Immunological Treatment of Cough Occurring in Dogs with Dirofilariosis

Mineo HAYASAKI, Akira HARAYAMA, Hideko SEKI, Katsuhiko KONNO, and Isamu OHISHI

Department of Internal Medicine and Parasitology, School of Veterinary Medicine, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

(Received 17 January 1991/Accepted 2 May 1991)

ABSTRACT. A persistent, spasmic and productive cough known as filarial cough often occurs in dogs with dirofilariosis, and has been considered to be the consequence of an allergic response to *Dirofilaria immitis*. Twenty-one dogs with filarial cough were subcutaneously injected with worm antigen (200 μg of protein concentration) extracted from adult *D. immitis* once a day for 5 days. These injections were effective for 17 (81%) of the dogs, resulting in a complete cure for 7 dogs and marked improvement for 10 dogs.—KEY WORDS: allergic cough, dirofilariosis, immunological treatment.

J. Vet. Med. Sci. 53(4): 651–653, 1991

A persistent cough in dogs infected with *Dirofilaria immitis*, which is empirically recognized by small animal clinicians, is called "filarial cough". This cough is characterized by a spasmic and productive cough and often show a poor response to treatment by antibiotics and the usual cough medicines. The underlying mechanism of this cough remains unknown. However, this cough might be a consequence of the allergic response to *D. immitis*, because it is now generally accepted that the clinical manifestations of parasitic disease result from immune responses to the worm antigens and not from a direct toxic effect of the worm *per se* [4]. It indicated the possibility of hyposensitization therapy for the cough.

The present paper reports that the filarial cough was cured while we were trying to inject the filarial antigen to the dogs with *D. immitis* infection in our immunological studies on canine dirofilariosis.

MATERIALS AND METHODS

Dogs: Twenty-one infected dogs with filarial cough, but no other clinical findings of dirofilariosis, were collected from the Veterinary Hospital of the School of Veterinary Medicine, Tokyo University of Agriculture and Technology, and were employed in this study. These dogs were diagnosed as having filarial cough, according to the results of clinicopathological examinations such as physiological findings, hematological and blood chemistry examinations, electrocardiography, radiography of the thorax and a blood-concentration test for microfilariae. The concentration method of fecal examinations indicated no eggs of intestinal parasites in their feces.

The dogs consisted of 17 males and 4 females, aged 3–16 years old; by breed, there were 18 mongrel dogs, 2 Shiba dogs and 1 beagle of these, there were 10 severe cases, 10 moderate cases and 1 mild case of the cough; the coughs were clinically categorized into three degrees of severity as mild, moderate and severe according to frequency, strength, depth and magnitude of the cough.

Antigen: Whole worm antigen extracted was used for immunological treatment. Equal numbers of adult male and female worms were collected from infected dogs and washed with saline several times. The fresh worms were homogenized by a tissue homogenizer and then an ultrasonicator. The homogenate was extracted overnight in PBS at 4°C, which was later delipidized with chilled ether by sequential centrifugation for 30 min at 5,000 × g. After the ether was discarded as the first supernatant layer, the extract of worms as the second supernatant layer was recovered and incubated for 1 hr at 37°C for evaporation of the remaining ether. This soluble extract was dialyzed against saline for 24 hr at 4°C and again centrifuged for 1 hr at 18,000 × g. After sterilization by a membrane filter with 0.22 μm pore size and subsequent lyophilization, the dry powder was divided into small bottles of 30 mg each under sterilized conditions and stored at −40°C until use.

Antigen treatment: The 21 dogs were subcutaneously injected with filarial antigen once a day for 5 days. Each injection contained 30 mg of dry powder of filarial antigen reconstituted with 1 m*l* of distilled water for the injection. Protein concentration in each injection was about 200 μg, as deter-

mined by the method of Lowry *et al.* [7].

Indirect hemagglutination (IHA) test: An IHA test using filarial antigen was also performed for monitoring the *D. immitis*-specific hemagglutinating antibody (IgG) by the method previously described [5].

Indirect fluorescent antibody (IFA) test: An IFA test, using a frozen cross section of female *D. immitis* as the antigen and FITC-labeled anti-dog IgG serum as the second antibody, was performed as previously described [6].

RESULTS

Antigen treatment: As shown in Table 1, the treatment was effective for 17 (81%) of the 21 infected dogs with 5 injections of antigen. In these 17 dogs, 7 dogs (33.3%) were completely cured with a disappearance of the cough and 10 dogs (47.6%) were markedly improved with an obvious reduction in severity of the cough. However, 4 dogs (19%) showed only a slight change in the cough. Seventeen of the above-mentioned dogs were monitored for prognosis. One dog exhibited the cough again after 1 month, but others did not suffer recurrence for 3 months or more after the treatment.

Table 1. Result of antigen treatment in dogs with filarial cough

Efficacy	No. of dogs treated	%
Complete cure	7	33.3
Marked cure	10	47.6
Less efficacy	4	19.0

Determination of IHA and IFA antibodies: The *D. immitis*-specific hemagglutinating antibody was also monitored in 6 of the 21 infected dogs by the IHA

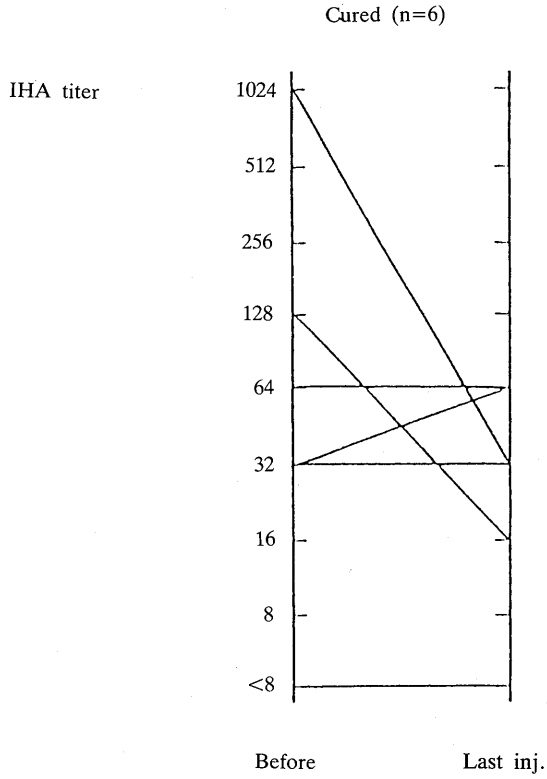

Fig. 1. Changes in indirect hemagglutinating antibody titer before and after antigen treatment for filarial cough.

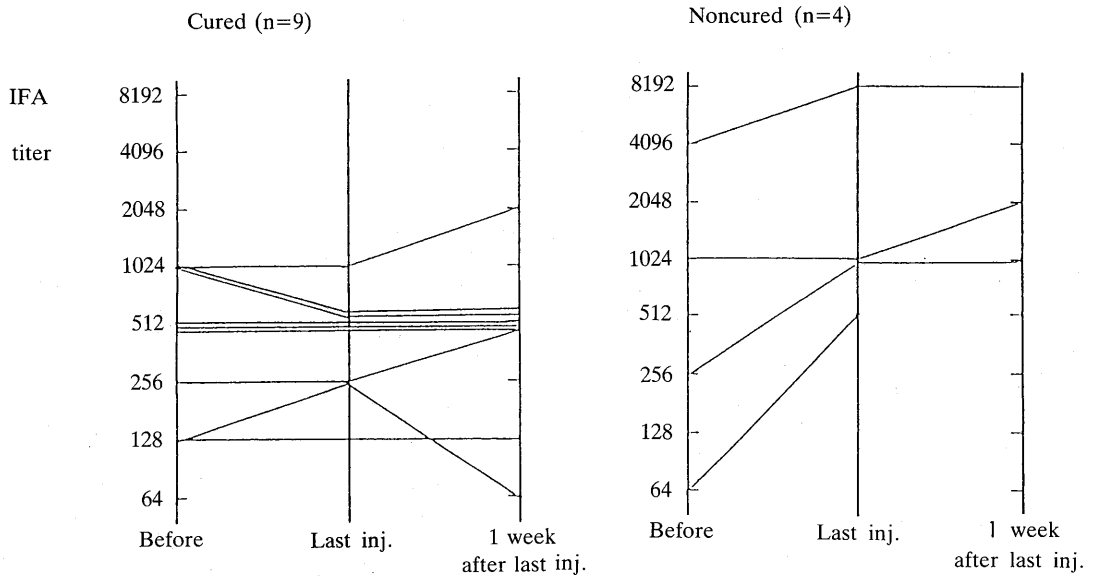

Fig. 2. Changes in indirect fluorescent antibody titer before and after antigen treatment for filarial cough.

test. Substantial change was not observed in the dogs, although 2 dogs showed a decrease of the titer from 1:1024 to 1:32 and from 1:128 to 1:16, respectively. There was no change in the other 4 dogs (Fig. 1). The *D. immitis*-specific IFA test was also used for monitoring the change of the IgG titer in 13 of the 21 infected dogs. However, a clear tendency was not observed (Fig. 2).

DISCUSSION

In general, an immunological hyposensitization was required to produce sufficiently the IgG-class antibody as a blocking antibody and reduce completely the allergic condition. However, in the present study, the effects of immunological treatment appeared within a short period and a high recovery rate (81%) was obtained. This strongly suggest that the injections of the worm antigen might suppress the incitement of the filarial cough, because no cough remedy was used in the cases employed in this study. Therefore, it seems likely that these injections induce hyposensitization to the cough.

The changes in IHA- and IFA-antibody titers varied and did not indicate a consistent trendy in the comparison before and after hyposensitization treatment. Contradictory results have also been reported [1–3], indicating that increase of the IgG level did not necessarily correlate with a successive hyposensitization therapy when measured by serological tests.

The results of the present study indicate the further investigation on the methods of hyposensitization with filarial antigen to have the complete cure of the filarial cough.

REFERENCES

1. Arbesman, C. E., Rose, N. R., and Kantor, S. Z. 1960. Immunologic studies of ragweed-sensitive patients. II. Effect of specific hyposensitization therapy on hemagglutinating antibody titers. *J. Allergy Clin. Immunol.* 31: 333–341.
2. Arbesman, C. E., Kantor, S. Z., Rapp, D., and Rose, N. R. 1960. Immunologic studies of ragweed-sensitive patients. III. Clinical aspects: The relationship of reagin and hemagglutinating antibody titers to results of hyposensitization therapy. *J. Allergy Clin. Immunol.* 31: 342–350.
3. Arbesman, C. E. and Reisman, E. 1961. Immunologic studies of respiratory ragweed pollen therapy. *J. Allergy Clin. Immunol.* 32: 435.
4. Haque, A. and Capron, A. 1986. Filariasis: Antigens and host-parasite interactions. pp. 317–402. *In*: Parasite Antigens (Pearson, T. W. ed.), Marcel Dekker, Inc., New York and Basel.
5. Hayasaki, M. 1981. Indirect hemagglutination test for diagnosis of canine filariasis. *Jpn. J. Vet. Sci.* 43: 21–26.
6. Hayasaki, M. 1983. Antigenicity of microfilarial and adult *Dirofilaria immitis* in indirect fluorescent antibody test. *Jpn. J. Vet. Sci.* 45: 113–115.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265–275.