Immunoblotting Analysis of Somatic Components of *Dirofilaria immitis*

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ABSTRACT. SDS-PAGE analysis of *Dirofilaria immitis* extracts demonstrated the complexicity of protein components of microfilariae, similar to that of adult male and female worms. Immunoblotting analysis using sera from microfilaremic and amicrofilaremic dogs with *D. immitis* infection suggest that antigenic components in the low molecular weight region may be related to the anti-microfilarial mechanism of the host.—KEY WORDS: antigen, *Dirofilaria immitis*, immunoblotting.
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Although the antigenic complexicity of *Dirofilaria immitis* was already pointed out [6, 7], analysis of worm components has so far not been made. In the present study, somatic protein components of *D. immitis* were compared for male, female and microfilariae.

Three kinds of worm derived extracts were prepared in this study. Fresh adult male and female worms were obtained from infected dogs and washed with saline several times. Microfilariae were also collected from the uterus of alive adult females. The worms were homogenized with chilled PBS (pH 7.2) by a tissue homogenizer and then an ultrasonicator. Following centrifugation for 60 min at 18,000 rpm, each supernatant was recovered as somatic extracted antigens of male worms (MEX), female worms (FEX) and microfilariae (MfEX). Thus, MEX and FEX may additionally contain a small amount of excretory and secretory (ES) products released from adult worms and FEX may also contains microfilarial protein. Protein concentrations were 1.4, 2.8 and 1.9 mg/m*l*, respectively as determined by the method of Lowry *et al.* [10].

Electrophoresis was performed as previously described with coomassie brilliant blue stain [7], using 12.5% or 7.5% acrylamide gel for analysis of low or high molecular weights. For band molecular weight determination, ten columns were simultaneously used for each analysis of antigens. Immunoblotting was conducted as previously described [7], following SDS-PAGE. Sera pooled from 6 microfilaremic dogs and 4 dogs with occult infection which is characterized by amicrofilaremic infection with mature males and fertile females were used. To determine the extent of immunoglobulin binding, peroxidase-conjugated goat anti-dog IgG (H+L) (Cappel Lab. Inc., Malvirn, PA) diluted 1:500 was used.

In analysis of extracted proteins by SDS-PAGE, many protein bands were observed at 12 to 247 kilodaltons (kDa) by means of densitometer determination (Fig. 1). There were 52 bands in MEX, 51 bands in FEX and 58 bands in MfEX as summarized in Table 1.

Band antigenicity for MEX, FEX and MfEX was assessed by immunoblotting. Many antigenic bands were noted at 20 to 240 kDa for microfilaremic sera. Twenty-one bands in MEX, 18 bands in FEX and 16 bands in MfEX were detected (Table 1). Bands in the high molecular weight region, approximately 60 to 240 kDa, were due to reactions stronger than those of bands in the low molecular weight region, below around 40 kDa (Fig. 2).

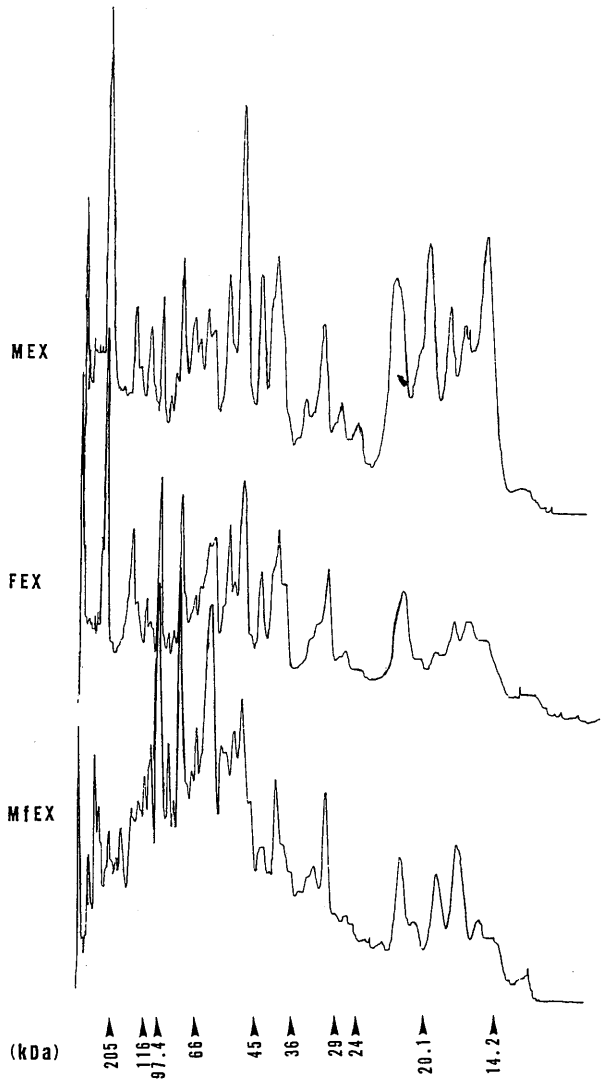

Fig. 1. Densitometric patterns (OD 610 nm) of protein bands of somatic extracts of *D. immitis*, detected by SDS-PAGE (12.5% gel) with coomassie blue staining. MEX: Male extract. FEX: Female extract. MfEX: Microfilaria extract.

On the other hand, sera from occult infected dogs recognized many antigenic bands as did also micro-

Table 1. Numbers of protein bands or antigenic bands in somatic extracts of *D. immitis*, detected by SDS-PAGE or immunoblotting

Bands	No. of bands		
	MEX	FEX	MfEX
Protein	52	51	58
Antigen to microfilaremic sera	21	18	16
Antigen to amicrofilaremic sera	25	32	26

MEX: Male extract. FEX: Female extract. MfEX: Microfilaria extract.

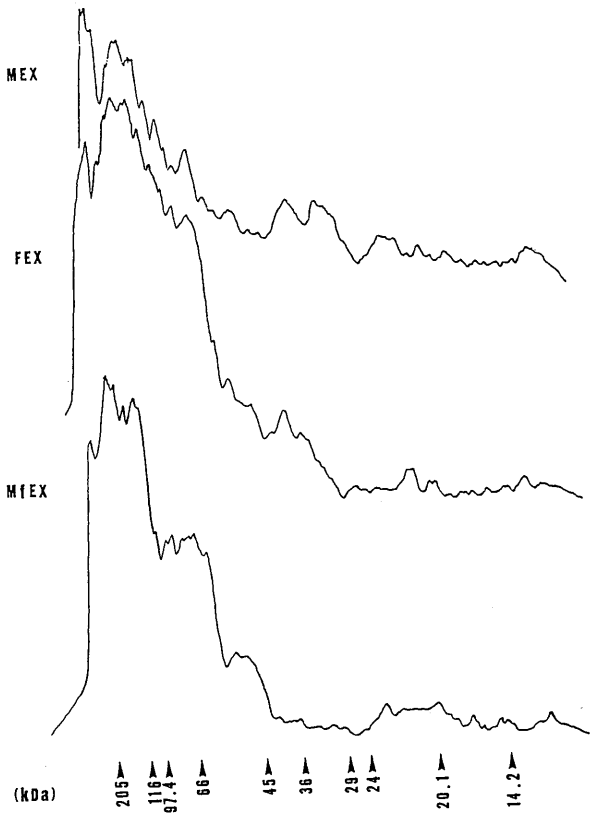

Fig. 2. Densitometoric patterns (OD 570 nm) of antigenic bands of somatic extracts of *D. immitis*, deteted by immunoblotting with sera from infected dogs with microfilaremia after SDS-PAGE (12.5% gel). MEX: Male extract. FEX: Female extract. MfEX: Microfilaria extract.

filaremic sera. Several bands in the low molecular weight and high molecular regions were quite easily recognized by amicrofilaremic sera, particularly a few bands were markedly observed in the low molecular weight region, below around 40 kDa, in the cases of FEX and MfEX (Fig. 3). Immunoblotting demonstrated 25 bands in MEX, 32 bands in FEX and 26 bands in MfEX (Table 1).

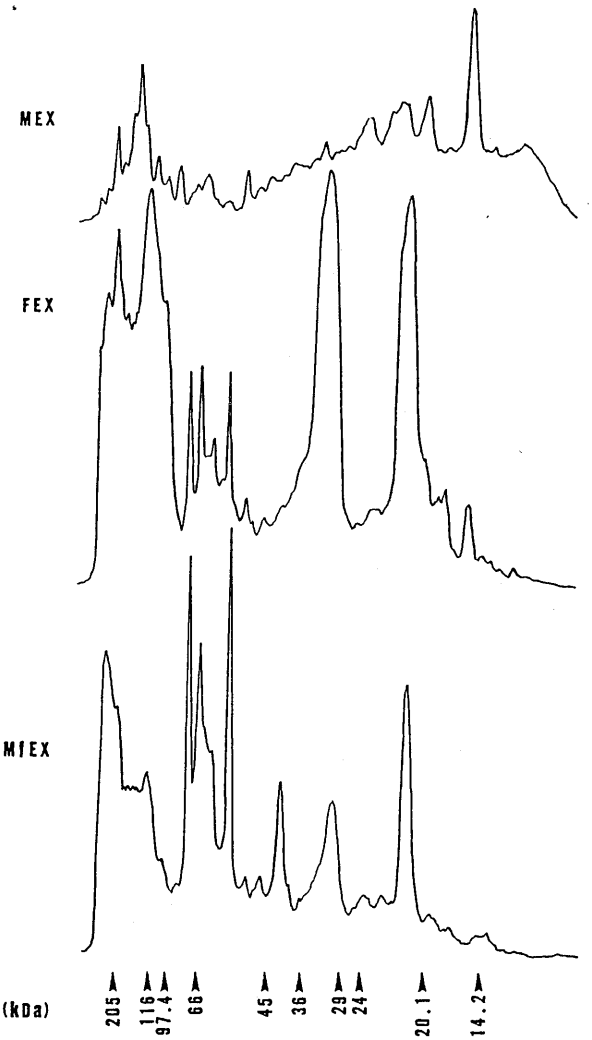

Fig. 3. Densitometoric patterns (OD 570 nm) of antigenic bands of somatic extracts of *D. immitis*, detected by immunoblotting with sera from infected dogs with amicrofilaremia after SDS-PAGE (12.5% gel). MEX: Male extract. FEX: Female extract. MfEX: Microfilaria extract.

The present results clearly demonstrated that MfEX possess many antigenic protein bands in somatic material as also do MEX and FEX. Still, microfilariae have very simple morphological structures and no digestive tract or genital grands. These data indicate the antigenic complexicity of microfilariae similar to those of adult worms. It seems that such complex antigenicity may refer a complexicity in biological metabolism of the microfilariae which may be acquired during biological evolution and serve to avoid immunological attack, as discussed already in our report [7].

In occult infection characterized by amicrofilaremic infection with mature males and fertile females, microfilariae may thus be killed in blood circulation after being released by a female. Such a killing of microfilariae from the circulation was associated with appearance of anti-

bodies to microfilarial surface [4, 14], although analysis of the surface antigen is not enough conducted.

The present data show that amicrofilaremic sera are more capable of recognizing antigenic bands than microfilaremic sera. The amicrofilaremic sera markedly react not only with high molecular weight material but also with low molecular weight materials in FEX and MfEX in contrast to microfilaremic sera. This finding indicates that the low molecular weight region of somatic protein may be related to the anti-microfilarial mechanism, as suggested in ES products (antigen), so far [1–3, 5, 8, 9, 11–13].

It seems likely that these reports may also support the importance of the low molecular weight antigens derived from *D. immitis* in the microfilaricidal immune response of the host.

REFERENCES

1. Devaney, E. 1987. *Parasitol. Immunol.* 9: 401–405.
2. Egwang, T. G. and Kazura, J. W. 1987. *Mol. Biochem. Parasitol.* 22: 159–168.
3. Ehrenberg, J. P., Tamashiro, W. K., and Scott, A. L. 1987. *Exp. Parasitol.* 63:205–214.
4. EL-Sadr, W. M., Aikawa, M., and Greene, B. M. 1983. *J. Immunol.* 130: 428–434.
5. Fujita, K. and Tsukidate, S. 1984. *Int. J. Parasitol.* 14: 547–550.
6. Hayasaki, M. 1983. *Jpn. J. Vet. Sci.* 45: 113–115.
7. Kaneko, H., Hayasaki, M., and Ohishi, I. 1990. *Jpn. J. Vet. Sci.* 52: 995–1000.
8. Kazura, J. W., Cicirello, H., and Forsyth, K. 1986. *J. Clin. Invest.* 77: 1985–1992.
9. Lal, R. B. and Ottesen, E. A. 1988. *J. Immunol.* 140: 2032–2038.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. *J. Biol. Chem.* 193: 265–275.
11. Maizels, R. M., Denham, D. A., and Sutanto, I. 1985. *Mol. Biochem. Parasitol.* 17: 277–288.
12. Scott, A. L., Diala, C., Moraga, D. A., Ibrahim, M. S., Redding, L., and Tamashiro, W. K. 1988. *Exp. Parasitol.* 67: 307–323.
13. Tamashiro, W. K., Ibrahim, M. S., Moraga, D. A., and Scott, A. L. 1989. *Am. J. Trop. Med. Hyg.* 40: 368–376.
14. Weil, G. J., Powers, K. G., Parvuoni, E. L., Line, B. R., Furrow, R. D., and Ottesen, E. A. 1982. *Am. J. Trop. Med. Hyg.* 31: 477–485.