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BRIEF COMMUNICATION

Schistosoma mansoni: DESCRIPTION OF A POTENTIALLY USEFUL MONOCLONAL ANTIBODY THAT RECOGNIZES SOLUBLE EGG ANTIGEN (SEA)

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KEYWORDS: *Schistosoma mansoni*; Monoclonal antibodies; Soluble egg antigen (SEA); Carbohydrate epitopes.

Many research groups have obtained monoclonal antibodies (MAbs) that react with SEA derived from *Schistosoma mansoni*. Most of them recognize carbohydrate epitopes that are shared by different developmental stages of the parasite[5, 6, 9]. Some of these reagents were shown to be useful to quantitate egg circulating antigens and can be considered a good assessment of infected individuals egg load. As a consequence, these assays are a potential diagnostic parameter for morbidity[7]. Also, they can be used to study the fate of the antigen in the host[1].

One of us have previously reported the production of an IgM MAb (3C6) that reacted with schistosomula, eggs and the inner layer of the gut from adult worms of *S. mansoni*[3]. It has been raised by immortalization of spleen cells of a seven-week-infected mouse. As a consequence, it represents an antibody produced under normal circumstances of infection, and the recognized epitope deserves investigation. The hybridoma 3C6 was recently re-cloned by limiting dilution generating a stable cell line called 3C6H6. Gel filtration-purified MAb was obtained from ascitic fluid and used in all experiments.

Immunoperoxidase reactions over liver tissue sections of infected hamsters showed a strong reactivity with egg antigens both inside and outside the egg shell (Fig. 1). When tested on adult worm sections MAb 3C6H6 bound to the digestive tract and tegument as previously shown[3]. ELISAs using purified SEA[2] and adult worm antigen (AWA)[4] showed a saturable reaction with SEA, and almost no recognition of AWA (Fig. 2).

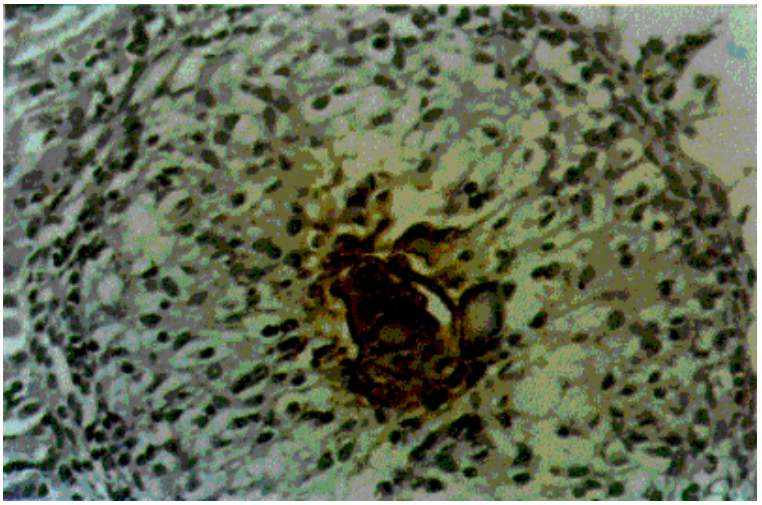

Fig. 1 - Immunoperoxidase of MAb 3C6H6 (5 µg/ml) on an infected hamster liver tissue section showing recognition of SEA diffusing from the egg shell. The aecond biotinylated antibody as well as avidin-biotin-peroxidase complex were from Vector Labs. (Vecstatin ABC kit). Diaminobenzydine diluted in PBS containing 0.005% H_2O_2 (v/v) was used as substrate, and hematoxylin as counterstain (400x magnification)

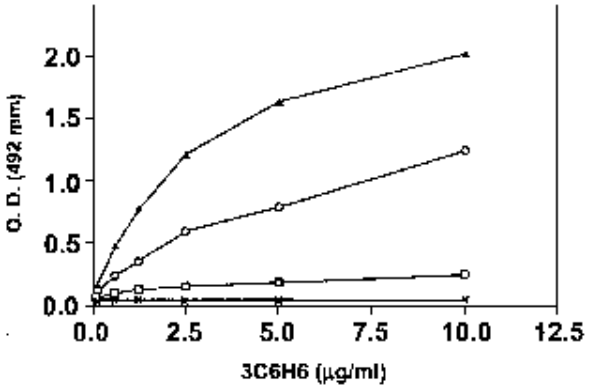

Fig. 2 - ELISA of MAb 3C6H6 tested in serial dilutions on solid phase coated with SEA (10 µg/ml)(t) and AWA (10 µg/ml)( ). 5mM (O) and 20 mM (x) $NaIO_4^-$ treated SEA were also tested. Second antibody was a goat anti-mouse IgM from Sigma, and the substrate employed was orto-phenylenediamine in citrate-phosphate buffer containing 0.03% H_2O_2 (v/v).

In order to partially characterize the chemical nature of the recognized epitope, SEA was treated by the sugar oxidant sodium periodate in different concentrations, according to WOODWARD, 1985[8]. Figure 3 depicts an immunoblotting assay of MAb 3C6H6 recognition of SEA transferred to nitrocellulose sheets and treated or not with $NaIO_4$. These results were confirmed in ELISA, as shown in figure 2.

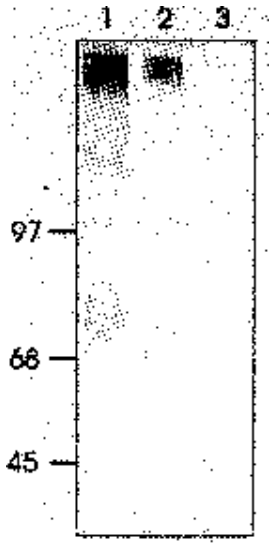

Fig. 3 - Immunoblotting of MAb 3C6H6 (10µg/ml) on SEA (10µg/slot) submitted to SDS-PAGE in a 10% gel using reducing conditions and transferred to nitrocellulose membrane. Before incubation with MAb 3C6H6, membrane strips were treated or not (lane 1) with $NaIO_4$ using the following concentrations: 5 mM (lane 2) and 20 mM (lane 3). The second antibody and the substrate employed were goat antimouse IgM alkaline-phosphatase conjugate and BCIP/NBT (from Sigma), respectively.

Taken together, these data demonstrate that the epitope recognized by MAb 3C6H6 presents a glycidic nature, and can be similar to one of the already described MAbs[5, 6, 9]. This reagent shows its usefulness in the recognition of antigen deposits trapped in glomeruli of infected experimental animals (DE BRITO, T. et al.; manuscript in preparation). Also, its previously shown reactivity with adult worms digestive tract and schistosomula opens the possibility of studies on protection immunity and/or its potential utilization on detection of circulating antigens.

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