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NOTE

Antigenicity of Microfilarial and Adult *Dirofilaria immitis* in Indirect Fluorescent Antibody Test

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Previously, Hayasaki [2] pointed out high antigenicity of the intrauterine microfilarial (I-Mf) antigen prepared from adult *Dirofilaria immitis* compared with circulating microfilariae (C-Mf), larvae and adult worms in immunological specificity and sensitivity by the indirect hemagglutination (IHA) test. More extensive study, however, is necessary for determining the antigenicity of I-Mf. The present study was undertaken, therefore, to clarify the antigenic activities of I-Mf, C-Mf and adult *D. immitis* by the indirect fluorescent antibody (IFA) test.

Serum samples were collected from two dogs experimentally infected with *D. immitis* on Days 0, 65 and 83 (dog No. 1 with IHA titers of 1:<8, 1:256 and 1:2048) and on Days 0 and 83 (dog No. 2 with IHA titers of 1:<8 and 1:128) after infection. IHA tests were performed by the method previously described [2] using I-Mf antigen. The procedure of the IFA test and preparation of frozen cross-sections of female *D. immitis* as an antigen were performed by the method of Ishii et al. [3] using fluorescein isothiocyanate conjugated anti-dog IgG rabbit serum (Miles Lab., Inc., Elkhart Ind.).

The two serum samples taken on Day 0 showed negative IFA titers of 1:16. In negative IFA, extremely weak fluorescence was observed on the muscle layer, but no specific fluorescence was found (Figs. 1 and 2). However, yellowish auto-fluorescence was obviously observed only in the lumen of the intestines. Reddish-brown auto-fluorescence was also barely observed on I-Mf, the muscle layer and the cuticle. In contrast, the three remaining serum samples taken on Days 65 and 83 all yielded IFA titers of 1:512. In positive IFA, specific fluorescence was detected on the lateral chord, the muscle layer, the intestines, the uterus, I-Mf, the ovary and the cuticle (Figs. 3, 4 and 5).

Intact I-Mf and C-Mf were thoroughly washed with phosphate buffered saline and tested for IFA. Specific fluorescence was observed on the surface of I-Mf (Fig. 6), but not on that of C-Mf. Thus, different antigenicities between I-Mf and C-Mf were confirmed not only by the IHA [2] but also by the IFA tests.

Several investigators used C-Mf of some filarial species as an antigen for the IFA test for diagnosis of infection with *Dirofilaria uniformis* [7], *D. immitis* [5], *D. repens* [5], *Dipetalonema* sp. [5], *Wuchereria bancrofti* [6] and *Onchocerca volvulus* [4]. These reports commonly indicated extremely low antigenicity of intact C-Mf and stressed the necessity of pretreatment with formalin or papain or by ultrasonication to utilize them for the IFA test. The low antigenicity of intact C-Mf

may have been caused by exposure to some unknown inhibitory substance(s) originating from host's blood protein after entering the circulation. Therefore, some pretreatment such as those mentioned above may be needed for activation of their antigenicity. On the other hand, since the body surface of I-Mf may possibly be immaculate, they probably exhibit a high antigenic reactivity. Wong [8] has reported in her study on experimental dirofilariasis in macaques that fluorescence was found on the muscle layer, the hypodermis and the cuticle in the cross-sections of *D. immitis*. These results resemble the present ones. On the other hand, in studies of filariasis other than *D. immitis*, little or no antigenicity has been reported on the cuticle or the ovary of *Litomosoides carinii* [3], or on the cuticle, the ova or I-Mf of *Onchocerca volvulus* [1]. The latter report, therefore, suggests the possibility that the excellent antigenicity of I-Mf is not necessarily common among filarial species.

Finally, it seems probable that I-Mf of *D. immitis* is applicable for immunological study of the infection with not only *D. immitis* but also other filarial species because of its high antigenicity.

References

1. Eyck, D. R. T. (1973). Exp. Prasitol. **34**, 154–161.
2. Hayasaki, M. (1981). Jpn. J. Vet. Sci. **43**, 21–26.
3. Ishii, A., Matsuda, H., Kamiya, M., and Kobayashi, J. (1969). Jpn. J. Parasitol. **18**, 1–7. (in Japanese).
4. Lucasse, C. (1962). Z. Tropenmed. Parasitol. **13**, 404–408.
5. Mantovani, A., and Sulzer, A. J. (1967). Am J. Vet. Res. **28**, 351–354.
6. Santos, L. G. D., Santos, D. S., and Azevedo, R. (1976). Ann. Trop. Med. Parasitol. **70**, 219–225.
7. Sadun, E. H. (1963). Exp. Parasitol. **13**, 72–82.
8. Wong, M. M. (1974). Trans. R. Soc. Trop. Med. Hyg. **68**, 479–490.

Explanation of Figures

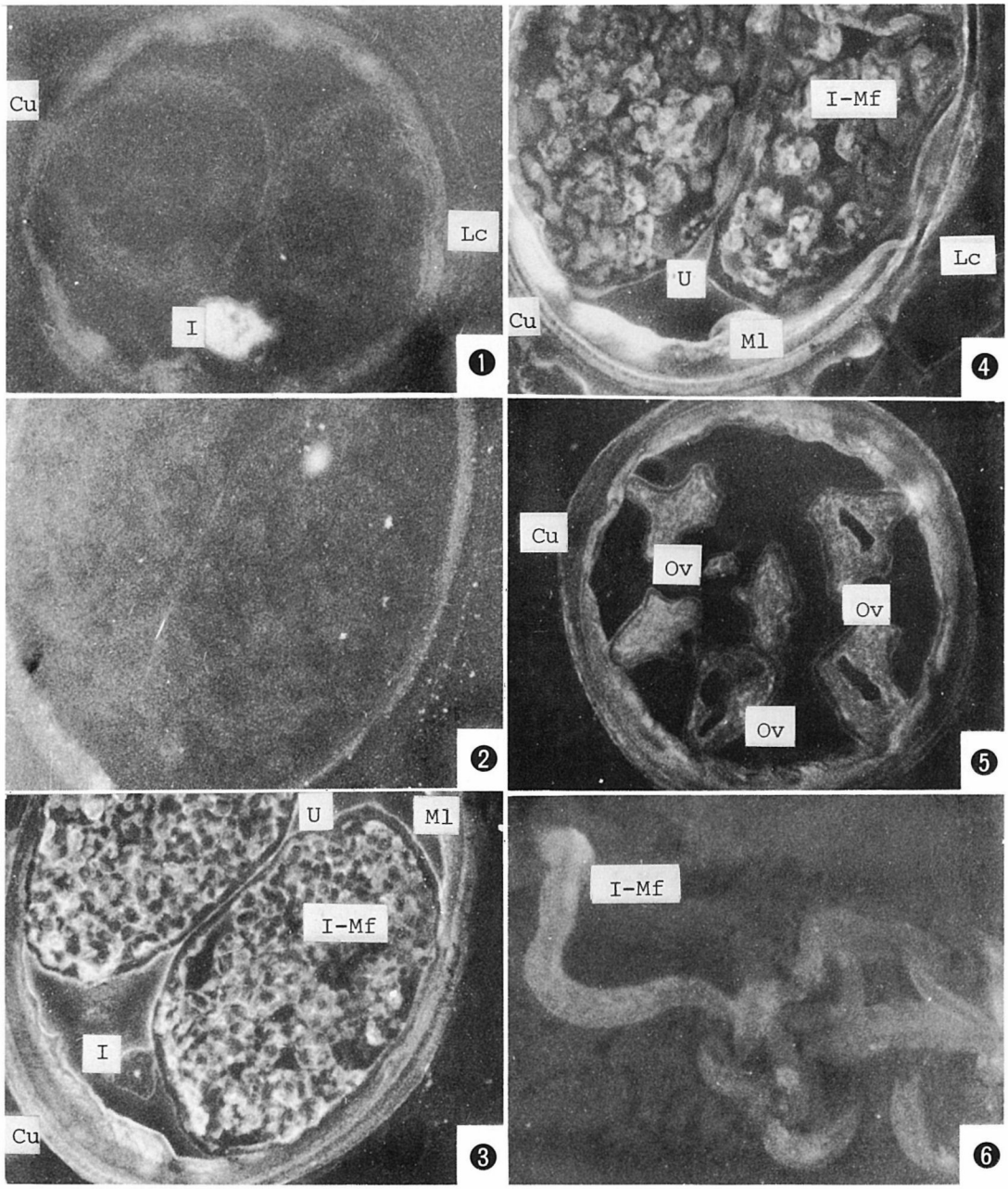
Figs. 1 and 2. Cross-section of *Dirofilaria immitis* allowed to react with a 16-fold dilution of each of non-infected dog sera collected from dog No. 1 (Fig. 1) and dog No. 2 (Fig. 2) on Day 0. 40×, 30 seconds exposure.

Figs. 3, 4 and 5. Cross-section of *Dirofilaria immitis* allowed to react with a 512-fold dilution of each of infected dog sera collected from dog No. 1 on Days 65 (Fig. 3) and 83 (Fig. 4) and dog No. 2 on Day 83 (Fig. 5). 40×, 30 seconds exposure.

Fig. 6. Intrauterine microfilariae of *Dirofilaria immitis* allowed to react with a 128-fold dilution of an infected dog serum collected from dog No. 2 on Day 83. 40×, 30 seconds exposure.

要　約

間接蛍光抗体法における犬糸状虫成虫とミクロフィラリアの抗原性（短報）：早崎峯夫（東京農工大学家畜内科学教室）——犬糸状虫雌虫の凍結切片を用いた間接蛍光抗体法において，寄生犬血清との間の陽性反応では，側線，筋層，消化管，子宮，子宮内ミクロフィラリア（Mf），卵巣およびクチクラに強い特異蛍光が認められた．いっぽう，雌虫から回収した intact な子宮内 Mf と寄生犬血清との間の反応においては特異蛍光が認められたが，寄生犬から回収した末梢血 Mf は寄生犬血清に対して陰性であった．



Cu: Cuticle
I: Intestine
I-Mf: Intrauterine microfilaria
Lc: Lateral chord
Ml: Muscle layer
Ov: Ovary
U: Uterus