A carbohydrate antigen of *Clonorchis sinensis* recognized by a species-specific mononal antibody

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Abstract: The enzyme-linked immunosorbent assay (ELISA)-inhibition test using a *Clonorchis sinensis* species-specific mouse monoclonal antibody (MAb). CsHyb 0605-23, showed increased specificity over the conventional ELISA used for serodiagnosis of clonorchiasis. To characterize the corresponding antigen further, the MAb was tested against polysaccharide, protein and glycolipid fractions obtained from a crude extract of *C. sinensis* adult worms, using chloroform, methanol and phenol extractions. Only the polysaccharide fraction was recognized by the MAb among those fractions. Mild oxidation of the antigen with sodium periodate showed decreased reactivity against the MAb. We concluded that the antigen and antigenic determinants recognized by the MAb are carbohydrates.

Key words: monoclonal antibody, *Clonorchis sinensis*, antigenic determinant, carbohydrate, polysaccharide

*Clonorchis sinensis* is one of the most important parasites of man in Korea. As previously reported, MAbs were developed for the serodiagnosis of clonorchiasis. The specificity of the serodiagnostic test was increased by using *C. sinensis* species-specific MAb, CsHyb 0605-23. Some characteristics of the corresponding antigen against the MAb were described in the previous report as follows. A very broad reacting band pattern with high molecular weight was present on Western blot analysis. The reacting antigen was observed in the fraction beyond the exclusion limit separated by Sephadex G-200 gel filtration. The antigen was shown to be distributed throughout the intestine and uterus of the adult worms by immunofluorescent microscopy. The isotype of the MAb was IgA (Yong et al., 1991).

Two hundred mg of lyophilized *C. sinensis* adult worms collected from an experimental rabbit's bile duct were extracted twice with chloroform/methanol (2:1) at 50°C, and centrifuged at 2,000 rpm for 10 min. The supernatant, containing glycolipid, was removed and evaporated at 50°C. Twenty-six mg of glycolipid was obtained from the supernatant fraction. The pellet was extracted with 45% aqueous phenol at 68°C for 30 min., and centrifuged at 10,000 rpm for 45 min. The aqueous phase containing polysaccharide was removed. Dialysis and lyophilization were done
subsequently, resulting in 5 mg of polysaccharide. The protein fraction was obtained from the phenolic phase (Folch et al., 1957; Sutherland and Wilkinson, 1971).

The MAb (Cshyb 0605-23), mouse immune serum, or non-immune mouse serum (negative control) were tested against 3 different fractionated antigens purified as above using ELISA. The immune serum demonstrated a high reactivity against both of the protein and polysaccharide fractions, indicating that the protein or the polysaccharide fractions could serve as antigens in clonorchiasis, as expected. However, the MAb reacted with the polysaccharide fraction only (Fig. 1). The species-specific antigen against the MAb was identified as a carbohydrate. The glycolipid fraction of C. sinensis did not demonstrate any noticeable reactivity to the immune serum or the MAb, indicating poor antigenicity of the fraction, even though the glycolipids are ubiquitous constituents of cell membranes that have many important biological activities, and are shown to have immunogenic properties as well as potential pathogenic

![Absorbance at 492 nm](image)

**Fig. 1.** Seroreactivity of BALB/c mice immunized with crude somatic antigens of C. sinensis adult worms against glycolipid, polysaccharide, or protein fractions of C. sinensis by ELISA. Fractionated antigens were reacted with MAb (Cshyb 0605-23), mouse immune serum, and non-immune mouse serum (negative control), respectively. The MAb reacted with the polysaccharide fraction only, whereas the immune serum showed a high reactivity with both the protein and polysaccharide fractions. The glycolipid fraction of C. sinensis did not show any noticeable reactivity to mouse immune serum or the MAb.

[Graph showing absorbance at 492 nm vs. sodium periodate concentration]

**Fig. 2.** The crude antigen of C. sinensis adult worms was coated on the ELISA plate, and treated with sodium periodate at 0, 5, 10, 15, 20 mM. The culture supernatant containing MAb was reacted. Reactivity against MAb decreased, and was dose-dependent.
carbohydrate antigens, as described in this study. Further characterization of carbohydrate antigens of *C. sinensis* are needed using more advanced biochemical or molecular biological methods.

**REFERENCES**


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=초록=

중특이 단세포균항체에 반응하는 간흡충의 당질항원 한 가지

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간흡충 두더 단세포균항체인 CaHyb 0605-23과 반응하는 간흡충 항원의 특성을 밝히기 위하여 간흡충의 성충 조합원을 당장질. 당장질. 당장질로 각각 분리한 후, 각각 항원으로 사용하여 단세포균항체와 호소면역흡착검사를 실시하였다. 그 결과, 오직 당장질분획만이 단세포균항체와 반응하였고, 당장합관을 sodium periodate를 사용하여 약하게 산화시키자 단세포균항체와의 반응도가 떨어졌다. 따라서 이 단세포균항체에 반응하는 간흡충의 항원 및 항원결합기는 당장질로 생각된다.