Recombinant cDNA Clones for Immunodiagnosis of Strongyloidiasis

Srinivasan Ramachandran, Robert W. Thompson, Albert A. Gam, and Franklin A. Neva

Because diagnosis of strongyloidiasis by stool examination is unreliable and because of the potential for serious disease in Strongyloides infections, there is need for improved diagnostic aids to facilitate recognition and treatment of this parasitic infection. Serologic testing, when available, requires antigen preparation from infected primates or dogs that can be difficult to maintain. Several recombinant clones from a cDNA library prepared from the infective stage of Strongyloides stercoralis were characterized. Serologic results indicate that the recombinant proteins were equally or more reactive than the larval somatic antigen. No cross-reactivity with recombinant antigen 5a was found with sera from patients with filarial or intestinal nematode infections. Recombinant antigens 5a and 12a detected parasite-specific IgE and IgG4 antibodies in Strongyloides-infected patients. Sequence analysis showed these antigens to be rich in proline and charged amino acids. Lack of homology from database searches suggests that the antigens are unique. These recombinant antigens should be useful in diagnostic and epidemiologic studies of strongyloidiasis.

Infection due to the intestinal nematode Strongyloides stercoralis occurs worldwide, although the prevalence is usually higher in the tropics. The biology and immunology of strongyloidiasis have been reviewed [1, 2]. Among the parasitic nematodes infecting humans, S. stercoralis is unique in possessing the ability to reinvade the same host—a process termed autoinfection. Clinical and laboratory evaluations of people who had been prisoners of war during World War II suggest that infections acquired in the prisoner-of-war camps of the Far East could continue at a chronic low level of autoinfection for several decades, perhaps indefinitely [3–5]. Although most of these infections are asymptomatic, they can become severe and complicated, involving multiple organs (hyperinfection syndrome), if chronically infected persons are immunosuppressed. This occurs especially if corticosteroids are given for treatment of other ailments or for organ transplantation [6, 7].

Coinfection with human T cell leukemia virus type I (HTLV-I) is another risk factor for activation of chronic strongyloidiasis and for interference with a response to treatment. Association of HTLV-I infection with strongyloidiasis has been noted in Japan [8], Jamaica [9], and the United States [10, 11]. Therefore, in instances in which chronic asymptomatic infection with S. stercoralis is a possibility, there is need for diagnostic tests that would facilitate recognition and treatment of this parasitic infection before immunosuppressive drugs are given.

Diagnosis of strongyloidiasis depends on the demonstration of larvae in fecal specimens. This is done by examination, using a microscope, of direct-smear samples or samples that have undergone various concentration procedures. However, negative results do not rule out infection because excretion of larvae is usually scanty and sporadic [12].

The development of an ELISA that uses a larval (L3) antigen was a major aid in the diagnosis of strongyloidiasis. The experience of several groups indicates that anti-L3 antibodies can be demonstrated in ~85% or more of parasitologically positive patients [13–16]. Recently, an immediate hypersensitivity skin-test using larval somatic and excretory/secretory antigens was developed that appears to be as sensitive and specific as the ELISA (Neva FA, unpublished data). However, the test is still restricted for use in research. The ELISA and the skin test are helpful in the diagnosis of strongyloidiasis; however, disadvantages of these diagnostic tests are (1) the need to maintain the parasitic infected dogs or primates, usually in an immunosuppressed state, in order to obtain enough larvae and (2) the fact that the antigens may cross-react with sera from patients with filarial infections. The availability of a recombinant antigen with comparable sensitivity and high specificity would facilitate immunodiagnosis of strongyloidiasis.

Recently, our laboratory constructed cDNA libraries from several stages of S. stercoralis [17]. Herein, we describe the isolation of a group of recombinant clones from an L3 infective stage cDNA library that show potential for sensitive and specific immunodiagnosis of chronic strongyloidiasis.

Materials and Methods

Patient sera for screening of cDNA library, Western blot analysis, and ELISA. Sera from patients with parasitologically proven infection were pooled for immunologic screening and tested individually [18]. The patients were clinically evaluated at the Clinical Center at NIH.
**Immunoscreening of the cDNA library.** The preparation of the library from the infective filariform (L3) stage of *S. stercoralis* has been described [17]. For immunoscreening, we used a previously described protocol [19]. In brief, ~200,000 plaques were screened after inducing the phage with 10 mM isopropyl-β-D-thiogalactoside (IPTG; Gold Biotechnology, St. Louis) for 3.5 h at 37°C. The plaque blots were blocked overnight with 2% casein (Casein Hammerstein; ICN, Aurora, OH) in 1× TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl) and then washed with TBST (1× TBS plus 0.05% Tween-20).

The pooled plaque was preabsorbed with 10 vol of saturated *Escherichia coli* lysate and subsequently diluted to 1:250 in 2% casein in 1× TBS. Blots were incubated with serum at a final dilution of 1:250 for 1 h at room temperature. The filters were washed several times with TBST and incubated with goat anti-human IgG–conjugated alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA) at a 1:50,000 dilution in TBST for 1 h at room temperature. The filters were washed with TBST, and the blots were developed with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate kit (Kirkegaard & Perry, Gaithersburg, MD).

Positive plaques were rescreened using the same procedure until a purified plaque was obtained. Bluescript SK+ plasmids containing the recombinant inserts were excised in vivo using an excision kit (Stratagene, La Jolla, CA), and the plasmid DNA was purified using the Qiagen (Chatsworth, CA) kit. Since the recombinant inserts were generally small, they were sequenced on both strands, using a combination of several primers (reverse, T3, SK+, and universal), by use of an ABI Prism automated sequencer (Applied Biosystems Division, Perkin-Elmer, Foster City, CA). Sequences were analyzed by use of software (Genetics Computer Group, Madison, WI) [20].

**Overexpression and purification of recombinant proteins.** The cDNA inserts were excised from Bluescript SK+ with *Eco*RI and *Xho*I (New England BioLabs, Beverly, MA), and the fragments were cloned into pET-30b (Novagen, Madison, WI), which has the same reading frame as the Bluescript plasmid at the *Eco*RI site. The pET-30b plasmid is a T7 RNA polymerase–based expression system for production of histidine-tagged recombinant proteins.

An overnight miniculture was prepared in Luria broth plus kanamycin (50 µg/mL) (LK) without induction. Larger cultures in LK were seeded with the miniculture (1:400) and grown with vigorous shaking until an *A*<sub>600</sub> of 0.6 was reached. Cultures were induced for the production of the recombinant proteins with IPTG at a *C<sub>f</sub>* of 100 (final concentration) of 1 mM and shaken vigorously for an additional 3 h. Cells were centrifuged and resuspended in 20 mM Tris-HCl, pH 7.9, and incubated with hen egg white lysozyme (Boehringer Mannheim, Indianapolis) at a *C<sub>f</sub>* of 100 µg/mL at 37°C for 30 min in the presence of protease inhibitor (Pefabloc; Boehringer Mannheim). Concentrated binding buffer was added to the paste to give a *C<sub>f</sub>* of 5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9. The total volume, which was 1/50 of the original culture volume, was sonicated at full amplitude with 30-s pulses on ice until the viscosity was reduced significantly.

The lysate was centrifuged at 39,000 g, solid guanidine-HCl was added to a *C<sub>f</sub>* of 6 M, and the pH was adjusted to 7.9 with 1 M sodium hydroxide. The final volume was adjusted with 8× binding buffer to 1/25 of the original culture volume. The lysate was incubated with Ni<sup>2+</sup>-sepharose resin with gentle shaking overnight at room temperature in a volumetric flask, which had five times the capacity of the volume of the protein solution. All steps of purification and dialysis were done at room temperature. Subsequently, the mixture was loaded onto a plastic column and washed with 10 column vol of 1× binding buffer plus guanidine-HCl (6 M), followed by 40 vol of 1× wash buffer (20 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9, plus 6 M guanidine-HCl).

The bound proteins were eluted by stripping the column with 10 column vol of 1× strip buffer (100 mM EDTA, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9). The eluate was dialyzed against 50 mM Tris-HCl, pH 7.5. The protein solution was concentrated by use of Centricron 10 (Amicon, Beverly, MA), and 1× binding buffer plus 6 M guanidine-HCl was added to a final volume of 10 mL.

Ni<sup>2+</sup>-sepharose resin was added, and the mixture was incubated with gentle rocking for 30–45 min at room temperature and then loaded onto a plastic column and washed as before. The column was stripped with 1× strip buffer plus 6 M guanidine-HCl.

Proteins were then dialyzed against stepwise decreasing concentrations of guanidine-HCl (6 M) to 0.5 M in 50 mM Tris-HCl, pH 7.5, to allow slow renaturation. Dialysis was done at each step for at least 3 h. Proteins were dialyzed against 1× PBS (8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, 2.7 mM KCl, 136 mM NaCl) several times and subsequently concentrated with Centricron 10 (Amicon). The concentration was estimated using the micro–bicinchoninic acid method (Pierce, Rockford, IL) [21]. A negative control antigen was prepared from plasmid pET-30b in *E. coli* BL21 (DE3) after induction and purified in the same manner.

**Western blots.** Purified recombinant proteins (1.35 µg/lane) were electrophoresed in 4%–20% gradient SDS–polyacrylamide gels (Novex, San Diego) under reducing conditions. Next, they were transferred to nitrocellulose membranes (BA85, Schleicher & Schuell, Keene, NH), using either a semidi blotter (W.E.P Company, Seattle) at constant current (300 mA) for 45 min or a wet blotter (Novex) according to the manufacturer’s protocol. The blots were subsequently blocked with 2% casein in 1× TBS overnight and then washed with TBST and incubated with primary antibody (for IgG: diluted 1:50 in 2% casein in 1× TBS; for IgE: diluted 1:20 in 5% bovine serum albumin [BSA] in 1× TBS) for 1 h at room temperature.

The secondary antibody, goat anti-human IgG (F<sub>c</sub>, fragment specific) conjugated to alkaline phosphatase, was used at a 1:3000 dilution. The signals in the IgE blots were amplified by use of a biotinylated goat anti-human IgE (cr) (Kirkegaard & Perry) at a 1:10,000 dilution in 5% BSA in 1× TBS for 1 h at room temperature. Blots were washed with TBST and incubated with streptavidin-conjugated alkaline phosphatase (Jackson ImmunoResearch) at a 1:1,000,000 dilution in 5% BSA in 1× TBS for 1 h at room temperature. Blots processed with rabbit antisera, the secondary antibody was goat anti-rabbit IgG (F<sub>c</sub>, fragment specific) conjugated to alkaline phosphatase (Jackson ImmunoResearch). Blots from negative control sera were processed and developed in the same manner.

**ELISA.** Box titration was used to determine the optimal concentration of the recombinant proteins used for coating. For IgG assays, recombinant antigens 5a and 12a were diluted to 0.25 µg/mL and 2.0 µg/mL, respectively, in 1× PBS and coated on Immulon-2 plates (Dynatech, Chantilly, VA) overnight. Next, the antigens were washed with PBST (1× PBS plus 0.05% Tween-20)
and blocked with 2% casein in 1× PBS for 1 h at 37°C. The control antigen was used at the same concentration as that for the recombinant antigens.

Each serum was tested at three 4-fold dilutions. Sera were mixed with 1 vol of E. coli 30b lysate at a C6 of 150 μg/mL and incubated at 37°C with shaking. The lysate was prepared from pET-30b in E. coli BL21 (DE3) by IPTG induction followed by resuspension in 1× PBS, sonication, and centrifugation at 39,000 g, as previously described for other antigens but without further purification. Subsequently, sera were diluted in 2% casein in 1× PBS to the required dilution. Plates were washed with PBST and incubated with sera for 1 h at 37°C. After being washed with PBST, the plates were incubated (1 h at 37°C) with goat anti-human IgG–conjugated alkaline phosphatase at a 1:5000 dilution in PBST.

After being washed with PBST, the plates were developed, using p-nitrophenol phosphate substrate (Kirkegaard & Perry) at a ratio of 1:2 to deplete them of IgG antibodies. Sera were subsequently diluted with E. coli lysate and 5% BSA in 1× PBS, as previously described. Signals were amplified by use of secondary biotinylated goat anti-human IgE at a 1:20,000 dilution in 5% BSA in 1× PBS. Plates were washed with PBST and incubated with streptavidin-conjugated alkaline phosphatase at a 1:1000 dilution in 5% BSA in 1× PBS. These dilutions were determined by box titration.

Antiserum against the recombinant antigen. Recombinant protein 5a (~300 μg) was purified by preparative SDS–PAGE, stained with Coomassie stain, and destained briefly. The 30-kDa band was excised from the gel, emulsified with complete Freund’s adjuvant, and injected subcutaneously at multiple sites into New Zealand White rabbits (Spring Valley, Woodbine, MD). Subsequently, two boosters were given with incomplete Freund’s adjuvant. Preimmune serum was used as a control on Western blots.

Results

Sequences. We obtained 10 clones that were consistently immunopositive after screening ~200,000 plaques of a cDNA library with pooled human serum from patients with parasitologically proven strongyloidiasis. The library was prepared from the infective (L3) stage of S. stercoralis. The 10 recombinant clones had insert sizes of 500–700 bp. Partial sequencing of the 5′ end of the cDNA inserts showed that 8 of the clones were related. The other 2 clones coded for a metal-independent β-galactose–binding lectin with high homology to lectins from Caenorhabditis elegans, Onchocerca volvulus, and other vertebrate lectins [22, 23], and they have two consensus amino acid motifs, HFNPRF and WGxExR. Although the lectin clones reacted strongly on Western blots, they were not specific for strongyloidiasis patients (data not shown). We could not identify homologous sequences from the database for the rest of the clones. The 3′ untranslated regions (3′ UTRs) and the inferred protein sequences of 4 of the 8 related clones (i.e., 5a, 8a, 12a, and 19a) are shown in figure 1. Other clones were smaller in size.

All of these clones are partial cDNAs because they lack the start codon for methionine. The sequence data suggest that although these clones are related to each other, they are distinct. However, clone 12a may be part of clone 19a due to partial reverse transcription. The putative polyadenylation signal was found 11 nucleotides upstream of the polyadenylation site, similar to that found in several C. elegans and O. volvulus genes (figure 1A).

The predicted protein sequences are rich in proline, basic (predominantly lysine) and acidic (glutamic acid) amino acids, and in several kinds of repeats (figure 1B). The N-terminal region (domain I) is rich in basic amino acids and is followed by a relatively neutral region, domain II, which is followed by a region rich in acidic amino acids, domain III.

Domain I consists of scrambled permutations of repeat motifs containing prolines and basic amino acids, predominantly lysines. Domain III, on the other hand, consists of only four amino acids (E, P, A, G) and regular repetitions of motifs, with proline repeated at every third position. Domain III also has glycines repeated sometimes at the third, sixth, or even at the ninth position, as in 12a/19a. The repeat position for glycine is always a multiple of 3.

The neutral domain II is conserved among these clones. Any potential differences in domain I cannot be addressed due to incomplete cDNA sequences, but differences in domain III and the C-terminal region after domain III constitute polymorphism. The differences between clones 5a and 12a/19a are due to the number of repeated motifs and substitutions in domain III and to substitutions in the C-terminal region. The same is true between clones 8a and 12a/19a. The differences between 5a and 8a are due only to the number of repeated motifs in domain III. The substitutions in domain III appear to be restricted because the only changes observed are glutamate for glycine and alanine. Sequence database searches show that these clones are highly similar to other proteins in the database that are also either proline rich, such as the repeats of circumsporozoite protein of Plasmodium berghei, or rich in basic amino acids, like histones from several species. However, the sequences of these S. stercoralis proteins appear to be unique.

Overproduction and purification of recombinant proteins and Western blot analysis. We expressed and analyzed 2 of the recombinant antigens (5a and 12a) from among those that showed the greatest sequence differences (figure 1B). The quality of the recombinant proteins produced and purified using the pET-30b system is shown in figure 2. The histidine-tagged small-molecular-mass protein (8 kDa) from the vector pET-30b was not completely excluded by the concentration procedure. Since a portion of this vector protein is fused to the recombinant proteins, the 30b antigen preparation was used as a negative control.
Figure 1. A. Multiple alignment of 3' untranslated regions (3' UTRs) from different recombinant clones. Arrows indicate start position of 3' UTRs immediately after termination codon and polyadenylation sites. Gaps (-) were introduced to maximize similarity. 3' UTRs of 5a and 8a and of 12a and 19a are almost identical. Bold indicates nucleotide signatures that are different between clones. Signatures can be used to group clones. Box indicates putative polyadenylation signal.

B. Multiple alignment of predicted protein sequences of recombinant clones. Three domains that radically differ in amino acid composition are marked. First amino acid in each sequence is combination of both linker sequence and parasite sequence. Thus, it should be ignored in sequence comparison. Gaps (-) were introduced to maximize similarity between sequences. Amino acid differences between clones in C-terminal region are shown in bold; differences can be used to group clones.

The predicted size of fusion protein 5a is 26 kDa, and that of 12a is 25 kDa. However, 30-kDa bands in protein 5a and 29-kDa bands in protein 12a were consistently positive on Western blots with patients' sera. Some of the low-molecular-mass bands are presumably due to degradation or prematurely terminated translation products (or both) because they also reacted positively with sera from some patients. None of these bands were seen with the normal negative serum pool.

The difference between the predicted and observed sizes of the recombinant antigens is presumably due to the highly charged repeated domains in these proteins [24]. A few additional host proteins also copurified in minor amounts. Western blot analysis with sera from 12 patients with chronic strongyloidiasis showed that all 12 had IgG and 9 had IgE antibodies against both recombinant antigens. Western blots with 3 representative serum samples are shown in figure 3. In both IgG and
IgE Western blots (figure 3A and B, respectively), recombinant antigen 12a appears less reactive than antigen 5a.

**ELISA.** Representative results for IgG antibodies reacting with recombinant antigens 5a and 12a in individual serum samples from 6 patients with chronic strongyloidiasis are shown in figure 4. Reactions to the control antigen, 30b, at concentrations equal to that of the recombinant antigens are also shown. Sera were tested at 4-fold dilutions, starting at 1:32. Optical density (OD) values of ≥0.6 were registered with all 6 sera at the lowest dilution tested. The reactions of these sera with control antigen and the reaction of the negative serum pool with both control and the recombinant antigens had, uniformly, ODs of <0.240. All serum samples previously found positive with the larval somatic antigen were also reactive with the recombinant antigens. OD values of the reactions of recombinant antigens were equal to or higher than the values of the reactions to larval somatic antigen. The degree of reactivity of individual sera to antigens 5a and 12a was not always equivalent.

Sera from patients with filarial infections produced results of particular interest when tested against the recombinant antigens, since these sera frequently cross-react with the larval somatic antigen. In this case, sera were tested at a lower dilution (1:8) to check for cross-reactivity. Nine of 10 filariae-infected patient sera (4 with loiasis, 2 with onchocerciasis, 1 with *Mansonella perstans*, 1 with *Mansonella ozzardi*, 1 with tropical pulmonary eosinophilia, and 1 with brugian filariasis and *Schistosoma mansoni* infection) that previously cross-reacted with larval somatic antigen failed to react with recombinant antigens 5a and 12a. Serum from a patient with *Loa loa* infection cross-reacted with antigen 12a.

Negative reactions (less than two times the control OD) to these recombinant antigens were also obtained with sera from 3 patients with multiple intestinal nematode infections (hookworm and *Trichuris* and *Ascaris* species) and from 3 patients with multiple intestinal and liver fluke infections (*Clonorchis*, *Opisthorchis*, and *Metagonimus* species). Sera from patients with these intestinal infections were chosen for use in checking for cross-reactivity because these infections are common in areas where *S. stercoralis* is prevalent. Serum from 1 patient infected with hookworm and *Trichuris* and *Ascaris* species reacted to antigen 12a at a serum dilution of 1:8.

For evidence of capacity to recognize parasite-specific IgE antibodies, the recombinant antigens were tested against a parasite-positive serum pool that had been depleted of IgG antibodies. Figure 5 shows the results of a box titration. It is apparent that both recombinant antigens reacted with the IgG-depleted serum. Sera from several patients, which were depleted of IgG in the same manner, were also reactive with the recombinant antigens.
Figure 6. Western blot analysis of larval somatic antigen probed with rabbit antiserum to recombinant antigen 5a. Lane A was loaded with 36 μg of larval somatic antigen; lane B was loaded with 1.35 μg of control antigen 30b; lane C was loaded with 1.35 μg of recombinant antigen 5a. Relevant bands in lane A are marked with arrows. Identical blot probed with preimmune serum from same rabbit did not show reactivity to any labeled bands.

size of antigen 5a is larger than the proteins in the somatic antigen preparation because of the 8-kDa portion from the pET-30b vector. The negative reaction of the preimmune serum (data not shown), the loss of reactivity to the recombinant antigens, and the four bands in the larval extract on preabsorption of the antiserum with excess of recombinant 5a but not with the negative control antigen (30b; data not shown) together indicate that the reaction is specific. The nature of the band just below the 29-kDa band in the larval somatic antigen lane in figure 6 is unclear because the reactivity to this band was not completely abolished by absorption with the recombinant 5a.

Discussion

Chronic strongyloidiasis is difficult to diagnose because the infection is usually asymptomatic. However, if infected patients become immunosuppressed as a result of intercurrent disease or the administration of drugs, such as corticosteroids, they may develop severe, life-threatening, hyperinfective strongyloidiasis. Hyperinfection or poor response to treatment of stron-
Strongyloidiasis may also occur in patients coinfected with HTLV-I retrovirus. Therefore, recognition and treatment of persons who are at greater risk for these complications of strongyloidiasis would be a sound preventive measure. Methods available for diagnosis include stool examination, ELISA, and an experimental skin test. If results of stool examination are negative, the next approach for diagnosis is the serologic test; the immediate hypersensitivity skin test is not generally available. However, these latter two diagnostic procedures depend upon the availability of parasite material from experimentally infected animals, such as dogs and primates, that are difficult to maintain or from an occasional heavily infected patient. The availability of sensitive and specific recombinant antigens that can be produced in large amounts would facilitate the use of serologic tests for diagnosis and epidemiologic surveys.

Herein, we describe a group of recombinant clones that could be used for diagnosis. The sequences of these clones appear unique; we could not identify any homologous protein to these antigens from the database. The presence of repeated motifs with regular periodicity and the restricted nature of substitutions suggest that these are structural proteins. There are several possibilities by which sequence polymorphisms in domain III could have arisen, such as from the artificial reverse transcription of an mRNA, from a heterogeneous population of mRNAs representing allelic variants of a single gene, from mRNAs of a multigene family, or from alternative splicing.

It is unlikely that the nucleotide substitutions observed between different mRNAs are cloning artifacts because 2 clones that were isolated independently and that coded for the galactose-binding lectin had identical sequences at the 3’ end. We analyzed, using computer programs, the mRNA sequences of the recombinant antigens 5a, 8a, 12a, and 19a for the potential of forming long secondary structures. The results revealed only very short motifs. Thus, the differences in the lengths of domain III between these clones do not appear to have arisen due to an artifactual reverse transcription of an mRNA with strong secondary structure.

The sequence differences due to scrambled permutations of similar amino acid repeat motifs among these clones, presence of multiple proteins in the larval extract, and the variations in the 3’ UTRs of the mRNAs taken together point to the polymorphic nature of this antigen. Further experiments are underway to analyze this polymorphism. Since these sequences consist of repeated motifs, variations could arise due to slippage-like mechanisms during DNA replication. This might explain the sequence differences due to deletions of several of these repeats. Our analysis using rRNA genes indicate that these mechanisms were active in generating molecular variations among Strongyloides species (Ramachandran et al., unpublished data).

IgG Western blot analysis using larval antigens has shown that most patients with strongyloidiasis have high levels of antibodies to 3 proteins from the larval extract, namely, the 41-, 31-, and 28-kDa proteins [25]. The antiserum against recombinant antigen 5a shows reactivity to multiple proteins of 29-, 30-, 31-, and 85-kDa from the larval extract. These reactions are specific because they can be abolished by absorption with excess of recombinant antigen 5a. Since this recombinant antigen is sensitive, having been recognized by sera from all strongyloidiasis patients tested so far, and shows no cross-reactivity to sera from patients with other nematode infections, it is likely that recombinant antigen 5a represents the 31-kDa protein. Our results also suggest that 2 proteins, 28- and 31-kDa, described by Conway et al. [25], are both related to 5a.

Recombinant antigen 5a was particularly sensitive as well as specific. Although antigen 12a is less specific, it was more reactive to some sera. Therefore, it could be useful in situations in which a concurrent filarial infection can be ruled out. For general serodiagnostic use, it may be advisable to pool multiple recombinant antigens. Different combinations of these antigens are currently being evaluated. The fact that IgG4 (data not shown) and IgE antibodies could be detected by these antigens in patients with chronic strongyloidiasis suggests that these antibodies might be useful for posttreatment monitoring and for development of recombinant skin test antigens.

Acknowledgments

We thank J. S. McCarthy and David Kaslow for useful suggestions, Tom Nutman for providing tropical pulmonary eosinophilia sera, and National Center for Biotechnology Information (NIH) for computational services.

References


