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Demonstration of the Cross-Reactivity among Antigens Extracted from Four Species of *Paragonimus* and Its Utilization for the Enzyme-Linked Immunosorbent Assay (ELISA)

SOMEI KOJIMA*, MASASHI KOBAYASHI† AND MUNEO YOKOGAWA†

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The enzyme-linked immunosorbent assay (ELISA) has been reported to be a useful tool for the immunodiagnosis of various parasitic diseases (Voller *et al.*, 1976, 1977; Kagan and Hillyer, 1981). We have also demonstrated the diagnostic value of the ELISA in Peruvian paragonimiasis (Yokogawa *et al.*, 1983). In general, the specificity as well as the sensitivity of antigen-antibody reactions is vital for any of the immunological diagnostic tests. However, materials for preparation of parasite antigens such as adult worms of *Paragonimus peruvianus* are not always available in most of institutes of parasitology in the world except those in Peru when the antigen is required. To overcome this kind of limitation, it seems to be worth examining whether or not an antigen extracted from the parasite concerned might be replaced by the antigen extracted from another species of the same genus. The present paper describes the cross-reactivity among antigens extracted from *P. peruvianus, P. westermani, P. miyazakii* and *P. ohirai,* and demonstrates that *P. westermani* derived antigens may substitute for *P. peruvianus* antigen for the detection of the antibody to the latter with the ELISA.

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* *Department of Parasitology, Shinshu University School of Medicine, Matsumoto 390, Japan.*
† *Department of Parasitology, Chiba University School of Medicine, Chiba 280, Japan.*

Materials and Methods

Serum samples and pleural exudates

Serum samples were obtained from students of primary and secondary schools and from inhabitants, who were either positive or negative for the skin test with *P. peruvianus* VBS antigen (see below), of the Condebamba district of Cajamarca, Northern Peru (Yokogawa *et al.*, 1983). Pleural exudates were obtained from a patient (M.F.) with proven paragonimiasis westermani (Yokogawa *et al.*, 1976).

Antigens

Veronal buffered saline extracts (VBS antigens) were prepared from adult worms of each of *P. peruvianus, P. westermani, Schistosoma japonicum,* and the Japanese strain of *Fasciola* sp. (Yokogawa *et al.*, 1955; Kaji *et al.*, 1983). Crude extracts of lyophilized adult worms of *P. westermani* and *P. miyazakii* were prepared with 0.05 M carbonate buffer, pH 9.6, after delipitization with chilled ethyl ether. An antigen was also extracted from *P. ohirai* adult worms with 0.1% NaCl solution according to the method of Tsuji (1974).

The enzyme-linked immunosorbent assay

(ELISA)

The ELISA was carried out by using 10 μg/ml of antigens for coating microtiter plates (M129 A, Dynatech Laboratories) (Kojima *et al.*, accepted; Yokogawa *et al.*, 1983). In some experiments, a solution containing 30 μg/ml of the antigens was used for coating the plates in order to increase the availability of cross reactive antigenic determinants. The results of the assay were expressed either as the antibody titer which was determined from the endpoint reaction or as the TS/NS ratio which was calculated by an equation:

$$\frac{\text{OD 450nm of a test sample (1:40 dilution) } -\text{mean OD of blanks}}{\text{OD 450nm of a pooled normal sample (1:40) } -\text{mean OD of blanks.}}$$

Results

In order to confirm the presence of cross-reactive antigens, serum samples which had been found to be positive for the ELISA with *P. peruvianus* antigen (Yokogawa *et al.*, 1983) were further examined with antigens extracted from *P. westermani, P. miyazakii* and *P. ohirai*. Results are summarized in Table 1, indicating that the majority of the test samples showed the same antibody titer or one dilution higher titer when *P. westermani* antigen was used instead of *P. peruvianus* antigen. A lower titer of the IgG antibody to *P. westermani* antigen was found in only one (No. 233) out of 16 samples. However, great variations in the antibody titer were observed when the antigen of *P. miyazakii* or *P. ohirai* was used for the ELISA. In addition, almost similar TS/NS ratio was obtained when comparison was made between VBS antigen of *P. peruvianus* and carbonate buffer extract of *P. westermani*, although there were a few exceptions which showed more than two times higher values with the latter antigen (Table 1, Nos. 190, 479 and 491).

Table 1 Cross reactivity among antigens extracted from adult worms of four species of *Paragonimus*

Serum No.	Titer (ELISA)				TS/NS ratio	
	Pp*	Pw†	Pm†	Po‡	Pp*	Pw†
233	320	160	160	320	11.0	13.0
188	320	320	160	320	13.5	10.5
273	1280	1280	1280	1280	28.7	30.0
291	1280	1280	1280	2560	23.3	22.3
360	1280	1280	640	640	25.5	22.5
373	320	320	160	80	8.5	9.5
854	320	320	640	640	17.0	17.0
1101	640	640	1280	1280	13.0	14.3
1104	640	640	1280	1280	14.3	15.7
183	160	320	320	320	11.0	13.0
190	320	1280	640	640	6.4	15.3
299	1280	2560	1280	1280	37.0	37.0
400	1280	2560	640	1280	17.5	21.0
479	160	320	640	640	9.3	18.5
491	160	320	320	640	9.8	23.0
605	320	640	640	320	15.8	29.0

* Veronal buffered saline extract of *P. peruvianus* (Pp).

† Carbonate buffer extract of *P. westermani* (Pw) or *P. miyazakii* (Pm).

‡ NaCl (0.1%) extract of *P. ohirai* (Po).

Further comparison was made in relation to TS/NS ratio and the IgG antibody titer by using a serum sample (No. 190) obtained from a Peruvian patient with proven paragonimiasis. The strongest reaction was observed with the carbonate buffer extract of *P. westermani*, although higher values in TS/NS ratio or higher ELISA titers were obtained with all antigens extracted from three species of *Paragonimus* other than *P. peruvianus* when compared with those obtained with *P. peruvianus* VBS antigen (Fig. 1). Furthermore, the presence of cross-reactivity among antigens extracted from these four species of *Paragonimus* was also demonstrated with pleural exudate of a Japanese patient with paragonimiasis westermani (Fig. 1). Again, the highest TS/NS ratio was obtained with the carbonate buffer extract of *P. westermani*, although just the same or similar ELISA titer was obtained when

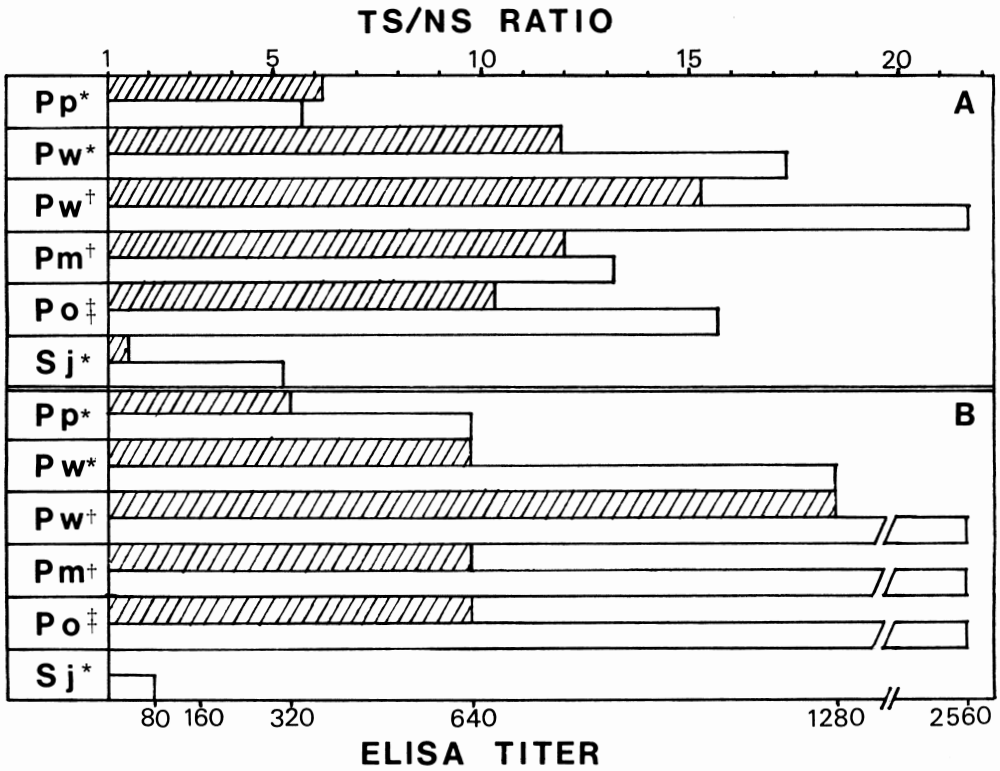

Fig. 1 Cross-reactivities among antigens extracted from four species of *Paragonimus*. The hatched bar indicates reactions of a serum sample (No. 190) from a patient with Peruvian paragonimiasis against antigens of *Paragonimus* spp. and *S. japonicum*, while the reactions of the pleural exudate from a Japanese patient with paragonimiasis westermani are shown by the open bar.

Pp*, Pw*, Sj*; VBS antigen of *P. peruvianus*, *P. westermani* or *S. japonicum*.

Pw†, Pm†; Carbonate buffer extract of *P. westermani* or *P. miyazakii*.

Po‡; 0.1% saline extract of *P. ohirai*.

TS/NS ratio (Panel A) and ELISA titer (Panel B); see Materials and Methods.

VBS or 0.1% NaCl extracts of *P. westermani, P. miyazakii* and *P. ohirai* were used (Fig. 1, panel B). However, there was no cross-reaction against *S. japonicum* VBS antigen (Fig. 1).

It was also demonstrated that the antigen-antibody reaction was identical when various dilutions of serum samples were applied for the ELISA plate coated with either of *P. peruvianus* VBS antigen or of the carbonate buffer extract of *P. westermani*. As shown in Fig. 2A, a serum sample (No. 273) showed an identical reaction curve to both of *P. peruvianus* and *P. westermani* antigens (shown with solid lines). Similar results were obtained with another sample (No. 605) which showed a higher titer (1:640) of the IgG antibody to *P. westermani* as compared with that to *P. peruvianus* (1:320) (Fig. 2A dotted lines).

On the other hand, cross-reactivities of a serum sample (No. 491; a suspected case of paragonimiasis with positive reactions to *P. peruvianus* antigens in the skin test, ELISA, complement fixation test and double diffusion in agar) against antigens extracted from other genera of trematodes

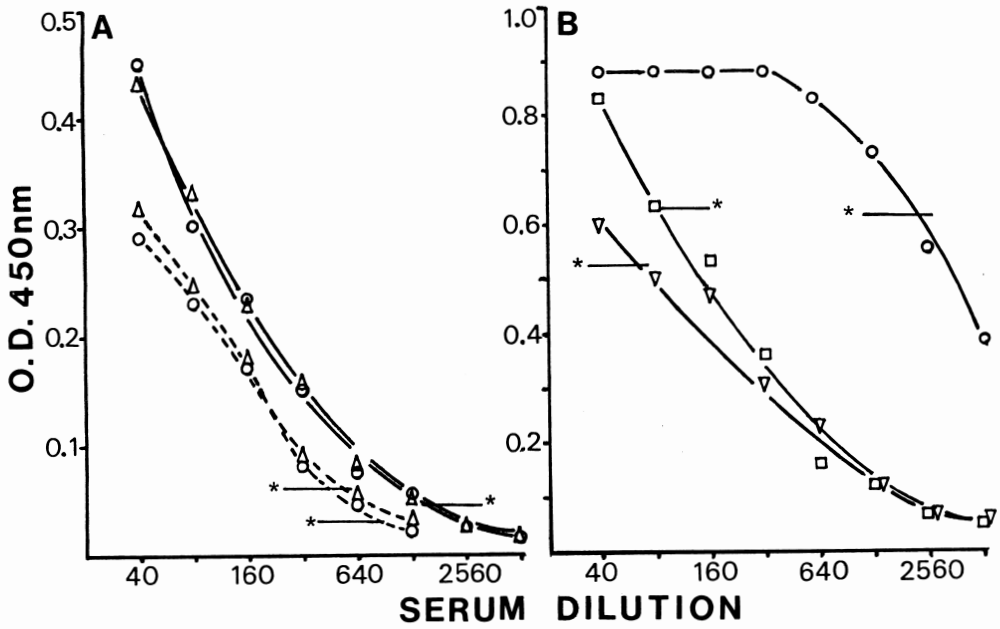

Fig. 2 The presence of common antigens between *P. peruvianus* VBS antigen and the carbonate buffer extract of *P. westermani*.

Various dilutions of serum samples were examined with the ELISA by using either of *P. peruvianus* (△) or *P. westermani* antigen (○). Panel A: Solid lines indicate reactions of a serum sample (No. 273) to these antigens, while dotted lines indicate those of another sample (No. 605). Panel B: Weak cross-reactions of a serum sample (No. 491) against VBS antigens of *Fasciola* sp. (□) or *S. japonicum* (▽) as compared with reactons against *P. westermani* antigen (○). Horizontal lines (*) represent the endpoint reactions.

such as *Fasciola* sp. or *S. japonicum* were found to be rather weak as compared with those against *P. westermani* antigen (Fig. 2B).

Discussions

The results described here demonstrate the presence of cross-reactive antigens among extracts obtained from adult worms of four species of *Paragonimus,* i.e., *P. peruvianus, P. westermani, P. miyazakii* and *P. ohirai.* The strongest and the most consistent results were obtained in terms of the IgG antibody titer or the TS/NS ratio when instead of *P. peruvianus* antigen, the carbonate buffer extract of *P. westermani* was used for coating the ELISA plates (Table 1 and Fig. 1). *P. westermani* VBS antigen was also found to be a proper substitute for *P. peruvianus* antigen because the difference of the ELISA titer was not significant (Fig. 1).

Moreover, it was shown that the antigen-antibody reaction was identical when the same serum samples were examined with *P. peruvianus* and *P. westermani* antigens for the ELISA (Fig. 2A). The higher titer of the IgG antibody to *P. westermani* antigen (1:640) in the sample No. 605 as compared with that obtained with *P. peruvianus* antigen (1:320) resulted from the difference of the positive limitation which was determined by the reaction of a pooled normal serum to the respective antigens. Indeed, by using *P. westermani* antigen, we have obtained exactly the same results as described by Yokogawa *et al.* (1983) in

the ELISA for the detection of the IgG antibody to *P. peruvianus* antigen. All of 50 samples positive for the ELISA with *P. peruvianus* antigen were also found to be positive with *P. westermani* antigen, while all of 75 negative samples were also negative with the latter antigen (Data not shown).

Quite recently, Hillyer and Serrano (1983) have demonstrated the presence of cross-reactive antigens among *P. westermani, S. mansoni* and *F. hepatica* adult worm extracts by Ouchterlony double immunodiffusion and ELISA. In addition, they have been able to induce protection to *S. mansoni* infection in mice by using *P. westermani* extracts for the vaccination. In our hands, however, cross-reactions of a serum sample from a suspected case of Peruvian paragonimiasis to antigens of *Fasciola* sp. or *S. japonicum* were apparently different from those to *P. westermani* antigen (Fig. 2B). The discrepancy between their results and ours may be due to different sources of the antisera used. They used hyperimmune rabbit sera for the demonstration of the cross-reactivity, while serum samples from suspected cases or from patients with paragonimiasis were used in the present study (Figs. 1 and 2).

From these results, it may be concluded that *P. westermani* antigens could substitute for *P. peruvianus* antigen for the detection of the antibody to the latter with the ELISA.

Summary

The presence of cross-reactive antigens was demonstrated among four species of *Paragonimus* (*P. peruvianus, P. westermani, P. miyazakii* and *P. ohirai*) by using the ELISA. The present results indicate that antigens extracted from *P. westermani* adult worms may substittue for *P. peruvianus* antigen in the ELISA.

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4種肺吸虫より抽出した抗原間の交叉反応性と ELISA への利用

小島荘明
(信州大学医学部寄生虫学教室)
小林　仁　横川宗雄
(千葉大学医学部寄生虫学教室)

一般に，寄生虫症の免疫診断法に関してはその感度とともに抗原抗体反応の特異性が重要な問題となる．しかし，ペルー肺吸虫のように材料の入手が困難な場合には，同属の他種虫体抗原をもって代用する必要が生じる．そこで，ペルー肺吸虫，ウエステルマン肺吸虫，宮崎肺吸虫，および大平肺吸虫の4種の成虫より抽出した抗原について，ELISA を用いて交叉反応性について検討した．その結果，ペルー肺吸虫抗原の代りにウエステルマン肺吸虫由来の抗原を用いれば，ELISA において最も一致した成績が得られることが判明した．