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Homocytotropic Antibody Formation in Rabbits implanted with *Dirofilaria immitis*

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The intradermal test (atopic type) using *Dirofilaria* antigen has been applied commonly for the diagnosis in human filariasis. It is assumed that the test is mediated by IgE antibody as reported by Ishizaka *et al.* (1966). Recently, increased serum immunoglobulin E (IgE) levels in human parasite infection has been shown by a number of workers. (Johansson and Mellbin, 1968; Hogarth-Scott, 1969; Ito *et al.*, 1972).

In the previous reports (Kobayashi *et al.*, 1969; Taniguchi, 1970; Hattori; 1970; Sumi, 1970, 1971), homocytotropic (Hc) antibodies were proved to be formed in rabbits and rats either infected or vaccinated with *Ascaris suum*, *Toxocara canis*, *Anisakis*. sp or *Dirofilaria immitis* and to be analogous to human IgE antibody.

The present experiments have been designed to determine the duration of Hc antibody formation and its properties in rabbits in which living *D. immitis* have been intraperitoneally implanted.

Materials and Methods

Antigen: *Dirofilaria immitis* adult worms obtained from the heart of the infected dogs, washed in physiological saline and homogenized with a glass tissue homogenizer. The homogenate was washed thrice with double volumes of acetone and then with several volumes of ether to remove the last traces of acetone. The washed homogenate was finally dried in vacuo. One gram of the dry powder was resuspended in 100 ml of phosphate buffered saline (PBS), incubated at 37°C for 30 min., and then kept overnight in a cold room. The suspension was centrifuged at 13,000 rpm for 30 min. and the sediment discarded. Protein concentration of the supernatant was adjusted to 1 mg P/ml with PBS and used as the saline-extracted antigen.

Living worms: Living worms were obtained from the heart of the infected dogs under sterile condition.

Experimental animals: Male albino rabbits weighing approximately 2 kg were used through the course of experiments.

Homologous passive cutaneous anaphylaxis (PCA): Homologous PCA was done according to the method reported by Zvaifler and Becker (1966). A sensitization period of 72 hrs. was used. Antigen challenge was performed by injection of 1 ml saline-extracted antigen and 1 ml of 1% Evans blue, intravenously. Blue spot of the skin was measured 45 min. after the antigen challenge. All PCA tests were done in duplicate. The PCA titre was determined by the highest dilution of antiserum giving the minimum positive reaction.

Immunization: Five rabbits were anesthetized with pentobarbital sodium and operated upon the limited region of the abdomen. Aseptically obtained worms were enclosed in the peritoneal cavity of rabbits; five male and 14 female worms were implanted in No. 6 rabbit, 3 and 2 in No. 7, 3 and 3 in No. 8, 3 and 7 in both Nos. 9 and 10, respectively. In rabbits Nos. 9 and 10 the worms were surgically removed 27 days after the implantation.

Treatment with mercaptoethanol or heat: A half ml of antisera was dialyzed against 250 ml of 0.1 M 2-mercaptoethanol for 3 hrs. at room temperature and then dialyzed

against 500 ml of 0.02 M iodoacetamide for 4 hrs. Before testing, samples were dialyzed against several changes of PBS for 18 to 24 hrs. at 4°C. Control samples were either dialyzed against PBS in place of mercaptoethanol and then treated with iodoacetamide as described above or dialyzed only against PBS. Antisera was also heated at 56°C in a water bath for 4 hrs., unheated samples served as controls.

Precipitating antibody: Precipitating antibody was measured by the ring test. The antibody titre was determined by the highest dilution of antisera giving minimum positive ring reaction.

Results

Hc antibodies appeared 9 days after implantation of the worms in all rabbits tested, and increased to the titre of 1 : 729 on the day 44. The PCA titres of sera of rabbits Nos. 6 and 8 increased up to 1 : 2916 on the day 48 after implantation and were maintained at this level for 142 days. In rabbits Nos. 9 and 10 from which worms were removed on the day 27, the PCA titres were gradually decreased on the 48 onward after implantation. In rabbit No. 10, no Hc antibody was detected on the day 212. In rabbit No. 6 which was given the greatest number of worms, the Hc antibody formation was markedly increased up to a PCA titre of 1 : 2916 on the 212 th day. Although the worms were not removed from rabbit No. 8 the Hc antibody formation decreased markedly 184 days after implantation (Fig. 1). The tendency of IgG antibody formation in sera of all animals irrespective of removal or reserve of the worms was similar. The IgG titres increased at 27 or 38 days after implantation, and maintained a plateau phase for about 114 days. Rabbit No. 6 possessed no IgG antibody at 184–212 days, but the Hc antibody showed a high titre of 1 : 2916 (Fig. 2).

The properties of Hc antibody: The Hc antibodies in rabbits sera obtained 14 days (rabbits Nos. 6–10), 44 days (Nos. 6–10), 100 days (No. 10), 184 days (No. 9) and 212 days (Nos. 6 and 8) after implantation were compared with regard to heat stability and the resistance to treatment with mercaptoethanol. The Hc antibodies in the early stages (14–44 days after) were highly heat-labile and mercaptoethanol-sensitive, but heat stability and mercaptoethanol resistibility increased as the days proceeded. With regard to rabbit No. 6 Hc antibody in the serum obtained on the 212th day was completely reactive in PCA test even after incubation of the serum at 56°C for 4 hrs. or merca-

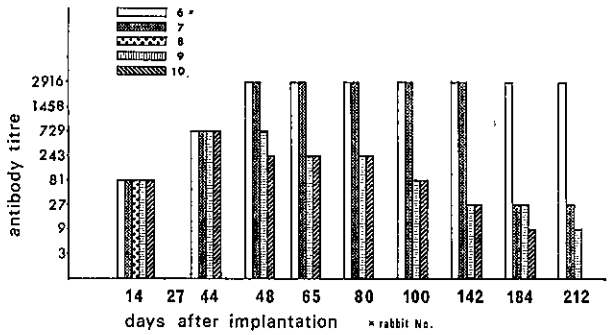

Fig. 1 Homocytotropic antibody formation in the rabbits implanted intraperitoneally with *Dirofilaria immitis* adult worms.

The determination of Hc antibody titre was made on deys 14, 44, 48, 65, 80, 100, 142, 184 and 212 after implantation. Implanted worms were surgically removed from rabbits Nos. 9 and 10 on day 27 after implantation. The rabbit No. 7 was sacrificed on day 14 by cardiac exsanguination and then serum was pooled.

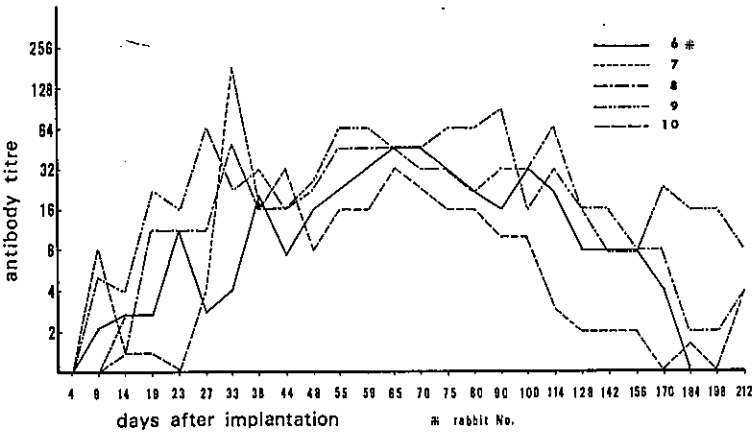

Fig. 2 Precipitating antibody formation in the rabbits implanted intraperitoneally with *Dirofilaria immitis* adult worms.
Figures on abscissa indicate days on which determination of precipitating antibody titre was made. Implanted worms were surgically removed from rabbits Nos. 9 and 10 on day 27 after implantation.

Table 1. Changes in properties of homocytotropic antibody treated with heat and mercaptoethanol

Rabbits used	Days after immunization	Treated with					
		Heat					Mercaptoethanol
		1/2	1	2	3	4 hrs.	(Alkylation and reduction)
No. 6-10	14	+*	−**	−	−	−	−
No. 6-10	44	+	−	−	−	−	−
No. 10	100	+	+	−	−	−	±
No. 9	184	+	+	−	−	−	+
No. 8	212	+	+	+	+	−	+
No. 6	212	+	+	+	+	+	+

* PCA, positive, ** PCA, negative
All of unheated samples showed positive by the PCA test.

ptoethanol treatment (Table 1).

Discussion

Sadun (1967) reported that rabbits infected with *Dirofilaria uniformis* were capable of producing Hc antibody for 28 weeks after the infection. Sumi (1970, 1970) reported that rabbits vaccinated with dry powder or extracts of *D. immitis* adult worms produced Hc antibodies for about 2 weeks, and those to which living adult *D. immitis* were intraperitoneally implanted, produced Hc antibodies for 4-80 days or onward.

The present study shows that *D. immitis* induced the formation of Hc antibody in all implanted rabbits for a long period. The Hc antibody formation lasted for about 6 months in a rabbit even after removal of the implanted worms on the day 27. The three other rabbits, from which the implanted worms were removed or not removed, produced Hc antibodies for at least 7 months. Rabbit No. 6 to which many worms had been implanted, produced a very high titre of Hc antibody (1 : 2916) during the entire test period.

These results indicated that the duration

of Hc antibody formation was correlated with the degree of antigen stimulation to the host. If the antigen stimulation was removed, the Hc antibody formation may terminate shortly after the removal.

The IgG antibody was produced in both infected and vaccinated animals. This IgG antibody is a blocking antibody on atopic type of allergy. Some investigators have reported that the intradermal test was available in the majority of cases of parasitosis. These results indicate that human parasitosis had a little or no IgG antibody instead of a very high titre of Hc antibody against this infection.

Rabbit No. 6 produced IgG antibody of extremely low titre, if any, and a very high titre of Hc antibody after 212 days. It is true of human parasitosis. It was tempting to speculate that the rabbit might induced immunotolerance with regard to IgG.

Strannegard and Bellin (1970) reported the formation of both early and late reagins in rabbits immunized with haemocyanin. These reagins were distinguishable among them by heat and mercaptoethanol treatment; early reagin was heat-labile and mercaptoethanol sensitive, whereas late reagin was heat stable and mercaptoethanol resistant. Each of reagins had one peak in PCA titre curve.

In our experiments, the appearance of the characteristics of early and late type of Hc antibodies was correlated with the time required since their formation was initiated, but the specific formation curves for early and late type of Hc antibodies were not shown. Further studies are necessary on this respect.

Summary

Living adult worms of dog filaria (*D. immitis*) were implanted into the peritoneal cavity of rabbits, and homocytotropic (Hc) and precipitating antibodies produced in the rabbits were studied. High titres of Hc and precipitating antibodies were produced in all rabbits tested and maintained for at least 6–7 months. In those cases the worms were not removed, the Hc antibody titre increased up to 1 : 2916 on 48 days after implantation. On the contrary, it decreased gradually in cases with worm removl. The titre and duration of Hc antibodies were correlated with the degree of antigen stimulation to the host. Heat stability and mercaptoethanol resistance of Hc antibodies were correlated with the time required for initial formation of both reagins.

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イヌ糸状虫 (*Dirofilaria immitis*) によるウサギの Homocytotropic (Hc) の産生について

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イヌ糸状虫 (*Dirofilaria immitis*) の生きた成虫をウサギの腹腔内に移植して，Hc 抗体の産生を経日的に sensitization period 72時間の homologous PCA 法でしらべた．その結果は Hc 抗体は200日以上の長期に亘つて産生された．またその変動は移植した虫体数の多いものが高値の抗体価を持続する傾向を示した．早期に移植虫体が除去された2例では Hc 抗体の，産生は持続したが，抗体価の低下傾向は，除去しなかつたものよりも速かつた．

Hc 抗体はその産生の初期のものは熱処理やメルカプトエタノール処理に対して，高い感受性を示したが，100日を経過して後は次第に耐性を増大した．