

# DETECTION OF *ANGIOSTRONGYLUS MALAYSIENSIS* CIRCULATING ANTIGEN USING MONOCLONAL ANTIBODY-BASED ENZYME-LINKED IMMUNOSORBENT ASSAY (MAb-ELISA)

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**Abstract.** Three MAbs 1C4.2D8, 1C4.2C4 and 1C4.1F5 were produced using sonicated adult worm antigens of *Angiostrongylus malaysiensis* and they were found to be secreters of IgG1. The MAbs 1C4.2C4 and 1C4.2D8 were found to react with antigens of *A. malaysiensis* and cross-react with the closely related *A. cantonensis* but not with other helminths. A total of 108 human sera collected from Orang Asli (aborigines) from Grik, in the State of Perak were tested for *A. malaysiensis* infection using the MAb-ELISA. MAb 1C4.1F5 and 25 (23%) were positive. Twenty of these positive samples were tested with the MAb 1C4.2D8 and none was found to be positive.

## INTRODUCTION

*Angiostrongylus malaysiensis* is the causative agent of angiostrongyliasis in Malaysia (Watts, 1969; Bisseru *et al.*, 1972). The disease first came to attention when Rosen *et al.*, (1967) reported an outbreak of eosinophilic meningoencephalitis due to *Angiostrongylus cantonensis* in Tahiti. Subsequently, hundreds of cases of eosinophilic meningoencephalitis have been diagnosed in Taiwan (Chen, 1979). The distribution of *A. malaysiensis* is widespread in Malaysia and a serological (ELISA) survey carried out using adult worm antigens has shown a 76% exposure rate to the parasite. In experimental animals, the migratory phase through the central nervous system of the infective stage and the development of the juvenile adult stage in the brain causes the pathology (Ambu *et al.*, 1985).

Various immunodiagnostic technics utilizing either crude or partially purified adult worm antigen of *A. cantonensis* have been used for the diagnosis of the infection (Welch *et al.*, 1980; Chen, 1986; Cheng *et al.*, 1989). The diagnosis of the disease in Malaysia may have been overlooked as clinical and laboratory findings are unreliable because of confusion with other central nervous system disorders. In order to overcome this problem an attempt was made to produce monoclonal antibodies (MAbs) for the diagnosis of *A. malaysiensis* infection.

The aim of the study was to produce monoclonal antibodies that could be used in the development of a specific and sensitive MAb-ELISA diagnostic system for the detection of *A. malaysiensis* infection in man.

## MATERIALS AND METHODS

Sonicated adult worm antigens of *A. malaysiensis* were used for the production of the MAbs. The MAbs were developed by fusing spleen cells from mice immunized with crude antigen of the adult worms with myeloma cells NS1 as described by Kohler and Milstein (1975). The immunoglobulin subclasses were identified using Iso Strips (Mouse Monoclonal Antibody Isotyping Kit, Boehringer-Mannheim, Indianapolis, USA).

Cross-reactivity studies on these MAbs to the closely related and more pathogenic *A. cantonensis* and other helminths were carried out using the indirect ELISA method (Voller *et al.*, 1976). The proteins of *A. malaysiensis* antigen were separated on SDS-polyacrylamide gel using the discontinuous system of Laemmli (1970). These proteins were electrophoretically transferred to a nitrocellulose membrane and the ascites containing the MAbs were then allowed to react with the proteins (Western blot analysis, Towbin *et al.*, 1979).

Development of a MAb-ELISA system for the detection of the *A. malaysiensis* antigen was carried out using the technic of Zheng *et al* (1987) with some modification.

## RESULTS

Three MAbs were produced - 1C4.1F5, 1C4.2C4 and 1C4.2D8-and their immunoglobulin subclasses were determined by using Iso-strips. They were found to be secreters of IgG1. The negative reference value for the test was obtained from sera of 30 healthy donors using the MAb-based ELISA. A test was considered positive when OD value was equal or more than the mean +3Std deviation of OD readings obtained from the controls.

The MAb 1C4.2D8 was found to be reactive to a 92 kDa band (Fig 1) and MAbs 1C4.1F5 and 1C4.2D4 reactive to 23 kDa band (Fig 2) of the sonicated adult worm antigens respectively. MAb

1C4.1F5 crossreacted with all other helminth antigens as shown in Table 2. MAbs 1C4.2C4 and 1C4.2D8 reacted with *A. malaysiensis* and cross-reacted with the closely related *A. cantonensis* but not with other helminth antigens such as *Toxocara canis*, *Ascaris lumbricoides*, *Schistosoma malaysiensis*, *Dirofilaria immitis* and *Septhacia muris*. The lowest level of antigen detectable by the assay was determined by using a known concentration of *A. malaysiensis* antigen. The antigen was diluted in pooled negative sera and then assayed using the MAb-based ELISA system. The lowest level of antigen detected at 1:100 dilution was 0.084 ug/ml (Table 1).

A total of 108 human sera collected from Orang Asli (Aborigines) from Grik, in the State of Perak were examined for *A. malaysiensis* infection using the MAb-ELISA. MAb 1C4.1F5 and 25 (23%) were considered positive cases (Table 3). Twenty of these positive samples were tested with the MAb 1C4.2D8 and none were found to be positive (Table 3).

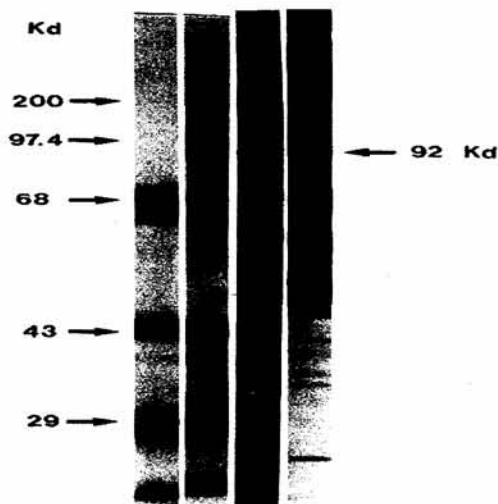


Fig 1 - Immunoblotting analysis of MAb 1C4.2D8 reacting to *A. malaysiensis* sonicated adult worm antigen.

Lane 1: Prestained low molecular weight protein standards - 2,850 to 43,000 daltons range.

Lane 2: Prestained high molecular weight protein standards - 14,300 to 200,000 daltons range.

Lane 3: MAb 1C4.2D8 reacting to *A. malaysiensis* adult worm antigen.

Lane 4: Polyclonal sera to *A. malaysiensis* adult worm antigen raised in rabbit reacting to its own antigen.

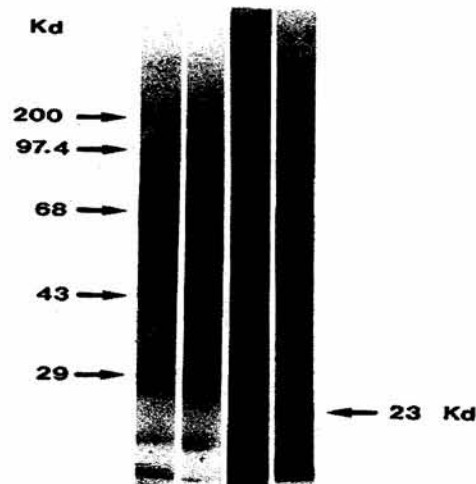


Fig 2 - Immunoblotting analysis of MAbs 1C4.1F5 and 1C4.2C4 reacting to *A. malaysiensis* sonicated adult worm antigen.

Lane 1: Prestained low molecular weight protein standards - 2,850 to 43,000 daltons range.

Lane 2: Prestained high molecular weight protein standards - 14,300 to 200,000 daltons range.

Lane 3 and 4: Monoclonal antibodies 1C4.1F5 and 1C4.2C4 reacting to *A. malaysiensis* adult worm antigen.

Table 1

Showing the cut-off point value and the detection limit of MAb-based ELISA using each of the MAb.  
The lowest level of antigen detectable by the assay was determined by using  
a known concentration of *A. malaysiensis* antigen.

MAbs	IgG subclasses	X-3 SD	Detection limit 1:100 (µg/ml)
1C4.2D8	IgG1	0.2033	0.084
1C4.2C4	IgG1	0.1261	0.084
1C4.1F5	IgG1	0.1303	0.084

SD = Standard deviation

Table 2

Reactivity of the MAbs 1C4.1F5, 1C4.2C4 and 1C4.2D8 with adult worm antigens of related  
and other helminths detected on ELISA (OD values were read at 492 nm).

Parasite adult worm antigen	Mean ELISA OD values of the MAbs		
	1C4.1F5	1C4.2C4	1C4.2D8
<i>A. malaysiensis</i>	0.668	0.526	0.913
<i>A. cantonensis</i>	0.629	0.259	0.237
<i>Toxocara canis</i>	0.596	0.016	0.017
<i>Ascaris lumbricoides</i>	0.683	0.013	0.021
<i>Schistosoma malaysiensis</i>	0.740	0.016	0.021
<i>Dirofilaria immitis</i>	0.492	0.012	0.012
<i>Septhacia muris</i>	0.385	0.016	0.013

ELISA OD values for the negative control (serum from negative mice) was 0.036. PBS control  
ELISA OD values were 0.024.

Table 3

Results showing total numbers of human sera from Grik tested for the presence of  
*A. malaysiensis* antigen using two of the MAbs by MAb-ELISA.

MAb used as capture antibody	No. of human sera tested	Positive	% Positive
1C4.1F5	108	25	23%
1C4.2D8	20	0	0

## DISCUSSION

Yii (1976) reported 125 cases of human infections in Taiwan who had mild to severe eosinophilic meningoencephalitis. Four of these patients died and 3 had permanent sequelae such as blindness. A case of eosinophilic meningitis in a young boy from Indonesia was reported by Lam *et al* (1990). Recently there has been a report of a yet undescribed species of *Angiostrongylus*, recovered from the eye of a man in Sri Lanka (Durette-Desset *et al*, 1993).

This is the first time monoclonal antibodies have produced for the diagnosis of *A. malaysiensis* infection in Malaysia. The first five cases of *A. malaysiensis* infection in Malaysia were detected parasitologically by Watts in 1969 and another single case by Bisseru in 1972. Subsequent to that no new infections in man were detected parasitologically. Recently, a serological survey employing ELISA with crude worm antigens and randomly collected patient sera showed 76% exposure rate to the parasite.

Patients who suffer from eosinophilic meningitis or meningoencephalitis rarely have a parasitologically confirmed infection and juvenile worms are difficult to recover following a spinal tap (Punyagupta, 1979; Cheng *et al*, 1984). Therefore, the development of specific and sensitive serological and molecular biology diagnostic techniques may be useful tools for the diagnosis of angiostrongyliasis.

Two kinds of MAbs have been produced against *A. cantonensis* by Ishida and Yoshimura (1992). Using Western blot analysis they have demonstrated that one MAb recognized a 16.1 kDa protein and the other a 85 kDa protein of young adult whole worm extract. Similarly, Shih and Chen (1991) produced two MAbs (*A. cantonensis*) secreting IgG and IgM classes and having tested it against 35 patient sera, found a positive reactive rate of 88%. In our study, the three kinds of MAbs that were produced against *A. malaysiensis* showed that 1C4.1F5, 1C4.2C4 were reactive to 23 kDa protein and 1C4.2D8 to the 92 kDa protein of the somatic adult worm antigen.

Using the MAb 1C4.2D8, we developed a MAb-ELISA system and found it to be sensitive in detecting *A. malaysiensis* adult worm antigens and no cross-reactivity was seen with any of the other helminth antigens tested. Using this system, twenty human sera (aborigene) from Grik, found to be positive with

1C4.1F5 were found to be negative when tested with 1C4.2D8. The MAbs were not evaluated for their sensitivity and detecting *A. malaysiensis* in patient sera at this stage as no parasitologically confirmed cases were available to be used as a gold standard. The MAb-ELISA system using MAb 1C4.2D8 developed for the detection of *A. malaysiensis* infection, may be used as a diagnostic test for patient treatment and as an epidemiological tool for the study of disease prevalence.

## ACKNOWLEDGEMENTS

The authors wish to thank the Director, Institute for Medical Research for permission to publish this paper. This study was funded by National Council for Research and Development, Malaysia.

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