration with diethylcarbamazine that are characteristic of cryptic infection, and had no circulating antigen detectable in their sera (13).

Isolation of antigen. Since *W. bancrofti* infective larvae are not readily available, L3 antigen extracts were prepared from the closely related filarial parasite *B. malayi*. L3 antigens from *B. malayi* used for two-dimensional electrophoresis were solubilized by using the cationic detergent cetyltrimethylammonium bromide (CTAB) at 1% concentration and pH 7.2. CTAB has been found to consistently solubilize more proteins than other solubilization agents do (12). L3 antigens used in

sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) were solubilized using 1% Nonidet P-40 and 0.5% SDS.

Two-dimensional gel electrophoresis. To purify the 43-kDa larval-stage antigen selectively recognized by infection-free, PI individuals, the L3 antigen extracts were separated by two-dimensional gel electrophoresis (28). Briefly, isoelectric focusing gels were made in glass tubing (140 by 1 mm). After polymerization, the CTAB-solubilized antigen extracts were loaded and the gels were run at 400 V for 12 h. The gels were removed and equilibrated in 5 ml of SDS sample buffer (10% [wt/vol] glycerol, 5% [vol/vol] β-mercaptoethanol, 2.3% [wt/vol] SDS, 0.0625 M Tris-HCl [pH 6.8]) for 30 min at room temperature. SDS–10% polyacrylamide gels (25) were cast for the second dimension, and the equilibrated first-dimension gel was placed carefully in the slot and sealed on the top with a 1% agarose solution to keep the isoelectric focusing gel in place. The SDS-polyacrylamide gels were electrophoresed at a constant current of 20 mA per gel until the dye front reached the bottom of the gel. The gels were either Coomassie blue stained or immunoblotted for further analyses.

Immunoblots. The SDS-polyacrylamide gels were transferred to nitrocellulose paper (46) and blocked with a 3% solution of skim milk powder in wash buffer (0.25 M Tris [pH 8.0], 0.2 M NaCl, 0.3% Tween 20, 0.05% Triton X-100). The primary human or rabbit antibody of interest was added, followed by goat anti-human or anti-rabbit immunoglobulin G conjugated to alkaline phosphatase (Jackson Immunoresearch Laboratories, Inc., West Grove, Pa.). The nitrocellulose filters were washed four times at 10-min intervals between each incubation. After the final wash, they were developed with 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) as the substrate and nitroblue tetrazolium as the chromogenic indicator (Kirkegaard & Perry, Gaithersburg, Md.). The reactions were stopped by washing the filters extensively with distilled water.

Immunization of rabbits. Spots corresponding to the 43-kDa antigen were isolated from 10 Coomassie blue-stained two-dimensional gels. The gel slices were frozen, emulsified with saline and RIBI adjuvant (RIBI Co., Hamilton, Mont.), and administered subcutaneously to a rabbit in four equal doses at weeks 0, 2, 4, and 6. The antiserum obtained at week 7 was used as the hyperimmune rabbit serum.

The rabbit anti-43-kDa antigen antibodies were further purified by adsorption to the 43-kDa antigen. Briefly, *B. malayi* L3 antigen extracts were separated by SDS-PAGE and immunoblotted. The blots were blocked with 3% skim milk powder and then incubated with the immune rabbit serum. The serum was affinity eluted from immunoblots corresponding to the regions of 40 to 45 kDa, using 0.1 M glycine–0.1 M sodium chloride (pH 2.6), and immediately neutralized with 2 M Tris (pH 8.0) (2). The affinity-eluted serum was fractionated with 50% ammonium sulfate, dialyzed against phosphate-buffered saline, and stored at −70°C until use.

Affinity purification of human and rabbit sera by using the recombinant antigen. Wild-type λgt11 and recombinant WbN43 phage were induced, and the proteins were blotted onto nitrocellulose filters. Serum from normal, PI, and infected individuals were adsorbed first onto the filters containing the wild-type λgt11 antigens to remove the nonspecific reactivity to λgt11 and then onto filters containing the WbN43 fusion protein. The human and rabbit sera showing affinity for WbN43 was eluted from the nitrocellulose filter as described previously (2).

Genomic library construction and immunoscreening. A genomic library of *W. bancrofti* was constructed in the phage expression vector λgt11 as described earlier (38). The library was screened with the rabbit anti-43 kDa antigen antibodies at a dilution of 1:100, and the positive recombinants were selected on the basis of an immunoenzyme visualization system using anti-rabbit immunoglobulin G conjugated to alkaline phosphatase (22). One recombinant, designated λWbN43, was plaque purified on the basis of its strong reactivity to the rabbit anti-43 kDa antigen antibodies.

Southern analysis. For Southern analysis, 10 μg of *B. malayi* DNA was digested with each of the restriction endonucleases *Bam*HI, *Eco*RI, *Hin*dIII, *Nco*I, and *Xba*I and probed with nick-translated DNA with a specific activity of $>10^8$ dpm/μg of DNA. Hybridization was carried out in the presence of 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate–0.5% SDS–5× Denhardt's solution–0.01 M EDTA–100 μg of denatured salmon sperm DNA per ml at 65°C for 12 to 16 h, followed by washes under stringent conditions (2× SSC–0.5% SDS for 5 min at room temperature, 2× SSC–0.1% SDS for 15 min, and 0.1× SSC–0.5% SDS for 2 h at 65°C) (26).

Slot blot analyses. The species specificity of the fragment encoding the chitinase-like region was carried out by slot blot hybridization (24). Five hundred nanograms each of the various filarial and nonfilarial DNAs (*B. malayi*, *B. pahangi*, *Dirofilaria immitis*, *Acanthocheilonema viteae*, *Litomosoides carinii*, *Onchocerca volvulus*, *Caenorhabditis elegans*, and human DNAs) were dotted onto nitrocellulose membranes and probed with the nick-translated *Eco*RI-*Nco*I (715-bp) fragment from WbN43 under conditions described above.

Subcloning and sequencing. For further characterization, the 2.3-kb *Eco*RI insert of λWbN43 was subcloned into the *Eco*RI site of pUC19 (53). The pUC19 subclone pWbN43 was mapped by using various restriction endonucleases, and three restriction deletion subclones were constructed. pWbN43 and the deletion subclones were sequenced in both directions by the dideoxy-chain termination method (41). The sequence was analyzed using the University of Wisconsin Genetics Computer Group package, version 7.0 (10).

Immunostaining. Live *B. malayi* L3 larvae and adult parasites were washed, fixed, embedded in paraffin, and sectioned into 5-μm sections. The sections were deparaffinized and immunostained with the primary rabbit antibodies as described previously (37).

Nucleotide sequence accession number. Nucleotide sequence data reported in this paper have been submitted to the GenBank data base under accession number L18869.

RESULTS

Isolation of the 43-kDa antigen and generation of rabbit antisera. To isolate the 43-kDa antigen, approximately 50 μg of *B. malayi* L3 antigen extract was subjected to two-dimensional electrophoresis and immunoblotted. The immunoblots were probed with serum from either a PI individual (Fig. 1A) or an infected individual (Fig. 1B). A 43-kDa antigen (Fig. 1A, arrow) with an isoelectric point (pI) of 4.6 was recognized differentially by the PI serum. From 10 similar two-dimensional gels which were Coomassie blue stained, the 43-kDa antigen spot was cut out and used for immunizing rabbits. Postimmune and preimmune rabbit sera were again tested on two-dimensional immunoblots containing L3 antigen extracts to demonstrate that the immune rabbit serum specifically recognized the 43-kDa antigen (data not shown).

Screening the *W. bancrofti* genomic expression library with anti-43-kDa antigen antibodies. Approximately 10^4 phage from the *W. bancrofti* genomic expression library in λgt11 were screened by using the rabbit anti-43-kDa antigen antibodies, and 15 positive phage were obtained. After four rounds of

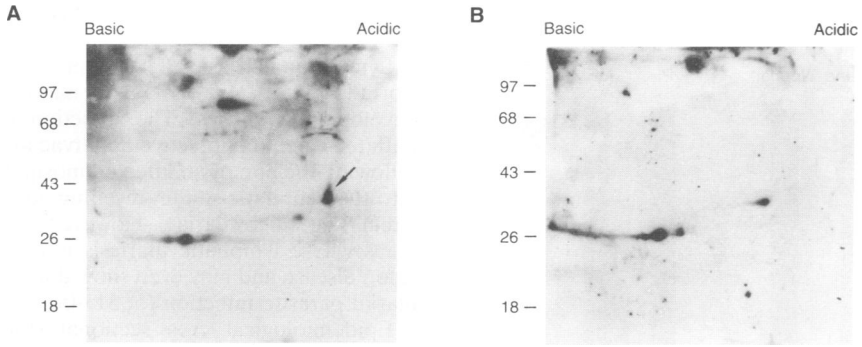

FIG. 1. Identification of the 43-kDa antigen. Fifty micrograms of the CTAB-solubilized *B. malayi* L3 antigen extract was separated by two-dimensional electrophoresis and immunoblotted. The blots were probed with sera from either PI individuals (A) or microfilaremic patients (B). The arrow indicates the position of the 43-kDa antigen. Positions of molecular mass standards (in kilodaltons) are indicated at the left of each panel.

screening and plaque purification, only 2 of 15 remained positive; one recombinant clone, designated λWbN43, was chosen for further characterization because of its stronger reactivity to the rabbit anti-43-kDa antigen antibodies. The other positive clone also had an identical insert size (2.3 kb) and hybridized to λWbN43.

Immunological characterization of the recombinant protein. The β-galactosidase fusion protein expressed by λWbN43 was analyzed on immunoblots by using human sera (PI and infected), rabbit antisera (pre- and postimmunization), or anti β-galactosidase antibodies. As shown in Fig. 2, anti-β-galactosidase antibodies (lane 1) recognized a fusion protein of approximately 140 kDa, indicating the size of the WbN43 expression product to be ~26 kDa. The same fusion protein was recognized only by PI patient sera (lane 2) or immune rabbit serum (lane 5), not by infected patient (nonimmune) sera (lane 3), normal human sera (lane 4), or preimmune rabbit serum (lane 6). The reactivity of this recombinant product was tested against each of the individual immune sera ($n = 5$) and infected patient sera ($n = 5$). The 140-kDa product was recognized by each of the immune sera and not by any of the infected patient sera (data not shown).

To identify the native parasite antigen recognized by antisera from the expressed λWbN43 recombinant protein, rabbit and human sera affinity purified on the recombinant protein were used to probe immunoblots containing *B. malayi* L3 antigen extracts. Figure 3 (lane 1) shows the Coomassie blue-stained gel containing the L3 antigen extract. Immune rabbit antiserum, affinity purified on the recombinant λWbN43 antigen (lane 2), but not the similarly affinity-purified preimmune serum (lane 3) recognized a 43-kDa protein band in the L3 immunoblots. Affinity-purified serum from a PI individual (lane 4) but not serum from an infected patient (lane 5) or normal human serum (lane 6) recognized the 43-kDa antigen in the immunoblots.

Subcloning and sequencing. The 2.3-kb insert of λWbN43 was subcloned into the *Eco*RI site of pUC19 and sequenced. The nucleotide sequence of the insert and the deduced amino acid sequence of the 5′ open reading frame (ORF) (684 bp, in frame with β-galactosidase) are shown in Fig. 4A. The WbN43 sequence showed significant sequence similarity to the chitinase sequences of *Serratia marcescens*, *Bacillus circulans*, *Streptomyces plicatus*, and *B. malayi*. The deduced amino acid sequence showed 46% identity (62% similarity) to *S. marcescens* chitinase A precursor, 23% identity (46% similarity) to *S. marcescens* chitinase B precursor, 24% (43% similarity) to *Bacillus circulans* chitinase A, 23% identity (46% similarity) to *Streptomyces plicatus* chitinase, and 23% identity (47% similarity) to *B. malayi* endochitinase precursor (MF1). The relationship among these chitinases is shown in Fig. 4B. While the WbN43 sequence contained other ORFs, none of these showed any similarity to any of the sequences in the GenBank (Los Alamos, N.Mex., and Mountain View, Calif.), EMBL Data Library (Heidelberg, Germany), and PIR (Washington,

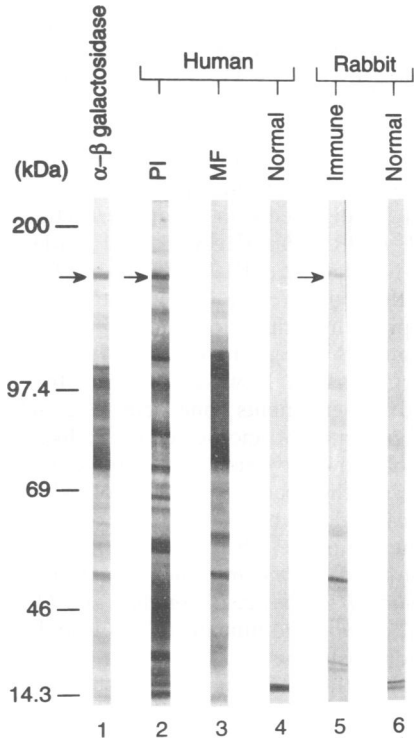

FIG. 2. Specificity of recognition of the recombinant λWbN43 fusion protein. The crude λWbN43 antigen preparation (~25 μg per lane) was reacted with anti-β-galactosidase antibody (lane 1), serum from a PI individual (lane 2) or a microfilaremic (MF) patient (lane 3), normal human serum (lane 4), immune rabbit serum (lane 5), or preimmune rabbit serum (lane 6). Arrows indicate positions of the recombinant fusion protein.

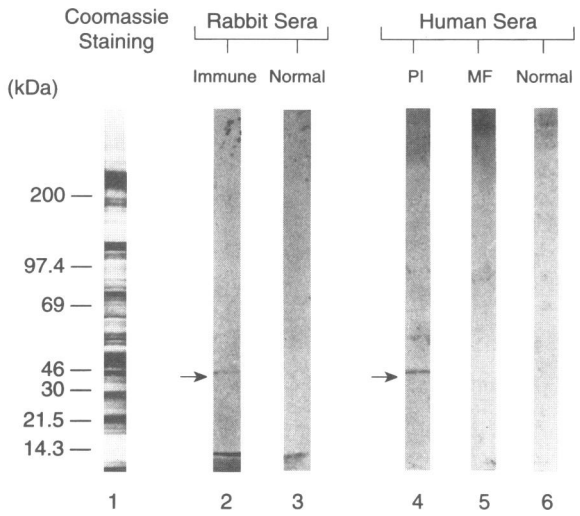

FIG. 3. Identification of the native WbN43 antigen. An immunoblot containing 25 μg of the CTAB-solubilized *B. malayi* L3 antigen extract per lane was reacted with affinity-purified immune rabbit serum (lane 2), preimmune rabbit serum (lane 3), PI serum (lane 4), microfilaremic (MF) patient serum (lane 5), or normal human serum (lane 6), each affinity purified on the λWbN43 recombinant antigen. Lane 1 shows the Coomassie blue-stained gel of the *B. malayi* L3 antigen extract. Arrows indicate the antigen of interest.

D.C.) data bases, nor were they in frame with β-galactosidase. Partial N-terminal sequencing of the expressed recombinant antigen gave an amino acid sequence identical to that of the deduced sequence of WbN43 coding for the chitinase-like antigen (data not shown); furthermore, partial sequencing of the λgt11 clone by using directional λgt11 primers showed the chitinase-like antigen to be in frame with β-galactosidase.

Southern analysis and species specificity. *B. malayi* genomic DNA (10 μg) was digested with various restriction endonucleases (*Bam*HI, *Eco*RI, *Hin*dIII, *Nco*I, and *Xba*I) and probed with the nick-translated *Eco*RI-*Nco*I (715-bp) fragment which encompasses the coding region for the chitinase gene. As seen in Fig. 5, the probe hybridized to single *Bam*HI, *Hin*dIII, *Nco*I, and *Xba*I fragments (the probe does not have the aforementioned restriction sites). However, the fact that the *Eco*RI digest yielded two fragments (one being a 2.3-kb fragment) indicates the presence of another chitinase-like sequence or the presence of a low-copy-number repetitive sequence within the gene. To test the species specificity of WbN43, slot blot analysis was carried out by using the nick-translated *Eco*RI-*Nco*I fragment as a probe. The probe hybridized only to DNA from *B. malayi* and *W. bancrofti*, not to any of several other filarial (*B. pahangi*, *A. viteae*, *D. immitis*, and *O. volvulus*) or nonfilarial (*C. elegans* and human) DNAs tested under highly stringent conditions (38).

Immunolocalization of the native WbN43 antigen. *B. malayi* adult female parasite tissue sections and L3 sections were probed with the rabbit serum affinity purified on the λWbN43 recombinant antigen. This rabbit serum localized the antigen to the intrauterine microfilariae (Fig. 6B) in the adult tissue section and to the tissues of the L3 parasites (Fig. 6D), while no reactivity was seen with the affinity-purified preimmune rabbit serum with either the adult (Fig. 6A) or L3 (Fig. 6C) tissue sections.

DISCUSSION

Lymphatic filariasis affects approximately 80 million people worldwide (52) and remains a major cause of morbidity in developing countries. The infection in the human host is initiated when the infective L3 larvae are deposited on the skin following the bite of an infected mosquito. These later develop into the lymphatic system-dwelling adult worms which release circulating microfilariae. However, all individuals living in areas where lymphatic filariasis is hyperendemic do not develop disease and may even show differential susceptibilities to filarial parasite infection (5, 31, 45).

Epidemiological cross-sectional studies to estimate age-specific rates of gain or loss of filarial infections have suggested that acquired resistance, if it occurs, is likely to be directed toward the developing larval stages of the parasite rather than adult worms (6, 7, 34, 47, 51). Such conclusions are consistent with studies of protective immunity in animal models. In these cases, a number of important observations have been made: (i) radiation-attenuated infective larvae confer protection to the vertebrate host against subsequent challenge infection in both permissive hosts (1, 8, 29, 44, 54) and semipermissive hosts (19); (ii) similar results have been seen with chemically abbreviated infections (3, 17) in which parasites that are killed by chemotherapeutic agents during the developing larval stages confer resistance to subsequent challenge; (iii) immunization with larval excretory-secretory products induces protection to challenge infection (39); and (iv) trickle infection experiments with *B. pahangi* in cats (8, 9) have shown that during the latter course of infection, the hosts are able to destroy most incoming larvae within 24 h of infection. Furthermore, in amicrofilaremic humans, the presence of antibodies to the surface of L3 suggests that larval killing may be occurring in human populations as well (20, 43).

Our earlier work on defining the existence of protective immunity in humans involved identifying a group of individuals living in an area where lymphatic filariasis is hyperendemic who were apparently infection free and, thus, PI (13). Qualitative analysis of the antibody responses of seven of these individuals to L3 antigens from *B. malayi* identified a 43-kDa antigen differentially recognized by the PI individuals. In the present study, antiserum was raised in rabbits against this 43-kDa antigen after it was isolated from preparative two-dimensional gels. This antiserum was then used to screen a genomic expression library of *W. bancrofti* in hopes of cloning and characterizing the analog of the 43-kDa antigen and identifying the epitope(s) of importance in the induction of protective immunity.

With this strategy, a recombinant clone designated λWbN43 was identified from ~10^4 phage screened from a *W. bancrofti* genomic expression library in λgt11. λWbN43 expressed a β-galactosidase fusion protein of ~140 kDa which showed differential and specific recognition by sera from PI individuals as had the native 43-kDa antigen on SDS-PAGE or two-dimensional immunoblots of *B. malayi* L3 antigen extracts earlier (13). However, this finding indicated that we had only a partial gene product expressed in WbN43.

WbN43 was found to contain a 2.3-kb insert, and sequence analysis showed the presence of a 684-bp 5′ ORF in frame with β-galactosidase. The deduced amino acid sequence from this ORF showed >40 to 60% sequence similarity (Fig. 4B) to bacterial chitinases (chitinases A and B of *S. marcescens* and chitinases from *Streptomyces plicatus* and *Bacillus circulans*) (4, 18, 23, 40, 48) and the MF1 (chitinase) protein of *B. malayi* (14). Of the seven highly conserved chitinase domains seen in the MF1 chitinase (14), WbN43 has five (one partial and four

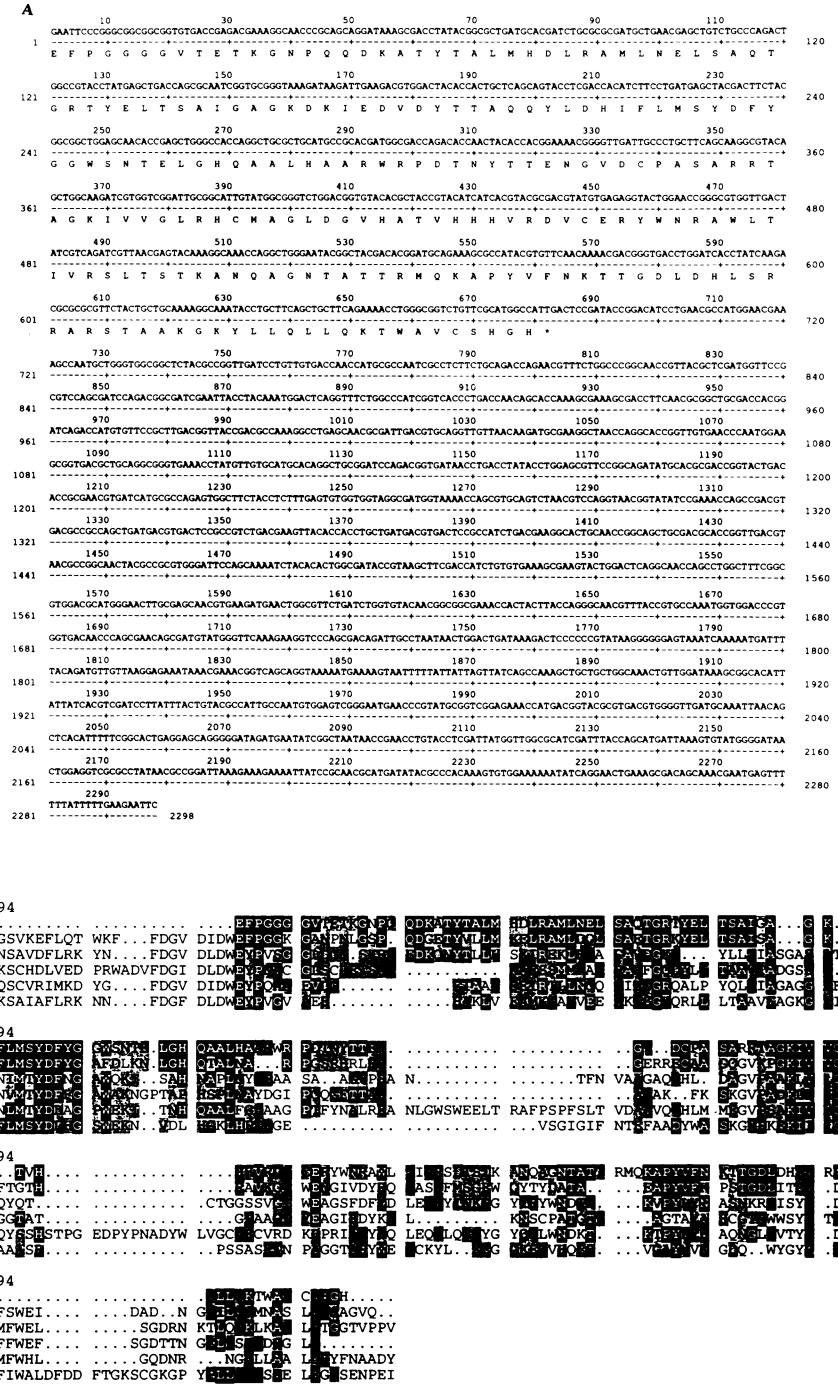

FIG. 4. (A) Complete nucleotide and deduced amino acid sequences of WbN43 (2.3-kb *W. bancrofti* genomic clone). (B) Alignment of the deduced 227-amino-acid sequence of WbN43 with the chitinase A precursor (*S. marcescens* A) and chitinase B precursor (*S. marcescens* B) sequences from *S. marcescens*, chitinase A1 precursor sequence from *Bacillus circulans*, chitinase 63 precursor from *Streptomyces plicatus*, and endochitinase precursor (MF1) from *B. malayi* (14). Alignments were calculated by the Genetics Computer Group (10) program PILEUP, using a gap weight of 3.0 and a gap increment weight of 0.2. (Dots represent gaps in the sequence.) Dark boxes with reverse fonts indicate identical amino acids at given positions, and the gray areas indicate conservative amino acid changes with respect to WbN43. The numbering scheme used is that of the *S. marcescens* chitinase A sequence.

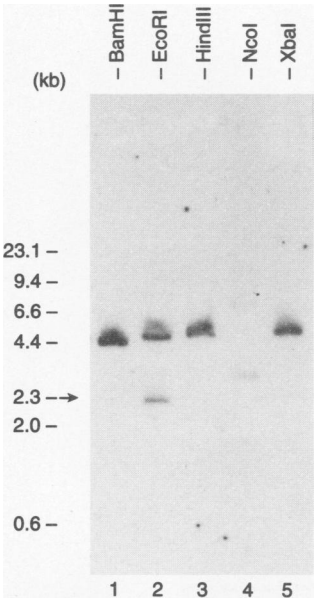

FIG. 5. Genomic organization of WbN43, determined by Southern blot analysis of *B. malayi* genomic DNA (10 μg) digested with *Bam*HI (lane 1), *Eco*RI (lane 2), *Hin*dIII (lane 3), *Nco*I (lane 4), and *Xba*I (lane 5). The blot was probed with the *Eco*RI-*Nco*I fragment (715 bp) of WbN43. The arrow indicates the position of the 2.3-kb *Eco*RI fragment.

full length) of these domains. It should be noted that MF1 had been isolated as the target molecule of a monoclonal antibody that mediated microfilarial clearance when infused into microfilaremic mice (15).

Chitinases are enzymes that hydrolyze chitin, a 1-4 β homopolymer of *N*-acetylglucosamine (11). It has been shown previously that some parasites require chitinase to degrade chitin-containing structures produced by the organism itself (*Onchocerca gibsoni* [16]) or its intermediate host (*Leishmania major* [42] or *Plasmodium* spp. [21]), while others depend on the chitinase of symbionts within the insect vector (*Trypanosoma* spp. [27]). Chitinase activity in *O. gibsoni* has been presumed to play a role in the remodeling of chitin fibrils in the developing eggshell or to free the developing microfilariae from their eggshells prior to birth (16). While brugian microfilariae do not hatch from the eggshells but rather reorganize and elongate this structure to form the sheath, it is possible that this activity involves chitinase.

Recent work has suggested that chitinases could be potential targets for blocking the transmission of these parasites. In *B. malayi*, it has been shown (15) that the MF1 (chitinase) antigen is expressed as microfilariae develop in the mosquito. This chitinase might be involved in microfilarial transmission by having a role either in the exsheathment process or during molting and development in the mosquito; alternatively, since the lectins in the mosquito midgut can block microfilarial invasion (35), it is possible that the *N*-acetylglucosamine

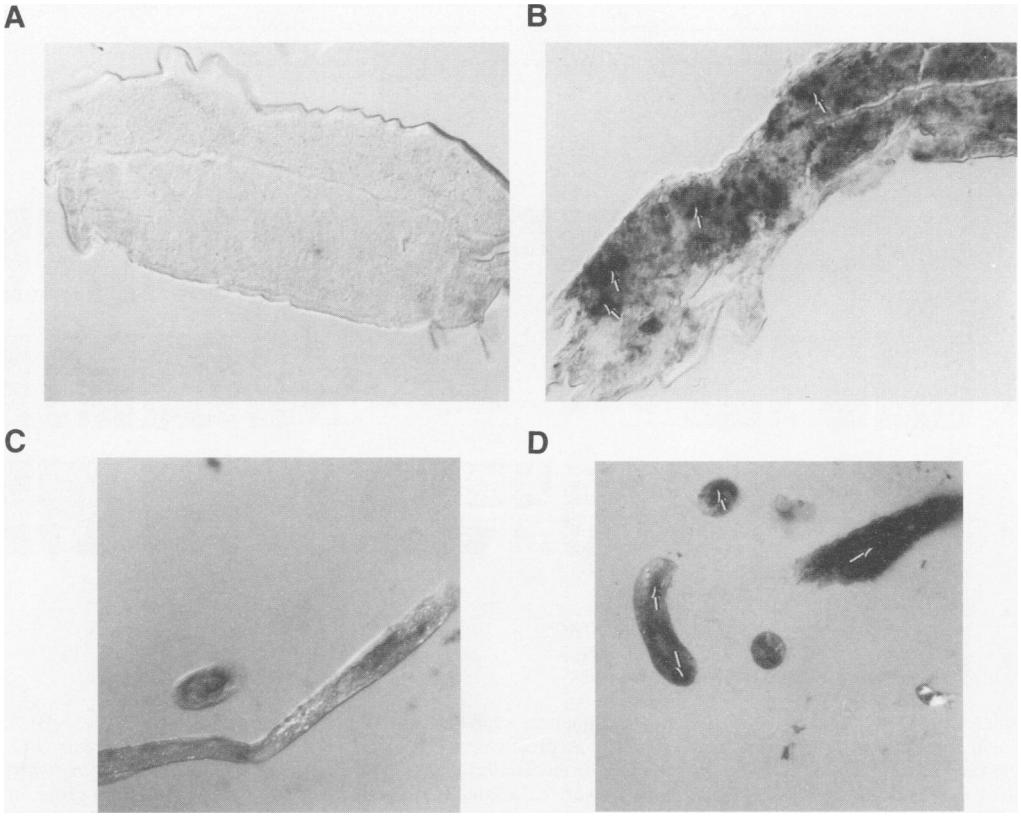

FIG. 6. Immunolocalization of the WbN43 antigen in the tissue sections of *B. malayi*. Adult parasite tissue sections were probed with preimmune rabbit serum (A) or immune rabbit serum (B), and L3 sections were probed with preimmune rabbit serum (C) or immune rabbit serum (D). Both immune and preimmune rabbit sera were affinity purified by using the recombinant WbN43 fusion protein and photographed with Nomarski optics (magnification, ×340). Intense labeling of only the intrauterine microfilariae is seen in the adult tissue section. The L3 sections are also intensely stained. Arrows indicate areas of localized antigen.

released by chitinase increases microfilarial infectivity by inhibiting specific lectins that block parasite invasion or later development in the thoracic muscles of the mosquito.

Chitinases are a variable class of proteins that have been shown to span a pI range from 3.8 to 9.9 and to have molecular masses ranging from 25 to 40 kDa in plants and fungi, from 21 to 57 kDa in bacteria, and up to 75 kDa in insects (11). The 43-kDa antigen has a pI of ~4.6 and a native molecular mass of 43 kDa, which is quite different from that of MF1 reported from *B. malayi* microfilariae. The presence of two bands seen in the *Eco*RI digests of the genomic DNA in the Southern blots (Fig. 5) could indicate the presence of different classes of chitinase-like genes with various degrees of homology, suggesting that WbN43 could be related, but not identical, to MF1 (15). Our results based on immunostaining also indicate that there may be different species of chitinases involved in the various life cycle stages. Rabbit anti-WbN43 antibodies showed staining only of the L3 tissue sections and microfilariae, not the adult tissue, an observation suggesting that there may be a spatial diversity in the expression of this protein.

Since vertebrate hosts do not contain chitin, they lack the metabolic enzymes required to degrade chitin, and the fact that there is a group of individuals in an area of hyperendemicity who are able to resist infection and mount a specific immune response to a chitinase-like 43-kDa antigen offers the possibility of an interesting candidate for vaccine development. While the current recombinant antigen is obtained from a genomic clone and may not be able to express the full-length protein, it has nevertheless been shown to be specifically recognized by the PI individuals. This antigen has also recently been subcloned, expressed, and purified in a bacterial expression system with a view to studying its protective potential in animal models. While this study cannot define the precise role of the chitinase-like 43-kDa L3 antigen in either parasite development or host immunity, its presence is consistent with the hypothesis that larval antigens are of paramount importance in the induction of protective immunity to filarial infection.

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