Identification of antigenic proteins from *Echinostoma caproni* (Trematoda) recognized by mouse immunoglobulins M, A and G using an immunoproteomic approach

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SUMMARY

Antigenic proteins of Echinostoma caproni *(Trematoda) against mouse IgM, IgA, IgG, IgG1 and IgG2a were investigated by immunoproteomics. Excretory/secretory products (ESP) of* E. caproni *separated by two-dimensional (2D) gel electrophoresis were transferred to nitrocellulose membranes and probed with the different mouse immunoglobulin classes. A total of four proteins (enolase, 70 kDa heat-shock protein (HSP-70), actin and aldolase) were accurately identified. Enolase was recognized in eight different spots of which seven of them were detected in the expected molecular weight and were recognized by IgA, IgG or IgG and IgG1. Another spot identified as enolase at 72 kDa was only recognized by IgM. Digestion with N-glycosidase F of the 72 kDa band rendered a polypeptide with an apparent molecular weight similar to that expected for enolase recognized by Western immunoblotting using anti-enolase antibodies. This suggests that glycosylated forms of enolase may be involved in the early thymus-independent responses against* E. caproni. *Early IgM responses were also generated by actin and the HSP-70 which suggests that these proteins are exposed early to the host and may be of importance in the parasite establishment. The IgA responses also appear to be mediated by the HSP-70 and aldolase which could be related with the close contact of these proteins with the host mucosal surface after secretion.*

Keywords actin, aldolase, Echinostoma caproni, *enolase, excretory/secretory products, heat-shock protein*

INTRODUCTION

Echinostomes are important parasitic flatworms invading domestic and wildlife animals and occasionally human beings (1). They are able to parasitize a wide range of invertebrate and vertebrate hosts in their life cycle making them a good model for the study of host–parasite interactions (2,3). Our group has used *Echinostoma caproni* (Trematoda: Echinostomatidae) as model for chronic and acute intestinal helminth infections. *Echinostoma caproni* is an intestinal trematode with no tissue phases in the definitive host (4). The use of this parasite as model is based on the observation that develops chronic or acute infections depending on the rodent host used. *Echinostoma caproni* is highly compatible in hamsters and mice, eliciting chronic infections (5,6). In contrast, the parasite is expelled in the rat within 7–8 weeks post-infection (wpi) (5). The host response to *E. caproni* has been studied by determining the immunoglobulin production and also by the analysis of the histopathological changes in the intestine of both high (hamsters and mice) and low (rats) compatible hosts (6–10). These studies confirmed the antigenic nature of excretory/secretory products (ESP) of *E. caproni*. However, the antigenic proteins of ESP involved in the host–trematode interactions remain to be characterized at the molecular level. In the related species *E. friedi*, several proteins were characterized by a proteomic approach (11), whereas only the glycolytic enzyme enolase has been accurately characterized in *E. caproni* (12).

A combination of proteomics and immune studies (immunoproteomics) has been proved to be a powerful tool for analysing immune responses at both the whole organism level and also in the characterization of individual antigenic proteins (13–15). To date, antigenic proteins of echinostomes have been poorly studied. Only scarce data produced by conventional SDS-PAGE (1-DE) immunoblot profiles are available (16,17). Furthermore, there are no reports on the antigens recognized by each immunoglobulin class. The

purpose of the present work is to identify the *E. caproni* major antigenic proteins recognized by IgM, IgA, total IgG, IgG1 and/or IgG2a in a highly compatible host such as the mouse. Moreover, the relationship between *E. caproni* antigens and the host immune mechanism is analysed.

MATERIALS AND METHODS

Parasites, experimental infections and sera

The species used in this study and the first and second intermediate snail hosts have been previously described (8). Encysted metacercariae of *E. caproni* were removed from the kidneys and pericardial cavities of experimentally infected *Biomphalaria glabrata* snails and used to infect ICR mice (*Mus musculus*). Male mice, weighing 32–40 g, were infected through a stomach tube with 75 metacercariae each of *E. caproni*. The animals were maintained under standard conditions with food and water *ad libitum*. The worm egg release was investigated daily in each infected animal, as described previously (18). Blood was collected weekly from each infected and control animal by cardiac puncture under anaesthesia. After clotting of the blood overnight at 4°C, serum was separated from the clot by centrifugation. The serum samples were stored at –20°C until use. A pool of sera collected at 1–2 wpi was used to investigate IgM response, whereas the remainder immunoglobulin classes were studied with a pool of sera from 5 to 7 wpi, coinciding with the maximal level of each immunoglobulin class (10).

Obtaining of ESP of *Echinostoma caproni*

To prepare ESP, adult worms of *E. caproni* were collected from the intestines of mice, thoroughly washed with PBS (pH 7·4), and maintained in a culture medium of PBS containing 0·8 mm phenylmethylsulphonyl fluoride, 100 U penicillin and 100 µg/mL streptomycin (all from Sigma, St. Louis, MO, UK), at concentrations of 10 worms/mL for 12 h at 37°C. After incubation, the medium was collected and centrifuged at low speed to remove larger debris, and the resulting supernatant was centrifuged at 15 000 *g* for 30 min at 4°C. Supernatants were concentrated using an ultrafiltration membrane with a cut off of 3000 Da (YM-3, Amicon, Beverly, MA). The protein content of the samples was determined by the Bradford method (Bio-Rad, Hercules, CA).

Polyclonal IgG anti-*Echinostoma caproni* enolase antibodies

Polyclonal IgG anti-*E. caproni* recombinant enolase anti-bodies were obtained by inoculating 2–3 kg New Zealand white rabbits with recombinant enolase of *E. caproni* (12). Recombinant enolase of 300 µg were emulsified in 0·3 mL of Freund's complete adjuvant and were used to hyperimmunize each rabbit by giving several inoculations. Rabbits were given similar inoculations on four dates, with two weekly intervals, using Freund's incomplete adjuvant. Blood was drawn 21 days after the final inoculation. Serum was pooled and the anti-*E. caproni* recombinant enolase globulins containing IgG were obtained by precipitation with 50% ammonium sulphate and then dialysed extensively against PBS.

1D and 2D-SDS-PAGE

One dimension (1D) SDS-PAGE in 10% gels was performed as described previously (11).

The 2D gel electrophoresis was carried out essentially as previously described (11), solubilizing protein samples in 7 m urea, 2 m thiourea, 4% CHAPS (w/v), 20 mm DTT and 2% (v/v) Biolytes 3–10 and bromophenol blue (all chemicals from Bio-Rad), and samples were then applied onto different linear ranges (pH 5–8; pH 3–6; pH 7–10) ReadyStrip™ IPG strips (7 and 11 cm long, Bio-Rad). Loads of 300 µg of ESP were subjected to isoelectric focusing on a Bio-Rad PROTEAN® IEF Cell at 20°C using the following program: (i) passive rehydration for 16 h; (ii) 300 V for 1 h (step and hold); (iii) 4000 V for 2 h (linear voltage ramping until reaching 4000 V) and (iv) 4000 V for 6·5 h (step and hold). After electrofocusing, the strips were reduced (2% DTT) and then alkylated (2·5% iodoacetamide) in equilibration buffer containing 6 m urea, 0·375 m Tris pH 8·8, 2% SDS and 20% glycerol, and the second dimension was performed using 10% polyacrylamide gels. Proteins from SDS-PAGE were stained with SYPRO Ruby or electroblotted onto a nitrocellulose membrane.

Western-blot analysis, quantification and stripping

Gels were transferred to nitrocellulose membranes in 20 mm Tris, 192 mm glycine, methanol 20% v/v, pH 8·3 as previously described (11). Filters were stained with Ponceau S (Sigma) for 10 min, and blocked for 3 h in 10 mm Tris–HCl, pH 7·4, 150 mm NaCl (TBS) containing 5% nonfat dry milk. After extensive washings with TBS containing 0·05% Tween-20 (TBST), blots were incubated with sera from infected mice at 1 : 200 dilution. After washings, the specific antigen proteins corresponding to each mouse immunoglobulin class were incubated further in an adequate dilution of horseradish peroxidase-conjugated antimouse IgM (OEM Concepts, TomsRiver, NJ), IgA (Nordic, Tilburg, The Netherlands), IgG (Bio-Rad), IgG1 (Nordic) and IgG2a (Nordic) for 2 h. After washing five times with TBS +3% BSA immune complexes were visualized using either Lumi-light

Western blotting substrate (Roche, Mannheim, Germany) or ECL^{TM} system (GE Healthcare, Little Chalfont, UK) following the manufacturer's instructions and analysed with a ChemiDoc^{TM} XRS system (Bio-Rad).

Filters were stripped at 50°C for 30 min in a solution containing 100 mM of 2-mercaptoethanol and 2% SDS in 62·5 mM Tris–HCl pH 6·7, followed by extensive washings and another blocking step previous to re-probing with sera from naïve mice.

In order to confirm the identity of the spots identified, membranes were also probed with the following antisera: Goat polyclonal antirabbit aldolase (AB1809 from Chemicon Int., Tamecula, CA); Rabbit polyclonal antihuman actin (A2066 from Sigma); Rabbit polyclonal antirecombinant *E. caproni* enolase (12); mouse monoclonal antibovine 70 kDa heat-shock protein (HSP-70) (Clone BRM-22, H5147 from Sigma). The bound antibodies were detected by incubating blots for 1 h at RT with horseradish peroxidase-conjugated antibodies: goat antirabbit IgG and goat antimouse IgG (from Bio-Rad) or donkey antigoat IgG (Santa Cruz Biotechnologies, Santa Cruz, CA) in TBST-1% BSA.

Mass spectrometry (MS) and identification

The spots that were recognized by any immunoglobulin class and also could be observed in the 2-DE gel were manually excised from the gel, washed twice with double-distilled water and digested with sequencing grade trypsin (Promega, Madison, WI) as described elsewhere (19). The digestion mixture was dried in a vacuum centrifuge, resuspended in 7 μL of 0·1% TFA, and 1 μL was spotted onto the MALDI target plate. After the droplets were air-dried at room temperature, 1 μL of matrix (5 mg/mL CHCA (Sigma) in 0·1% TFA-ACN/H_2O (1 : 1, v/v)) was added and allowed to air-dry at room temperature. MALDI MS and MS/MS data were acquired with a 4700 Proteomics analyser (Applied Biosystems, Foster City, CA). The samples without a positive identification were analysed by LC/MS/MS. Peptide separation by LC-MS/MS was performed using an Ultimate nano-LC system (LC Packings, Amsterdam, The Netherlands) and a QSTAR XL Q-TOF hybrid mass spectrometer (MDS Sciex, Applied Biosystems, Concord, Canada). Samples (5 μL) were delivered to the system using a FAMOS autosampler (LC Packings) at 30 μL/min, and the peptides were trapped onto a PepMap C18 precolumn (5 mm 300 m i.d.; LC Packings). Peptides were then eluted onto the PepMap C18 analytical column (15 cm 75 m i.d.; LC Packings) at 200 nL/min and separated using a 55 min gradient of 15%–35% ACN. The QSTAR XL was operated an information-dependent acquisition mode, in which a 1-s TOF MS scan from 400 to 2000 m/z, was performed, followed by 3-s product ion scans from 65 to 2000 m/z on the two most intense doubly or triply charged ions. External calibration of the MALDI TOF instrument was performed using the 4700 Cal Mix (Applied Biosystems) according to the manufacturer indications. For MS/MS calibration, the fragmentation of Angiotensin I included in the 4700 Cal Mix was used. The QSTAR-XL TOF was calibrated with a mixture of CsI and cPDI inhibitor.

Database search

Database search on Swiss-Prot and NCBInr databases was performed using MASCOT search engine (Matrix-Science, London, UK). Searches were done with tryptic specificity allowing one missed cleavage and a tolerance on the mass measurement of 100 p.p.m. in MS mode and 0·8 Da for MS/MS ions. Carbamidomethylation of Cys was used as a fixed modification and oxidation of Met and deamidation of Asn and Gln as variable modifications. A protein identification was considered accurate when at least three peptides were identified with an overall MASCOT score > 50.

Deglycosylation

In order to deglycosylate one of the spots identified as enolase, ESP were 1D electrophoresed and the gel was excised in the molecular weight (MW) range of 70–75 kDa. These products were digested in a tube using recombinant N-glycosidase F (Roche) according to the manufacturer's instructions.

RESULTS

2-DE of ESP of *Echinostoma caproni*

The ESP of *E. caproni* were first electrofocused using 3–10 linear immobilized pH gradient strips and electrophoresed in 5%–20% gradient polyacrilamide gels. Staining of these gels allowed the detection of about 54 spots, most of them located between pH 5 and 8 and MW range of 20–140 kDa (data not shown). Five batches of *E. caproni* proteins were separated by 2-DE and the average position and number of spots determined with PDQuest^{TM} 2-D Analysis Software (Bio-Rad). The protein patterns were very similar, thereby confirming a highly reproducible method for the establishment of species-specific protein detection and proteome maps.

Once we had determined the spot pI and MW, and in order to improve the spot resolution and detection, the ESP were electrophoresed in 5–8 IPG strips and ran in a second dimension in 12% polyacrilamide gels, resolving in more than 88 spots after Sypro Ruby staining. The MW range of the spots was 23–136 kDa (Figure 1).
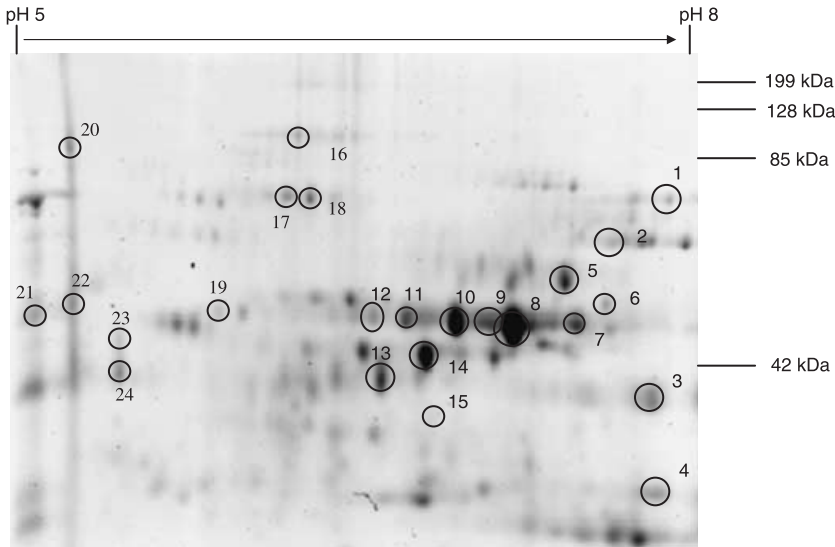
Figure 1 Representative Sypro Ruby staining of analytical 2-DE gels of *E. caproni* ESP using pH 5–8 strips. Numbered circles indicate the spots that were analysed by mass spectrometry. Identification of the spots can be found in Table 1.

Antigenic profiles of ESP of *Echinostoma caproni*

To investigate the antigenicity of *E. caproni*, identical gels in the pH range 5–8 were transferred onto nitrocellulose membranes and studied for IgM, IgA, IgG, IgG1 and IgG2a detection. Spots were recognized by all the immunoglobulin classes, except by IgG2a. A total of 24 spots were recognized, though one of them (spot 4) was simultaneously recognized by sera from naïve animals and, thus, discarded for further analysis. The profile was specific for each isotype (Figure 2; Table 1):

IgM (Figure 2a): A total of five major spots (spots 15, 17, 18, 19 and 20), whose MW range was 31–110 kDa and pI range 5·2–6·6, were recognized by IgM. Sera from naïve animals did not recognize any spot (data not shown).

IgA (Figure 2b): A total of nine spots (spots 1–6, 9, 16 and 18) were recognized by IgA in the pI and MW ranges of 6·1–7·7 and 38–122 kDa, respectively. From these spots, spot 4 was also recognized by negative sera and discarded (data not shown). Moreover, one of the IgA-recognized spots (18) was simultaneously recognized by IgM.

IgG (Figure 2c): Eighteen spots were recognized by total IgG and 12 of them were analysed by MS/MS since they were also detected in the 2-DE gel (spots 5, 7, 8, 10–14 and 21–24). These spots were located in the MW range from 40 to 146 kDa and pI range 5·0–7·4. One of the IgG-recognized spots (5) was simultaneously recognized by IgA. Sera from naïve animals did not recognize any spot (data not shown).

IgG1 (Figure 2d): Two spots (spots 8 and 10) were recognized by IgG1 in the pI range of 6·8–6·9 and weighing 46–47 kDa. Negative sera did not recognize any spot (data not shown). All the spots specifically recognized by IgG1 were simultaneously recognized by total IgG.

Identification of antigens

All the protein spots detected in the 2-DE gel and specifically recognized by any of the immunoglobulins were analysed by MALDI-MS and some of them were identified by their PMFs and MS/MS (Table 1). Comparing the resulting PMF and peptide fragmentation to protein databases, we were able to accurately identify several spots (Table 1). However, two of these proteins (SJCHGCO1653 and Annexin A5) were not considered for further analysis due to the low coverage observed. Considering the remaining identified spots, eight (7,8,9,10,11,12,17,20), were identified as enolase, covering from 17% to 31% of the molecule. Western immunoblot using polyclonal antibodies raised against recombinant enolase of *E. caproni* revealed a number of spots located in the pI range of 5·9–7·3 (Figure 3a). Interestingly, two of the spots recognized by the sera from infected animals (spot 17 by IgG and spot 21 by IgM) and identified as enolase were not recognized by the polyclonal antibodies. Regarding the spot 17, the immunogeneicity of this isoform seems to be affected by the different pI. The remaining spot identified as enolase (spot 17) observed at 72 kDa was not recognized by the antibody. In order to determine post-traslational modifications, such as glycosylation, and to confirm the identity of this spot, ESP of *E. caproni* were 1D electrophoresed and the gel was excised in the MW range of 70–75 kDa. The excised materials were digested with N-glycosidase F, subjected to electrophoresis again and transferred onto nitrocellulose. Western immunoblot using the antienolase antibody revealed a band with an apparent MW of 47 kDa as expected for an enolase (Figure 3b).

A spot (numbered as 3) recognized by IgA was identified as homologous to *S. mansoni* aldolase, covering 18% of the
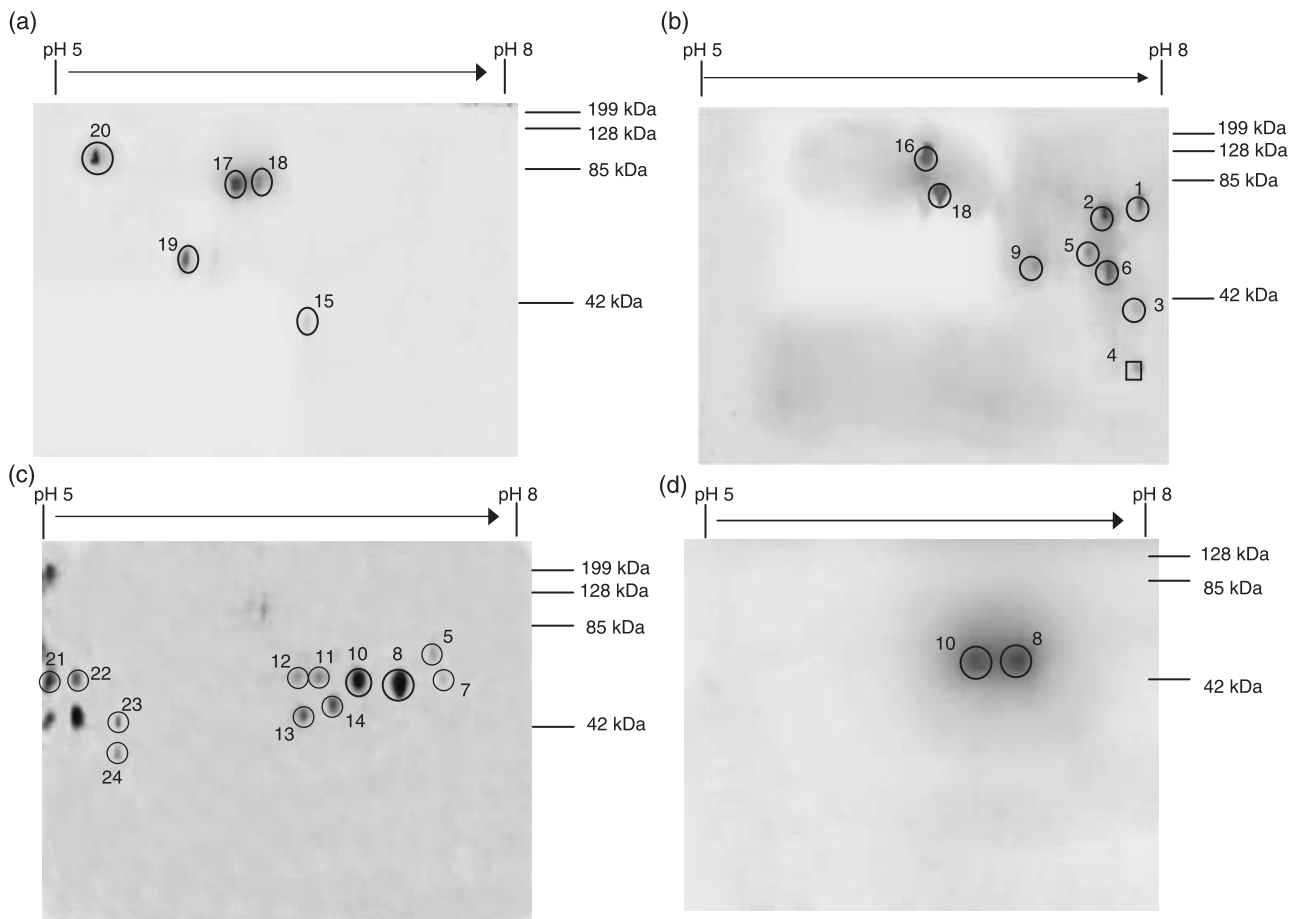
Figure 2 2-DE immunoblot of *E. caproni* ESP with mouse IgM (a), IgA (b), total IgG (c) and IgG1 (d). Numbered circles indicate the spots that were analysed by mass spectrometry. Identification of the spots can be found in Table 1. Spot number 4 (within a square) was also recognized by negative sera and discarded for further analysis.

molecule. As shown in Figure 4(c), commercially available polyclonal antibodies against rabbit muscle aldolase detected a group of spots of an apparent MW of 40 kDa and pI of 7·7, demonstrating its presence in ESP of *E. caproni.*

Another prominent spot of 70 kDa and pI 6·1 (numbered as 18) and recognized by IgM and IgA was found to be homologous to *S. mansoni* HSP-70 with a coverage of 18%. The identity of this protein was confirmed by immunoblot using commercially available anti-HSP-70 antibodies. A group of spots of about 70 kDa and pI range of 5·8–6·1 were immunodetected (Figure 4b).

The spot numbered as 19, with an apparent molecular mass of 42 generated a peptide that exhibited a 100% of identity with *S. japonicum* actin. This spot was strongly recognized by IgM and its identity was confirmed by Western immunoblot using anti-actin antibodies (Figure 4c). Moreover, additional spots were detected at 42 kDa and pI range of 5·8–6·0.

DISCUSSION

In the present study, we have identified some of the most antigenic proteins in *E. caproni* infections using an immuno-proteomic approach. It is known that *E. caproni* induces significant antibody responses in its vertebrate host (7,10,20–22), but little is known about the antigenic proteins eliciting these responses. Identification of these proteins may be of importance to gain further understanding on the host–parasite relationships in intestinal helminth infections.

From the total spots observed on the 2-DE profile of ES products of *E. caproni*, 23 were specifically recognized by different immunoglobulin classes of sera from experimentally infected mice. From those spots a total of 13 spots, corresponding seven different proteins, were identified. Two of these proteins (SJCHGCO1653 and Annexin) were not considered for further analysis due to the low coverage observed. However, it should be considered that the low

Table 1 Identification of antigenic *E. caproni* proteins by PMF and MS/MS. Peptide sequences were used to search the Swiss-Prot/TrEMBL databases by MSift (protein Prospector) and BLASTp (NCBI)

Spot no.	MW (kDa) exp/theo	pI exp/theo	% of coverage (No. of matched peptides)	MASCOT score	Description	Species (Accesion no.)	Identify by[a]	Ig class recognition
3	38/40	7·7/5·8	18 (6)	86	Aldolase	*S. mansoni* (P53442)	M	Ig A
5	54/27	7·2/5·8	< 10 (2)	103	SJCHGC01653 fragment of Aldehyde dehydrogenase	*S. japonicum* (AAW25914)	LC	Ig G/Ig A
6	51/41	4·4/5·9	< 10 (1)	56	Annexin	*S. mansoni* (AAC79802)	LC	Ig A
7	46/47	7·3/6·6	17 (8)	195	Enolase	*E. caproni* (CAK47551)	M	Ig G
8	46/47	6·9/6·6	28 (14)	359	Enolase	*E. caproni* (CAK47551)	M	Ig G/Ig G1
9	47/47	7/6·6	15 (5)	205	Enolase	*E. caproni* (CAK47551)	M	Ig A
10	47/47	6·7/6·6	26 (12)	344	Enolase	*E. caproni* (CAK47551)	M	Ig G/Ig G1
11	47/47	6·5/6·6	22 (8)	193	Enolase	*E. caproni* (CAK47551)	M	Ig G
12	47/47	6·5/6·6	22 (10)	295	Enolase	*E. caproni* (CAK47551)	M	Ig G
18	70/70	6·1/5·4	18 (15)	238	HSP-70	*S. mansoni* (P08418)	M	Ig M/Ig A
17	72/47	6/6·6	31 (12)	272	Enolase	*E. caproni* (CAK47551)	M	Ig M
19	47/42	5·8/5·3	18 (5)	87	Actin-1	*S. mansoni* (P53470)	M	Ig M
21	48/47	5/6·6	15 (7)	159	Enolase	*E. caproni* (CAK47551)	M	Ig G

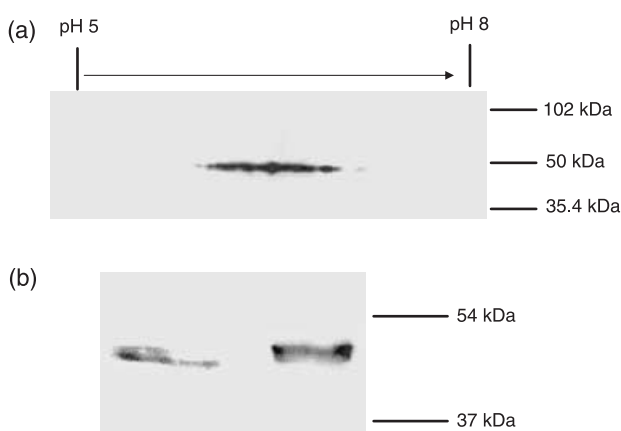[a]M, MALDI TOF/TOF; LC, LC/MS/MS.

Figure 3 2-DE immunoblot of *E. caproni* ESP with antienolase antibodies: (a) 2-DE immunoblot of *E. caproni* ESP; and (b) 1-DE immunoblot of *E. caproni* ESP ranging from 70 to 75 and digested with recombinant N-glycosidase F (lane 1) using *E. caproni* ESP as control (lane 2).

number of sequences of *Echinostoma* spp. deposited in the databases prevented us from obtaining accurate identification of more proteins. To further confirm the identity of those proteins, Western immunoblot analyses were carried out using available antibodies. Most of the identified proteins appear to be involved in the process of parasite invasion and establishment. Among these proteins a group of proteins specific for either IgM, IgA, IgM and IgA, IgG or IgG/IgG1 were recognized by the sera of infected mice.

Enolases appear to be the most antigenic proteins in ESP of *E. caproni*. A total of eight spots were identified as enolase. The presence of enolase in ESP of *E. caproni* has been previously reported (12). Enolases are traditionally thought to be cytosolic, but recently they have been shown to be secreted and present on the external surfaces of different trematode species such as *Fasciola hepatica* (23), *Schistosoma bovis* (24,25) and the related species *E. friedi* (11), although they lack the classic signal peptide for surface location (12,26). The fact that enolases induce significant
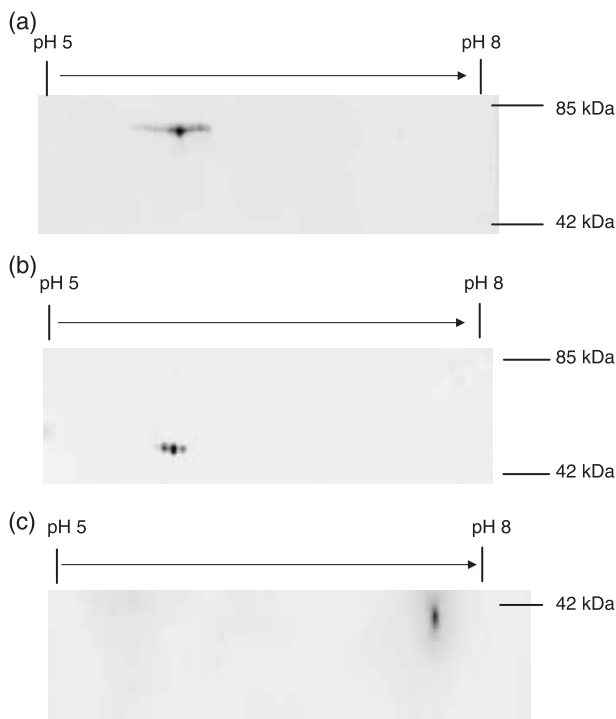
Figure 4 2-DE immunoblot of *E. caproni* ESP with anti-HSP-70 (a), antiactin (b) and antialdolase (c) antibodies.

antibody responses supports the notion that they are secreted during infections. This may be of importance since enolase of helminths binds plasminogen and may play a role in the degradation of the host's extracellular matrix (12,23,25,27).

The identity of six of the enolase spots was confirmed by Western immunoblotting using polyclonal antibodies raised against recombinant enolase. These enolase spots showed a MW similar to that expected. The spot 17 showed a MW similar to that expected though it was located at pI 5. This fact suggests that the pI affect the immunogeneicity of this spot. The remaining spot identified as enolase showed an apparent MW of 72 kDa and it was not recognized by the anti-enolase antibody. According to our results, the increased MW observed in this molecule appears to be related to glycosylation modifications. After N-glycosidase digestion and subsequent Western immunoblotting of the ESP in the MW range 70–75 kDa, the enolase was detected at the expected MW. The glycosylation of enolases has been previously reported in other trematodes such as *S. bovis* (28). In our case the glycosylation of this isoform of *E. caproni* enolase appears to affect the target epitopes recognized by the antirecombinant enolase antibodies.

Interestingly, the glycosylation of enolase also affects the antibody response against *E. caproni* in mice. The nonglycosylated enolase isoforms were recognized by IgG, IgG/IgG1 or IgA. This suggests that these isoforms could be involved in the antibody responses during chronic phases of the infection. In contrast, the glycosylated form of enolase only generated IgM responses. It is known that *E. caproni* induces early IgM responses in mice and rats and this can be related to thymus-independent antigens in *E. caproni* adult worms (10). In this context, our results support the idea that glycosilation of enolase can be one of the responsible mechanisms of these early IgM responses.

IgM responses in *E. caproni* infections are also generated by actin and HSP-70. The presence of actin in the ESP and the surface of helminths is well documented (11,29,30) and may be of importance in the host–parasite interactions. In *E. friedi* infections an increased amount of actin has been correlated with early expulsion of this parasite in rats (11). The antigenicity of the actin is not surprising since it is exposed early to the immune system because it forms part of the spines of the collar and surface of the echinostomes (31,32). The 70 kDa parasite stress protein, HSP-70, is regarded to play an important role in the survival strategy by which hosts down-regulate parasiticidal mechanisms. In echinostome infections, parasite HSP-70 could be involved in the establishment of chronic infections by favouring host local inflammatory response (9,11).

The IgA responses appear to be mediated by HSP-70, one of the isoforms of enolase and aldolase. This fact could be related to the close contact of these proteins with the host mucosal surface after secretion. As mentioned above, the HSP-70 is secreted by echinostomes in highly compatible hosts and may affect the host local response (11). Enolase and aldolase bind and enhance the activation of plasminogen, which may be useful for the parasite establishment (12,28). Moreover, aldolase is a glycolytic enzyme identified in parasite proteomes interacting with molecules like actin, which seems to be essential for parasite motility (33).

In conclusion, antigenic protein expression profiling by immunoproteomics may provide a better understanding of the intestinal helminth infection processes. In this study, several antigenic proteins were recognized on *E. caproni* immunoblot profiles against mice IgM, IgA, IgG and IgG1. The present work reveals that host humoral immune response for *E. caproni* is focused mainly on proteins related to survival strategies such as enolase aldolase, HSP-70 and actin.

ACKNOWLEDGEMENTS

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