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BRIEF COMMUNICATION

PRODUCTION OF MONOCLONAL ANTIBODIES ANTI-*Taenia crassiceps* CYSTICERCI WITH CROSS-REACTIVITY WITH *Taenia solium* ANTIGENS

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SUMMARY

We describe the production of the potential monoclonal antibodies (MoAbs) using BALB/c mice immunized with vesicular fluid (VF)-Tcra (*T. crassiceps*) antigen. Immune sera presented anti-VF-Tcra (<20kD) IgG and IgM antibodies with cross-reactivity with *T. solium* (Tso) antigen (8-12, 14, and 18 kD). After cell fusion, we selected 33 anti-Tcra and anti-Tso reactive IgM-clones and 53 anti-Tcra specific IgG-clones, 5 of them also recognizing Tso antigens. Two clones identified the 8-14 and 18kD peptides of VF-Tcra.

KEYWORDS: *Taenia solium*; *Taenia crassiceps*; Monoclonal antibody; Cysticercosis.

The teniasis-cysticercosis complex is a public health problem in many countries, with neurocysticercosis (NC) being the most severe form caused by the presence of *Taenia solium* cysticerci (Tso) in the central nervous system. The search for antibodies in serum and cerebrospinal fluid (CSF) using antigens obtained from Tso has been efficiently utilized for diagnosis[4,12,13]. Monoclonal antibodies (MoAbs) anti-Tso have been used to detect antigens in CSF samples from patients with NC[2,3] or to obtain specific antigens by immunoaffinity[9]. The production of sufficient amounts of antigens from Tso cysticerci is impaired by the difficulty in obtaining parasites from infected swine, with the need to investigate alternative parasites. Cross-reactivity between Tso and *Taenia crassiceps* cysticerci (Tcra) obtained by intraperitoneal inoculation of mice has been reported[6,7,10,13].

In the present study we describe the cross-reactivity of the antibodies produced by mice immunized with Tcra and the production of anti-Tcra MoAbs. The total Tso antigen (T-Tso) and the vesicular fluid antigen of Tcra (VF-Tcra) were prepared as described before[13]. By SDS-PAGE, the T-Tso antigen presented various peptides of 8 to 158kDa and the VF-Tcra antigen was rich in <20kDa peptides (Figure 1). Low molecular weight peptides of Tcra[6] and of Tso[4,12] antigens have been reported to be immunodominant in cysticercosis.

For cell fusion, female BALB/c mice were immunized with 50 µg VF-Tcra and 30 days later received a booster containing double the initial concentration. The animals were bled before and at different days after immunization. IgM and IgG antibodies were detected in the immune sera by ELISA with the Tcra and Tso (cross-reactivity) antigens starting on the 15th day, with IgM presenting a second peak after the booster. The blots showed that IgM and IgG antibodies identified the 12-14 and 18kDa peptides of VF-Tcra and also cross-reacted with the peptides of 8-12kDa, 14 and 18 kDa of the T-Tso antigen (Figure 1).

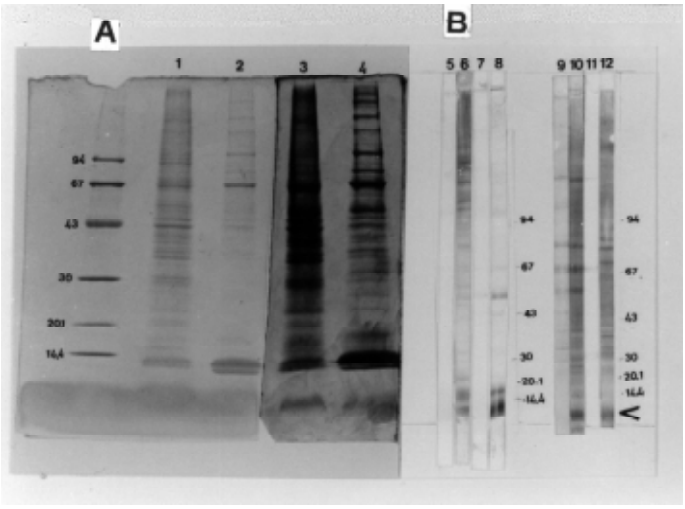

Fig. 1 - **A**: Tso (1, 3) and Tcra (2, 4) peptides identified by SDS-PAGE [Coomassie Blue (1, 2) and silver nitrate (3, 4) staining]. **B**: Immunoblotting of Tcra (5-8) and Tso (9-12) antigens recognized by IgM (5, 6, 9, 10) and IgG (7, 8, 11, 12) antibodies from serum of mice immunized with VF-Tcra. Lanes 5, 7, 9, 11: before immunization; lanes 6 and 10: 37th day after immunization; lanes 8 and 12: 83rd day after immunization.

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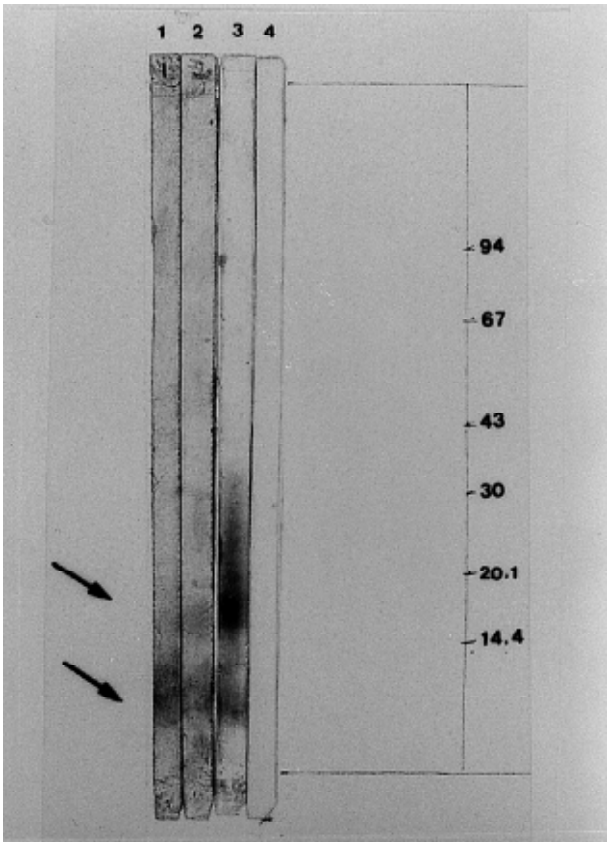

Fig. 2 - Immunoblotting with VF-Tcra antigen of the supernatant of 3B7 (1) and 3E2 (2) clones, immune serum from mice (3) and culture medium (4).

HAYUNGA *et al.*[5], reported that sera from swine immunized with Tso, from cattle immunized with *T. saginata*, and from human cysticercosis recognized the <12kDa peptide of the VF-*T. hydatigena*, and the sera from swine also identified the <12kDa peptide of Tcra.

Cell fusion was performed with 1.5 x 10^8 immunized mouse spleen cells and 1.5 x 10^7 P_3X_{63}-Ag8.653 myeloma cells and the cells were distributed in four 96-well microplates. The supernatants from two (2x96) plates were assayed after 11 days by ELISA and the results showed 33 IgM-clones with reactivity to Tcra and Tso antigens and 53 IgG-clones anti-Tcra, 5 of them also recognizing Tso antigens.

On the 13th day after fusion, we selected 72 clones from the remaining two plates (2x96) with cross-reactivity to T-Tso antigens using anti-Ig conjugate. These 72 clones were distributed in three 24-well plates for expansion in a larger volume of culture medium. Nine days after the expansion we selected 24 clones by ELISA using anti-Ig conjugate. The 3B7 and 3E2 clones identified the 8-14 and 18kDa peptides of VF-Tcra antigen (Figure 2).

We did not find out any other publication reporting a MoAb to VF-Tcra with cross-reactivity with Tso. For the study of the teniasis-cysticercosis complex, other authors have reported production of MoAb such as anti-Tso to detect swine cysticercosis[11], specific anti-Tso eggs[8], and anti-Tso cross-reacting with *T. saginata* and *T. taeniaeformis*[1].

In conclusion, our results and the successful production of anti-Tcra MoAbs cross-reacting with Tso antigens indicate their potential usefulness in the detection of antigens in samples from human and/or swine cysticercosis. The MoAbs could also be utilized for the purification of cross-reacting antigens of VF-Tcra to be used in immunologic tests to detect anti-Tso cysticercus antibodies.

RESUMO

Produção de anticorpos monoclonais anti-cisticercos de *Taenia crassiceps* com reatividade cruzada com antígenos de *Taenia solium*

É descrita a produção de potenciais anticorpos monoclonais (MoAbs) usando camundongos BALB/c imunizados com antígenos de líquido vesicular de *T. crassiceps* (VF-Tcra). O soro imune apresentou anticorpos IgM e IgG anti-VF-Tcra para os peptídeos <20kDa, e com reatividade cruzada com peptídeos 8-12, 14 e 18kDa de *T. solium* (Tso). Após a fusão, foram selecionados 33 clones IgM com reatividade anti-Tcra e anti-Tso e 53 clones IgG com reatividade específica, sendo que destes, 5 apresentaram reatividade cruzada com antígeno de Tso. Dois clones identificaram os peptídeos 8-14 e 18kDa de VF-Tcra.

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