Identification and partial characterization of a myosin-like protein from cysticerci and adults of *Taenia solium* using a monoclonal antibody

J. AMBROSIO[1]*, M. CRUZ-RIVERA[1], J. ALLAN[2], E. MORÁN[1], K. ERSFELD[2] *and* A. FLISSER[1]

[1] *Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, México, 04510 D.F.*
[2] *Department of Biological Sciences, University of Salford, Salford M5 4WT, UK*

(*Received 2 October 1996; revised 21 November 1996; accepted 21 November 1996*)

SUMMARY

The host–parasite relationship in taeniosis due to *Taenia solium* is practically unknown. Monoclonal antibodies were prepared against whole extracts of adult *T. solium* parasites and evaluated with tapeworms recovered from experimentally infected hamsters and with cysticerci from naturally infected pigs. With one antibody, mAb 4B3, it was possible to identify, purify and partially characterize a *T. solium* myosin. Some findings indicate that it corresponds to conventional myosin or myosin type II such as: purification with KCl, high molecular weight, size, structure (dimeric protein with globular and long tail portions), reaction with commercial anti-myosin antibodies, distribution in muscle fibres of parasites and cross-reactivity with antibodies against paramyosin from *T. solium* cysticerci. The reaction of the mAb was only with taeniids and not with other parasites. Also myosin was detected in faeces of infected animals and in supernatants of parasite cultures. Its presence in biological fluids may be useful for diagnosis of infected hosts.

Key words: cysticerci, immunofluorescence, myosin, *Taenia solium*, taeniosis, tapeworms.

INTRODUCTION

Taenia solium (Cestoda) causes 2 diseases: cysticercosis and taeniosis. The former is a public health problem in many developing countries, while the latter is a benign asymptomatic disease that, although it is the main risk factor for acquiring cysticercosis (Sarti *et al*. 1992), is not considered a public health problem. There are practically no studies of taeniosis probably because the only host is man. A few years ago an experimental model of taeniosis was developed in hamsters (Allan *et al*. 1991) mainly with the purpose of standardizing a better diagnosis of this tapeworm infection by an ELISA for detection of antigen in faeces (Allan *et al*. 1990). This assay resulted in a high sensitivity and a good specificity except that it does not discriminate *T. solium* from *T. saginata*. In order to further increase the specificity we have prepared monoclonal antibodies (mAb) against adult *T. solium*.

Myosin and actin, the classical muscular proteins have not been identified and characterized in cestodes and their role in the host–parasite relationship is unknown (Smyth & McManus, 1989). There are indirect data from morphological observations which indicate that the distribution of several muscle fibres is related to evagination of cysticerci (Slais, 1982) and to fixation and stabilization of adult parasites to the mucosa in the presence of intestinal movements (Lumsden & Hildreth, 1983). Also, muscle fibres participate in proglottid movement for their release from the host and probably for the release of eggs (Lumsden & Hildreth, 1983; Smyth & McManus, 1989). Here we report the characterization of myosin from *T. solium* identified with mAb 4B3.

Only 1 protein has been thoroughly studied in *T. solium*: Antigen B (AgB) because it is immunodominant in human neurocysticercosis (Flisser, Woodhouse & Larralde, 1980; Guerra *et al*. 1982; Laclette, Merchant & Willms, 1987). The C terminal portion inhibits complement activity because it binds to C1q (Laclette *et al*. 1992) and the N terminal induces a strong antibody response (Laclette, J. P., personal communication), Ag B binds to collagen (Plancarte, Flisser & Larralde, 1983), is apparently secreted (Laclette *et al*. 1987) and is present on other cestodes (Olivo, Plancarte & Flisser, 1988). Furthermore a great similarity with paramyosin has also been described (Laclette *et al*. 1991) and, in the present study, cross-reactivity of AgB with mAb 4B3 was found.

* Corresponding author: Departamento de Microbiología y Parasitología, Facultad de Medicina, Edificio A, 2^{0}. Piso, Ciudad Universitaria, México, 04510 D.F.
Tel: +525 6232467. Fax: +525 6232382.
E-mail: jrah@servidor.unam.mx.

https://doi.org/10.1017/S0031182097008822 Published online by Cambridge University Press

MATERIALS AND METHODS

Experimental model

Golden hamsters (*Mesocricetus auratus*), 10 weeks old of both sexes were treated with 30 mg/kg of praziquantel (Cysticid, Merck) 1 week before they were infected with cysticerci. Animals were maintained with sterilized acid tap water *ad libitum* and fed with commercial rodent balanced food (Nutricubos para animales de laboratorio, Ralston Rations). Infection of animals was performed by oral administration of 5 *T. solium* cysticerci recovered from fresh pork meat. In order to maintain the infection hamsters were immunosuppressed with 2 mg of methyl prednisolone acetate per animal (Depomedrol, UpJohn) administered at days 1 and 15 after infection. At 30 days post-infection hamsters were humanely euthanized with ether and intestinal tapeworms were recovered and used as required. Faeces from non-infected and infected hamsters were collected weekly post-infection and kept frozen until use.

Parasite tissues

Cysticerci were obtained from pigs and adult parasites were recovered from hamsters. Parasites were washed exhaustively with phosphate-buffered saline, pH 7·2 (PBS), whole cysticerci, the scolex and some proximal and distal proglottids were covered with an embedding solution (Tissue-Tek Miles Scientific Laboratories), immediately frozen by immersion in liquid nitrogen and stored at −20 °C until use. For immunofluorescence studies sections 7–10 μm were obtained in a cryostat (American Optical Corporation) at −20 °C.

Parasite extracts

Taenia solium tapeworms and cysticerci as well as adult *T. saginata* worms were washed 5 times with PBS, homogenized with 3 M KCl in a chilled Polytron (Brinkman Instruments), extracted at 4 °C with slow stirring and centrifuged at 2000 ***g*** for 30 min at 4 °C. The supernatant was recovered, dialysed against PBS, centrifuged at 24000 ***g*** for 60 min at 4 °C, filtered with a 0·22 μm pore membrane and concentrated under positive pressure with nitrogen (Flisser *et al.* 1975; Allan *et al.* 1990). Protein concentration in each extract was measured (BioRad Protein Assay) and antigens were kept in aliquots at −20 °C until use. Protease inhibitors (1·1 mM *p*-hydroxymercuribenzoate, 3·4 mM phenylmethylsulfonylfluoride, 2·5 mM ethylenediaminomethane tetraacetic acid, EDTA) were added to extracts during preparation. Crude parasite extracts of adults of *T. saginata*, *Fasciola hepatica*, *Macracanthorynchus hirudinaceus* and *Ascaris suum* were obtained from G. Avila, UNAM, México following the method described for cysticerci as well as for the extracts from *T. taeniaeformis* cysticerci and adults that were obtained from A. Plancarte, UNAM, México. Parasite extracts from *Trichinella spiralis* were obtained from D. Correa, INDRE, México and *Leishmania mexicana* parasites were obtained from E. Melendro, UNAM, México.

Excretion/secretion antigens from adult parasites

Fresh *T. solium* adults were washed 5 times with sterile PBS mixed with a diluted antibiotic–antimicotic solution (Gibco) and kept *in vitro* using RPMI 1640 medium (Gibco) supplied with HEPES buffer (0·2 %), glutamine (2 mM), sodium pyruvate (1 mM) and sodium bicarbonate (0·007 %) during 2 days under standard conditions (37 °C, 5 % CO_2 and 95 % relative humidity). Afterwards culture medium was removed and concentrated (× 10) under positive pressure, the protein concentration was measured and the solution was kept at −20 °C in aliquots. Excretion/secretion (ES) from *T. solium* cysticerci were obtained from D. Correa, INDRE, México.

Myosin isolation

Parasite extracts and ES were processed in order to obtain semi-purified myosin (Dowben, 1969; Giese, 1973). Extracts were mixed with 0·2 M NaCl, homogenized and centrifuged (12000 ***g***) during 30 min at 4 °C. Pellets were recovered in ice in 0·6 M KCl, pH 6·5 and centrifuged as above. Supernatants were recovered and used as the myosin solution. To obtain myosin from ES, culture medium was mixed with 0·6 M KCl and centrifuged as above. Protein concentration was determined and myosin solutions were kept at −20 °C in aliquots.

Myosin antigenic analysis

The specificity of myosin was tested in ELISA using the myosins obtained from ES products and extracts of *T. solium* and from *T. saginata* adults as well as one commercial myosin from porcine muscle (Sigma). Three different antibodies were used: mAb 4B3 which was produced against *T. solium* adults (see below), a polyclonal antibody against *T. solium* AgB donated by A. Landa, UNAM, México and a commercial anti-myosin (smooth and skeletal muscle from bovines) antiserum produced in rabbits (Sigma).

Myosin structure

Electron transmission microscopy was performed with purified filaments of myosin. For this, semi-purified myosin was mixed with a low salt buffer

https://doi.org/10.1017/S0031182097008822 Published online by Cambridge University Press

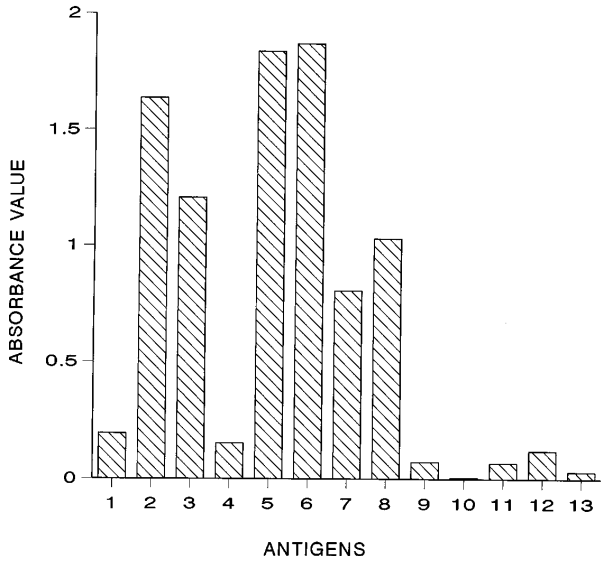

Fig. 1. Specificity of mAb 4B3 for several parasite antigens as tested by ELISA. *Taenia solium* cysticerci (1), tapeworms obtained from humans (2) and from infected hamsters (3), ES of cysticerci (4) and tapeworms (5), *T. saginata* adults (6), *T. taeniaeformis* cysticerci (7) and adults (8), *Fasciola hepatica* (9), *Trichinella spiralis* (10), *Macracanthorhynchus hirudinaceus* (11), *Ascaris suum* (12) and *Leishmania mexicana* (13). Antigens were adsorbed to ELISA plates and incubated with the supernatant of cultured hybridoma 4B3. Reactions were developed with anti-mouse IgG coupled to peroxidase, OPD and H_2O_2. Absorbance values were obtained at 492 nm.

solution (2-mercaptoethanol, EDTA and distilled water), extracted overnight at 4 °C and centrifuged in an Eppendorf microfuge at 13000 ***g*** for 30 min at 4 °C (Hermanson, Laframboise & Daood, 1991). The pellet containing purified myosin filaments was dissolved in 50 μl of PBS and an aliquot was stained with 1 % uranyl acetate on a copper support (300 mesh) previously treated with Formvar membrane and evaporated charcoal and observed in an electron transmission microscope (JEOL JEM-1200 EXII) at $\times$ 40000–60000 using 100 kV.

Monoclonal antibodies against adult T. solium

Monoclonal antibodies were prepared according to Goding (1983), Harlow & Lane (1988) and Craig, MacPherson & Nelson (1986) with minor modifications. Balb/c mice were immunized i.p. with 0·2 mg of an adult *T. solium* extract obtained from hamsters using Freund's complete (once) and incomplete (twice) adjuvant (Sigma) and a final intravenous booster with the extract. After 4 immunizations (10–14 day intervals) spleen cells were removed and fused with murine myeloma cells (X63/Ag8.653) using polyethylene glycol 4000 (Gibco). Optimem I culture medium (Gibco) complemented with 5 % inactivated foetal bovine serum (FBS, Gibco), 1 % antibiotics (streptomycin/penicillin 1000000 I.U.), 2 mM L-glutamine, 0·0045 mM azaserine, 0·075 mM hypoxanthine (HAT medium) were used to culture cells for 3 weeks under standard conditions (37 °C, 95 % relative humidity and 7 % CO_2). Surviving cells were cultured for 2 more weeks in the same complemented medium but without azaserine. Afterwards, the supernatant was recovered and tested by ELISA as described below. Cells of selected wells were cloned by limiting dilution and maintained in Optimem medium complemented with 5 % FBS and 1 % antibiotics. Cells were stored frozen in liquid nitrogen. All further assays were performed with culture supernatants as source of mAb.

Characterization of mAb 4B3

mAb 4B3 was selected because of its high recognition of adult *T. solium* antigens. Immunoglobulin (Ig) class and subclass were determined by ELISA using Immulon IV immunoplates (Dynatech Laboratories), culture supernatants were used as antigen, PBS–Tween 20 as blocking agent and several commercial anti-mouse Ig antibodies conjugated with peroxidase (1:500, Sigma). Reactions were developed with 5-aminosalicylic acid (Sigma) and 0·005 % (v/v) H_2O_2. Absorbance values were obtained at 450 nm after 25 min.

Specificity of mAb 4B3

ELISA was performed with antigenic extracts obtained from *T. saginata*, *T. taeniaeformis*, *F. hepatica*, *T. spiralis*, *M. hirudinaceus* and *A. suum* at 25 mg/ml and, in the case of *L. mexicana*, with 10 million parasites/ml which were adsorbed to Maxisorb (Nunc) plates overnight. Plates were blocked with 3 % commercial unfatted milk (Sveltys) in PBS–Tween 0·3 %, washed 3 times with PBS–Tween, incubated with mAb supernatants for 1 h at room temperature, washed again, incubated for 1 h with a commercial anti-mouse Ig antibody conjugated with peroxidase (1:500), washed and developed with substrate solution (0·4 mg/ml) *o*-phenylenediamine (OPD, Sigma) diluted in citrate buffer with 30 % (v/v) H_2O_2. Reaction was stopped with 2 M sulfuric acid after 5 min and absorbance values were measured at 492 nm.

Antigens recognized by mAb 4B3

Antigen characterization was performed by Western blot. *T. solium* extracts were separated by electrophoresis in SDS–polyacrylamide gels (5 and 10 %) under reduced or non-reduced conditions (Mighty Small SE250, Hoeffer) during 2 h at 100 V as described (Harlow & Lane, 1988). The gel was transferred to nitrocellulose membrane (NCM)

https://doi.org/10.1017/S0031182097008822 Published online by Cambridge University Press

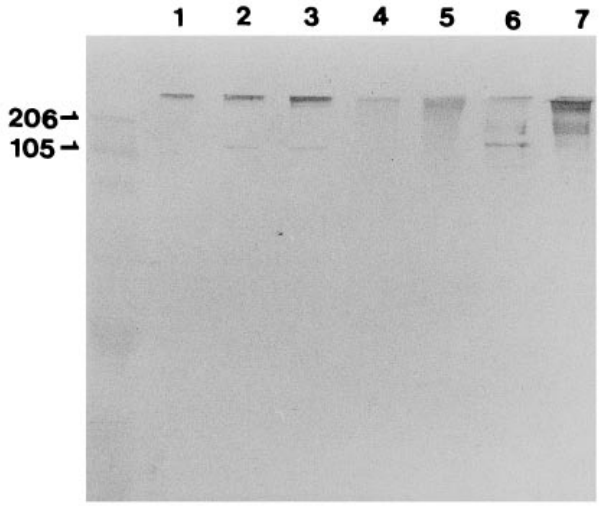

Fig. 2. Western blot characterization of *Taenia solium* parasite antigens by mAb 4B3. Whole extracts of adults recovered from humans (1, 2, 3), ES of adults from hamsters (4, 5) and extracts of cysticerci (6, 7) were fractionated using 10% SDS–PAGE gels under non-reduced (odd lanes) or reduced (even lanes) conditions. Western blot was performed on NCM and developed using antibodies as described for ELISA with diaminobenzidine as substrate. High range pre-stained protein molecular weight standards were used (the 2 highest bands are indicated).

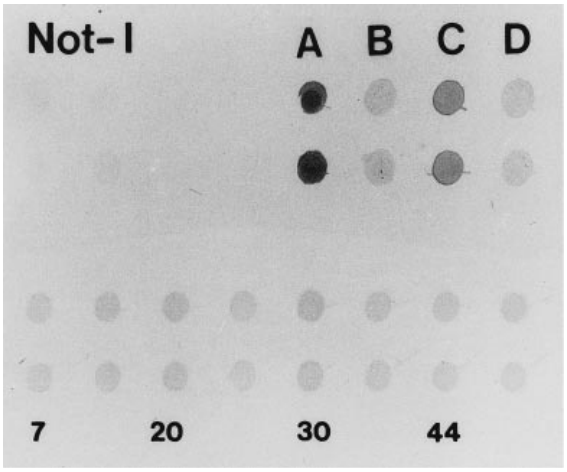

Fig. 3. Detection of secreted faecal antigens by mAb 4B3. Faecal supernatants corresponding to days 7, 14, 20, 27, 30, 39, 44 and 50 post-infection from infected and from non-infected hamsters (Not-I) were analysed by dot blot. As positive controls, extracts of tapeworms from humans (A), hamsters (B), ES from adults (C) and cysticerci (D) were used. Antibodies and substrates were used as in ELISA.

(Schleicher & Schuell, 0·2 μm, Hoeffer transfer equipment) at 100 V for 1 h according to the method described by Towbin, Staehelin & Gordon (1979), with recommended modifications (Harlow & Lane, 1988). High range pre-stained molecular weight markers were used (Gibco). Strips were processed as for the ELISA described above but the substrate used was 3,3′-diaminobenzidine (DAB, Sigma) solution (0·5 mg/ml) with 30% (v/v) H_2O_2. After 5 min incubation the reaction was stopped.

Detection of selected antigens with mAb 4B3

Dot blot assays were performed according to Chaicumpa *et al.* (1992). ES and faeces from infected and non-infected hamsters were used as samples (5–10 μl) and adsorbed to NCM using a dot apparatus (BioRad). Faeces were treated with PBS–0·3% Tween as described by Allan *et al.* (1990) for the ELISA for coproantigen detection. Non-specific reaction between secondary antibody and faecal samples was eliminated by adsorption of non-infected hamster faecal supernatants with conjugated anti-mouse IgG (3 times). Antigen samples adsorbed to NCM were processed as described for ELISA but using shorter incubation times (30 min).

Immunohistochemical recognition of parasite tissue

Immunofluorescence assays were performed following similar steps to the ELISA. Sections were blocked with PBS-unfatted milk, incubated in a humid chamber with mAb for 1 h, washed and further incubated with anti-mouse IgG coupled to fluorescein isothiocyanate (IgG–FITC, 1:50, Sigma) in the presence of Evan's blue (1:5000) as a counter-stain. Observations were made in an epifluorescence microscope (Nikon) using ×10, 40, 60 and 100 objectives and filters with a range between 490 and 517 nm. All observations were registered on colour film (Fujicolor) ASA 1600.

RESULTS

The mAb 4B3 was IgG1 that recognized antigens from *T. solium* ES, cysticerci and adults, *T. saginata* and *T. taeniaeformis* but not with trematodes such as *F. hepatica*, nematodes such as *T. spiralis*, *M. hirudinaceus* and *A. suum* or the protozoan parasite *L. mexicana* (Fig. 1). Recognition by Western blot or mAb 4B3 of antigens from adult, cysticerci and ES from *T. solium* showed that mAb reacted mainly with 2 bands, one above 206 kDa and a thin band at 105 kDa. Recognition did not change in reduced or non-reduced conditions (Fig. 2). Faecal supernatants from infected hamsters also reacted with mAb 4B3 (Fig. 3). Immunohistochemical results are shown in Figs 4 and 5. In a low magnification of a cysticercus section, fluorescence was located mainly in the bladder wall and in the spiral canal (Fig. 4A) while at high magnification muscle fibres located in the spiral canal with an ordered distribution of muscle fibres (Fig. 4B) and below the tegumental surface in the bladder wall were stained without any ordered

https://doi.org/10.1017/S0031182097008822 Published online by Cambridge University Press

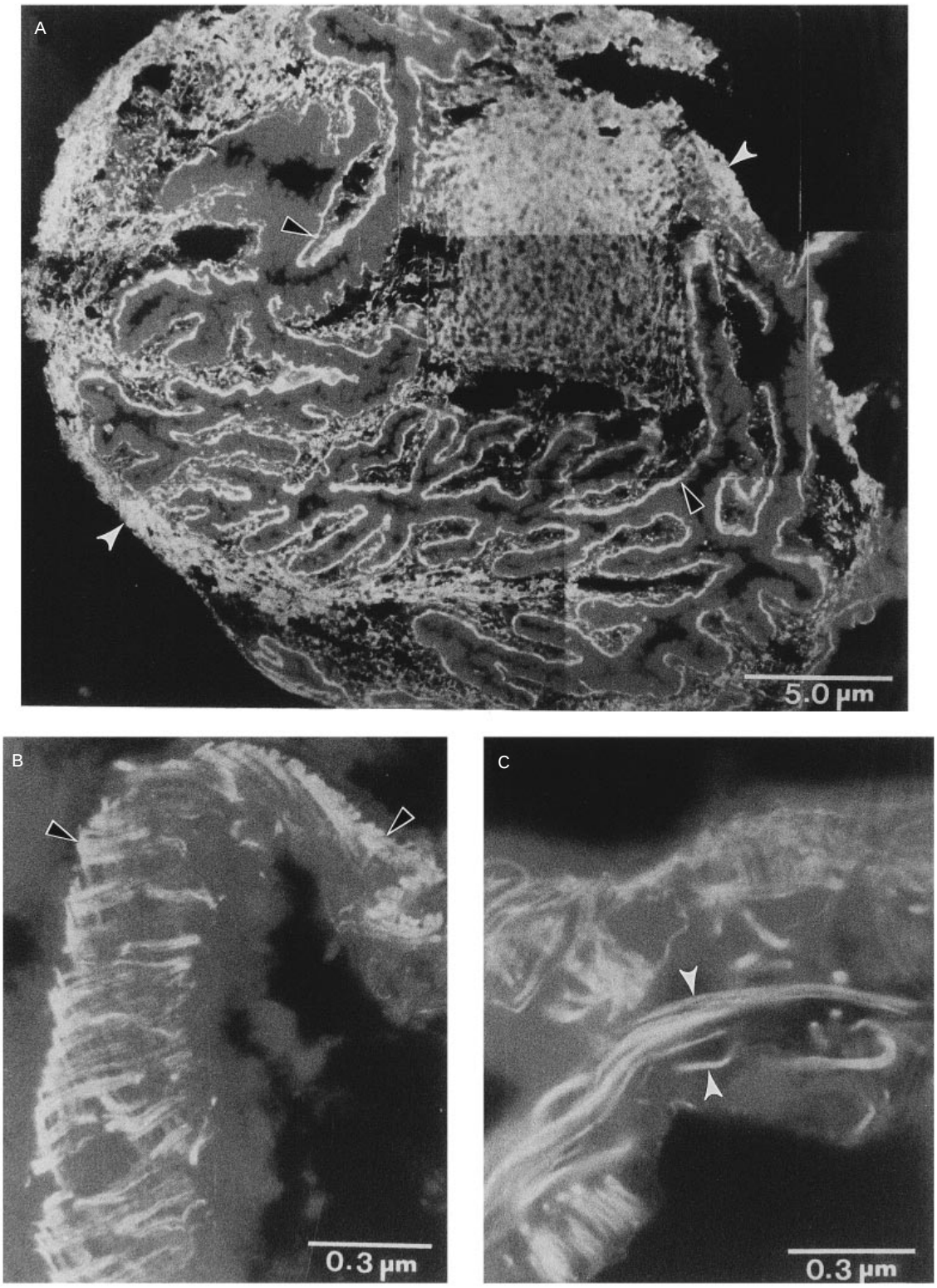

Fig. 4. Recognition of mAb 4B3 to parasite tissue. (A) Photographic composition in order to show a whole section of a cysticercus. Intense stain is seen in the spiral canal (black arrows with white border) and in the cysticercus tissue (white arrows). (B) Closer observation of the spiral canal (black arrows with white border). (C) Closer observation of the vesicular bladder wall (white arrows). The intense stain is specific for the mAb and the pale diffuse stain corresponds to background tissue.

distribution (Fig. 4C). In the adult parasite fluorescence was intense in the suckers where a random distribution of aggregated fluorescence was seen (Fig. 5A and B); in the neck fluorescence was located in long filaments that apparently connect the scolex to strobila (Fig. 5C) and intense stain was also seen

https://doi.org/10.1017/S0031182097008822 Published online by Cambridge University Press

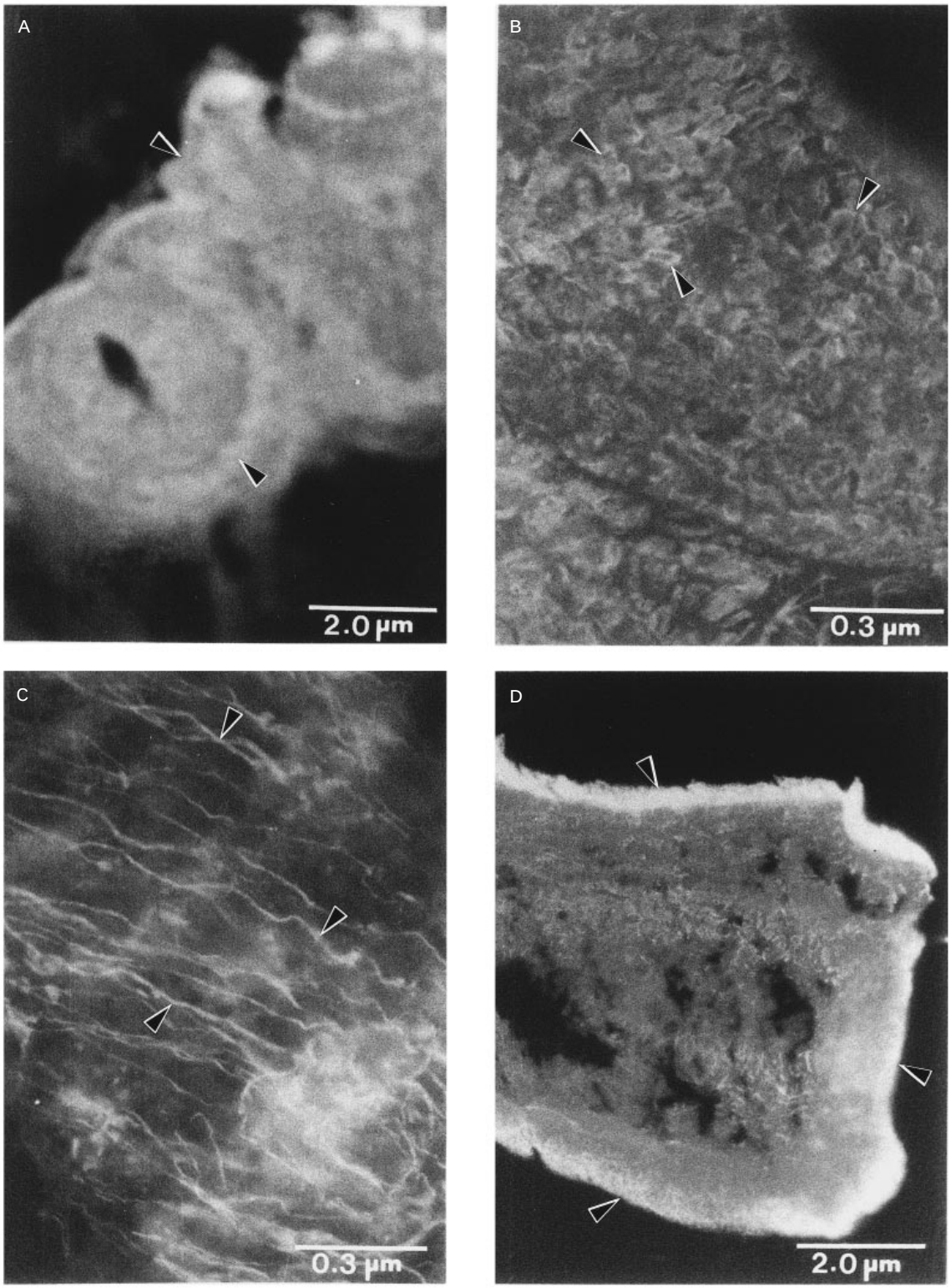

Fig. 5. Recognition of mAb 4B3 to tapeworm tissue: (A and B) Scolex. (C) Neck. (D) Proglottid. Arrows point to positive immunofluorescence.

in the border of proglottids (Fig. 5D). Myosin from different parasites was recognized by mAb 4B3, by anti-Ag B polyclonal antibodies and by commercial anti-myosin (Fig. 6), apparently mAb 4B3 recognized a common antigenic determinant in all myosins tested with high reactivity, except ES myosin that was not recognized by anti-Ag B Ab.

Western blot analysis of ES myosin with mAb 4B3 showed that one 206 kDa fraction was recognized (Fig. 7) and electron transmission microscopy revealed that ES myosin filaments have similar ultrastructure to conventional myosin or myosin II as double heads, neck, long coil and its size (inserts in Fig. 7).

https://doi.org/10.1017/S0031182097008822 Published online by Cambridge University Press

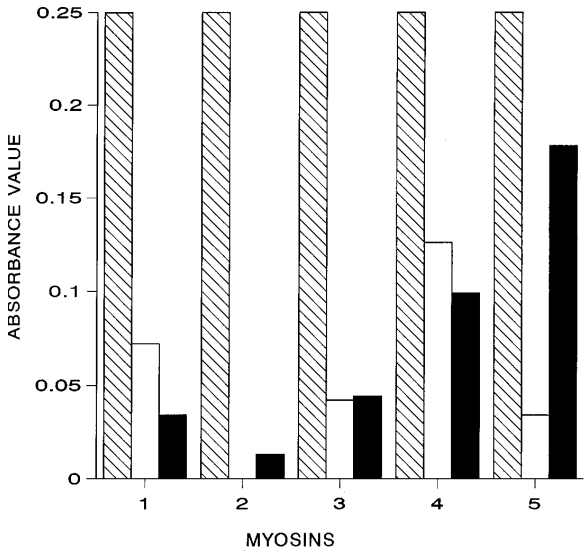

Fig. 6. Antibody recognition of parasite myosins. Myosin extracts (0·3 M KCl) were tested by ELISA using mAb 4b3 (▨), polyclonal antibodies against Antigen B (□) and commercial antibodies against smooth and skeletal bovine myosin (■). Purified myosin samples were from cysticerci extract (1), ES products (2), tapeworms from infected hamsters (3), *Taenia saginata* tapeworms (4) and commercial myosin from porcine muscle (5).

DISCUSSION

The data obtained in this study show that mAb 4B3 recognizes myosin in all samples obtained from *T. solium*. Myosin was defined by the following parameters: purification with KCl (Dowben, 1969; Giese, 1973), high molecular weight (> 206 kDa) (Darnell, Baltimore & Lodish, 1990; Giese, 1973), distribution of fluorescence in parasite tissue at the level of muscle fibres (Newport *et al.* 1987), appearance and size of purified myosin filaments in electron microscopy (Darnell *et al.* 1990) and recognition of 206 kDa myosin filaments with mAb 4B3. Electron microscopy indicated that the structure of myosin in solution corresponds to conventional myosin or myosin II (Darnell *et al.* 1990) and that it is present as homodimeric protein according to its high molecular weight (Dowben, 1969; Giese, 1973).

The fact that mAb 4B3 recognized myosin in *T. solium* adult and cysticercus suggests that it is the same type of myosin or at least they share an epitope, as defined by ELISA, Western blot and immunohistochemistry. Similarity among myosins was further seen in the cross-reactivity of mAb 4B3 with other taeniids, but not with the nematodes assayed or with the trematode and the protozoan parasite.

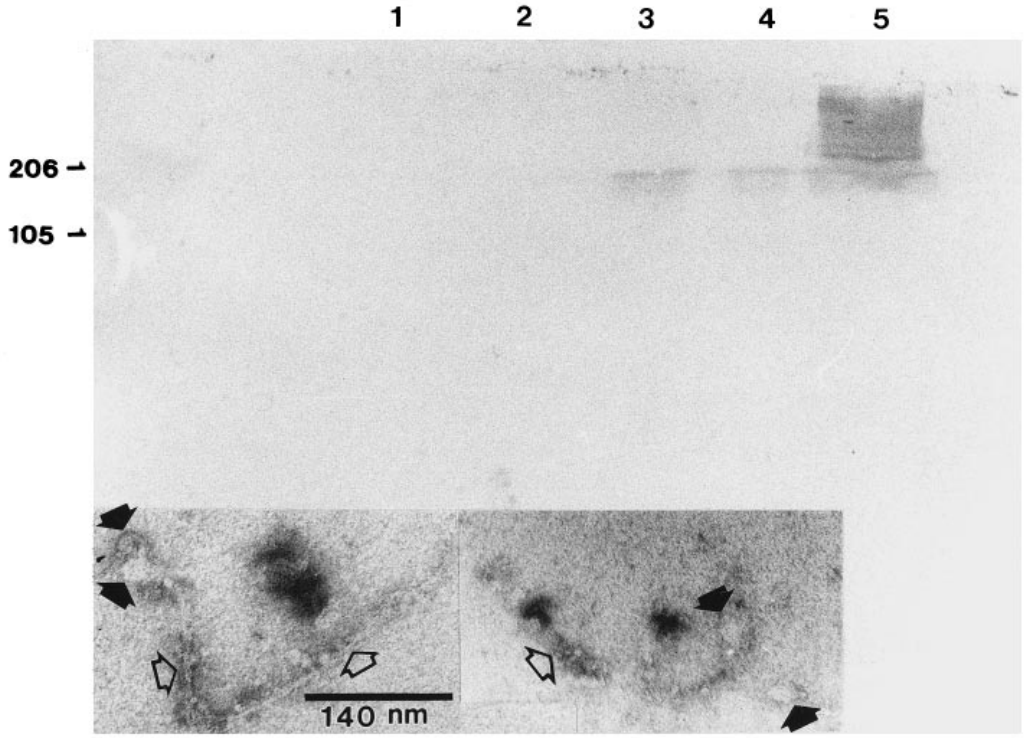

Fig. 7. Western blot analysis of tapeworm *Taenia solium* myosin. Samples were processed by 10% SDS–PAGE under reduced conditions and Western blot analysis. Different sources of myosin were analysed by Western blot against mAb 4B3. Intestinal hamster tissues (1), commercial pig myosin (2), filaments purified from ES products (3), ES products (4) and tapeworm extract from hamsters (5). No reaction was seen with intestinal myosins and the reaction with pig myosin, although present, was very faint. Purified myosin filaments from ES obtained from tapeworms were identified by electron transmission microscopy at 100 keV using uranyl stain (inserts). Myosin heads are indicated with full arrows, myosin tails with empty arrows.

https://doi.org/10.1017/S0031182097008822 Published online by Cambridge University Press

Interestingly a commercial myosin obtained from skeletal pig muscle reacted intensely by ELISA and by Western blot mAb 4B3 recognized native myosin and the heavy chain fraction (data not shown).

In the case of cysticerci, an additional band was seen by Western blot, its molecular weight (105 kDa) corresponds to AgB (Guerra *et al*. 1982) as well as its histological distribution (Laclette *et al*. 1995), suggesting that paramyosin or AgB is recognized by mAb 4B3 which is specific for myosin. A similar finding between paramyosin and myosin was recently described in *Brugia malayi* (Schmitz *et al*. 1996). Myosin in *T. solium* seems to be distributed along the surface of proglottids in the adult parasite which is also evident from the metacestode stage where the spiral canal was labelled in the outer surface that will become the adult parasite. This distribution could be related with growth and support of a long parasite in the intestine where it has to remain in the presence of vigorous peristaltic movements. In the neck, myosin distribution might help fasten the large strobilum to the head as well as support nascent proglottids as suggested by the labelling of the long fibres.

T. solium myosin was detected with mAb 4B3 in secretion and excretion products and in extracts from tapeworms recovered from humans as well as in faeces from infected hamsters. This is an important finding because it may be used as a marker of infection for more specific diagnostic assays of the intestinal adult parasite. Detection of myosin in serum by a mAb has been described in association with cardiac diseases (Katoh *et al*. 1995*a*, *b*) and antimyosin antibodies have been found in schistosome and filarial infections (Newport *et al*. 1987; Raghavan *et al*. 1992). The ELISA for coproantigen detection in taeniosis applied to humans (Allan *et al*. 1990) was standardized with anti-*T. solium* antibodies raised using whole adult parasites minced in 3 M KCl where parasite myosins are purified (Giese, 1973). This assay has high sensitivity but it does not differentiate between *T. solium* and *T. saginata*. By raising antibodies against different regions of adult *T. solium* myosin the technique might become highly specific, because specific non-conserved regions of myosin have been found in all myosins studied (Katoh *et al*. 1995*a*, *b*; Kimura *et al*. 1991; Newport *et al*. 1987; Ruppel & Spudich, 1995). We are currently working on this aspect.

This work was supported by the European Community contract CI1*CT940081, a PAPED-UNAM contract 012302 and a PAPIIT-UNAM contract IN209994, J.A. was supported by a CONACyT fellowship, M.C.R. was supported by a PAPIIT-UNAM fellowship, J.C.A. was supported by a Wellcome Trust Tropical Fellowship, Royal Society and Mexican Academy of Sciences, Y. Medina (INDRE) for training in monoclonal technology, M. T. Merchant (Fac. Medicina, UNAM) for electron transmission microscopy.

REFERENCES

ALLAN, J. C., AVILA, G., GARCIA-NOVAL, J., FLISSER, A. & CRAIG, P. S. (1990). Immunodiagnosis of taeniasis by coproantigen detection. *Parasitology* **101**, 473–477.

ALLAN, J. C., GARCIA DOMINGUEZ, C., CRAIG, P. S., ROGAN, M., LOWE, B. S. & FLISSER, A. (1991). Sexual development of *Taenia solium* in hamsters. *Annals of Tropical Medicine and Parasitology* **85**, 573–576.

CHAICUMPA, W., YUWAPORN, R., BURI, D., CHONGSUA, M. & ECHEVERRIA, P. (1992). Diagnosis of typhoid fever by detection of *Salmonella typhi* antigen in urine. *Journal of Clinical Microbiology* **30**, 2513–2515.

CRAIG, P. S., MACPHERSON, C. N. L. & NELSON, G. S. (1986). The identification of eggs of *Echinococcus* by immunofluorescence using anti-oncospheral monoclonal antibody. *American Journal of Tropical Medicine and Hygiene* **35**, 152–158.

DARNELL, G., LODISH, H. & BALTIMORE, D. (1990). *Molecular Cell Biology*, 2nd edn. Scientific American Books, New York.

DOWBEN, R. M. (1969). *General Physiology. A Molecular Approach*. Harper & Row Publishers, Inc. USA.

FLISSER, A., TARRAB, R., WILLMS, K. & LARRALDE, C. (1975). Immunoelectroforesis y doble immunodifusión en el diagnóstico de la cisticercosis cerebral humana. *Archivos de Investigación Médica* **6**, 1–13.

FLISSER, A., WOODHOUSE, E. & LARRALDE, C. (1980). Human cysticercosis: antigens, antibodies and non-responders. *Clinical and Experimental Immunology* **39**, 27–37.

GODING, W. J. (1983). *Monoclonal Antibodies : Principles and Practice*. Academic Press, Orlando, Florida.

GIESE, A. C. (1973). *Cell Physiology*, 4th edn. W. B. Saunders Company, Philadelphia.

GUERRA, G., FLISSER, A., CAÑEDO, L. & LACLETTE, J. P. (1982). Biochemical and immunological characterisation of antigen B purified from cysticerci of *Taenia solium*. In *Cysticercosis : Present States of Knowledge and Perspectives* (ed. Flisser, A., Willms, K., Laclette, J. P., Larralde, C., Ridaura, C. & Beltran, F.), pp. 437–451. Academic Press, New York.

HARLOW, E. & LANE, D. (1988). *Antibodies: a Laboratory Manual*. Cold Spring Harbor Laboratory, USA.

HERMANSON, J. W., LAFRAMBOISE, W. A. & DAOOD, M. J. (1991). Uniform myosin isoforms in the flight muscles of little brown bats, *Myotis lucifugus*. *Journal of Experimental Zoology* **259**, 174–180.

KATOH, H., SUZUKI, T., HIROI, Y., OHTAJI, E., SUZUKI, S., YAZAKI, Y. & NAGAI, R. (1995*a*). Diagnosis of aortic dissection by immunoassay for circulating smooth muscle myosin. *Lancet* **345**, 191–192.

KATOH, H., SUZUKI, T., YOKOMORI, K., SUZUKI, S., OHTAJI, E., WATANABE, M., YAZAKI, Y. & NAGAI, R. (1995*b*). A novel immunoassay of smooth muscle myosin heavy chain in serum. *Journal of Immunological Methods* **185**, 57–63.

KIMURA, A., TSUJI, T., MATOBA, R., FUJITAMI, N., OHMORI, K. & MATSUMURA, S. (1991). Tissue-specific and non-tissue specific heavy chain isoforms of myosin in the brain as revealed by monoclonal antibodies. *Biochimica et Biophysica Acta Protein Structure and Molecular Enzymology* **1118**, 59–69.

LACLETTE, J. P., LANDA, A., ARCOS, L. & WILLMS, K. (1991). Paramyosin is the *Schistosoma mansoni* (Trematoda) homologue of antigen B from *Taenia solium* (Cestoda) *Molecular and Biochemical Parasitology* **44**, 287–296.

LACLETTE, J. P., MERCHANT, M. T. & WILLMS, K. (1987). Histological and ultrastructural localization of antigen B in the metacestode of *Taenia solium*. *Journal of Parasitology* **73**, 121–129.

LACLETTE, J. P., SHOEMAKER, CH. B., RICHTER, D., ARCOS, L., PANTE, N., COHEN, C., BING, D. & NICHOLSON-WELLER, A. (1992) Paramyosin inhibits complement C1. *Journal of Parasitology* **148**, 124–128.

LACLETTE, J. P., SKELLY, P. J., MERCHANT, M. T. & SHOEMAKER, CH. B. (1995). Aldehyde fixation dramatically alters the immunolocalization pattern of paramyosin in platyhelminth parasites. *Experimental Parasitology* **81**, 140–143.

LUMSDEN, R. D. & HILDRETH, M. B. (1983). The fine structure of adult tapeworms. In *Biology of the Eucestoda*. Vol. 1, (ed. Arme, C. & Pappas, P. W.), pp. 190–205. Academic Press, London.

NEWPORT, G. R., HARRISON, R. A., MCKERROW, J., TARR, P., KALLESTAD, J. & AGABIAN, N. (1987). Molecular cloning of *Schistosoma mansoni* myosin. *Molecular and Biochemical Parasitology* **26**, 29–38.

OLIVO, A., PLANCARTE, A. & FLISSER, A. (1988). Presence of antigen B from *Taenia solium* cysticercus in other Platyhelminthes. *International Journal for Parasitology* **18**, 543–546.

PLANCARTE, A., FLISSER, A. & LARRALDE, C. (1983). Fibronectin-like properties in antigen B from the cysticercus of *Taenia solium*. *Cytobios* **36**, 83–93.

RAGHAVAN, N., MAINA, C. V., FITZGERALD, P. C., TUAN, R. S., SLATKO, B. E., OTTESEN, E. A. & NUTMAN, T. B. (1992). Characterisation of a muscle-associated antigen from *Wuchereria bancrofti*. *Experimental Parasitology* **75**, 379–389.

RUPPEL, K. M., & SPUDICH, J. A. (1995). Myosin motor function: structural and mutagenic approaches. *Current Opinions in Cellular Biology*. *Cytoskeleton* **7**, 89–93.

SARTI, E., SCHANTZ, P. M., PLANCARTE, A., WILSON, M., GUTIERREZ, I. O., LOPEZ, A. S., ROBERTS, J. & FLISSER, A. (1992). Prevalence and risk factor for *Taenia solium* taeniasis and cysticercosis in humans and pigs in a village in Morelos, México. *American Journal of Tropical Medicine and Hygiene* **46**, 677–684.

SCHMITZ, K. A., HALE, T. J., RAJAN, T. V. & YATES, J. A. (1996). Localization of paramyosin, myosin, and a heat shock protein 70 in larval and adult *Brugia malayi*. *Journal of Parasitology* **82**, 367–370.

SLAIS, J. (1982). Morphology of the scolex of cysticercus cellulosae in brain cysticercosis. In *Cysticercosis : Present States of Knowledge and Perspectives* (ed. Flisser, A., Willms, K., Laclette, J. P., Larralde, C., Ridaura, C. & Beltran, F.), pp. 235–299. Academic Press, New York.

SMYTH, J. D. & MCMANUS, D. P. (1989). *The Physiology and Biochemistry of Cestodes*. Cambridge University Press, Cambridge.

TOWBIN, H., STAEHELIN, T. & GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. *Proceedings of the National Academy of Sciences, USA* **76**, 4350–4354.