

Analysis of taeniid antigens using monoclonal antibodies to *Echinococcus granulosus* antigen 5 and antigen B

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Abstract. Antigens derived from *Echinococcus granulosus*, *Taenia hydatigena* and *T. pisiformis* cyst fluids, *T. solium* cysticerci, *E. multilocularis* protoscoleces and *E. vogeli* cyst membranes were examined by enzyme-linked immunosorbent assay (ELISA) and immunoelectrophoresis (IEP) using four monoclonal antibodies (mAb) to *E. granulosus* antigen 5 (Ag5) and antigen B (AgB). Anti-Ag5 mAbs 24.14 and 61A12 reacted strongly with *T. hydatigena* and *T. pisiformis* cyst fluids and, to a lesser degree, anti-AgB mAbs 31.15 and 39B3 also displayed some reaction with these antigens in ELISA. The formation of a modified arc 5 band between Anti-Ag5 mAbs and *T. hydatigena* cyst fluid (THCF) in IEP further confirms the existence of Ag5 in *T. hydatigena* cyst fluid. However, the inability of THCF and *T. pisiformis* cyst fluid (TPCF) to form an AgB band as well as that of TPCF to form an arc 5 band with mAbs in IEP does not exclusively prove the lack of AgB in THCF and TPCF or the lack of Ag5 in TPCF. The absence of a reaction of mAbs with *T. solium*, *E. multilocularis* and *E. vogeli* antigen preparations in ELISA or IEP would suggest that these mAbs may recognise epitopes different from those of *T. solium*, *E. multilocularis* and *E. vogeli* parasites; this might be exploited for specific differentiation of *E. granulosus*.

Taeniid cestodes (family Taeniidae) are a group of closely related parasites of animals and man. The unilocular hydatid parasite, *Echinococcus granulosus*, is one of the most important among taeniid cestodes. It not only causes economical losses in animal production but also poses health problems to humans. Taeniid cestodes other than *E. granulosus* are also of importance in that the larvae of these parasites often infect the same host as

does *E. granulosus*, and they are often immunologically indistinguishable from *E. granulosus*.

Since the identification of antigen 5 (Ag5) and antigen B (AgB) in *E. granulosus* cyst fluid, the specificity of hydatid disease diagnosis in man and animals has been greatly improved (Capron et al. 1967; Varela-Díaz and Coltorti 1976). Nonetheless, species-specific detection of taeniid infections has been complicated by the findings that anti-Ag5 antibodies exist in sera from patients infected with *E. multilocularis*, *E. vogeli* and *Taenia solium* cysticercosis (Varela-Díaz et al. 1977b, 1978; Yarzabal et al. 1977; Schantz et al. 1980) and in sera from sheep infected with *T. hydatigena* and *T. ovis* (Varela-Díaz et al. 1977a; Yong and Heath 1979). Furthermore, an antigen similar to Ag5 of *E. granulosus* has been demonstrated in *T. hydatigena* cyst fluid (Yong et al. 1978; Liu et al. 1992d) and an antigen that shares similar features with *E. granulosus* AgB has also been detected in *T. hydatigena* and *T. pisiformis* cyst fluids (Liu et al. 1992d).

With monoclonal antibodies (mAbs) to *E. granulosus* Ag5 and AgB being produced in this laboratory, a study was carried out to examine antigens prepared from *E. granulosus*, *T. hydatigena* and *T. pisiformis* cyst fluids, *T. solium* cysticerci, *E. multilocularis* protoscoleces and *E. vogeli* cyst membranes through the use of these mAbs, the aim being to identify species-specific epitopes of *E. granulosus* for improved hydatid disease diagnosis.

Sheep hydatid cyst fluid (SHCF) from *E. granulosus*-infected sheep livers and lungs was prepared as described elsewhere (Liu et al. 1992a). *T. hydatigena* cyst fluid (THCF) was processed from sheep omentum or mesentery containing *T. hydatigena* cysts from a local abattoir (Blayney, Australia). *T. hydatigena* cysts were removed from the surrounding tissues and cut open in a petri dish. The pooled cyst fluid (THCF) was centrifuged, filtered and dialysed as for SHCF. For the preparation of *T. pisiformis* cyst fluid (TPCF), *T. pisiformis* eggs were derived from *T. pisiformis* gravid worms maintained in experimental dogs at the Veterinary Clinical Centre. Rabbits were orally infected with 2500 *T. pisiformis*

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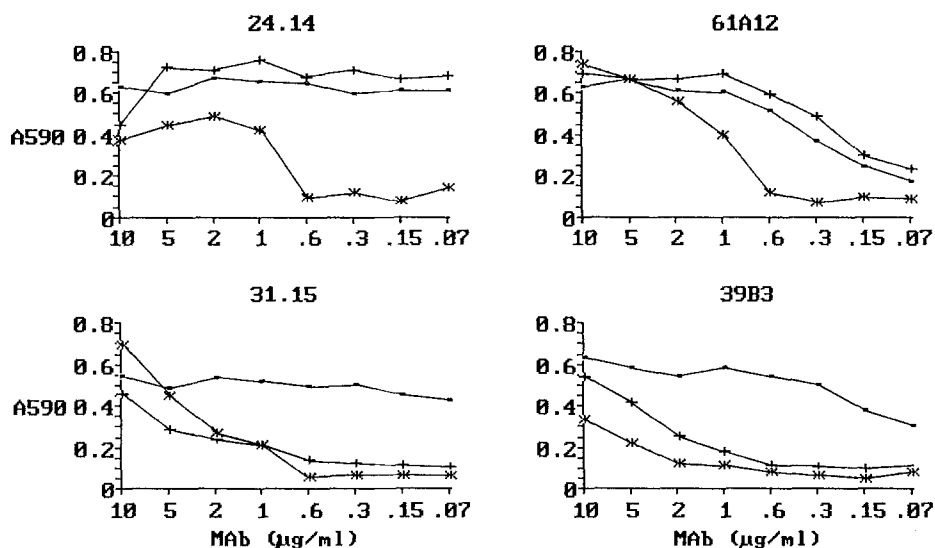


Fig. 1. Examination of taeniid antigens in ELISA. *Echinococcus granulosus* (SHCF) and *Taenia hydatigena* (THCF) antigens at 10 µg/ml, *T. pisiformis* (TPCF) antigen at 20 µg/ml, and mAb concentrations ranging from 10 to 0.07 µg/ml were used. The test was read at an absorbance wavelength of 590 nm. The heading 24.14 indicates mAb 24.14 reacting with SHCF (■), THCF (+) and TPCF (*)

mis eggs each for at least 4 months. Cyst fluid was collected from *T. pisiformis* cysts found in the omentum and pelvic cavity of the infected rabbits. The pooled cyst fluid (TPCF) was processed as described above for THCF.

For the preparation of *T. solium* antigen (Tso), 10 mg freeze-dried *T. solium* cysticerci, kindly supplied by Dr. P. Schantz, Centres for Disease Control, Atlanta, was mixed with 2 ml 0.02 M TRIS buffer (pH 8) containing protease inhibitors. It was sonicated and processed according to the method of Jenkins and Rickard (1985). For the preparation of *E. multilocularis* antigen (Em), 10 mg freeze-dried *E. multilocularis* protoscoleces, also provided by Dr. P. Schantz, was mixed with 2 ml 0.02 M TRIS buffer (pH 8) containing protease inhibitors and processed as described for Tso. For the preparation of *E. vogeli* antigen (Ev), 10 mg freeze-dried *E. vogeli* cyst membranes, also supplied by Dr. P. Schantz, was mixed with 2 ml 0.02 M TRIS buffer (pH 8) containing protease inhibitors and processed as described above for Tso. Rabbit and sheep antisera were raised against the above-mentioned antigens using the method described by Liu et al. (1992b).

Monoclonal antibodies (mAbs) to *E. granulosus* Ag5 and AgB were produced and purified as described elsewhere (Lightowlers et al. 1989; Liu et al. 1992a). The specificity of anti-Ag5 and -AgB mAbs was initially determined by their formation of typical immunoelectrophoretic arc 5 and AgB bands and immunoprecipitation band patterns with SHCF antigens (Lightowlers et al. 1989). Two mAbs (24.14 and 61A12, of Ig_{2a} and Ig_{2b} subclasses, respectively) that may recognise a similar epitope of Ag5 and two mAbs (39B3 and 31.15, of Ig_{2a} and Ig_{2b} subclasses, respectively) that may be against two different epitopes of AgB (Liu et al. 1992c) were used in this study. MAb and SP2 myeloma ascites were tested undiluted in immunoelectrophoresis (IEP), and protein A-purified mAb and normal mouse IgG were used in enzyme-linked immunosorbent assay (ELISA).

The ELISA used in the present study was similar to that described by Liu et al. (1992b). The optimal con-

centrations of SHCF, THCF, TPCF, Tso, Em and Ev were determined in ELISA checkboards using rabbit antisera to these antigens. SHCF and THCF at 10 µg/ml and other antigens at 20 µg/ml (total protein) were applied to 96-well plates. This was followed by the addition of mAb or normal mouse IgG at concentrations ranging from 10 to 0.07 µg/ml. After the addition of urease-labeled sheep anti-mouse IgG conjugate (CSL, Australia), the assay was developed using urease substrate and read at an absorbance wavelength of 590 nm. The IEP technique carried out in the present study was a modified version of the method described by Varela-Díaz and Coltorti in 1976 (Liu et al. 1992b). SHCF, THCF, TPCF, Tso, Em and Ev antigens were concentrated to about 50 mg/ml by lyophilisation or Centricon filter units (Amicon) for use in IEP.

As shown in Fig. 1, when used to analyse taeniid antigens in ELISA, all four mAbs were shown to react strongly with SHCF, for which these mAbs were selected. Although mAbs 24.14 and 61A12 displayed a strong cross-reaction with THCF, they also showed some binding with TPCF. Some cross-reaction was observed between THCF and mAbs 31.15 and 39B3 at 5–10 µg/ml, but the binding of these mAbs with TPCF was marginal. Mabs 24.14 and 39B3 did not bind with Tso, Em or Ev, whereas mAbs 61A12 and 31.15 displayed a low level of binding with these three antigens (Fig. 2). Normal mouse IgG did not react with any of the antigens tested (data not shown).

Whereas mAbs to *E. granulosus* Ag5 (24.14 and 61A12) and AgB (31.15 and 39B3) were found to form typical immunoelectrophoretic arc 5 and AgB bands with SHCF (Lightowlers et al. 1989), only anti-Ag5 mAbs 24.14 and 61A12 formed a modified arc5 with THCF in IEP (Fig. 3). Anti-AgB mAbs 31.15 and 39B3 did not show any reaction with THCF. None of the four mAbs reacted with TPCF, Tso, Em or Ev antigens (data not shown). SP2 ascites showed no reaction with any of the antigens tested (data not shown).

Antigen sharing among various taeniid cestodes has frequently been reported. *T. hydatigena* cyst fluid has

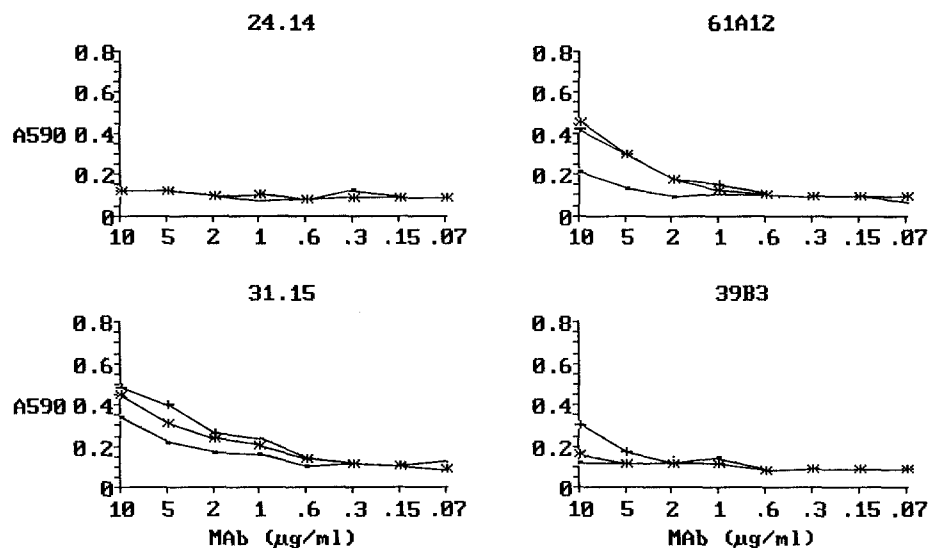


Fig. 2. Examination of taeniid antigens in ELISA. *Taenia solium* (Tso) *Echinococcus multilocularis* (Em) and *E. vogeli* (Ev) antigens at 20 μg/ml and mAb concentrations ranging from 10 to 0.07 μg/ml were used. The test was read at an absorbance wavelength of 590 nm. The heading 24.14 indicates mAb 24.14 reacting with Tso (■), Em (+) and Ev (*)

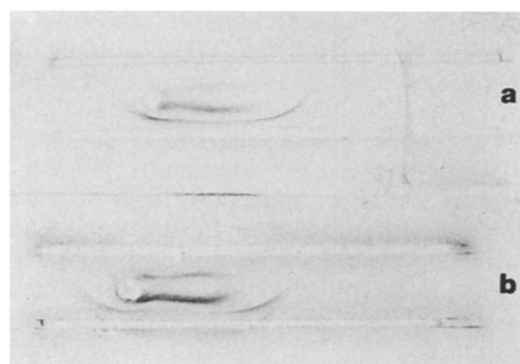


Fig. 3a, b. Examination of *Taenia hydatigena* cyst fluid (THCF) in IEP. Top trough a mAb 24.14; b mAb 61A12. Bottom trough reference sheep anti-SHCF serum

been shown to share antigens with *E. granulosus* cyst fluid (Varela-Díaz et al. 1977a; Yong et al. 1978) and with *T. ovis* (Varela-Díaz et al. 1972). A close antigenic relationship between *T. hydatigena* and *T. solium* metacystodes has also been demonstrated (Herbert and Osberg 1975).

It is apparent from the present study that the cyst

fluids of *T. hydatigena* and *T. pisiformis* contain antigenic epitopes similar to Ag5 of *E. granulosus* as shown by the cross-reaction between anti-Ag5 mAb and THCF and TPCF in ELISA. The formation of an arc 5 band between THCF and anti-Ag5 mAbs in IEP further confirms the presence of Ag5 of *E. granulosus* in THCF (Liu et al. 1992d). Nevertheless, the absence of an arc 5 band in IEP between TPCF and anti-Ag5 mAbs does not exclusively prove the lack of hydatid Ag5 in TPCF. It might reflect that Ag5 in TPCF, as detected by anti-Ag5 mAbs in ELISA, does not possess the same structure as Ag5 of THCF or SHCF and is thus incapable of forming an arc 5 band in IEP with mAbs 24.14 and 61A12. This point is further supported by a recent study in which it was found that rabbit antiserum to TPCF was capable of forming an arc 5 band with SHCF or THCF, whereas TPCF itself failed to form an arc 5 band with rabbit antisera to SHCF or THCF in IEP (Liu et al. 1992d). In addition, the discrepancy observed using TPCF in ELISA and IEP may also have been due to the comparatively lower sensitivity of the latter test. The low degree of reactivity between anti-AgB mAbs and THCF and TPCF in ELISA suggests that there are some components in THCF and TPCF that may share part of the AgB epitope with SHCF, but this similarity is not strong enough to enable the formation of an AgB band with mAbs in IEP. It could also be due to the possibility that mAbs 31.15 and 39B3 may be directed to antigenic epitopes of AgB that are not exactly shared by the equivalent molecule in THCF and TPCF. Nonetheless, rabbit sera raised against THCF or TPCF readily formed an AgB band with SHCF (Liu et al. 1992d).

The lack of reaction of all mAbs with Tso, Em and Ev antigens indicates that the epitopes identical to those recognised by these mAbs are not detected on antigens prepared from *T. solium* cysticerci, *E. multilocularis* protoscoleces and *E. vogeli* cyst membranes. Perhaps this could be exploited for species differentiation of *E. granulosus* from these taeniid parasites.

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