

## The 10 kDa protein of *Taenia solium* metacestodes shows genus specific antigenicity

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**Abstract:** Genus specific antigenicity of the 10 kDa protein in cyst fluid (CF) of *Taenia solium* metacestodes was demonstrated by comparative immunoblot analysis. When CFs from taeniid metacestodes of *T. saginata*, *T. solium*, *T. taeniaeformis* and *T. crassiceps* were probed with specific monoclonal antibody (mAb) raised against 150 kDa protein of *T. solium* metacestodes, specific antibody reactions were observed in 7 and 10 kDa proteins of *T. solium* and in 7/8 kDa of *T. saginata*, *T. taeniaeformis* and *T. crassiceps*. The mAb did not react with any protein in hydatid fluid of *Echinococcus granulosus* and *E. multilocularis*. This result revealed that the 10 kDa peptide of *T. solium* metacestodes and its equivalent proteins of different *Taenia* metacestodes are genus specific antigens that are shared among different *Taenia* species.

**Key words:** *Taenia* metacestodes, cyst fluid, monoclonal antibody, genus-specific protein

Neurocysticercosis (NCC), an infection of the central nervous system caused by *Taenia solium* metacestodes, is recognized as a major cause of neurological disease worldwide. The disease is now regarded as one of the most important emerging diseases together with other viral and drug resistant bacterial infections (White, 1997).

Neuroimaging findings from several neurological patients provide a clue leading to the

diagnosis of NCC. In many patients diagnosed with NCC, however, supplementary immunological tests were necessary to support the diagnosis because neuroimaging findings often failed to distinguish NCC from other brain diseases (Chang et al., 1988). A specific antigen is important for undertaking the serological tests. Cyst fluid (CF) of *T. solium* metacestodes is currently recognized as the most reliable sources of diagnostic antigens (Cho et al., 1988; Yang et al., 1998). Recently, a 10 kDa peptide of 150 kDa protein in the CF appeared to be sensitive and specific in diagnosing active NCC (Yang et al., 1998; Chung et al., 1999).

Many investigators have been searching for a substitute antigen in serodiagnosis of NCC since *T. solium* metacestodes are difficult to collect even in highly endemic areas. In addition, CFs of *T. crassiceps*, *T. saginata* and *T. hydatigena* have been regarded as substitute antigens, because they were found to

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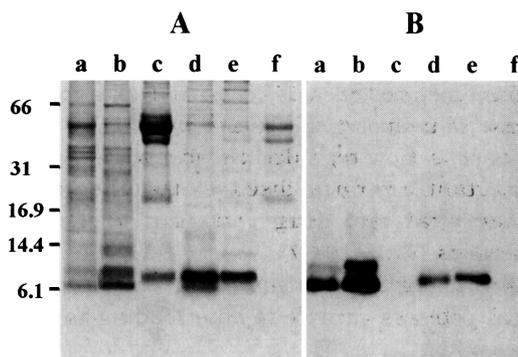
share common immunological responses against NCC (Hayunga et al., 1991; Zarlenga et al., 1994; Bueno et al., 2000). However, to understand the common antigenicity shown among different species, further studies are required with respect to the common epitopes which are shared not only by CF of *T. solium* and other *Taenia* species but also by hydatid fluid (HF) of *Echinococcus granulosus* and *E. multilocularis*. In the present study, we have examined whether the 10 kDa glycoprotein of *T. solium* metacestodes shares common antigenicity in CF/HF of several different taeniid metacestodes.

CF of *T. solium* metacestodes was collected from naturally infected pigs as described previously (Yang et al. 1998). Metacestodes of *T. saginata* were collected from an experimental calf which was challenged with 100,000 viable eggs. Metacestodes of *T. taeniaeformis* and *T. crassiceps* were harvested from laboratory maintained rats and mice, respectively. HF of *E. granulosus* was obtained from a patient with cystic echinococcosis. HF of *E. multilocularis* was collected from an experimental rat. The crude CFs and HFs were centrifuged at 20,000 *g* for 1 hr and the supernatant was used as antigens. All procedures were carried out at 4°C. They were stored at -70°C until use further.

Monoclonal antibody (mAb) was generated by immunizing BALB/c mice using the 150 kDa protein from native CF of *T. solium* metacestodes (Cho et al., 1988). Antibody secreting cells specific to 7, 10 and 15 kDa of 150 kDa proteins were selected and expanded. IgG fractions were purified by Protein-A Sepharose 4B affinity column (Pharmacia, Piscataway, NJ, USA). CFs and HFs from different species were separated by 10% Tricine SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The blot was incubated with mAb diluted at 1:200. Peroxidase conjugated anti-mouse IgG (whole molecule, Cappel, PA, USA) was used in dilution of 1:1,000. The blot was developed with 4-chloro-1-naphthol chromogen (Sigma, St. Louis, MO, USA).

Fig. 1A shows a protein profile resolved by Tricine SDS-PAGE. CF of *T. saginata* metac-

estodes exhibited four major bands between 40-60 kDa, but no prominent proteins in a low molecular weight range were observed. Only the 7 and 10 kDa proteins could be faintly identified (lane a). CF of *T. solium* metacestodes demonstrated several protein bands including 7, 10, and 15 kDa as major constituents (lane b). Those of *T. crassiceps* and *T. taeniaeformis* showed similar profiles in which 7 or 8 kDa protein was the major component (lanes d and e). HF of *E. granulosus* and *E. multilocularis* exhibited major bands at 52 and 43 kDa. In addition, a 8 kDa protein, which may be a subunit of *E. granulosus* antigen B (Fernandez et al., 1996), was observed in *E. granulosus* (lane c). The same blot was probed with generated mAb. As shown in Fig. 1B, CF of *T. solium* metacestodes reacted strongly to 7 and 10 kDa proteins (lane b). CF from *T. saginata* reacted strongly to a 7 kDa protein while reacting weakly to a 10 kDa protein (lane a). CFs of *T. taeniaeformis* (lane d) and *T. crassiceps* (lane e) showed reactive band each at 7 or 8 kDa with minor difference in their molecular weights. In contrast, the mAb did not recognize any reactive band in HFs of *E. granulosus* or of *E. multilocularis* (lanes c and



**Fig. 1.** (A) Analysis of the CF proteins resolved by 10% Tricine SDS-PAGE stained with Coomassie blue. (B) Immunoblot analysis of CF/HF from metacestodes of different taeniid cestodes with anti-150 kDa mAb of *T. solium* metacestodes. The CFs are probed with 1:200 diluted antibody. CFs from metacestodes of *T. saginata* (lane a), *T. solium* (lane b), *T. taeniaeformis* (lane d) and *T. crassiceps* (lane e) show immunoreactive bands at around 7-10 kDa. On the contrary, those from *E. granulosus* (lane c) and *E. multilocularis* (lane f) do not show positive reactions.

f).

The present observation demonstrated clearly that the 7 or 8-10 kDa proteins are common components present mostly in CFs of different *Taenia* species metacestodes. As shown in Fig. 1A, there were two distinctive bands corresponding to 10 and 7 kDa in molecular weights in CF of *T. solium* metacestodes while only one band, approximately 7 or 8 kDa in size, was found in other *Taenia* species. The proteins had previously been described as 10 kDa possibly due to a different degree of glycosylation as well as to a low resolving power of conventional SDS-PAGE that failed to accurately determine the molecular weights (Hayunga et al., 1991; Zarlenga et al., 1994; Chung et al., 1999). In fact, the proteins were assumed to be 7 or 8 kDa judged by Tricine SDS-PAGE as shown in this study.

The mechanism of common antigenicity shared between the 10 and 7 kDa proteins in CF of *T. solium* metacestodes is poorly understood. A possible explanation is that the two proteins may be a subunit of the 150 kDa protein complex which shares a high degree of sequence homology. Elucidation of molecular information of these related genes will likely to resolve this hypothesis.

The 10 kDa antigen identified in previous and present studies (Yang et al., 1998; Chung et al., 1999) seems to be same with the 8 kDa peptide described by Rodriguez-Canul et al. (1998). It is also possible that the 10 kDa protein is a reduced form of glycoprotein of 24 and 39-42 kDa (Tsang et al. 1989) as analyzed by Plancarte et al. (1999). Clearly, the 10 kDa antigen of *T. solium* metacestodes or 7 or 8 kDa proteins with similar diagnostic properties present in other *Taenia* species could be valuable in strengthening clinical diagnosis of NCC. With this 10 kDa protein, the differential diagnosis of cysticercosis from both cystic and alveolar echinococcosis may be possible.

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