

The Change of the Indirect Hemagglutination Titers in Mice Infected with *Dirofilaria immitis* Microfilariae following the Administration of Diethylcarbamazine

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Abstract: About 30,000 microfilariae of *D. immitis* were inoculated into ICR mice subcutaneously. The mice were injected with DEC at 300mg/kg/day for 3 consecutive days, then the IHA test was carried out until 6 weeks after DEC treatment. Before DEC treatment, the IHA titers in two of six mice was 1:240 and those in the other four was 1:120. One week after DEC treatment, the IHA titer began to rise in most mice and at 4 weeks, those in all mice reached maximum. During the observation period, the maximum titers in four of six mice was 1:960 and those in the remaining two was 1:480.

Key words: *Dirofilaria immitis*, Mice, DEC, IHA

INTRODUCTION

DEC is widely used for treatment of filariasis. However, it has been reported that patients with onchocerciasis experience severe inflammatory reactions following the administration of DEC (Buck et al., 1974; Bryceson et al., 1977; Taylor et al., Sakamoto et al., 1984). With regard to the relations between inflammatory reactions and immunological conditions in the patients with onchocerciasis, there are some reports which describe the changes of level of immunoglobulin and circulating immune complexes in the patients following DEC treatment (Guerra-Caceres et al., 1980; Greene et al., 1983). On the other hand, the lack of a small laboratory host animal model presents some difficulties to the study of human onchocerciasis. Although rodents have been used as proxy

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hosts (Nelson et al., 1966; Rabalais, 1974; Aoki et al., 1980), the model of onchocercal microfilariae in rodents has not been fully utilized for further studies on onchocerciasis. Recently, Sakamoto et al (1984) reported that *Dirofilaria immitis* microfilariae survived in mice for several days when microfilariae were inoculated subcutaneously and that this model was useful for study of skin-dwelling filariasis. In this paper, the results of the IHA test was carried out to improve understanding of the immunological changes in mice infected with *D. immitis* microfilariae and treated with DEC are reported.

MATERIALS AND METHODS

Blood containing numerous microfilariae was collected from a dog infected with *D. immitis*, and was mixed with 0.83% NH_4Cl solution to allow complete haemolysis. After repeated centrifugation at 300g force for 10 min. at 5C and washing with Hanks' solution, solution containing 30,000 microfilariae was inoculated into ICR mice subcutaneously in the inguinal region once a week for 10 consecutive weeks. DEC treatment was started from one day after the final inoculation. Mice were injected with DEC at 300mg/kg/day for 3 consecutive days intraperitoneally. The IHA test was applied to determine the change of antibodies to *D. immitis*. Sheep red blood cells (SRBC) were washed in Alserver's solution, and formalinized, were mixed with an equal volume of 0.0025% tannic acid solution and incubated in a water bath at 37C for 5 min. The crude antigen was extracted from adult *D. immitis* with 0.015M phosphate buffered saline (pH 7.2). The formalinized SRBC were sensitized with antigen. For the purpose of the IHA test, blood samples were collected from the eyes of mice with filter paper just before treatment, and 24 hours, 1, 2, 3, 4, 5, and 6 weeks after the final treatment. The filter papers were dried, and stored at -20C until used. The eluate obtained by soaking the filter papers in 0.4ml of 0.015M PBS (pH 7.4) was used in the IHA test.

RESULTS

Table 1 shows the results of the IHA test before and after DEC treatment. Before DEC treatment, the IHA titers in two of six mice was 1:240 and those in the remaining four was 1:120. 24 hours after the final treatment, the IHA titer rose in one mouse but decreased in another. However, the other 4 mice did not show any change of the IHA titers. At one week, all of the mice except one showed higher titers compared with those before treatment. The IHA titer of one mouse reached maximum at 2 weeks (1:480), and then maintained the same level until 6 weeks. At 4 weeks, the IHA titers of all mice reached maximum, which were then maintained at the highest level until 6 weeks. During the observation period, the maximum titers in four of six mice were 1:960 and those in the remaining two were 1:480.

Table 1. The change of the IHA titers in mice after DEC treatment.

Mouse No.	Before	Days after the final DEC treatment						
		24h	1w	2w	3w	4w	5w	6w
1	120	240	240	240	480	960	960	960
2	120	120	240	240	480	480	480	480
3	240	240	240	480	480	960	960	960
4	120	120	240	480	480	480	480	480
5	240	120	480	480	960	960	960	960
6	120	120	240	480	960	960	960	960

DISCUSSION

In the present study, the maximum titer recorded in the IHA test was 1:240 when about 30,000 microfilariae were inoculated subcutaneously once a week for 10 consecutive weeks. Benjamin et al (1976) reported that when *Matomys natalensis* was infected with *Brugia pahangi*, IHA antibody was detected at 9 days after infection, and the maximum titer was 1:512 at 87 to 94 days. With regard to IHA antibodies in their paper, it is possible that adult worms stimulate immunological mechanisms in host. On the other hand, in this experiment, it was described that even when microfilariae only were inoculated, IHA antibodies were detected although the levels of titers were low.

Other studies have been made of the relationship of microfilarial density to antibody titers. In patients with onchocerciasis, the level of IHA antibody had a tendency to increase with the intensity of the infection expressed as microfilarial density (Ikeda et al., 1979). On the other hand, Franks (1979) reported the presence of soluble filarial antigen in the sera of patients with raised microfilaraemia from *Wuchereria bancrofti*. Desowitz and Una (1976) reported the presence of soluble antigen in the sera of two dogs with high level of microfilaraemia of *D. immitis*. In this study, the maximum titer was not high level before DEC treatment. This might be caused by the small number of microfilariae inoculated.

In the present study, the level of IHA antibody began to increase on the first week in four of six mice and reached maximum by the fourth week in all mice after the final DEC treatment. Gurra-Caceres et al (1980) reported that there were no changes in titer of anti-microfilarial antibodies found using ELISA until 24 hours after DEC treatment in three patients with onchocerciasis. On the other hand, Carme et al (1982) reported that free serum antigen was increased from 6th days in rats infected with *Litomosoides carinii*, and that the antigen remained detectable for two to three weeks after DEC treatment. In the present study, although the IHA titers were low levels before DEC treatment (maximum: 1:240), a remarkable increase was found in all mice examined after

treatment (maximum : 1:960). Greene et al (1983) reported that patients with onchocerciasis who did not have increased circulating immune complexes (CIC) before treatment developed high levels of ClqBA after treatment. In contrast, the slight increase in CIC levels at the end of treatment for the initially increased group in the Greene report was not statistically significant. Although some of the mechanisms possibly involved in the absence of host response are discussed, including anti-complement factors, poor antigenicity, acquisition of host antigen, immune tolerance and blocking antibodies (Henson et al., 1979), it seems that an immunization with a proper quantity of antigen is an important factor in changing the IHA titers after DEC treatment.

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Dirofilaria immitis ミクロフィラリア感染マウスに於ける Diethylcarbamazine 投与時の IHA titer の変動

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Dirofilaria immitis ミクロフィラリア30,000隻を6匹のICRマウスのソ径部皮下に毎週1回10週間接種後DEC 300mg/kgで3日間治療した。DEC投与前のIHA titerの最高値は1:240であったが投与後1週目より titer は上昇し始め4週目には全てのマウスが最高値(4匹は1:960, 2匹は1:480)を示し6週までの観察期間では titer は最高値を維持した。

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