第26巻 第4号　　寄生虫学雑誌　　昭和52年8月 (1977)

# Immunological Observations on Seven Cases of Eosinophilic Meningoencephalitis Probably Caused by *Angiostrongylus cantonensis* in Okinawa, Japan

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(Received for Publication; February 14, 1977)

## Introduction

*Angiostrongylus cantonensis* is primarily a parasite of rodents (Mackerras and Sanders, 1955), and it has attracted notice as an important agent of eosinophilic meningoencephalitis which occurs on many Pacific islands and in Southeast Asia (Alicata, 1962; Rosen *et al.*, 1967; Punyagupta *et al.*, 1970). The disease is considered to be produced by invasion into the central nervous system of the 3rd-stage larvae in the main ways of the ingestion of molluskan intermediate hosts or other paratenic hosts and characterized by significant meningitic signs and abnormal findings in cerebrospinal fluid such as increasing of the fluid pressure and protein concentration as well as, most constantly, eosinophilic pleocytosis.

In spite of these clinical findings, the disease is often difficult to distinguish from a variety of other diseases affecting the central nervous system. In addition, for the direct diagnosis, worms of *A. cantonensis* must be found in the cerebrospinal fluid but this has been rather exceptional (Hsieh, 1967). Although some immunological tests such as skin test and complement fixation test have been used for the indirect diagnosis (Alicata and Brown, 1962; Anderson *et al.*, 1962; Kagan and Zaiman, 1964), the results have been equivocal. For the immunological diagnosis, it is considered that various immunological aspects in this disease must be first clarified. Notwithstanding that more than 2,000 cases of the disease have been reported in the literature, the cases examined immunologically are considerably few at the present time.

Recently, the authors had an opportunity to examine immunologically seven suspected cases of angiostrongyliasis occurred in Okinawa, and the results obtained are as follows.

## Materials and Methods

1) Collection of worms:

Third-stage larvae of *A. cantonensis* were obtained from naturally infected land snails, *Achatina fulica*, collected in southern Taiwan. Twenty albino rats, weighing 100 ~200 g each, were infected with 100 larvae. The rats were sacrificed at 50 days after the infection, and the adult worms were collected from their lungs.

Adult *Ascaris lumbricoides* var. *suum* were collected from intestines of slaughtered swines, *Dirofilaria immitis* from hearts of stray dogs, *Paragonimus westermani* from lung of an experimentally infected dog,

*Echinoccocus multilocularis* from livers of infected gerbils and *Clonorchis sinensis* from livers of infected rats, respectively.

2) Preparation of whole worm extracts:

Freshly collected worms were washed several times with phosphate buffered saline (PBS, *i.e.* 0.005 M phosphate buffer containing 0.15 M NaCl) pH 7.2, homogenized and lyophilized. Then an excess of a cold acetone was added to the dry powder for delipidization. After centrifugation at 8,000 rpm for 30 minutes, an excess of PBS was added to the sediment and well stirred for 48 hours at 4 C. The mixture was then centrifuged at 10,000 rpm for 30 minutes, and the supernatant fluid was concentrated, dialyzed against PBS and stored at −20 C.

3) Preparation of antisera to *A. cantonensis*:

A rabbit, weighing 3,000 g, was used to prepare the immune serum to the whole worm extract of *A. cantonensis*. One milliliter of the extract (10 mg of proteins) was emulsified in an equal volume of Freund's complete adjuvant (Difco) and injected subcutaneously four times at intervals of 7 days. The rabbit was bled 10 days after the last injection, and the serum was collected.

The sera from five rats infected with *A. cantonensis* were also used as antisera to *A. cantonensis*. These sera were collected 50 days after the infection, pooled and stored at −20 C.

4) Collection of patient sera and cerebrospinal fluids:

Seven cases of eosinophilic meningoencephalitis occurred in Okinawa and examined immunologically by the authors are listed-up in Table 1. At the first examinations, the patients elapsed about 4 years and 5 months in case 1, 11 months in case 2, 40 days in cases 3 and 4, 33 days in case 5, and 10 days in case 7 after the suspected infection. Since the patient of case 6 was only one-year-old, infection source and date of infection of this case could not be supposed. The sera of four cases were further collected as follows: at 72, 84, 128 and 180 days in cases 3 and 4, at 44 and 73 days in case 5, and at 17, 24, 32, 39, 46, 53 and 67 days in case 7. The cerebrospinal fluids were also collected from cases 5, 6 and 7.

5) Gel-diffusion techniques:

Double-diffusion was performed according to the method of Ouchterlony (1958) by using 1.2% agarose (Beheringwerke) in Velonal-HCl buffer (pH 8.6, $\mu$=0.05) on a 5×8 cm glass slide. The depth of the gel was approximately 1.5 mm. Precipitin bands were allowed to develop for 48 hours at room temperature. The slide was then washed for 7 days in several changes of normal saline and dried under filter paper. Staining was done with Amidoblack 10 B.

Immunoelectrophoresis was done according to the method of Scheidegger (1955) using the same gel-plate as above. A potential of 80 V was maintained across the slide for 4

Table 1 Seven cases of eosinophilic meningoencephalitis probably due to *A. cantonensis* in Okinawa

| Case No. | Age, Sex | Date of admission to hospital | Suspected source of infection | Reporter |
|---|---|---|---|---|
| 1 | 47, ♀ | Oct., 1970 | *Deroceras laeve* | Yonamine (not reported) |
| 2 | 34, ♀ | 11, May, 1974 | *Laevicaulis alte* | Nakamoto *et al.*, 1974 |
| 3 | 38, ♀ | 21, Feb., 1975 | *Bufo asiaticus* | Kinjo *et al.*, 1975 |
| 4 | 20, ♂ | 21, Feb., 1975 | *Bufo asiaticus* | Kinjo *et al.*, 1975 |
| 5 | 53, ♀ | 23, Oct., 1975 | *Achatina fulica* | Nakamoto, 1976 |
| 6 | 1, ♀ | 27, Dec., 1975 | unknown | Ashimine, 1976 |
| 7 | 49, ♂ | 4, Feb., 1977 | *Achatina fulica* | Hanada (not reported) |

Cases 1 and 2: swallowing of a slug as medicine
Cases 3 and 4: swallowing of fresh liver of a toad as medicine
Cases 5 and 7: handling or eating of the snails

hours and precipitin bands were allowed to develop for 48 hours at room temperature. The drying and staining procedures were similar to the above.

6) Indirect hemagglutination test (IHA test) :

The test was almost the same as the method of Jacobs and Lunde (1957) with sheep erythrocytes treated with 1 : 100,000 tannic acid. The antigen which was purified as already described (Sato, 1975) was used at a protein concentration of 200 $\mu$g/ml to sensitize the sheep erythrocytes. All sera were inactivated at 56 C for 30 minutes and then absorbed with non-treated sheep erythrocytes to remove natural antibodies. Dilutions of sera were made with PBS containing 0.6% inactivated normal rabbit serum. For estimation of antibody titer, a microtiter plate with V-bottom was used. The test-sera were diluted with a calibrated diluter in a serial 2-fold dilution, and then a drop of 1% sensitized cell suspension was added to each well with a calibrated dropper. The plate was shaken to suspend the cells and was allowed to settle for 4 hours at room temperature before the resulting patterns of cells at bottoms were read.

7) Skin test :

Antigen employed in the test was purified with the antibody-immunoadsorbent columns (Sato *et al.*, 1974). The test was performed by the intradermal injection of the antigen (0.3 $\mu$g of protein nitrogens) in 0.02 ml of saline on the volar surface of the forearm. The diameter of the wheal and erythema were measured at 15 minutes after the injection. A positive reaction in the test was considered to be a wheal of 9 mm or more, or a erythema of 20 mm or more in mean diameter (Ishizaki *et al.*, 1961).

8) Mercaptoethanol treatment of sera and cerebrospinal fluids :

The sera and cerebrospinal fluids were

Table 2 Results of the gel-diffusion techniques on seven suspected cases of angiostrongyliasis

| Case No. | Time elapsed after the suspected infection | Ouchterlony reactions against whole worm extract of : | | | | | | No. of immunoelectrophoretic precipitin bands to whole worm extract |
|---|---|---|---|---|---|---|---|---|
| | | *A. cantonensis* | *A. lumb. suum* | *D. immitis* | *P. westermani* | *C. sinensis* | *E. multilocularis* | |
| 1 | 4 years & 5 months | (−) | (−) | (−) | (−) | (−) | (−) | 0 |
| 2 | 11 months | (−) | (−) | (−) | (−) | (−) | (−) | 0 |
| 3 | 40 days | (+) | (+) | (+) | (−) | (−) | (−) | 6 |
| | 72 days | (+) | | | | | | 6 |
| | 84 days | (+) | | | | | | 5 |
| | 128 days | (+) | | | | | | 3 |
| | 180 days | (+) | | | | | | 1 |
| 4 | 40 days | (+) | (−) | (−) | (−) | (−) | (−) | 3 |
| | 72 days | (+) | | | | | | 3 |
| | 84 days | (+) | | | | | | 3 |
| | 128 days | (+) | | | | | | 1 |
| | 180 days | (+) | | | | | | 1 |
| 5 | 33 days | (+)(+)* | (+) | (+) | (−) | (−) | (−) | 5 |
| | 44 days | (+)(+)* | | | | | | 5 |
| | 73 days | (+)(+)* | | | | | | 5 |
| 6 | 20 days** | (+)(+)* | (+) | (−) | (−) | (−) | (−) | 5 |
| 7 | 10 days | (−) | (−) | (−) | (−) | (−) | (−) | 0 |
| | 17 days | (+)(−)* | (−) | (−) | (−) | (−) | (−) | 1 |
| | 24 days | (+)(+)* | (−) | (+) | (−) | (−) | (−) | 2 |
| | 32 days | (+) | | | | | | 4 |
| | 39 days | (+) | | | | | | 4 |
| | 46 days | (+) | | | | | | 5 |
| | 53 days | (+) | | | | | | 4 |
| | 67 days | (+) | | | | | | 3 |

( ) : result of serum, ( )* : result of cerebrospinal fluid, ** Time elapsed after the admission to hospital

diluted 1 : 4 with PBS and dialyzed overnight at room temperature against PBS containing 0.1 M 2-mercaptoethanol (2-ME). The 2-ME was removed prior to antibody assey by further dialysis against several changes of PBS for 18 hours at 4 C.

## Results

1) Examinations by the gel-diffusion techniques :

The sera of above-mentioned cases were examined by the Ouchterlony method and immunoelectrophoresis, and the results obtained are summarized in Table 2. By the Ouchterlony method, the sera of cases 3, 4, 5 and 6 showed marked positive reactions at the first examination, whereas, the sera of cases 1 and 2, 4 years and 5 months and 11 months after the suspected infection respectively, did not develop any precipitin band (Fig. 1, A). The results of periodical examinations of case 7 are represented in Fig. 1, B. The positive reaction first appeared at 17 days and continued following 50 days. When the sera of five cases, which showed positive reactions in the Ouchterlony method, were further examined by immunoelectrophoresis, 5 or 6 precipitin arcs in cases 3, 5 and 6 were similar to those of infected rats, while only 3 arcs were observed in case 4 (Fig. 2). During the period of 180 days of the examination in cases 3 and 4, number of precipitin arcs was decreased gradually with the lapse of time (Table 2), and all of the arcs except only one, as seen in Fig. 2, disappeared at 180 days in both cases. The sera of case 7 were also periodically examined and the results are revealed in Fig. 3. In this case, the number of precipitin arcs was only one at 17 days but it was increased up to 5 arcs within following 29 days.

The cerebrospinal fluids of cases 5, 6 and 7 were examined by the Ouchterlony method and the antibodies could be detected in all

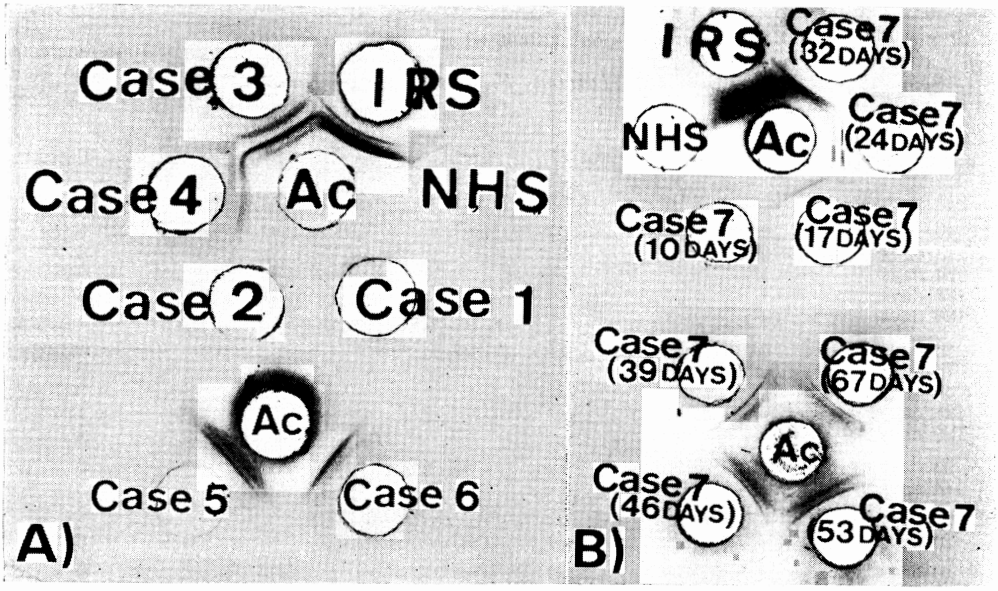


Fig. 1 A) Ouchterlony reactions of six cases against whole worm extract of *A. cantonensis*. B) Ouchterlony reactions of case 7 showing periodical changes of precipitin bands against whole worm extract of *A. cantonensis*.

Ac : whole worm extract of *A. cantonensis*, IRS : serum of rat infected with *A. cantonensis*, NHS : normal human serum, Case 1, 2, 3, 4, 5, 6 and 7 : sera of cases 1 at 4 years and 5 months, 2 at 11 months, 3 and 4 at 40 days, 5 at 33 days, 6 at 20 days* and 7 at 10, 17, 24, 32, 39, 46, 53 and 67 days after the suspected infection or admission to hospital*.

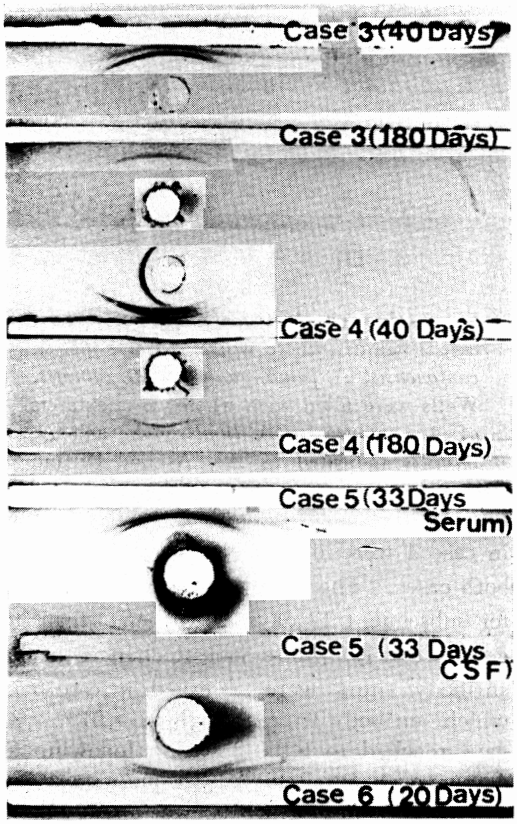


Fig. 2 Immunoelectrophoretic analysis of cases 3, 4, 5 and 6. Wells were filled with the whole worm extract of *A. cantonensis* and troughs with sera or cerebrospinal fluid (CSF) of these cases as indicated.

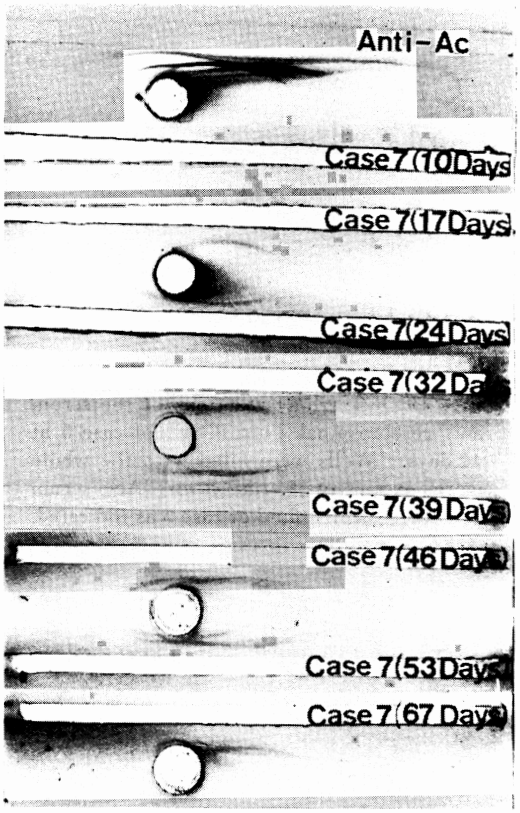


Fig. 3 Immunoelectrophoretic analysis of case 7. Wells were filled with the whole worm extract of *A. cantonensis* and troughs with the sera of case 7 periodically collected during 10 to 67 days after the suspected infection and antiserum of rabbit immunized with the whole worm extract (Anti-Ac).

specimens except one of case 7 at 17 days (Table 2). The intensity of the precipitin bands of the cerebrospinal fluids, however, was generally weaker than that of the sera (Fig. 4). When the antibodies in the cerebrospinal fluid and the serum of case 5 at 44 days were analyzed immunoelectrophoretically, the numbers of precipitin arcs were 5 in the serum and only 2 in the cerebrospinal fluid. This result is also shown in Fig. 2.

Cross-reactions against *D. immitis* and *A. lumb. suum* were observed in cases 3 and 5, against *A. lumb. suum* and *E. multilocularis* in case 6, and against *D. immitis* in case 7 (Fig. 5). The reactions against antigens of other helminthic species in cases 4, 6 and 7 were weaker than those against *A. cantonensis*, however, the reactions against *D. immitis* in case 3 were considerably strong as well as against *A. cantonensis*. When the reactions of case 3 were analyzed by the immunoelectrophoresis (Fig. 6), 6 precipitin arcs were demonstrated against *A. cantonensis* but only 3 arcs against both *A. lumb. suum* and *D. immitis*.

2) Examinations by the hemagglutination test :

Antibody levels estimated by the IHA test with the sera and cerebrospinal fluids of seven cases are summarized in Table 3. In six cases examined within 11 months after the suspected infection, the sera showed

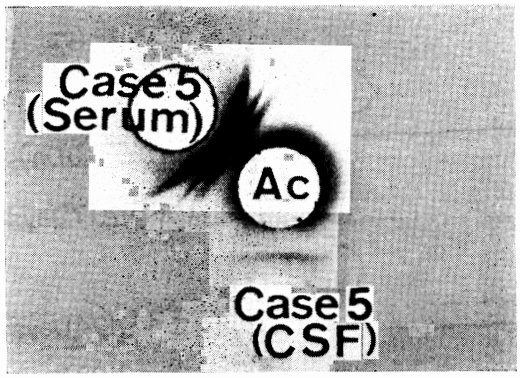


Fig. 4 Ouchterlony reactions of the serum and cerebrospinal fluid (CSF) of case 5 at 44 days. Wells were filled with the whole worm extract of *A. cantonensis* (Ac), serum and cerebrospinal fluid of case 5 as indicated.

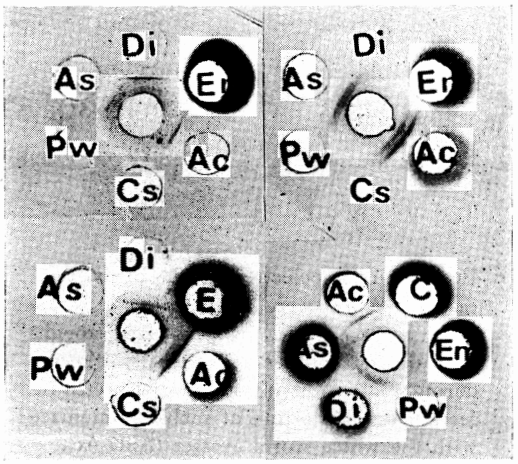


Fig. 5 Ouchterlony reactions of cases 3, 5, 6 and 7 against whole worm extracts of six helminthic species. 3, 5, 6 and 7: sera of cases 3, 5, 6 and 7, Di, As, Pw, Cs, Ac and Em: Whole worm extracts of *D. immitis*, *A. lumb. suum*, *P. westermani*, *C. sinensis*, *A. cantonensis* and *E. multilocularis*, respectively.

positive antibody levels over ×16. Although the positive reaction in case 2, 11 months after the infection, was significantly weak, other sera of cases 3, 4, 5 and 6 showed high levels of titer over ×256. In cases 3 and 4, the titers of ×4,096 in case 3 and of ×256 in case 4 were detected at first 40 days and peak titers of ×8,192 in case 3 and of ×512

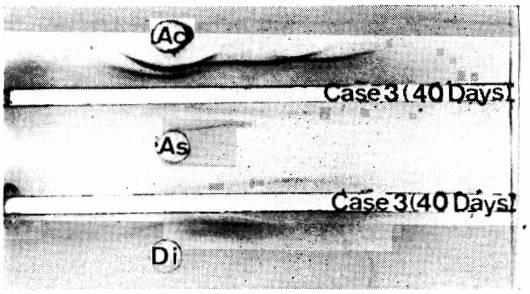


Fig. 6 Immunoelectrophoretic analysis of case 3 against whole worm extracts of *A. cantonensis*, *A. lumb. suum* and *D. immitis*. Wells were filled with whole worm extarcts of *A. cantonensis*, *A. lumb. suum* and *D. immitis* indicated as Ac, As and Di and troughs with serum of case 3 at 40 days.

in case 4 were obtained at next 72 days in both cases. The peak titers were maintained for subsequent 12 days and then began to decline slowly. More violent changes in the antibody titers were observed in case 5 in which antibody titer as high as ×512 at 33 days reached to ×2,048 within following 11 days and then fell to ×1,024 at 73 days. In case 6, the titer of ×8192 was already detected at 20 days after the admission to hospital. The antibody response in early stage of infection could be examined in case 7. In this case, low titer of ×16 at 10 days rose steadily to a peak titer of ×2,048 at 39 days, maintained subsequent 7 days and then fell to ×1,024 at 53 and 67 days. The antibodies were also detected by the IHA test in the cerebrospinal fluids of cases 5, 6 and 7, but its antibody titers were considerably lower than those of the sera. In addition to the low titer level, the appearance of antibodies in the cerebrospinal fluid of case 7 was later than that in the serum (Table 3).

In order to study the effect of the 2-ME treatment on the antibody activity, the sera and cerebrospinal fluids were treated with 0.1 M 2-ME and antibody levels were compared before and after the treatment. As seen in Table 3, the 2-ME treatment was somewhat effective in the specimens collected within 40 days. In the specimens of case 5 collected at 33 days, the titers fell from

Table 3 IHA titers of seven suspected cases of angiostrongyliasis

| Case No. | Time elapsed after the suspected infection | IHA titers | |
|---|---|---|---|
| | | Before 2-ME treatment | After 2-ME treatment |
| 1 | 4 years & 5 months | <16 | ** |
| 2 | 11 months | 64 | 64 |
| 3 | 40 days | 4,096 | 2,048 |
| | 72 days | 8,192 | 4,096 |
| | 84 days | 8,192 | 8,192 |
| | 128 days | 4,096 | 4,096 |
| | 180 days | 1,024 | 1,024 |
| 4 | 40 days | 256 | 128 |
| | 72 days | 512 | 512 |
| | 84 days | 512 | 512 |
| | 128 days | 256 | 128 |
| | 180 days | 256 | 256 |
| 5 | 33 days | 512(256) | 256(64) |
| | 44 days | 2,048(512) | 1,024(256) |
| | 73 days | 1,024(256) | 1,024(256) |
| 6 | 20 days* | 8,192(1024) | 2,048(256) |
| 7 | 10 days | 16 | <16 |
| | 17 days | 64(<16) | <16(**) |
| | 24 days | 128(32) | 32(**) |
| | 32 days | 512 | 256 |
| | 39 days | 2,048 | 1,024 |
| | 46 days | 2,048 | 2,048 |
| | 53 days | 1,024 | 1,024 |
| | 67 days | 1,024 | 1,024 |

* Time elapsed after the admission to hospital, ** not examined
( ) Titers in the cerebrospinal fluids

Table 4 Results of the skin test of five suspected cases of angiostrongyliasis

| Case No. | Time elapsed after the suspected infection | Skin reactions Wheal / Erythema (mm) |
|---|---|---|
| 1 | 4 years & 5 months | 12×11/30×25 |
| 2 | 11 months | 10× 8/30×25 |
| 3 | 40 days | 15×11/20×20 |
| 4 | 40 days | 15×11/40×25 |
| 5 | 33 days | 10× 9/31×29 |

×512 to ×256 in the serum and from ×256 to ×64 in the cerebrospinal fluid. The 2-ME treatment also decreased antibdy activities of case 6 from ×8,192 to ×2048 in the serum and from ×1,024 to ×256 in the cerebrospinal fluid. In case 7, the antibody titers of low levels in the sera collected within 17 days were completely destroyed by the treatment. Contradictory, the 2-ME treatment did not so much effect on the antibody activities in the specimens collected at 72 days or more.

3) Examinations by the skin test :

In Table 4, the results of the intradermal skin test with the purified antigen on 5 patients are shown. Strong positive reactions over 9 mm in mean diameter of wheal and 20 mm in erythema were observed on these 5 patients even on a patient who elapsed so long as 4 years and 5 months and whose serum showed positive reacton neither in the Ouchterlony method nor in the IHA test.

## Discussion

After the report of Rosen *et al.* (1967), *Angiostrongylus cantonensis*, a Metastrongylid lungworm of rodents, has received much attention as the causative agent of human eosinophilic meningoencephalitis in the Pacific islands and Southeast Asia. In Japan, three cases of eosinophilic meningoencephalitis probably due to *A. cantonensis* were first reported in 1970 (Simpson *et al.*, 1970), suc-

cessively nine similar cases occurred untill the present time in Okinawa (Ashimine *et al.*, 1970; Yonamine and Ashimine, 1972; Nakamoto *et al.*, 1974; Kinjo *et al.*, 1975; Ashimine, 1976; Nakamoto, 1976). These twelve cases including unreported three cases were summarized in a review by Otsuru (1977). On the other hand, a number of reports have been published concerning the distribution and incidence of the parasite in its definitive and intermediate hosts in Japan (Nishimura, 1966; Ohbayashi and Orihara, 1968; Hori *et al.*, 1969; Hori and Kusui, 1972; Hori *et al.*, 1973; Hori *et al.*, 1974; Asato and Kishimoto, 1976; Sano *et al.*, 1977).

In endemic areas, it has been considered that the diagnosis of the disease is, in general, not so difficult because of the characteristic clinical syndrome such as appearance of meningitic signs, eosinophilic pleocytosis in the cerebrospinal fluid and so on. These symptoms, however, do not always occur uniformly and are of various grades. Therefore, the disease may be mistaken for a variety of other disease affecting the central nervous system even in the endemic areas. For example, in our survey conducted in Taiwan, suspected Japanese encephalitis cases were examined for angiostrongyliasis by the IHA test and a high positive rate was obtained among the negative cases in the hemagglutination inhibition test for Japanese encephalitis (Suzuki *et al.*, 1973; Otsuru *et al.*, 1977).

Introduction of some suitable immunodiagnosis for angiostrongyliasis has been desired, but relatively little information is available on it. In previous papers, the authors reported the purification of specific antigen for immunodiagnosis by using the immunoadsorbent (Sato *et al.*, 1974; Sato, 1975) and it was successfully used as a skin test antigen in an epidemiological survey conducted in Taiwan and Okinawa (Chen *et al.*, 1974; Otsuru *et al.*, 1977) and as an IHA test antigen in an experimental study of infected animals (Sato, 1975). Fortunately, seven suspected cases of angiostrongyliasis occurred in Okinawa were immunologically examined with these purified antigens.

The sera of five out of these seven cases, examined within 180 days after the suspected infection, showed positive reactions in the gel-diffusion test. When these positive cases were examined immunoelectrophoretically, four cases developed 5 or 6 precipitin arcs in their maximum numbers showing similar patterns to those of infected rats. Another positive case, however, developed only 3 arcs. This difference may be related to the dose of infected larvae. Even in the same case, the number of immunoelectrophoretic precipitin arcs were different as the course of time after infection. In two cases examined throughout 180 days, their maximum numbers of precipitin arcs were already obtained at the first 40 days, and it decreased to only one at 180 days. On the other hand, in a case periodically examined during 10 to 67 days, the number of precipitin arcs increased from one at 17 days to five at 46 days. Although three antigenic components specific for *A. cantonensis* have been identified by immunoelectrophoresis (Tsuji, 1975), we could not determine whether those specific bands were included in the precipitin arcs obtained with the patient sera in the present study. Many cross-reactive components have been observed among the whole worm extracts of *A. cantonensis*, *A. lumb. suum*, *T. canis* and *D. immitis* (Borthemy *et al.*, 1972; Tsuji, 1975), and in our examinations, the sera of patients also cross-reacted against whole worm extracts of *A. lumb. suum*, *D. immitis* and/or *E. multilocularis*, though the cross-reactions were generally weak and the numbers of immunoelectrophoretic precipitin arcs were less than that with *A. cantonensis*. So it should be emphasized that the Ouchterlony method may be not sufficient itself, and further demonstration of immunoelectrophoretic pattern similar to that of infected rat may be necessary for the diagnosis. However, it seems that the appearance of such immunoelectrophoretic pattern in human infection needs one month or more

after the infection. The identification of precipitin bands specific for *A. cantonensis* among the precipitin arcs developed by patient serum may be also very important for the diagnosis, but it is the subject for a future study.

The IHA test is a sensitive and advantageous test for detection and estimation of antibodies, and it was successfully used for detection of serum antibodies in rats infected with *A. cantonensis* (Kamiya and Tanaka, 1969; Sato, 1975). By this method, the sera of 6 cases examined within 11 months showed positive antibody response. Among these positive cases, the serum of a case elapsed 11 months showed considerably low titer of ×64, however, high titer levels over ×256 were detected in other 5 cases and persisted over 6 months in 2 out of these cases. The antibody response in early stage of infection could be examined about a case in which low titer of ×16 at 10 days rose rapidly to a peak titer of ×2,048 within following 29 days. From the results, it was considered that the antibodies with the IHA test may rise rapidly within 1 month and began to diminish during 6 to 12 months after the infection. Therefore, it may be very important for the diagnosis to demonstrate the violent change of antibody titer within 1 month at least by means of two-point examinations at a certain interval. Antibodies were also detected in the cerebrospinal fluids by the test, but its appearance was later and its levels were considerably lower than those of the sera. In an experimental infections to monkeys, it has been reported that the antibodies with IHA test were first provoked in the cerebrospinal fluids and its titers were generally higher than those of sera (Chen *et al.*, 1973). The results obtained in our human examinations were different from this results, and the reasons why this differences occurred are obscure.

The 2-ME sensitivity of the sera and cerebrospinal fluids were tested in an attempt to distinguish between 2-ME sensitive (IgM) and 2-ME resistant (IgG) antibodies. The antibody activities were somewhat destroyed by the 2-ME treatment in the specimens collected within 40 days, however, the treatment did not effect on antibody activities in the specimens collected at 72 days or more. From the results, it was considered that the 2-ME sensitive IgM antibodies in the early stage of infection were low levels and it may be tranferred to the 2-ME resistant IgG antibodies within 2 months in the disease.

There are several reports concerning the skin test for angiostrongyliasis in man using adult worm extract (Alicata and Brown, 1962; Kagan and Zaiman, 1964). However, they were unable to obtain conclusive result because of many cross-reactions with other helminthic antigens and non-specific reactions due to the complex nature of the antigens used. The majority of investigators now agree that the test should by of limited value to exclude the doubt of the disease only in the case showing negative reaction. In the present study, five cases examined by the skin test showed strong positive reactions even on the case elapsed so long as 4 years and 5 months after the suspected infection and did not show any positive reaction in the gel-diffusion test and the IHA test with its serum. On the other hand, Suzuki *et al.* (1974) carried out the test with the same antigen on 21 cases of eosinophilic meningoencephalitis occurred in Taiwan, and indicated that the antibody responsible for the skin test came to be detected about 10 to 30 days after the onset of the disease. In addion to this negative reaction within 10 days or more after the onset of disease, remaining of positive reaction for a long time after disease indicates more difficulty in the practical use of the test for the diagnosis of each case, and the test may be still of value as a screening test in epidemiological survey.

As discussed above, the immunologically characteristic findings in the disease seemed to occur within about 1 month after the infection, and it was considered that the change of antibody levels with the IHA test in early stage of infection and the demonstra-

tion of immunoelectrophoretic pattern similar to that of infected rat or the identification of precipitin arcs specific for *A. cantonensis* are reliable for the diagnosis of this disease.

## Summary

For the immunological diagnosis of angiostrongyliasis, the immunological examinations by the gel-diffusion techniques, the indirect hemagglutination test and the skin test were carried out on seven suspected cases of angiostrongyliasis occurred in Okinawa Prefecture.

Out of seven cases, four cases examined within 6 months after the suspected infections showed positive reactions in all of these tests except the skin test which could not apply to 2 cases. However, one of other 2 cases examined at 11 months did not show positive reaction in the gel-diffusion test. Another case elapsed 4 years and 5 months showed positive reaction only in the skin test.

In immunoelectrophoretic analysis of 5 cases, immunoelectrophoretic patterns of 5 or 6 precipitin arcs were similar to those of infected rats in four cases, but another case developed only 3 precipitin arcs. The numbers of immunoelectrophoretic precipitin arcs varied as the laps of time after infection in 3 cases periodically examined, and their maximum numbers of precipitin arcs occurred at about 1 month or more. Cross-reactions with the antigens of other parasites, such as *A. lumb. suum*, *D. immitis* and *E. multilocularis*, were often observed.

In the indirect hemagglutination test, titers of high level near to the baseline titer to a plateau were detected within 1 month or more in 5 cases and it were persisted over 6 months in 2 cases. The serum of a case elapsed 11 months showed considerably low titer. From these results, the antibodies may rise rapidly within 1 month and it began to diminish during 6 to 12 months after the infection.

The antibodies were also detected in the cerebrospinal fluids of 3 cases with the gel-diffusion test and the indirect hemagglutination test, however, its appearance was later and its levels were lower than those of the sera.

Positive skin reactions were observed on all of examined five patients even on a patient who elapsed so long as 4 years and 5 months and whose serum showed positive reaction neither in the gel-diffusion test nor in the indirect hemagglutination test.

From the results obtained, it is considered that the immunologically characteristic and reliable findings for the immunodiagnosis may occur within 1 month after infection and that the demonstration of immunoelectrophoretic pattern similar to that of the infected rat, as well as the identification of specific band for *A. cantonensis*, and the violent change of antibody titers in early stage of infection may be very important for the immunodiagnosis.

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# 沖縄における広東住血線虫症疑いの好酸球性脳脊髄膜炎7症例の免疫学的検討

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広東住血線虫症の免疫学的診断を目的として，沖縄県で発生した本症疑いの好酸球性脳脊髄膜炎7症例を免疫学的に検討した．

これら7例のうち，感染をうけたと推定される日から6カ日以内に検査した5例は，ゲル内沈降反応，間接赤血球凝集反応において著明な陽性反応を示したほか，検査できなかつた2例を除く3例では皮内反応においても陽性反応が示された．他方，11カ月を経過した1例では，間接赤血球凝集反応と皮内反応は陽性であつたが，ゲル内沈降反応は陰性であった．また，4年5カ月を経過した他の1例では皮内反応のみが陽性であつた．

免疫電気泳動法による検査では，4例が5～6本の沈降線を形成し，そのパターンは感染ラツトのそれに類似していたが，1例では3本の沈降線を認めたのみであつた．また，同一症例において沈降線数は感染後の日数経過とともに増減がみられ，最高の沈降線数は感染後40日前後に認められた．

間接赤血球凝集反応では，5例において感染後1カ月内外で平衡状態に近い高抗体価が検出され，このうち2例では6カ月にわたつて維持された．他方，11カ月経過した1例での血清抗体価は著しく低く，これらの結果より，著しい抗体価の上昇が1カ月以内に認められ，6～12カ月の間に消退すると考えられた．

本線虫抗原に対する抗体は，3例の髄液中にも認められたが，その出現と抗体価は血清中のそれに比べて遅く，低い値であつた．

皮内反応による陽性反応は検査した5例すべてに認められ，この中には4年5カ月を経過していて，その血清がゲル内沈降反応，間接赤血球凝集反応のいずれにおいても陽性反応を示さない症例も含まれている．

以上の結果より，本症の免疫学的診断は，有効な免疫学的所見が感染後1カ月前後に出現すると推定されるので，この時期に感染ラツトと類似する免疫電気泳動パターンを証明することや，本線虫に特異的な沈降線を同定すること，さらに間接赤血球凝集反応によつて抗体価の大きな変動を捉えることなどが重要と考えられた．