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Identification and Preliminary Evaluation of a Novel Recombinant Protein for Serodiagnosis of Strongyloidiasis

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Abstract. *Strongyloides stercoralis* is a human parasite that can cause a long-term infection. In immunosuppressed patients, strongyloidiasis may be fatal when there is overwhelming autoinfection resulting in the migration of large numbers of larvae through many organs. Definitive diagnosis is still a challenge, and a combination of symptoms, microscopic identification, and serology test results are often used to arrive at a clinical decision. However, intermittent larval excretion, low parasite burden, and occult infections are challenges with parasitological diagnosis of infection with *S. stercoralis*. Meanwhile, serologic tests using immunoglobulin G and parasite antigen extract have problems of cross-reactivity with other helminthic infections. Recombinant antigen-based serodiagnosis is a good alternative to overcome the laboratory diagnostic issues. Herein, we report on the isolation of cDNA clone encoding an antigen of potential diagnostic value identified from immunoscreening of a *S. stercoralis* cDNA library. The translated protein had highest similarity to *Strongyloides ratti* immunoglobulin-binding protein 1. The recombinant antigen produced, rSs1a, was assessed using western blot and enzyme-linked immunosorbent assay. The latter showed 96% diagnostic sensitivity and 93% specificity; thus, rSs1a has good potential for use in serodiagnosis of human strongyloidiasis.

INTRODUCTION

Strongyloidiasis is a human parasitic disease, mainly caused by the pathogenic species *Strongyloides stercoralis*. The parasite is commonly found in the tropics, subtropics, and warm temperate regions especially in resource-poor countries with inadequate sanitary conditions.[1] The parasite infects humans percutaneously when the filariform larvae (third-stage larvae [L3]) enter the human body via skin penetration from contaminated soil,[2] by oral ingestion of the larvae,[3,4] or rarely by person-to-person transmission.[4] Nosocomial transmission was also suggested in institutionalized individuals with mental retardation.[3]

Infection by this parasite is often overlooked because of the difficulty in measuring the infection as well as the complex diagnostic methods required to find it. Hence, strongyloidiasis is also known as "the most neglected of the neglected tropical diseases."[5] Unlike some other parasitic diseases, strongyloidiasis is capable of causing long-term infection as well as fatal consequences in humans. This is due to the phenomenon of autoinfection which allows sequential rounds of infection within the same host in the absence of external sources of infection.[6] Autoinfection is thought to be the main reason for undetected persistence of this parasite over decades in immunocompetent individuals, with the current record of chronic infection of 75 years.[7] Unregulated autoinfection can lead to fatal hyperinfection syndrome in immunocompromised patients. Strongyloidiasis is becoming an important parasitic disease as it accounts for 60–85% mortality rate among immunocompromised patients[8] and has contributed to a 16.7% mortality rate in patients requiring hospitalization.[9]

The biggest challenge in the management of strongyloidiasis is its diagnosis as *Strongyloides* infection has neither a reliable clinical marker that can easily be used for diagnosis and monitoring nor a dependable standard method to rule out the infection. Definitive diagnosis of strongyloidiasis is usually made by a combination of clinical signs, symptoms, microscopic identification of larvae in the stool, and/or serologic test results. The sensitivity of stool microscopy is compromised by the intermittent larval excretion and low parasite burden. Meanwhile, the available commercial serologic tests are based on native parasite antigen extracts that frequently cross-react with other helminthic infections.

Symptomatic patients often suffer from uncharacterized symptoms manifesting in skin, abdomen, or the respiratory system. Because most of the patients are asymptomatic, strongyloidiasis patients are frequently underdiagnosed and misdiagnosed. Therefore, there is an urgent need for more research to improve the diagnosis of strongyloidiasis to avoid the serious outcomes and progression of the disease.

In this regard, recombinant protein can be produced in large amounts and with high purity, and may replace crude parasite extract as antigen to detect *Strongyloides* infection. Studies on the production of recombinant antigens isolated from a cDNA library prepared from the infective stage of *S. stercoralis* have been published by Siddiqui et al.,[8] Ramachandran et al.,[10] and Ravi et al.[11] Thus far, the NIE recombinant protein (https://www.ncbi.nlm.nih.gov/protein/5669875) is the most promising infection marker and has been tested in an enzyme-linked immunosorbent assay (ELISA).[12] The availability of more candidate diagnostic markers will be useful in developing a highly specific and sensitive serodiagnostic assay for strongyloidiasis.

Herein, we report on the isolation of a DNA clone encoding an antigen of potential diagnostic importance identified from immunoscreening of *S. stercoralis* cDNA library. This clone was reactive with patient sera and did not react with negative sera when probed with IgG4 antibody. The corresponding purified recombinant protein was evaluated by western blot and ELISA.

METHODS

Serum samples. All serum samples used in this study were archived and anonymized samples from serum bank at Institute for Research in Molecular Medicine (INFORMM).

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Ethical clearance for use of these samples was obtained from Universiti Sains Malaysia (USM) Human Research Ethics Committee (USM/JEPeM/17050273). The serum samples were divided into two groups. Group A (*N* = 24) consisted of samples that were positive for anti-*Strongyloides* antibody using a commercial ELISA kit (IVD Research Inc., Carlsbad, CA) and an *in-house* ELISA[13], and their corresponding stool samples were positive by microscopy and/or real-time polymerase chain reaction (PCR). Meanwhile, group B serum samples (*N* = 168) were seronegative by both serological tests, and/or their corresponding stool samples were negative by microscopy and/or real-time PCR. This control group comprised serum samples from healthy donors living in *Strongyloides*-endemic area (*N* = 17) and patients with the following infections: ascariasis (*N* = 7); mixed hookworm and trichuriasis (*N* = 7); hookworm (*N* = 17); trichuriasis (*N* = 4); schistosomiasis (*Schistosoma mansoni*) (*N* = 27); lymphatic filariasis (*N* = 62); trichostrongylus (*N* = 5); toxocariasis (*N* = 8); amoebiasis (*N* = 5); toxoplasmosis (*N* = 6); giardiasis (*N* = 1); and malaria (*N* = 2). With regard to lymphatic filariasis, the serum samples came from 33 *Brugia malayi* and 29 *Wuchereria bancrofti* patients, respectively.

Pre-adsorption of serum samples for immunoscreening. Before performing immunoscreening, an equal volume of serum samples from group A (*N* = 5) and group B (*N* = 5) were pooled separately and pre-adsorbed against two kinds of *Escherichia coli* XL1-Blue antigen preparations to remove cross-reactive antibodies, that is, whole cell pellet at 100 mg per tube and 250 µL lysate (500 µg) per 100 µL of beads. The latter was immobilized onto 0.5-µm microsphere beads (Bangs Laboratories Inc., Fishers, IN) at a dilution of 1:400, at 4°C, overnight.

A total of 30 µL pooled serum samples of group A and B were each incubated consecutively with the two *E. coli* antigen preparations for 24 hours at 4°C on a rotary shaker. Each step was repeated twice and thimerosal (0.05% [v/v]) was added to prevent bacterial growth. The final recovered pre-adsorbed serum sample was centrifuged and kept at −80°C. The efficiency of serum pre-adsorption at each step was assessed by IgG-ELISA.

Indirect ELISA. Each well of the high-binding microtitre plate (Nalgene Nunc Int., Rochester, NY) was coated with 100 µL of *E. coli* XL1-Blue lysate antigen at a concentration of 5 µg/mL diluted in 0.06 M carbonate buffer (pH 9.6) and incubated overnight at 4°C. Next day, the plate was incubated at 37°C for 1 hour and washed five times for 5 minutes each with 200 µL of phosphate-buffered saline with 0.05% Tween 20 (PBS-T) at 37°C with shaking at 700 rpm. After the fifth wash, the wells were blocked with 200 µL of 3% (w/v) bovine serum albumin (BSA). After 1-hour incubation at 37°C and a washing step, 100 µL/well of pre-adsorbed serum sample at 1:100 dilution was added and incubated at 37°C, 2 hours, 300 rpm. Anti-human IgG-HRP (Invitrogen, Carlsbad, CA) at 1:2,000 was then added (200 µL/well) and the plate was incubated at 37°C for 30 minutes. After another washing step, 100 µL/well of 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) substrate (Roche Diagnostics GmbH, Mannheim, Germany) were added and incubated for 30 minutes. The absorbance readings were read with an ELISA Multiskan spectrophotometer (Thermo Scientific, Waltham, MA) at 405 nm (test filter) and 490 nm (reference filter).

cDNA library immunoscreening. The *S. stercoralis* cDNA library was made from a mixture of L3 and adult worms of the helminth. It was constructed in λTriplEx2 vector by Clontech Laboratories Inc. (Mountain View, CA). Before immunoscreening, the phage cDNA library was serially diluted with 1× lambda dilution buffer to produce around 300 to 500 well-separated plaques per plate. A volume of 10 µL of the phage at dilution of 10^4 was added to 600 µL of *E. coli* XL1-Blue cell at an optical density at 600 nanometers (OD_{600nm}) of 0.5. Following incubation at 37°C for 15 minutes, the mixture was poured into melted Luria–Bertani (LB)/$MgSO_4$ soft top agarose and plated onto pre-warmed LB/$MgSO_4$ agar plate at 37°C for 4–6 hours. When the plaques became visible, nitrocellulose membrane (Millipore, Bedford, MA) impregnated with 10 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was overlaid on top of the agar and incubated for 4–6 hours at 37°C. Marking positions were asymmetrically made on the nitrocellulose membrane and to the side of the plate for easy reference during plaque coring.

Subsequently, the blot was washed three times, 5 minutes each with tris-buffered saline with 0.05% Tween 20 (TBS-T) (20 mM Tris, 150 mM NaCl, 5% [v/v] Tween-20), followed by blocking with diluted Superblock™ (Pierce Biotechnology, Rockford, IL) for 1 hour at room temperature. After a washing step, the blot was incubated with pooled positive pre-adsorbed serum samples in tris-buffered saline (TBS) (1:100), overnight at 4°C. The next day, the blot was washed and incubated with monoclonal mouse anti-human IgG4-HRP (Invitrogen) at 1:2,000 dilution in TBS-T for 2 hours at room temperature. The signal of the reaction was detected using chemiluminescence substrate (Thermo Scientific) and developed on an X-ray film. Dark spots on the film represented reactive phage clones.

The location of the corresponding phage clone on the plate was then identified and cored out as a gel plug. The phage was allowed to diffuse in 200 µL 1× lambda dilution buffer overnight at 4°C, recovered on the following day by centrifugation at 15,000 × *g* for 5 minutes. Randomly selected clones were rescreened in the secondary immunoscreening following the same procedure as described previously to obtain single isolated clone. Subsequently, a tertiary immunoscreening was performed whereby each clone was incubated with individual positive (*N* = 14) and negative (*N* = 24) pre-adsorbed serum samples for assessment of the diagnostic sensitivity and specificity of the clone. The nitrocellulose membrane was divided into six sections, each for a different serum sample, including positive and negative serum controls.

Sensitivity refers to the number of serum samples from group A that reacted with the protein expressed by a particular cDNA clone. Specificity refers to the number of serum samples from group B that did not react with the protein expressed by a particular cDNA clone.

In vivo excision, plasmid extraction, and sequencing analysis. The potential phage cDNA clones from the tertiary immunoscreening were in vivo excised from the lambda vector to form phagemids containing the cloned inserts, thus allowing conversion of cDNA clones (in the form of phagemids) into plasmids. To do this, 200 µL overnight culture of *E. coli* BM25.8 was combined with 150 µL of eluted plaque identified from the tertiary immunoscreening and incubated at 31°C for 30 minutes without shaking. Then, 400 µL of LB broth was added to the cell suspension and further incubated for an additional 1 hour at 31°C with shaking at 225 rpm. Using a sterile glass spreader, 5 µL of the cell suspension was spread on LB/ampicillin plate and incubated at 37°C overnight. The

isolated colony was picked out and the recombinant DNA plasmid was purified using QIAprep Spin Miniprep Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions, then sent for sequencing using vector-specific primers. Searches for sequence identity were performed using National Center for Biotechnology Information and other public databases for nematodes such as nematode.net (http://nematode.net/NN3_frontpage.cgi) and Wellcome Trust Sanger Institute (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/strongyloides).

Expression and purification of recombinant protein. The selected insert DNA sequence was custom cloned (EPOCH Life Science Inc., Missouri City, TX) into pET28b expression vector with dual tags, that is, thioredoxin and histidine. In our laboratory, it was used to transform *E. coli* host cells BL21 (DE3) and protein expression was performed in 2 L of terrific broth supplemented with 50 µg/mL kanamycin. The culture was grown at 37°C with shaking at 200 rpm until the OD_{600nm} reached 0.6 to 0.7. At that point, 1 mM IPTG was added and the culture was further incubated for 4 hours at 30°C.

Cells were then harvested by centrifugation (10,000 × *g*, 30 minutes at 4°C) and the cell pellet was resuspended in cold lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, and pH 8.0) with lysozymes (Amresco, Solon, OH) at a final concentration of 0.5 mg/mL and protease inhibitors cocktail (1 mL cocktail in a final volume of 25 mL lysis buffer) (Roche Diagnostics GmbH). After 30 minutes' incubation on ice, the cell lysate was disrupted by sonication (Misonix Sonicator 3000; Cole-Parmer, Vernon Hills, IL) for four cycles of 30 seconds/cycle at an output of 4.5 Hz on ice. The supernatant was treated with 0.5 µg/mL DNase1 (Amresco), incubated at 4°C for 30 minutes, and then centrifuged at 10,000 × *g* for 30 minutes. The filtered lysate was incubated with nickel-nitrilotriacetic acid resin slurry (QIAGEN GmbH) on a rotator for 1 hour before affinity chromatography. A gradient washing was performed using three concentrations of phosphate buffers (50 mM NaH_2PO_4, 300 mM NaCl, and pH 8.0) with imidazole concentration of 20–40 mM, and the target protein was eluted with buffer containing 250 mM imidazole. Up to 10–15 eluted fractions (500 µL each) were collected, and the purity of protein in each fraction was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Fractions with high-purity proteins were pooled and concentrated using Vivaspin column with 3,000 kDa cut-off (GE Healthcare, Buckinghamshire, United Kingdom). The protein concentration was determined using Bio-Rad Protein assay reagent (Bio-Rad, Hercules, CA) and then stored at −80°C.

Protein verification by matrix-assisted laser desorption/ionization-time-of-flight/time-of-flight (MALDI-TOF/TOF). The target protein band from the SDS gel was excised manually, cut into small pieces, and kept in a microfuge tube containing 100 µL of distilled water before sending for analysis using MALDI-TOF/TOF 5800 analyzer mass spectrometer (AB Sciex, Framingham, MA). The peptide spectra obtained from this analysis were searched using ProteinPilot software against SwissProt, and specific nematode databases of *S. stercoralis* and *Strongyloides ratti* downloaded from Wellcome Trust Sanger Institute website, http://www.sanger.ac.uk/research/initiatives/globalhealth/research/helminthgenomes. The database search was performed using MASCOT search engine version 2.1 (Matrix Science Ltd., London, United Kingdom). In addition, the search was also performed using INFORMM database (InformmDB), a collection of closed database that we created, which consisted of 50 protein sequences of recombinant proteins produced by researchers at our institute.

The presence of the recombinant protein was also determined using western blot probed with anti-His-HRP. The electrophoresed recombinant protein was transferred onto nitrocellulose membrane in alkaline transfer buffer (25 mM Tris-base, 190 mM glycine, 10% [v/v] methanol) using the Bio-Rad Trans-Blot®SD Semidry Transfer Cell (Bio-Rad). The membrane was blocked with 1% (v/v) alkaline-soluble casein-blocking solution (CalBioChem, San Diego, CA) for 1 hour at room temperature. After three washes with TBS-T (0.5% [v/v]) at 5 minutes each, the membrane was incubated with monoclonal anti-His-HRP (Thermo Scientific) diluted at 1:2,000 for 1 hour at 4°C. Following one additional washing step, signal reaction was developed using chemiluminescence substrate.

Evaluation of the antigenicity of the recombinant protein by IgG4-ELISA. The diagnostic sensitivity and specificity of the target protein were evaluated by IgG4-ELISA using the panel of serum samples as described earlier. A similar procedure of ELISA was performed as described earlier, except that the microtiter plate was coated with the target recombinant protein at 10 µg/mL, primary antibody at 1:50 and anti-human IgG4-HRP at 1:1,000 dilution, with phosphate-buffered saline as the diluent. The cut-off value (COV) of the test was determined by receiver operator characteristic curve analysis of the ELISA data obtained from testing of all serum samples (N = 192). In parallel, antigenicity of the target recombinant protein was evaluated by western blot (as described above) using the following parameters: 20 µg protein per lane, primary antibody at a dilution of 1:100, and anti-human IgG4-HRP at 1:2,000 dilutions, with TBS-T as the diluent.

RESULTS

Pre-adsorbed serum sample for immunoscreening. Enzyme-linked immunosorbent assay revealed that the immunoreactivity of the pre-adsorbed serum samples progressively decreased with each round of adsorption; the decrease was particularly notable after the first adsorption step using the *E. coli* pellet. The ELISA OD of the final pre-adsorbed sample was below 0.1, which was at least 10 times less than the OD of non-adsorbed serum samples, showing the efficiency of serum pre-adsorption process in eliminating contaminant antibodies to *E. coli* in the serum samples.

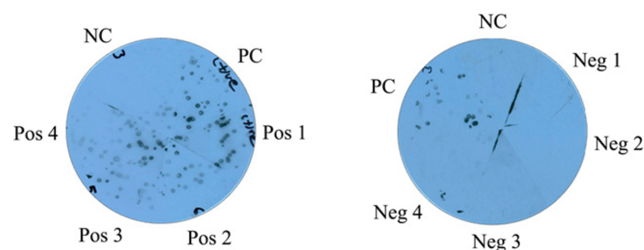

FIGURE 1. Representative images of IgG4-tertiary immunoscreening for evaluation of diagnostic sensitivity and specificity of clone Ss1a using individual positive and negative pre-adsorbed serum samples. NC = negative control serum; Neg = serum from group B; PC = positive control serum; Pos = serum from group A. This figure appears in color at www.ajtmh.org.

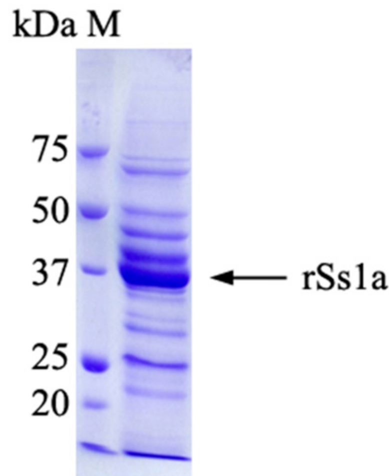

FIGURE 2. SDS-PAGE analysis of purified rSs1a; M: Precision Plus Protein™ Unstained Standard Marker (Bio-Rad). This figure appears in color at www.ajtmh.org.

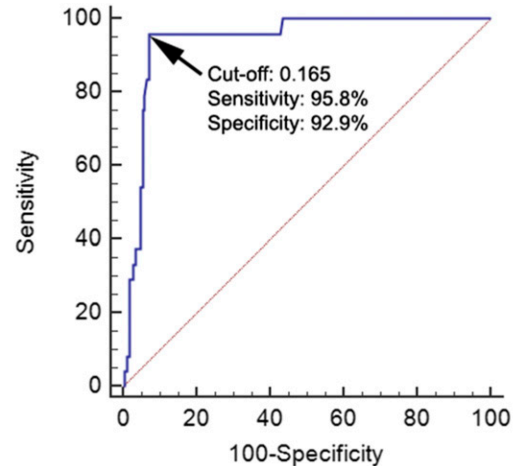

FIGURE 4. Receiver operating characteristic curve of rSs1a IgG4-enzyme-linked immunosorbent assay for the detection of strongyloidiasis (area under the curve = 0.942, 95% confidence interval = 0.90–0.97, $P < 0.0001$). This figure appears in color at www.ajtmh.org.

Immunoscreening of the cDNA library. A total of 150 IgG4-detected cDNA clones were successfully isolated from 10 series of primary immunoscreening, of which approximately 10,000 recombinant phage cDNA library clones were screened using pooled pre-adsorbed positive serum samples. Of these, 20 were randomly selected and carried through to secondary and tertiary immunoscreenings. After the tertiary screening using individual positive and negative pre-adsorbed serum samples, seven were found to be of good diagnostic potential, with diagnostic sensitivity and specificity ranging from 64–100%. The cDNA clone with the highest potential was identified as clone Ss1a, with sensitivity and specificity of 100% and 87%, respectively. Representative images of IgG4-tertiary immunoscreening are shown in Figure 1.

Cloning, expression, and purification of rSs1a protein. Sequence analysis of the gene insert of clone Ss1a showed 99% similarity to *S. stercoralis* genome assembly *S. stercoralis* PV0001, scaffold SSTP_contig0000018 (Identities = 598/601 [99%], Gaps = 2/601 [0%]) with accession number LL999076.1. Analysis of the translated protein showed the highest similarity to *S. ratti* immunoglobulin-binding protein 1 (BiP) (identities = 188/247 [76%], positives = 225/247 [91%], and gaps = 1/247 [0%]) with accession number CEF66010.1.

SDS-PAGE analysis showed that the thickest band had molecular mass of 37 kDa (Figure 2). Four bands (including three above the thickest band) showed reactivity when tested against anti-His-HRP western blot (Figure 3A). MALDI-TOF/TOF identified the thickest band (~37 kDa) as thioredoxin fusion protein when searched against Swissprot, with protein and peptide scores of 127 and 80, respectively. The same search of the InformmDB database, gave protein and peptide scores of 152 and 78 showing hits to the amino acid sequence of rSs1a. Because the predicted molecular mass of the recombinant protein is 49 kDa, rSs1a on SDS-PAGE was a truncated protein. The other three protein bands above rSs1a band showed hits for *E. coli* proteins.

Western blot and IgG4-ELISA. The above finding was substantiated by the results of rSs1a IgG4-western blot using serum samples, which showed that only the 37-kDa protein band was reactive with 11 positive serum samples (Group A). A specificity of 93% was obtained when rSs1a was tested with 30 negative serum samples from Group B samples. The two cross-reactive serum samples were from an amoebiasis patient and a healthy person. A representative image of rSs1a IgG4-western blot is shown in Figure 3B. Receiver operator characteristic curve analysis (Figure 4) of the data ($N = 192$)

FIGURE 3. Representative image of western blot analysis of purified rSs1a: (**A**) probed with anti-His-HRP (His); (**B**) probed with positive serum samples from Group A (lane 1) and control serum samples from Group B: lanes 2–5: healthy individuals; lanes 6–12: mixed hookworm infection and trichuriasis; lane 13: hookworm; lane 14: ascariasis; lanes 15–17: trichuriasis; lane 18: toxocariasis; lanes 19–20: amoebiasis; lanes 21–22: schistosomiasis; lane 23: lymphatic filariasis; and M: Precision Plus Protein Unstained Standard Marker (Bio-Rad). This figure appears in color at www.ajtmh.org.

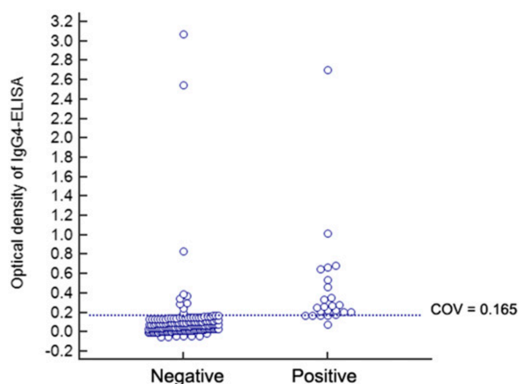

FIGURE 5. Scattergraph plot of optical density (OD) readings of IgG4-enzyme-linked immunosorbent assay (ELISA) using the positive and control serum samples. An OD reading of 0.165 was used as the cutoff value (COV) to discriminate between positive and negative results. This figure appears in color at www.ajtmh.org.

from the IgG4-ELISA revealed a COV of 0.165, which produced the highest sensitivity and good specificity, with area under the curve of 0.942 (95% confidence interval = 0.90–0.97, $P < 0.0001$). Evaluation of the antigenicity of rSs1a by IgG4-ELISA showed a diagnostic sensitivity of 96% (N = 24) and a diagnostic specificity of 93% (N = 168). Figure 5 shows a scatter plot of the OD readings obtained in the ELISA.

DISCUSSION

The use of immunological screening of cDNA libraries in identifying species-specific genes has been reported by a number researchers for the past 30 years. This approach has identified antigenic proteins in several parasitic diseases such as toxoplasmosis,[14] anisakiasis,[15] trichinellosis,[16] and cystic echinococcosis.[17] With regard to strongyloidiasis, various approaches have identified clones from *S. stercoralis* cDNA library. Using a PCR-generated probe to screen a *S. stercoralis* cDNA library, Siddiqui et al.[18] reported a full-length cDNA clone producing the γ subunit of *S. stercoralis* isocitrate dehydrogenase (nicotinamide adenine dinucleotide) designated as SsICDH, a zinc finger protein designated as SsZFP[8] and a clone containing the complete coding sequence of a nuclear hormone receptor designated as SsNHR.[19] Using a similar approach, Gallego et al.[20] reported a cDNA clone encoding a novel aspartic protease, which was named as SsPep. However, the immunoscreening approach is preferable because it involves expressing fusion proteins by the recombinant phage bacteria/virus, and thus the selection of specific reactive clones can be achieved using serum samples of infected patients. For example, Ramachandran et al.[10] and Siddiqui et al.[8] reported two recombinant proteins, namely 5a and 12a, the former being related to the NIE-recombinant protein.[11] NIE has been applied to luciferase immunoprecipitation systems,[21] Luminex[22] assay, and a one-step sandwich IgG immunoassay.[12]

In the present study, reactive clones were identified by immunoscreening of a *S. stercoralis* cDNA library constructed from L3 and adult worms. Anti-L3 antibodies have been demonstrated in ~85% or more of parasitologically positive patients.[10] The larvae are metabolically active, and therefore they produce proteins and secretions that can be recognized by the host immune system.

On infection with *S. stercoralis*, the host responds by producing different isotypes of immunoglobulins, that is, IgA, IgE, IgM, IgG, and the subclasses of IgG antibody. IgA and IgE play an important role in modulating larval output.[23] The latter is downregulated with the increasing duration of the infection. Secretion of IgM usually indicates acute infection, whereas IgG is the most abundant circulating antibody produced during chronic infection.[24,25] The problem with IgG is the possibility of cross-reaction with filarial antigens.[26] In relation to this, many studies have focused on studying the role of the IgG subclasses, namely, IgG1, IgG2, IgG3, and IgG4, in providing protective immunity against strongyloidiasis.[6,27] IgG1 and IgG4 are the major components of the immunoglobulin response, the latter acts as a blocking antibody to specific anti-IgE antibody.[23] IgG4 has also been reported to play a prominent role in chronically infected patients, with high titers found in non-cured strongyloidiasis patients under albendazole treatment.[28–30] Another important feature of IgG4 is its high specificity when tested with serum samples from other helminth infections, as compared with that of IgG antibody.[13] Thus, in the present study, the antigen encoded in cDNA clone Ss1a was identified as having diagnostic potential because of its recognition by IgG4 subclass antibodies in patient sera.

The rSs1a protein is identical to the BiP of *S. ratti* and also known as glucose-regulated protein-78. It is a highly conserved and multifunctional protein, that acts as an essential chaperone protein for the assembly of immunoglobulin molecules.[31] Investigations into the role of BiP in mammalian cells suggested that this protein is involved in protecting cells from endoplasmic reticulum stress[32]; others have suggested that BiP is involved in immunoglobulin light chain folding and disulfide bond formation, with a function as a receptor regulating the coagulation cascade and cellular proliferation.[31]

IgG4-western blot and IgG4-ELISA using the purified rSs1a protein showed that the protein has good diagnostic potential with sensitivity of 100% and 96%, respectively, and 93% specificity. Thus, rSS1a merits further studies to validate its diagnostic value, using a much large number of serum samples from various endemic regions.

CONCLUSION

This study has identified rSs1a as a novel *S. stercoralis* recombinant protein that may serve as potential diagnostic reagent for serodiagnosis of strongyloidiasis. This may help to improve serodiagnosis of human strongyloidiasis, and for epidemiological studies of the disease.

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Disclaimer: R. N. and N. A. are named as inventors in a patent titled "*Strongyloides stercoralis* protein and/or corresponding DNA and RNA sequences for application in diagnosis" filed in Malaysia (PI 2015002836) and at PCT (PCT/MY 2016/050053).

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