The Korean Journal of Parasitology
Vol. 34, No. 4, 279-281, December 1996

☐ **Brief Communication** ☐

A carbohydrate antigen of *Clonorchis sinensis* recognized by a species-specific monolonal 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 serodiagnotic 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

• Received Sep. 20 1996, accepted Nov. 16 1996.

• This study was supported through a teaching assistant research grant by Yonsei University College of medicine, 1996.

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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 effectors in some infections (Klatser *et al.*, 1985; Sut *et al.*, 1990).

Mild periodate oxidation has been shown to cleave carbohydrate vicinal hydroxyl groups without altering the structure of the polypeptide chain (Mates & Steiner, 1978). It was observed that periodic acid oxidation of flagellar glycoproteins abolishes binding of a group of putative anti-carbohydrate MAbs by dose-dependent inhibition using enzyme-linked immunoassays (Woodward *et al.*, 1984). In this study, we tried to determine the carbohydrate or peptide characteristic of the antigenic determinant by similar methods. After coating an ELISA plate with crude antigens (20 μg/ml), wells were treated with sodium metaperiodate at 0, 5, 10, 15, 20 mM, respectively, for 1 hour. The MAb was reacted subsequently. Results showed that the decrease in absorbance was dose-dependent (Fig. 2).

In conclusion, the antigen and antigenic determinants recognized by one of the *C. sinensis* species-specific MAbs are carbohydrates. Although research on infectious organisms principally focuses on protein antigens, there are also important

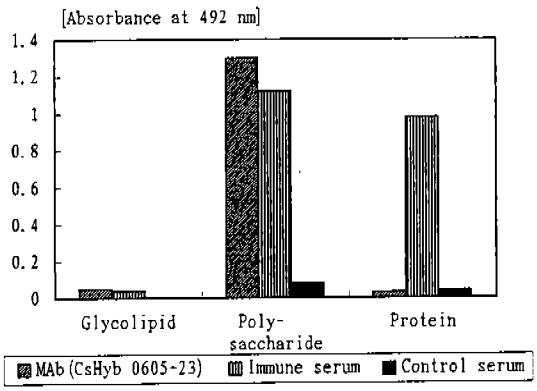

Fig. 1. Seroreactivity of BALB/c mice immunized with crude somatic antigens of *C. sinensis* adult worms against glycolipid, polysaccharide, or protein fractions of *C. sinensis* by ELISA. Fractionated antigens were reacted with MAb (CsHyb 0605-23), mouse immune serum, and non-immune mouse serum (negative 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.

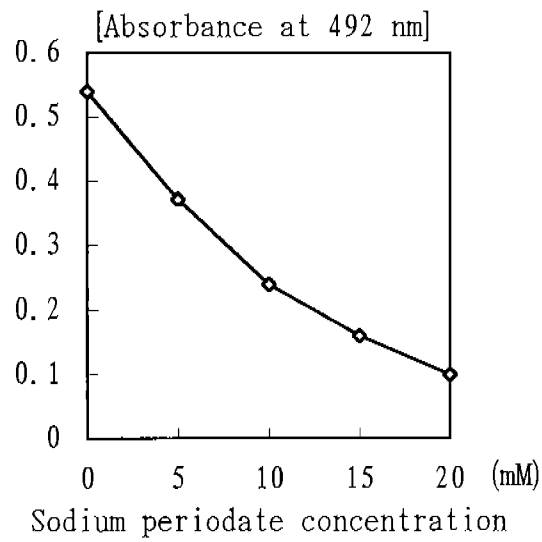

Fig. 2. The crude antigen of *C. sinensis* adult worms was coated on the ELISA plate, and treated with sodium periodate at 0, 5, 10, 15, 20 mM. The culture supernatant containing MAb was reacted. Reactivity against MAb decreased, and was dose-dependent.

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

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226:** 497-509.

Klatser PR, Madeleiney YL, Wit DE, Kolk AHJ (1985) An ELISA-inhibition test using monoclonal antibody for the serology of leprosy. *Clin Exp Immunol* **62:** 468-473.

Mates MJ, Steiner LA (1978) Antisera to frog immunoglobulins cross-react with a periodate-sensitive cell surface determinant. *Nature* **273:** 761-763.

Sut A, Sirugue S, Sixou S, Lakhdar-Ghazal F, Tocanne JF, Lanceelle G (1990) Mycobacteria glycolipids as potential pathogenicity effectors: alteration of model and natural membranes. *Biochemistry* **29:** 8498-8502.

Sutherland IW, Wilkinson JF (1971) Chemical extraction methods of microbial cells. In: Methods in Microbiology (Norris, JR, Ribbons DW ed.), Vol. 5B p345-383, Academic Press, London, U.K.

Woodward MP, Young Jr WW, Bloodgood RA (1984) Carbohydrate epitopes on flagellar membrane glycoconjugates. *J Cell Biol* **99:** 176a.

Yong TS, Im KI, Chung PR (1991) Analysis of *Clonorchis sinensis* antigens and diagnosis of clonorchiasis using monoclonal antibodies. *Korean J Parasitol* **29:** 293-310.

=초록=

종특이 단세포군항체에 반응하는 간흡충의 당질항원 한 가지

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간흡충 특이 단세포군항체인 CsHyb 0605-23과 반응하는 간흡충 항원의 특성을 밝히기 위하여 간흡충의 성충 조항원을 당지질, 당질, 단백으로 각각 분리한 후, 각각 항원으로 사용하여 단세포군항체와 효소면역흡착검사법을 실시하였다. 그 결과, 오직 당질분획만이 단세포군항체와 반응하였다. 당질항원을 sodium periodate를 사용하여 약하게 산화시키자 단세포군항체와의 반응도가 떨어졌다. 따라서 이 단세포군항체에 반응하는 간흡충의 항원 및 항원결정기는 당질로 생각된다.

〔기생충학잡지 34(4): 279-281, 1996년 12월〕