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Specific Monoclonal Antibodies to *Strongyloides stercoralis*: A Potential Diagnostic Reagent for Strongyloidiasis

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SUMMARY **In this study, specific hybridomas secreting monoclonal antibodies (MAb) to antigen of *Strongyloides stercoralis* filariform larvae were produced. Specific epitopes targeted by the MAb were protein in nature and located *in situ* in the internal content of the filariform larvae of the parasite but not in the esophagus. The MAb reacted to the homologous antigen in an indirect ELISA but did not reveal any reaction to the SDS-PAGE separated-homologous antigen in a Western blot analysis (WB) suggesting a conformational epitope specificity. The MAb were of IgG1 isotype which is the isotype known to have high affinity to this epitope so they were used in a dot-ELISA to detect the antigen of the parasite. The assay could detect the epitopes in 78 ng or more of the crude filariform larval extract but did not reveal any positive result when applied to detect antigen in stool samples of parasitologically confirmed strongyloidiasis patients. The negative antigen test results can be explained as follows. Either the MAb were filariform stage-specific and thus did not recognize the rhabditiform larval antigen mainly contained in the patient's stool or the amounts of antigen in the stool samples were too small and/or unevenly dispersed. In the latter instance, the MAb developed in this study would have a diagnostic potential if used in an immunological test design where more volume of fresh stool sample could be accommodated in the test, *e.g.* a sandwich plate ELISA.**

Strongyloides stercoralis, a round worm that infects human, has both free living and parasitic life cycles. The unique characteristic that separates this parasite from almost all other worms that infect man is its capacity to replicate within the host causing autoinfection that persists for many years.[1,2] There appear to be three possible outcomes of strongyloidiasis in infected individuals, depending on the host's immune system and the parasite's ability to evade the host's immune responses. These are (1) elimination of infection, (2) chronic asymptomatic infection of the gastrointestinal tract in immunocompetent hosts that may remain undetected for several decades,[3] and (3) in immunocompromised hosts, disseminated (pulmonary) hyperinfection that can develop due to accelerated reproduction of the parasite caused by autoinfection[4-9] which has a high mortality.[10,11]

Strongyloidiasis is one of the most difficult parasitic infections to be diagnosed.[4,7] Standard parasitological methods, *e.g.* direct fecal smear and formalin-ether concentration technique, have low sensitivity even after repeating the examination several times. This is because the larval output is minimal and irregular in the majority of infected individuals.[4,12] Baermannization and agar-plate cultures[4,13] are more sensitive but time-consuming.[14]

Various immunological techniques have been used for serodiagnosis of strongyloidiasis by detection of antibodies in sera of suspected individuals. When crude extracts of the parasite, *e.g.* filariform larvae, were used as antigen, false positive results were found in sera of patients infected with other parasites,[1,15,16,17] such as hook worms, *Trichuris*, filarial worms, *Schistosoma*, *Paragonimus* and *Echinococcus*. Therefore, attempts have been

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made to identify and characterize highly specific *S. stercoralis* antigenic components with the aim of exploiting them in immunodiagnosis.[4] Alternative approaches for diagnosis of current strongyloidiasis would be through directly detecting parasite antigen(s) or DNA in the specimens. DNA technology, *e.g.* PCR or DNA hybridization, is hampered by the requirement of expensive reagents and equipment. Moreover, it is known that stool specimens contain PCR inhibitor(s) and thus often render false negative results by PCR. Specific monoclonal antibodies against the parasite antigens would offer high specificity and a uniform detection reagent compared to an assay using polyclonal antibodies. Such an antigen detection test could also serve as a tool for monitoring the status of a patient after receiving therapy as well as in an epidemiological study on current infections.

In this study, hybridomas secreting monoclonal antibodies specific to *S. stercoralis* protein antigen have been produced. Experimental details of hybridoma production, the *in situ* anatomical locations of epitopes of the monoclonal antibodies and the diagnostic potential of the antibodies as a detection reagent are reported herein.

MATERIALS AND METHODS

Preparation of *S. stercoralis* antigen

The filariform larvae of *S. stercoralis* were obtained from the cultures of human feces containing *S. stercoralis* rhabditiform larvae by the polyetylene-tube method.[18] The larvae were washed four times with distilled water (DW) then lyophilized. The dried larvae were ground in a glass tissue grinder in DW containing protease inhibitors, *i.e.* 10 mM phenylmethysulfonyl fluoride (PMSF), 10 mM tosylamide-2-phenylchloromethyl ketone (TPCK) and 10 mM ethylene diamine tetraacetic acid (EDTA),[19] at 4°C in an ice bath. The preparation was sonicated at 20 kHz per second for 10 minutes at 4°C. Then the homogenate was allowed to stand on ice for 1 hour with occasional agitation, after which it was centrifuged at 10,000 × *g* for 30 minutes at 4°C. The protein content of the supernatant, *i.e.* crude somatic extract (CE), was determined using protein assay reagents (Bio-Rad Chemical Co., USA) and bovine serum albumin (BSA) fraction V (Sigma Chemical Co., USA) as the standard. The dry weight of the preparation was also determined. The CE was kept in small aliquots at –70°C in lyophilized form until use.

Preparations of heterologous antigens

Heterologous antigens were prepared from various helminthes and protozoa as well as from stool samples of healthy, parasite-free individuals. They were used for screening of specific hybridomas to *S. stercoralis* antigens. The list of these antigens is given in Table 1.

Mouse immunization

Three adult BALB/c mice (6-8 weeks old at the start of immunization) were immunized intraperitoneally with CE. Blood samples were taken from the retro-orbital plexus of each mouse prior to the antigen injection and the serum samples were collected and subsequently used as negative controls. CE (100 μg) dissolved in 200 μl of normal saline solution (NSS) was emulsified in an equal volume of complete Freund's adjuvant (Sigma Chemical Co., USA); the emulsion was injected intra-peritoneally (IP) into each mouse. Four booster doses were given at two-week intervals with 200 μg of the same antigen emulsified in 200 μl of incomplete Freund's adjuvant (Sigma Chemical Co., USA). Two weeks after the fourth booster, the mice were bled via the retro-orbital plexus. The blood was collected and checked for antibody titers against the homologous antigen by using an indirect enzyme-linked immunosorbent assay[20] and Western blot analysis. The mouse with the highest antibody titer against the homologous antigen was chosen as a source of immune spleen cells for fusion with myeloma cells while the other immune mice were bled and their sera were pooled and used as positive control serum (PS). Three days before the cell fusion, a final intravenous (IV) booster was given to the selected mouse using the same dose of antigen in 0.2 ml of NSS.

An indirect ELISA for detecting mouse antibodies

An indirect ELISA was used for checking antibody titers in mouse immune sera, screening for antibodies in spent media of the hybrid cells, cross-reactivity checking of antibodies secreted by the hybridomas and for quantitative determination of monoclonal antibodies. It was performed as follows: antigen at 10 μg dry weight/ml carbonate-bicarbonate buffer (100 μl), pH 9.6, was added into each well of the microtiter plates; the plates were incubated at 4°C overnight. The unbound antigen was washed away using phosphate buffered saline, pH 7.4 (PBS), containing 0.05% Tween-20 (PBS-T). The uncoated sites were blocked with 1% bovine serum albumin (BSA), 0.2% gelatin in PBS, for 1 hour at 37°C. After washing as above, 100 μl of the individual antibody preparations were added to the appropriate antigen coated wells. Blank wells containing either PBS or spent medium of the P3x-63Ag8.-653 myeloma cells instead of the antibodies were included in each plate. The plates were incubated at 37°C for 1 hour, then 100 μl of 1:1,000

Table 1 Homologous and heterologous antigens used for checking cross-reactivity of the antibodies produced by hybridomas

Name of parasite	Developmental stage		Source
	Adult	Larva	
NEMATODES			
1. *Strongyloides stercoralis*	-	Filariform larvae	*In vitro* culture of rhabditiform larvae obtained from stools of infected children[15]
2. Human hookworms	-	√	*In vitro* cultured ova obtained from stools of infected children
3. *Gnathostoma spinigerum*	-	3rd stage larvae	Livers of naturally infected eels [25]
4. *Trichinella spiralis*	-	√	Experimentally infected mice [26]
5. *Angiostrongylus cantonensis*	√	-	Experimentally infected rats [27]
6. *Ascaris lumbricoides*	√	-	Stools of patients
7. *Toxocara canis*	√	-	Autopsy from dogs
8. *Dirofilaria immitis*	√	-	Autopsy from dog's heart
9. *Capillaria philippinensis*	√	-	Stools of infected individuals after purgation
CESTODES			
10. *Taenia saginata*	√	-	Stools of infected individuals after purgation
11. *Echinococcus granulosus*	-	√	Hydratid fluid
12. Cysticercus cellulosae	-	√	Atopsy from hill tribe swine
13. *Hymenolepis nana*	√	-	Stools of infected individuals after purgation
TREMATODES			
LIVER FLUKES			
14. *Ophisthorchis viverrini*	√	-	Hamsters experimentally infected with metacercariae[24]
15. *Fasciola gigantica*	√	-	Autopsy from cow
16. *Echinostoma malayanum*	√	-	Stools of infected individuals after purgation
17. *Echinostoma revolutum*	√	-	Stools of infected individuals after purgation
MINUTE INTESTINAL FLUKES			
18. *Haplorchis taichui*	√	-	Stools of infected individuals after purgation
BLOOD FLUKES			
19. *Schistosoma japonicum*	√	-	Mice infected with cercariae obtained from infected snails[28]
20. *Schistosoma mansoni*	√	-	Mice infected with cercariae obtained from infected snails[28]
21. *Schistosoma mekongi*	√	-	Mice infected with cercariae obtained from infected snails[28]
PROTOZOA			
22. *Toxoplasma* tachyzoites	√	-	Experimental infected mice
23. *Plasmodium falciparum*	√	-	Blood stage culture
24. *Plasmodium vivax*	√	-	Blood of infected individuals
25. *Entamoeba histolytica*	√	-	*In vitro* cultured trophozoites

goat anti-mouse immunoglobulin-horseradish peroxidase conjugate (Dakopatt, Glostrup, Denmark) was added and the plates were further incubated as above. After the final wash, 100 µl of freshly prepared peroxidase substrate was added to all wells. The enzymatic reaction was allowed to occur at room temperature in the dark for 30 minutes; the reaction was stopped by adding 50 µl of 1 N NaOH. The optical density (OD) of the content of each well was determined at 492 nm against that of the blank wells using an ELISA reader (Labsystem, Multiscan *Ex*, Finland). A result was declared positive when the read-out OD was ≥ 0.05. One indirect ELISA unit (EU) was the smallest amount of the antibody preparation that still

gave a positive result.

Hybridoma production

The P3x-63-Ag8.653 myeloma cells were used for hybridoma production. The cell line is a non-Ig producing variant. These particular cells lack the hypoxanthine-guanine phosphoribosyl transferase enzyme (HGPRT deficient) and thus they are resistant to 8-azaguanine. Hybridomas produced from these parental cells can be selected by hypoxanthine, aminopterin and thymidine (HAT) medium[21] or medium containing azaserine supplemented with hypoxanthine (HA medium). In preparation for the cell fusion, the selected immunized mouse was bled via the retro-orbital plexus; the serum sample was subsequently used as an immune serum (IS). The mouse was then sacrificed by cervical dislocation, the exterior of the mouse was soaked in 70% ethyl alcohol and the spleen was removed aseptically. The spleen was washed 3 times with RPMI-1640 in three petri-dishes. All fatty tissue was trimmed off and the spleen was minced in the serum free-RPMI medium to yield a single cell suspension [by filtering the cell preparation through a 70 μm-cell strainer (Falcon B ecton Dickinson Labware, USA) in order to remove any tissue debris and big clumps]. The cells were suspended in 20 ml of the RPMI-1640 medium in a 50 ml sterile tissue culture tube (Costar Co., USA), washed once by pelleting at 200 × *g* centrifugation and were then resuspended in 10 ml of the same medium before checking their viability by using the trypan blue exclusion method. Cells with over 95% viability were used for the cell hybridization.

Myeloma cells were added to a tube of spleen cells (the ratio of myeloma cells: spleen cells was approximately 1:10). The cells were gently mixed and centrifuged at 200 × *g* at room temperature for 10 minutes and the supernatant was removed. One and a half ml of a 50% solution (w/v) of PEG-4,000 (Sigma Chemical Co., USA) in NSS, prewarmed at 37°C, was added to the cell pellet with regular agitation to stir up the cells in the pellet. The tube was immediately transferred to a 37°C water bath with constant shaking for 90 seconds. The mixture was then gradually diluted by dropwise addition of 20 ml of serum-free RPMI-1640 medium over a period of 10-15 minutes while agitating the tube. The cell preparation was centrifuged at 200 × *g* at room temperature for 10 minutes and the supernatant was discarded. Warm hypoxanthine-azaserine selective medium was added to the cell pellet to achieve a cell concentration of approximately 5×10^5 cells/ml. The cell preparation was distributed in 200 μl aliquots into the wells of 96-well tissue culture plates (Costar, USA). The plates were incubated at 37°C in a humidified CO_2 incubator and inspected daily for cell growth by inverted microscope. Replacement of the used culture medium in the wells by fresh medium was performed at appropriate times. This was accomplished by careful removal of about 0.1 ml of the medium from each well and adding an equal volume of fresh medium. After 7-10 days and when the hybrids had grown sufficiently, 0.1 ml of the cultured supernatant from each well was collected into a properly labeled vial and tested by the indirect ELISA against the homologous antigen for the presence of antibodies. Cells from the antibody positive wells were subsequently cloned by limiting dilution technique using spleen cells of a non-immune BALB/c mouse as feeder cells. To perform the limiting dilution technique, the cells of the positive wells (polyclones) were mixed gently, then 10 ml of the cells were pipetted into 5 ml of RPMI medium supplemented with 10% BS to make approximately 20-200 cells/ml. The cell preparation was plated out (100 μl/well) into rows A and B of the feeder cell plate. The remaining volume of about 2.5 ml was diluted with 7.5 ml RPMI medium supplement with 10% BS and plated out (100 μl/well) into rows C and D of the plate making 5-50 cells/ml (0.5-5 cells/well). The remaining volume was diluted in the same way to a concentration of approximately 1.25-12.5 cells/ml and 0.3-3 cells/ml, respectively. One hundred μl aliquots of each cell suspension were plated in the rows E, and F and G, and H, respectively. The plates were incubated at 37°C in a humidified 5% CO_2 incubator. The growth of the clones were observed daily using an inverted microscope. Approximately one week after cloning, colonies were noticed. The spent medium from wells with single clones was

Table 2 List of parasites found in patients of group 2

Number of patients	Name of parasite
2	*Entamoeba histolytica*
3	*Giardia lamblia*
3	*Gnathostoma spinigerum*
5	Hookworm(s)
5	*Opisthorchis viverrini*
2	*Taenia saginata*
5	*Trichinella spiralis*

tested for specific antibodies by the indirect ELISA. The cells of which the spent medium showed positive antibody activity were transferred and expanded in 24-well tissue culture plates. The same strategy was used for recloning to ensure monoclonality of the hybridomas. Recloning was repeated until all wells in the plate contained single hybridoma colonies. Then the clones were nurtured further in 75 mm^3 tissue culture flasks. The cultures in the flasks were maintained for an experimental growth phase. The supernatants of the cultures were checked for cross-reactivity against heterologous antigens and determined for isotypes as well as for antibody titers against the homologous antigen using the indirect ELISA.

Characterization of antibodies secreted by the hybridomas

Western blot analysis

CE components that had been resolved in the polyacrylamide gel by electrophoresis were electroblotted onto a sheet of nitrocellulose paper (NCP, Costar Scientific Corp., USA)[22] After blotting, the empty sites on the NCP were blocked by soaking the membrane in a blocking solution (3% BSA, 0.5% gelatin in PBS) at room temperature with gentle rocking for 1 hour. The NCP was then washed to remove the excess BSA and gelatin with three changes of the washing buffer (PBS-T) over a period of 15 minutes. It was then placed in a solution of the antibody preparation (monoclonal antibodies; positive control serum; PS or immune mouse serum; IS) for 1 hour at room temperature on a rocking platform. Thereafter the NCP was washed three times with the washing buffer and then put in a solution of anti-mouse immunoglobulin-horseradish peroxidase conjugate (1:1,000 in PBS, pH 7.4) (Dakopatts, Glostrup, Denmark) for an hour at room temperature with continuous shaking. After rigorous washing (5 times for 5 minutes each) with the washing buffer, the NCP was placed in a freshly prepared substrate solution (0.2% 2, 6- sodium dichlorophenol indophenol in M/15 phosphate buffer, pH 7.6 with H_2O_2) until the bands of immune complexes appeared. Then the NCP was washed with DW until the background was cleared.

Isotyping of the monoclonal antibodies

Isotypes of MAbs were determined by Mouse Typer kit (Bio-Rad, USA) which could determine mouse isotypes and sub-isotypes into IgG1, IgG2a, IgG2b, IgG3, IgM, IgA, kappa (κ) and lambda (λ) chains by ELISA.

Indirect immuno-alkaline-phosphatase staining

The filariform larvae of *S. stercoralis* were obtained from the cultures of human feces containing *S. stercoralis* rhabditiform larvae by the polyethylene-tube method. They were fixed in a cold alcohol-formalin solution overnight, dehydrated with increasing concentrations of ethanol, cleared with xylene, and embeded in paraffin. Five μm-sections were prepared, mounted on glass slides, dried at 60°C for 10 minutes and stored at 4°C prior to use.[23] The sections were placed in an oven at 60°C for 30 minutes and deparaffinized immediately with two changes of xylene, passed through two changes of absolute ethanol and 95% ethanol (3 minutes each step). They were rinsed gently under running distilled water, soaked in 0.1 M PBS for 5 minutes and blocked with 20% normal rabbit serum and 1% bovine serum albumin (BSA Fraction V, Sigma Chemical Co., USA) in PBS, for 30 minutes in a humidified chamber at room temperature. The serum was then blotted away with filtered paper; 100 μl of the mouse monoclonal antibodies to be tested were applied directly to the worm sections on each slide and incubated at room temperature for 30 minutes in a humid box. Then the slides were gently rinsed with PBS and placed in three changes of the buffer bath for 3 minutes each. The rabbit-anti-mouse immunoglobulins-alkaline phosphatase conjugate (Dakopatts) at dilution 1:1,000 in 0.2% BSA (about 100 μl) was added to cover the section. The preparation was kept for 30 minutes at room temperature, and the slides were then washed as above and placed in 0.15 M Tris buffer, pH 9.6. The substrate solution was prepared by mixing one volume BCIP/NBT (Kirkegaard and Perry Laboratories, USA) with two volumes of the 0.15 M Tris buffer, pH 9.6 and 100 μl of this substrate solution was applied onto each worm section on slides. After the slides were kept at room temperature for 30 minutes in the dark, the reaction was stopped by rinsing the slides with distilled water. [24]

Monoclonal antibody based-dot-ELISA for detecting *S. stercoralis* antigen

CE of the filariform larvae was serially diluted in a two-fold manner from 2 mg/ml to 7.8 μg/ml in phosphate buffered saline, pH 7.4. All antigen dilution at 2.5 μl aliquots were individually dotted onto a strip of NCP (5 μg/dot to 0.0195 μg/dot) and allowed to air-dry. A negative control, consisting of extracts of other parasites was included on the same NCP strip. The NCP strip was placed in a blocking solution (3% BSA, 0.5% gelatin in PBS, pH 7.4) at room temperature for 30 minutes, and was then washed with washing buffer three times. The NCP was incubated with MAb for 1 hour at room temperature, washed as above,

and incubated with rabbit anti-mouse Igs-alkaline phosphatase conjugate (1;1,000) for 1 hour at room temperature. The NCP was washed thoroughly with the washing buffer as above and then once with the substrate buffer before incubating with freshly prepared substrate solution until purplish blue spots were clearly seen. The reaction was stopped by rinsing the NCP with DW. Positive results could be identified with the naked eye, as purplish blue spots, while the negative reaction remained colorless.

Fecal samples and detection of *S. stercoralis* coproantigen

Forty fecal samples were collected from three groups of individuals; 10 patients with parasitologically confirmed *S. stercoralis* only (group 1), 25 patients with other parasitic infections (group 2) (Table 2) and 5 normal individuals whose stool samples contained no parasites at the time of the sample collection (group 3). For detection of the parasites, all stools were examined using a stool egg count and formalin-ether concentration methods. Stool samples of each subject were collected and examined individually on three consecutive days and two smears of each sample were examined. All stool samples were also subjected to the polyethylene tube culture method and other aliquots of the ones that were positive for the *S. stercoralis* filariform larvae were included in group 1.

Fecal samples of all three groups were extracted individually using 0.1 M-citrate buffer, pH 5.0, contained in 5% bovine serum albumin (BSA). To 1 g of specimen, 4 ml of the buffer were added, mixed thoroughly and then centrifuged at 200 × *g* for 10 minutes. The supernatant was collected and stored in 1 ml aliquots at –20 °C until used in the dot-ELISA for detecting *S. stercoralis* antigen.

RESULTS

After the fifth intraperitoneal injection with the immunogen, individual mice were bled and their serum samples were tested by the indirect ELISA against the homologous antigen. It was found that mice no. 1, 2 and 3 had ELISA titers of 1:6,400, 1:12,800 and 1:6,400, respectively. Mouse no. 2 was used as the source of immune splenocytes for the hybridoma production while mice no. 1 and 3 were bled and their serum samples were collected, pooled and used as positive control serum (PS).

A total of 1.55×10^8 spleen cells were recovered from the no. 2 mouse; they were fused with 1.55×10^7 myeloma cells using 50% PEG 4,000 in NSS as a fusogen. Aliquots of 200 µl containing 10^5 cells were distributed into wells in 96 wells-tissue culture plates. From a total of 1,840 wells, 1,395 wells (75.81%) contained growing hybrids; among them cells in 24 wells were positive for antibodies to *S. stercoralis*. Cells from wells which grew well and the spent culture medium gave an optical density of the indirect ELISA higher than 0.1 were monocloned by limiting dilution method. Spent medium of the growing hybrids were checked for cross-reactivity of the monoclonal antibodies against the panel of heterologous antigens listed in Table 1 and also the extracts of stool samples from parasite free-individuals. Finally, two monoclones (hybridomas) of which the secreted monoclonal antibodies did not cross-react to any of the heterologous antigens, were obtained. They were designated clones 19F11 and 4C8. Both clones secreted κ light chains and γ1 heavy chains. At the maximum phase of growth, the indirect ELISA titer of the spent culture medium of both clones was 1:64 against the homologous antigen.

Fig. 1 shows Western blot results of the IS and monoclonal antibodies (MAb) secreted from clone 19F11 which was selected for further study on the basis of its rapid growth as compared with the clone 4C8 which grew slower. MAb of clone 19F11 did not reveal any immune complex band against the SDS-PAGE separated-CE of *S. stercoralis* filariform larvae while the IS revealed several bands against the same.

Fig. 2 shows the immuno-alkaline phosphatase reactivity of MAb 19F11 on sections of filariform larvae of *S. stercoralis*. Although the MAb 19F11 did not show bands in the WB, they reacted readily *in situ* with epitopes of the *S. stercoralis* larvae. The reaction was found mainly at internal structures, *i.e.* intestine; however, the contents of the esophagus of the filariform larvae were unstained.

The MAb 19F11 based-dot ELISA was used to determine the concentration of *S. stercoralis* antigen using specific MAb. CE of the filariform larvae, serially diluted in a two-fold manner from 5 µg/dot to 0.019 µg/dot in PBS were spotted (2.5 µl per dot) onto a strip of NCP. The smallest amount of the CE which gave a positive dot-ELISA was 78 ng (Fig. 3).

When the MAb based-dot-ELISA was used to detect *S. stercoralis* antigen in the stool samples of all the subjects of the three groups, none of them gave a positive result.

DISCUSSION

Strongyloidiasis is a potentially dangerous parasitic infection of humans especially in the immunocompromised host.[29] Conventional diagnostic methods for parasitic infections, *e.g.* direct stool smear or formalin-ether concentration method,

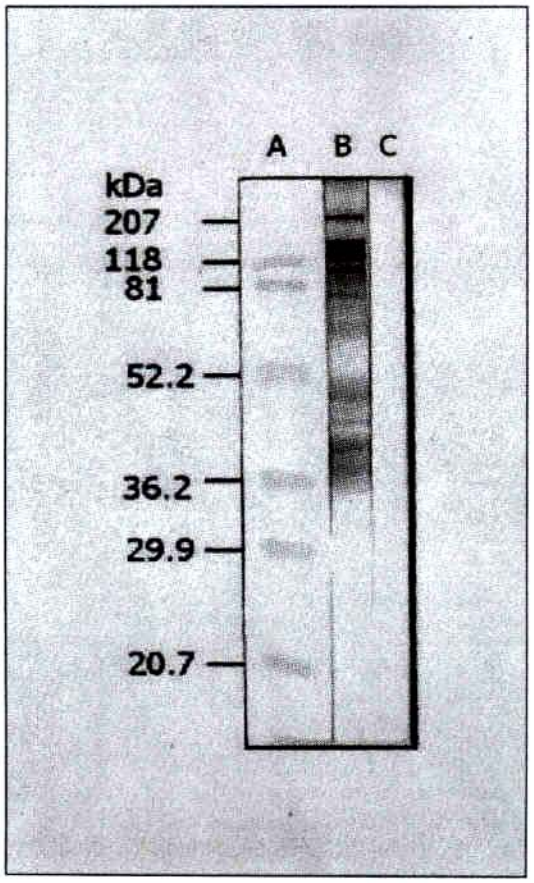

Fig. 1 Western blot showing patterns of immune mouse serum (IS) and MAb of hybridoma clone 19F11 (MAb 19F11) against SDS-PAGE separated-extract of filariform larvae of *S. stercoralis* (CE). Lane A, Molecular weight markers (kDa); Lane B, Immune mouse serum (IS); Lane C, MAbs of hybridoma clone 19F11. The MAb did not react with the CE in WB (no reaction is seen)

are often insensitive owing to a scant and inconsistent presence of the parasite larvae in the stool samples of the majority of infected patients.[29,30] For a higher accuracy, repeated examinations of several aliquots of several stool samples obtained from the same individual on several consecutive days must be done.[29] Other methods, such as duodenal intubation with biopsy and Enterotest™ may be used to collect the patient's specimens which are then subjected to parasitological diagnostic methods such as the Baermanized technique and culture. These procedures have been reported to be more sensitive than the conventional direct stool smear method.[30] However, such treatments are cumbersome and unpleasant to the patients which prevents their routine use.[30] Diagnosis by antibody detection, *e.g.* indirect ELISA,[1,15,16,17,29,31] has been hampered by the immunological cross-reactivity among helminthes. The assay's sensitivity and specificity vary depending upon the kind of antigens use and the standard method to which the developing assay is compared, amongst many other attributes. The DNA techniques, *e.g.* PCR and DNA hybridization, are laborious, expensive and may be insensitive, owing to the presence of inhibitors for PCR in the specimens. As such, alternative methods have been sought for strongyloidiasis diagnosis. One approach is an antigen detection assay using a specific antibody as an antigen capture tool.

In this study, a hybridoma secreting monoclonal antibodies specific to antigen of *S. stercoralis* filariform larvae was produced through fusion of splenocytes of a mouse, while was immune to the filariform larval antigen, and mouse myeloma cells. The hybrid secreted kappa, IgG1 immunoglobulins, which is the isotype known to have a high binding affinity to their epi-

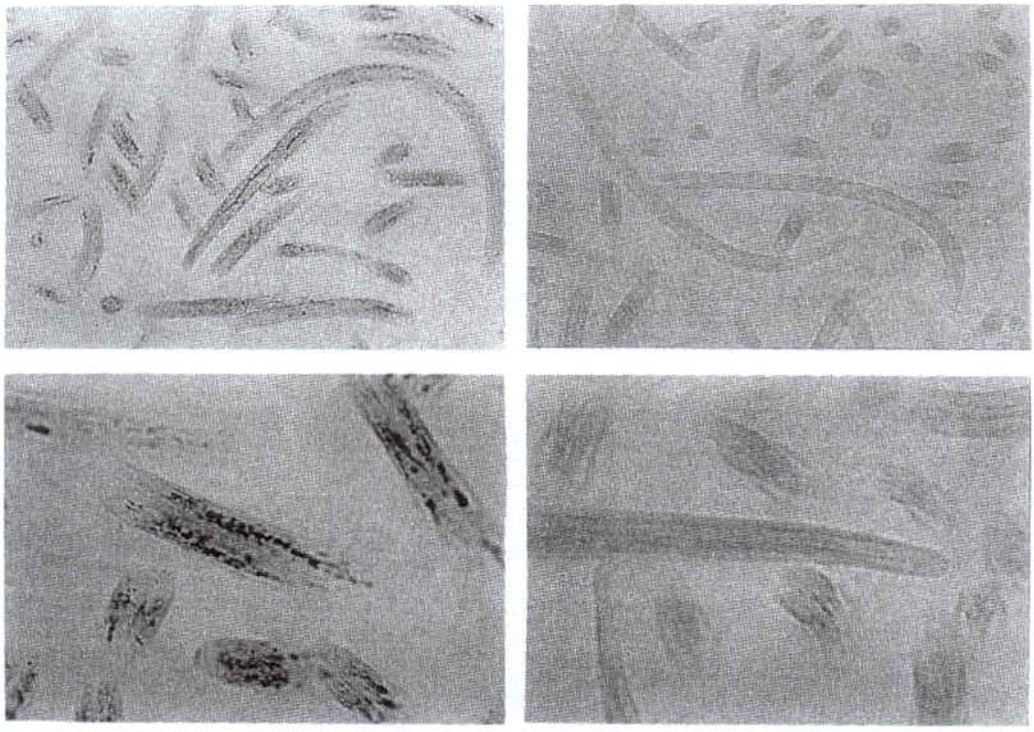

Fig. 2 Immuno-alkaline phosphatase reactivities of MAb 19F11 on sections of filariform larvae of *S. stercoralis*. A, Section of filariform larvae incubated with MAb 19F11 (400×); B, Section of filariform larvae incubated with PBS (negative control) (400×); C, Section of filariform larvae incubated with MAb 19F11 (1,000×); D, Section of filariform larvae incubated with PBS (negative control) (1,000×).

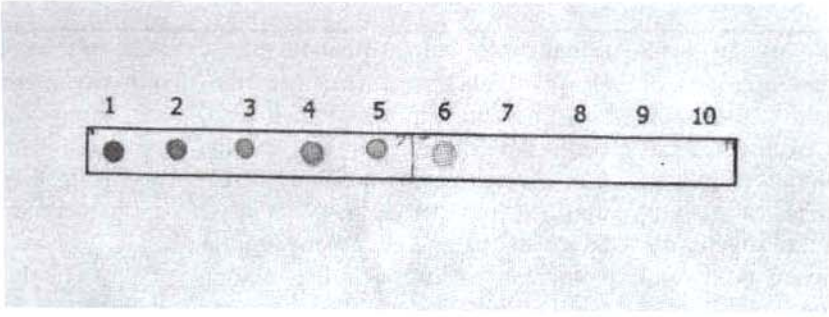

Fig. 3 MAb-based dot-ELISA for detection of various amounts of CE. 1-9, 5 μg/dot-0.019 μg/dot, respectively; 10, negative control. The lowest amount of CE which could be detected by the dot-ELISA was 78 ng.

topes. The hybrid was stable, *i.e.*, at the stationary phase of cell growth of several subcultures, the spent culture fluids consistently yielded an indirect ELISA titer of 1:64 to the homologous antigen. However, the antibodies were non-reactive in Western blot analysis against the SDS-PAGE separated- homologous antigen, in spite of the fact that the immune serum (IS) of the same mouse from which the hybridoma acquired its antibody encoding gene was reactive and revealed several bands with the same antigen (Fig. 1). The different reactivities of the MAb observed in the indirect ELISA and in the Western blot analysis can be explained. In the latter, the CE in the SDS solution containing a reducing reagent, *i.e.* 2-mercaptoethanol, was heated to 100°C for a few minutes prior to subjecting to electrophoresis. Such treatment may denature the epitopes by destroying the conformational configuration required for specific binding to the paratopes on the respective antibody molecules rendering them non-reactive in the WB. The natural configuration of the epitopes required for paratope binding are better preserved in the CE used in the indirect ELISA than in the WB. Moreover for WB, after the SDS-PAGE, the separated components were electrotransblotted onto the nitrocellulose membrane before reacting with the antibody. It is unknown how this treatment affects the epitope orientation on the membrane; such procedure may or may not alter the physical properties such as hydrophobicity versus hydrophilicity of the components. Usually, the reaction of monoclonal antibodies and polyclonal antibodies, such as the IS, to an antigen are different. Antisera, like our IS, which were raised against the native proteins, almost always contain at least some antibodies which recognize denatured antigens while the rest are reactive to the natural epitopes. It is most likely this fact that allows the polyclonal antibodies to function in the WB. In marked contrast to the polyclonal antibodies, the monoclonal antibodies against the native antigens may or may not recognize the denatured product. If they were not reactive to the denatured product, such as after the SDS-PAGE, no antigen-(monoclonal) antibody reaction would result.[32] However, data concerning this point in the literature are scarce, but it would be expectable that polyclonal antibodies (like the IS) are more reliable in the WB than monoclonal antibodies (like the MAb 19F11).

The MAb 19F11 were reactive to the intestinal content of filariform larvae of the parasite (Fig. 2). The epitope is protein in nature (the proteinase-K treated-CE did not react to the MAb 19F11 in the indirect ELISA; data not shown). The epitopes are not found in the esophagus of the worm and only exist in small quantities *in situ* (Fig. 2). This might be one of the reasons why the MAb based-dot-ELISA was negative in all of the parasitologically confirmed strongyloidiasis stools tested. The lowest limit of the dot-ELISA for CE was 78 ng (the amount of epitopes in this amount of CE is not known). Moreover, the epitopes may not be evenly dispersed in the patient's stool. Aliquots used for examining for parasites and the aliquot collected for antigen detection were different. The negative antigen test results may also be due to the filariform larval-stage specificity of the MAb, while most antigen in the stools of the infected individuals would be from rhabditiform larvae. In this study, the stool samples which were used in the dot-ELISA were mixed individually with 0.1 M citrate buffer, pH 5.0, containing 5% BSA. The acid buffer was intended to decrease the activity of pH-specific intestinal proteases while the BSA served as excess protease substrate. The solution was one of the two best stool diluents recommended by Viscidi *et al.*[33] The fecal antigen might be even more diluted by the buffer treatment. Moreover, the stool extracts were subsequently stored at –20°C for many months before used in the antigen detection by the dot-ELISA. All of the above mentioned conditions could account for the insensitivity of the antigen test. It is expected that the sensitivity of the assay could be improved by using fresh stool samples and immunological test versions where a higher volume of the antigenic preparation can be tested, *e.g.* sandwich ELISA performed in ELISA plates (where upto 250 µl of sample can be used instead of the 2.5 µl used in the dot-ELISA). Thus, eventhough the antigen detection using the MAb based-dot-ELISA was not successful in the present study, the MAb so-produced still has a diagnostic potential.

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