[Jpn. J. Parasitol., Vol. 37, No. 5, 347–352, October, 1988]

# An Antigenic Component for the Serodiagnosis of Paragonimiasis Miyazakii

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(Received for publication; June 20, 1988)


## Abstract

Antigenic components useful for serodiagnosis of paragonimiasis miyazakii were studied using immunoblotting.

When the cysteine proteinase inhibitor, [L-3-trans-carboxyoxiran-2-carbonyl]-L-leucyl-agmatin (E-64), was added, antigen degradation was prevented and antibody detection was markedly improved.

Components of *Paragonimus miyazakii* antigens with molecular weights of 27 kD, 33–37 kD and 120 kD, were detected by sera of hosts infected with *P. miyazakii* from an early stage of infection. The 27 kD and 33–37 kD components were commonly detected by sera of hosts infected with *P. westermani*. On the other hand, the 120 kD component was specifically detected by sera of hosts infected with *P. miyazakii* yet not with *P. westermani*. Although some *Fasciola* sp. or *Schistosoma japonicum* infected hosts possessed cross reacting antibodies to one or two of the *P. miyazakii* antigenic components, the reactions were so weak that they could easily be distinguished from those of *P. miyazakii* infected host sera. Consequently, the 27 kD and 33–37 kD components are considered to be useful antigens for the serodiagnosis of paragonimiasis, and the 120 kD component for specifically identifying paragonimiasis miyazakii.


**Key words:** *Paragonimus miyazakii, Paragonimus westermani,* immunoblotting, cysteine proteinase inhibitor (E-64), serodiagnosis

## Introduction

Serodiagnosis of parasitic infections is widely used, since parasites or their eggs are not always detectable by other methods.

In the case of paragonimiasis, diagnostic methods such as the double diffusion test (Yogore *et al.* 1965), complement fixation test (Yokogawa 1956), passive hemagglutination test (Yokogawa 1956) and enzyme linked immunosorbent assay (ELISA) (Yokogawa *et al.* 1983) have been developed.

Antibodies to antigens of the infecting *Paragonimus* species as well as other species are detected in sera of paragonimiasis patients. This was taken advantage of by obtaining paragonimus antigens for ELISA in order to diagnose paragonimiasis caused by another *Paragonimus* species for which antigens are difficult to obtain (Kojima *et al.* 1983). However, this cross reaction renders serodiagnosis ambiguous for discriminating between *Paragonimus* species differing in their sensitivity to therapeutic drugs such as Bithionol and newly developed Praziquantel (Yokogawa *et al.* 1980).

Recently, we found that endogenous cysteine proteinase in some trematodes, including *P. westermani* and *P. miyazakii,* was responsible for degrading antigens during the heating process required for sodium dodecyl sulfate acrylamide gel electrophoresis (SDS-PAGE) (Itoh *et al.* in preparation). We also found that [L-3-trans-carboxyoxiran-2-carbonyl]-L-leucyl-agmatin (E-64), a cysteine proteinase inhibitor (Hanada *et al.* 1978a, b), prevented degradation. These findings enabled us to analyze antigenic components of lung

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flukes for serodiagnosis using immunoblotting.

In the present paper, we showed that detection of antibodies to paragonimus antigens by immunoblotting is significantly improved by addition of E-64. We also looked into utilizing components of paragonimus antigens for serodiagnosis of the parasitosis using the rat-*P. miyazakii* system. Three paragonimiasis miyazakii cases were studied as well.

## Materials and Methods

*Parasites and antigen preparations*

Metacercariae of *P. westermani* were obtained from freshwater crabs (*Geothelphusa dehaani*) collected at Kashiwane and Togane in Gifu Prefecture (Shiwaku *et al.* 1986). Adult worms were obtained from the lungs of two dogs which were orally administered the metacercariae 5 months previously. Dog #1 was given 25 and dog #2, 15 metacercariae. 16 and 8 adult worms were recovered respectively. Blood collected from the dogs was used as a source of *P. westermani* infected dog sera.

Five rats (Wistar strain female rats, 4 weeks old) were each orally administered five *P. miyazakii* metacercariae obtained from crabs collected at Dojima, Shizuoka Prefecture. Ten weeks later, infecting worms were counted and the presence of lung cysts was observed. Recovered adult worms of *P. miyazakii* were used as a source of antigens. Five worms were recovered from rats #1 and #3, three worms from rat #2, and one worm from rats #4 and #5. No cysts were observed in rats #4 and #5.

Adult worms of both paragonimus species were homogenized in 0.05 M phosphate buffer, pH 7.4. After sonication, the homogenates were centrifuged at 3,500 x g, 4°C for 5 min and the supernatants were used as a source of antigens. The protein concentration was measured as described by Bradford (1976) and adjusted to 10 mg/ml. The solutions were kept at −80°C until use.

*Serum samples*

*P. westermani* infected dog sera were obtained as described above. *P. miyazakii* infected rat sera were collected ten weeks after infection. Sera of rats #2 and #3 collected three and five weeks after infection were also used. Two serum specimens of paragonimiasis miyazakii patients #1 and #3 were used (one of the sera was kindly supplied by Prof. T. Araki, Nara Medical University). Pleural exudate of a paragonimiasis miyazakii patient #2 was also used. Sera from the following animals were used as sources of other trematode infected hosts; *Schistosoma japonicum* infected mice, a *Clonorchis sinensis* infected rabbit and *Fasciola* sp. infected cattle.

*SDS-PAGE*

Antigen solutions were mixed with one fifth volumes of 10mM E-64 and kept at room temperature (23°C) for 15 min. An equal volume of sample buffer (2.5% SDS, 25% glycerol, 2.5% 2-mercaptoethanol in 0.125 M Tris-HCl buffer pH 6.8) was then added. The solutions were placed in a boiling water bath for 3 min, and then applied to an 8–16% gradient polyacrylamide gel (80x80 mm) prepared according to Laemmli (1970).

*Immunoblotting*

Immunoblotting was carried out as described by Towbin *et al.* (1979). After incubation with serum samples (x25 diluted), IgG antibodies attached to components on the membrane were detected by peroxidase conjugated anti-IgG antibodies corresponding to the host species.

## Results

*Effect of E-64 on the Detection of Antibodies by Immunoblotting*

As shown in Fig. 1, the addition of E-64 considerably enhanced the clarity of many bands, especially in the higher molecular weight region.

*Cross-reactions between* P. miyazakii *Adult Worm Antigens and Sera of Other Trematode Infected Hosts*

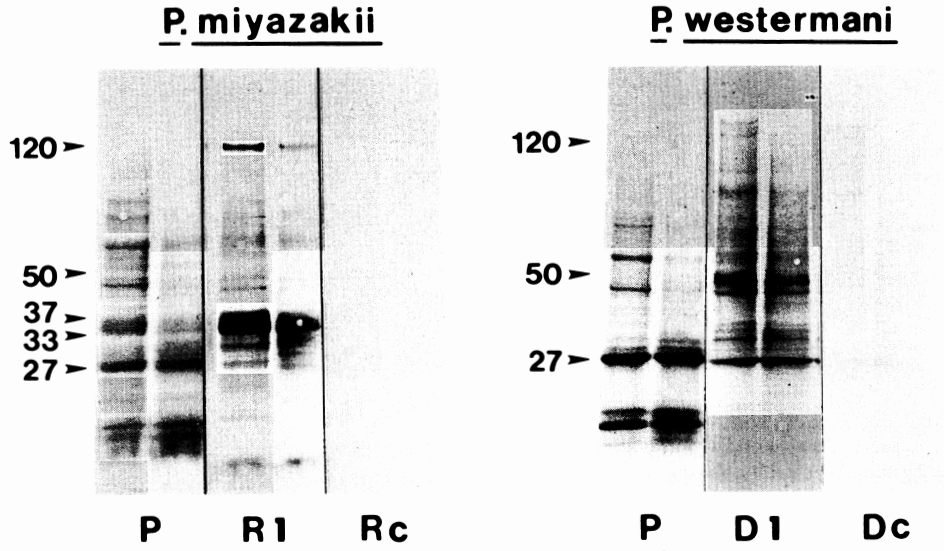


Fig. 1. Effect of E-64 in the detection of IgG antibodies to *P. miyazakii* and *P. westermani* antigens. Each antigen solution was mixed with E-64 (left lanes) or with distilled water instead of E-64 (right lanes). P: protein staining, R1: serum of rat #1, Rc: normal rat serum, D1: serum of dog #1, Dc: normal dog serum.

Antibodies raised in hosts infected by trematodes such as *Fasciola* sp., *C. sinensis* and *S. japonicum* were studied by immunoblotting to determine their cross-reactivity to *P. miyazakii* adult worm antigens. As shown in Fig. 2, cross-reacting antibodies to a few components were detected in sera of two out of ten mice infected with *S. japonicum* and in one out of five cattle infected with *Fasciola* sp. No antibody was detected in a *C. sinensis* infected rabbit. The presence of antibodies in these sera to each infecting parasite antigens was confirmed by immunoblotting (data are not shown).

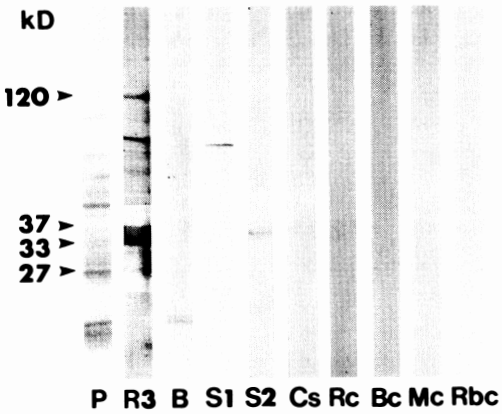


Fig. 2. IgG antibodies to *P. miyazakii* antigens in sera of trematode infected hosts. P: protein staining, R3: serum of rat #3 collected 10 weeks after infection, B: serum of *Fasciola* sp. infected cattle, S1 and S2: sera of *S. japonicum* infected mice, Cs: serum of *C. sinensis* infected rabbit, Rc: normal rat serum, Bc: normal bovine serum, Mc: normal mouse serum, Rbc: normal rabbit serum.

*Cross-reactions between* P. miyazakii *and* P. westermani

Sera and pleural exudate of *P. miyazakii* infected hosts contained cross-reactive antibodies to *P. westermani* antigenic components. Similarly, antibodies to *P. miyazakii* antigenic components were detected in *P. westermani* infected host sera (Fig. 3). Each serum, however, reacted with its own infecting species antigens in greater number and much more intensely. The 120 kD component of *P. miyazakii* was specifically detected by *P. miyazakii* infected host sera and pleural

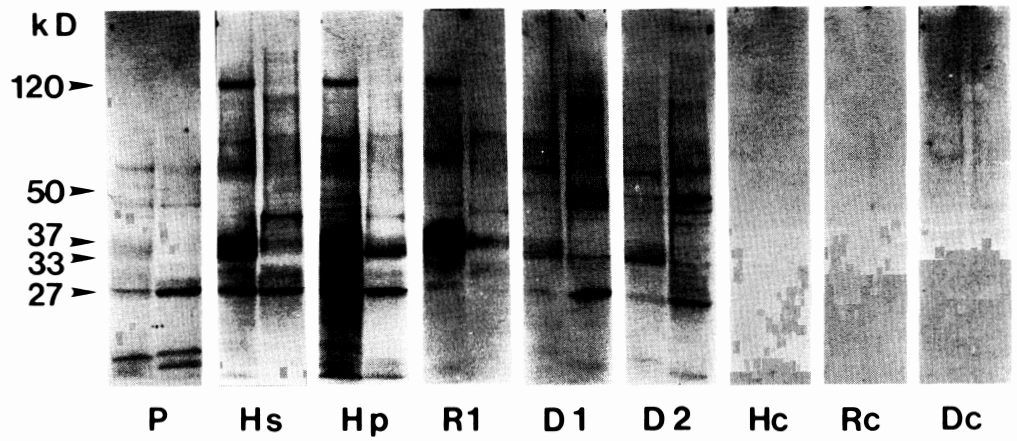


Fig. 3. Cross-reactions of IgG antibodies against *P. miyazakii* (left lanes) and *P. westermani* (right lanes) antigens. P: protein staining, Hs: serum of paragonimiasis miyazakii patient #1, Hp: pleural exudate of paragonimiasis miyazakii patient #2, R1: serum of rat #1, D1 and D2: sera of dogs #1 and #2, Hc: normal human serum, Rc: normal rat serum, Dc: normal dog serum.

exudate, but not by *P. westermani* infected sera.

On the other hand, the 27 kD and 50 kD components of *P. westermani* were the major components detected by sera of *P. westermani* infected hosts. The 27 kD component cross-reacted with *P. miyazakii* infected sera, but the 50 kD component was detected only by *P. westermani* infected sera.

*Appearance of Antibodies to the 120 kD component after* P. miyazakii *infection*

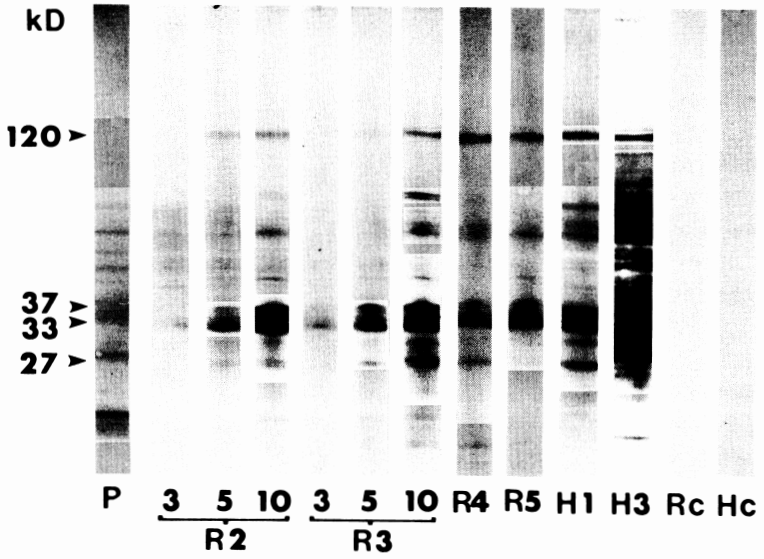


Fig. 4. Appearance of IgG antibodies to *P. miyazakii* antigens. P: protein staining, R2 and R3: sera of rats #2 and #3 collected at 3, 5 and 10 weeks after infection, R4 and R5: sera of rats #4 and #5, H1 and H3: sera of paragonimiasis miyazakii patients #1 and #3, Rc: normal rat serum, Hc: normal human serum.

Fig. 4 shows that IgG antibodies to the 120 kD component of *P. miyazakii* antigens appeared 5 weeks after infection. Rats harboring only one fluke without cyst formation also produced antibodies to the 120 kD component (R4 and R5). Antibodies to the 120 kD component were detected in sera from paragonimiasis miyazakii patients as was also observed in sera of rats infected with the parasite.

## Discussion

Immunoblotting was used to search out useful antigens for serodiagnosis of paragonimiasis miyazakii. Addition of E-64 was essential in our studies, since a marked improvement in antibody detection was achieved with its use.

The presence of cross reacting antigens between some trematodes such as *P. westermani, S. mansoni,* and *F. hepatica* was demonstrated by Ouchterlony immunodiffusion and ELISA using hyperimmunized sera (Hillyer & Serrano, 1983). These cross-reacting antigens, however, might be poorly recognized by hosts in the infectious state. Levels of antibody to these antigens are too low to be measured with the system used here, since our results showed only a slight cross-reaction between *P. miyazakii* antigens and sera of hosts infected with *S. japonicum* or *Fasciola* sp.

Cross-reactions between *P. westermani* or *P. miyazakii* infected sera and *Paragonimus* antigens, previously reported by Kojima *et al.* (1983), were confirmed by immunoblotting. The 27 kD component was common to both *Paragonimus* antigens. Common antigenicity was also observed in the 33–37 kD components of *P. miyazakii.* Each host, however, showed a stronger antibody response to common antigens of infecting *Paragonimus* species than to antigens of another species. Based on this, it is speculated that even in the same molecule, some epitopes may be common while others may be species specific.

Antibodies to the 27 kD, 33–37 kD and 120 kD components were detected at the early infectious stage in host sera infected with *P. miyazakii.* This was the case even in hosts harboring a single worm without cyst formation. Other trematode infected host sera did not contain cross-reacting antibodies to these components. Our observations show that the 27 kD and 33–37 kD components are useful antigens for diagnosis of paragonimiasis.

The 120 kD component of the *P. miyazakii* antigen was considered to be *P. miyazakii* specific: this component was not contained in *P. westermani* antigens, was specifically detected by *P. miyazakii* infected host sera, yet not detected by closely related *P. westermani* infected host sera.

The 27 kD component of *P. westermani* was reported as a standard antigen for diagnosis of the parasitosis (Sugiyama *et al.*, 1987). The 50 kD component, however, was detected by sera of *P. westermani* infected hosts while not by *P. miyazakii* infected host sera, and may be a more suitable antigen for serodiagnosis of *P. westermani.*

In conclusion, the 27 kD and 33–37 kD components are useful antigens for diagnosis of paragonimiasis, and the 120 kD component is a candidate for serodiagnosis of paragonimiasis miyazakii and distinguishing infecting *Paragonimus* species which differ in their sensitivity to therapeutic drugs.

### Acknowledgments

We are grateful to Professor T. Araki, Nara Medical University, for kindly supplying serum of a paragonimiasis miyazakii patient.

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