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Research Note

Cross-reactivity in the Sera of Patients with Human Pulmonary Dirofilariasis by Means of a Mixed Passive Hemagglutination (MPHA) Test, in Comparison with an Enzyme-linked Immunosorbent Assay (ELISA)

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To diagnose human pulmonary dirofilariasis, some immunological tests have been applied. Of them, an enzyme-linked immunosorbent assay (ELISA) was considered to be most useful because of its high sensitivity, though the cross-reactivity to other helminthic infections such as *Ascaris* and *Toxocara* was seen (Sato *et al.*, 1985; Glickman *et al.*, 1986). Recently, the authors first used a mixed passive hemagglutination (MPHA) test for the diagnosis of human pulmonary dirofilariasis and recognized that it was a useful aid as well as the ELISA (Ohnishi *et al.*, 1987; 1988).

In the present study, the cross-reactivity of the patients' sera to other helminthic antigens was investigated by the MPHA test in comparison with the ELISA. The sera tested were obtained from eleven proven cases of pulmonary dirofilariasis and 324 healthy persons as controls. The control sera were used to set the diagnostic values by both methods. The specific antibodies of IgG class were measured by the MPHA test using IgG cell kits (Biotec Corp., Tokyo) and by the ELISA using HRP-conjugated anti-human IgG solution (Cappel Corp.) as described previously (Ohnishi *et al.*, 1988). The antigens tested were 16 preparations: *Dirofilaria immitis* female adults extract (FEX), male adults extract, female adults excretory-secretory product (FES) and male adults excretory-secretory product; *Ascaris suum* FEX; *Anisakis* sp. larvae extract (LEX); *Toxocara canis* embryonated eggs extract; *Trichinella spiralis* LEX; *Trichuris vulpis* FEX; *Ancylostoma caninum* FEX; *Gnathostoma hispidum* LEX; *Angiostrongylus cantonensis* FEX; *Metastrongylus apri* FEX; *Paragonimus miyazakii* adults extract (AEX); *Fasciola hepatica* AEX; *Spirometra erinacei* plerocercoids extract. The excretory-secretory products were prepared by the culture of adult worms in Eagle's MEM solution for one week at 37°C. These freeze-dried antigens were used at the concentration of 1.25 μg/ml for the MPHA test and 5 μg/ml for the ELISA. MPHA titers were represented as reciprocals of the highest serum dilutions giving completely agglutinating reactions and the diagnostic values were tentatively given at 1:400 or greater. ELISA titers were expressed as OD values after correcting with the formula of Ohnishi *et al.* (1988) as follows:

$$\text{Corrected log (OD value of sample serum)} = \log(\text{OD value of sample serum}) \times \frac{\log(\text{OD value of positive control serum})}{\log(\text{OD value of positive reference serum})}$$

The cutoff titers were defined as greater than the mean values plus 3SD in controls.

The results of the MPHA test were summarized in Table 1. The FES antigen was the most sensitive of four crude antigens derived

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Table 1. Frequency distributions of antibody titers in the sera of controls and patients with pulmonary dirofilariasis by means of a mixed passive hemagglutination (MPHA) test using various antigens

Antigens used		Reciprocal MPHA titers					Positive rates*	
		≦100	200	400	800	1600≦	Patients	Controls
D. immitis	FEX	301†	20(1)‡	4(3)	6(3)	4(4)	90.9	1.2
	MEX	65(9)	5(1)	2(1)			9.1	1.6
	FES	57	8	4(3)	3(2)	6(6)	100	3.0
	MES	66(1)	5(4)	2(2)	2(2)	2(2)	54.5	0
A. suum	FEX	44	9	11(6)	5(2)	4(3)	100	14.5
Anisakis	LEX	115(6)	2(2)	1(1)	2(2)		27.3	0
T. canis	EEX	64(6)	4(3)	3(2)			18.2	1.7
T. spiralis	LEX	68(9)	3(1)	2(1)			9.1	1.6
T. vulpis	FEX	64(11)	2				0	0
A. caninum	FEX	68(10)	3(1)	1			0	1.6
G. hispidum	LEX	79(5)	5(1)	2(2)	3(3)		45.5	0
A. cantonensis	FEX	66(6)	2(2)	3(3)	1		27.3	1.6
M. apri	FEX	69(9)	2(1)	2(1)			9.1	1.6
P. miyazakii	AEX	73(11)					0	0
F. hepatica	AEX	75(11)					0	0
S. erinacei	PEX	63	2(1)	3(2)	4(4)	4(4)	90.9	1.5

* 1:400 or greater.

† No. of sera examined.

‡ No. of cases with pulmonary dirofilariasis were included in parentheses.

Abbreviations: FEX and MEX, Female and male adults extract; FES and MES, Female and male adults excretory-secretory products; LEX, Larvae extract; EEX, Embryonated eggs extract; AEX, Adults extract, PEX, Plerocercoids extract.

from *D. immitis*. All of the 11 patients were positive with titer of 1:400 or greater. Some of the patients' sera cross-reacted with other helminthic antigens such as *Ascaris, Spirometra, Gnathostoma, Anisakis* and *Angiostrongylus*. The ELISA also showed strong reaction of the FES antigen, but the sensitivity of the ELISA declined slightly because of the high cutoff titer (Fig. 1). The positive rates against heterogeneous nematode antigens except for *Toxocara* antigen were low. The cross-reactivity to the *Spirometra* antigen in the ELISA was strongly seen.

The clarification of the cross-reactivity in the patients' sera has been remained unsolved in the present study. Based on the present results, however, the MPHA test had high sensitivity using *Dirofilaria* FEX antigen as well as the ELISA. The procedure of the MPHA test is simple and reading of the result with the naked eye is easy (Shibata *et al.*, 1981; Ohnishi *et al.*, 1987; 1988). In addition, the MPHA test has advantages to examine simultaneously 96 samples using various antigens in one microtiter plate at the same time and to detect other classes of specific antibodies using respective cell kits (Sugishita & Hirayama, 1986).

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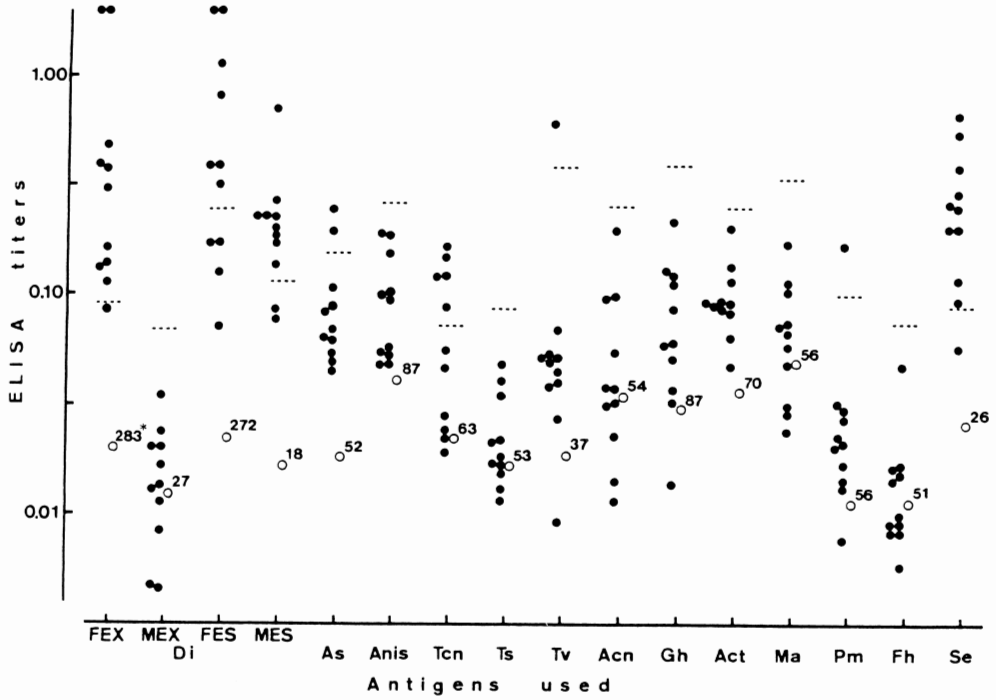

Fig. 1 Cross-reactivity in the sera of controls and patients with human pulmonary dirofilariasis by means of ELISA using various antigens. (●), Each serum of patient with human pulmonary dirofilariasis; (○), Mean values in controls (*, No. of sera examined); - - - , Cutoff titers as mean values + 3SD in controls.

Abbreviations: Di, *D. immitis*; As, *A. suum*; Anis, *Anisakis* sp.; Tcn, *T. canis*; Ts, *T. spiralis*; Tv, *T. vulpis*; Acn, *A. caninum*; Gh, *G. hispidum*; Act, *A. cantonensis*; Ma, *M. apri*; Pm, *P. miyazakii*; Fh, *F. hepatica*; Se, *S. erinacei*; FEX and MEX, Female and male adults extracts; FES and MES, Female and male adults excretory-secretory products.

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