

DIAGNOSIS OF CLONORCHIASIS BY ELISA-INHIBITION TEST USING A *CLONORCHIS SINENSIS* SPECIFIC MONOCLONAL ANTIBODY

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Abstract. The ELISA-inhibition test using *Clonorchis sinensis* specific monoclonal antibody (CsHyb 0605-23) for diagnosis of clonorchiasis was carried out. It demonstrated sensitivity and high specificity in comparison with the conventional ELISA.

INTRODUCTION

Clonorchis sinensis is a common parasite of man which is largely confined to areas in Eastern Asia, including Japan, China, Taiwan, North Vietnam and South Korea. It causes various pathological changes in the bile duct and surrounding liver tissues. Immunological studies on clonorchiasis, especially research on specific antigens of *C. sinensis* is of value not only for elucidating characteristics of infection with this fluke, but also for diagnosis.

Monoclonal antibodies having high affinity for specific antigenic determinants (Köhler and Milstein, 1975) have been used to study parasite immunology. Reports on monoclonal antibodies produced against *C. sinensis* have been rare.

The present study was carried out to analyze *C. sinensis* antigens recognized by monoclonal antibodies and to set up an ELISA-inhibition test to improve the specificity of immunodiagnostic tests.

MATERIALS AND METHODS

Hybridomas were produced by fusion between spleen cells of Balb/c mice immunized with *C. sinensis* antigens and plasmacytoma cells (P₃-X63-Ag 8.V653) originally from the Balb/c mice. Four specific monoclonal antibodies were selected from the hybridomas. Characteristics of these monoclonal antibodies and antigenic determinants recognized by them were studied. An ELISA-inhibition test was set up using one

monoclonal antibody (CsHyb 0605-23) and it was compared to a conventional ELISA (Fig 1).

RESULTS

The results obtained in this study were as follows:

1. Twenty-nine clones secreting monoclonal antibodies against *C. sinensis* antigens were produced and 8 were determined to be specific. Others cross-reacted with various parasite antigens.

2. Six clones were determined to be secreting monoclonal antibodies against exposed antigenic determinants of natural infection.

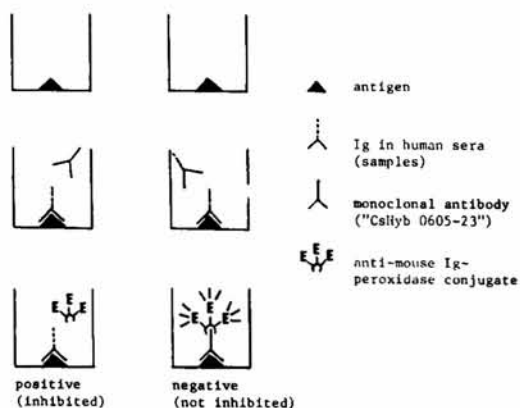


Fig 1 - ELISA-inhibition test using specific monoclonal antibody ("CsHyb 0605-23") to detect antibodies against *Clonorchis sinensis* in the human sera.

3. Isotypes of 4 selected specific monoclonal antibodies were IgG1 (CsHyb 0714-20, CsHyb 0714-25), IgG2b (CsHyb 0605-10), and IgA (CsHyb 0605-23).

4. By enzyme-immunoelectrotransfer blot, CsHyb 0714-20 and CsHyb 0605-10 were each found to be reactive against 10 kDa and 34 kDa epitopes.

5. The antigenic determinant recognized by CsHyb 0714-20 was revealed to be present at the surface and parenchyma of a fluke by indirect immunofluorescent assay. CsHyb 0605-10 and CsHyb 0714-25 were reactive to antigens in the parenchyma and intestines. CsHyb 0605-23 was found to recognize the antigenic determinant around the uterine eggs.

6. Four of the specific monoclonal antibodies tested reacted to the early eluted fractions of *C. sinensis* antigens separated by Sephadex G-200 gel filtration.

7. The epitopes recognized by four monoclonal antibodies were determined to be proteins which were destroyed by treatment with pronase or trypsin.

8. Using the conventional ELISA, 75% of patients with clonorchiasis were positive, while 7.1% of the normal controls and 37.5% of sera from patients with paragonimiasis were considered false positives. However, by the ELISA-inhibition test using a *C. sinensis* monoclonal antibody (CsHyb 0605-23), 77.1% of clonorchiasis cases were determined to be positive. There were no false negative reactions from the normal controls or the paragonimiasis cases.

In conclusion, the ELISA-inhibition test using CsHyb 0605-23 had more sensitivity and higher specificity compared to the conventional ELISA (Figs 2 and 3).

DISCUSSION

Clonorchiasis is an important human parasitic disease in East Asia (Rim, 1986). In Korea, it is thought that about 2 million people are infected with the parasite.

A number of attempts have been made to use immunological tests for the diagnosis of the

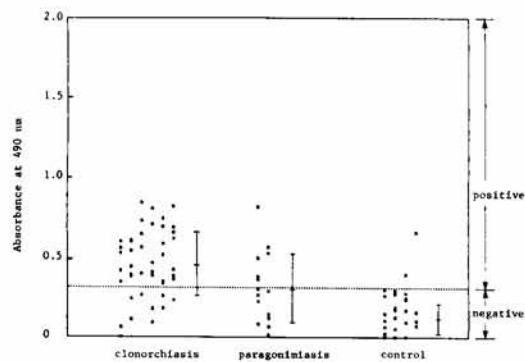


Fig 2 - Distribution of ELISA values of the sera in clonorchiasis and paragonimiasis cases against *Clonorchis sinensis* antigens.

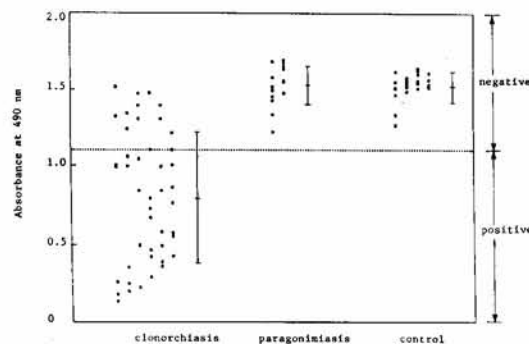


Fig 3 - Distribution of ELISA-inhibition test values of the sera in clonorchiasis and paragonimiasis cases using monoclonal antibody ("CsHyb 0605-23") against *Clonorchis sinensis* antigens.

disease, such as CF, IFA (Cho and Soh, 1974) and ELISA (Lee *et al*, 1981; Yang *et al*, 1983). Some were of value, but the cross-reaction with other parasites or diseases were a problem (Cho and Soh, 1976; Choi, 1975). The ELISA-inhibition test using a specific monoclonal antibody has been used in the diagnosis of a variety of infectious diseases (Klaster *et al*, 1985; Cabrera *et al*, 1989). Using CsHyb 0605-23, produced and used in this study in the ELISA-inhibition test, there was no cross-reaction with antigens of other helminths. It reacted to the early fractions of *C. sinensis* antigens separated by Sephadex G-200 gel filtration. Antigenic determinants recognized by this monoclonal antibody were located mainly

around the uterine eggs and were protein in nature.

The results showed that the sensitivity of this system was almost the same as the conventional ELISA, and the specificity was determined to be excellent. Performing the ELISA-inhibition test was easy and did not require more time than the conventional ELISA. Moreover, it did not require any laborious steps to purify specific antigens to increase the specificity of an immunodiagnostic test. Therefore, the ELISA-inhibition test using specific monoclonal antibodies can be applied in the diagnosis of various parasitic diseases.

REFERENCES

- Cabrera Z, Parkhouse RME, Forsyth K, Gomez Priego A, Pabon R, Yarzabal L. Specific detection of human antibodies to *Onchocerca volvulus*. *Trop Med Parasitol* 1989; 40:454-9.
- Cho KM, Soh CT. Evaluation of the indirect fluorescent antibody test with adult worm antigen for the immunodiagnosis of clonorchiasis. *Yonsei Rep Trop Med* 1974; 5:45-6.
- Cho KM, Soh CT. Indirect fluorescent antibody test for the serodiagnosis of paragonimiasis and clonorchiasis. *Yonsei Rep Trop Med* 1976; 7:26-39.
- Choi WY. Indirect fluorescent antibody test for diagnosis of clonorchiasis. *Korean Cent J Med* 1975; 28:533-7 (in Korean).
- Klaster PR, Madeleiney YL, Wit DE, Kolk HJ. An ELISA-inhibition test using monoclonal antibody for the serology of leprosy. *Clin Exp Immunol* 1985; 62:468-73.
- Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256:495-7.
- Rim HJ. The current pathobiology and chemotherapy of clonorchiasis. *Korean J Parasitol* 1986; 24 (suppl):1-141.
- Lee JK, Min DY, Im KI, Lee KT, Soh CT. Study of enzyme-linked immunosorbent assay (ELISA) method in serodiagnosis of *Clonorchis sinensis* infection. *Yonsei J Med Sci* 1981; 14:133-46 (in Korean).
- Yang JS, Lee JS, Rim HJ. The use of ELISA in the diagnosis of human clonorchiasis. *Korea Univ Med J* 1983; 20:201-10 (in Korean).