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Serodiagnosis of Avian Schistosome Dermatitis by Indirect Fluorescent Antibody (IFA) Test Using Cercariae as Antigen

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Introduction

Schistosome dermatitis is known to be a maculopapular skin eruption resulted from the penetration of the skin by cercaria of certain species of nonhuman schistosomes. This schistosomes are world widely distributed and affect those who work or swim in fresh or sea water.

The etiology and epidemiology of schistosome dermatitis were first described by Cort (1928) in the United States. In Japan, avian schistosome dermatitis has been found among farmers working in paddy-fields as reviewed by Oda (1973). But recently, it is pointed out that some of agricultural chemicals or sometimes drainage from factories may produce dermatitis as well, since a lot of chemicals have been applied to paddy-field. The diagnosis of schistosome dermatitis is usually made by history of contact with cercariae-infested water and by cutaneous rash. However in most cases, it is rather difficult to find avian schistosome cercariae or the infected vector snails from the paddy-fields. It is therefore hard to distinguish this from other dermatitis resulted from chemicals by clinical findings alone.

Sadum *et al.* (1960, 1962) used the fluorescent antibody technique for the serological diagnosis of schistosomiasis mansoni, and found that the cuticle of the cercariae were consistently and specifically reactive with the positive serum. Therefore, it seems to be of interest to try an application of the technique for the diagnosis of avian schistosome dermatitis.

The aims of the present study were; (a) to determine the applicability of the indirect fluorescent antibody technique for the diagnosis of schistosome dermatitis in experimental condition; and (b) to evaluate technique as a screening test in epidemiologic studies of schistosome dermatitis.

Materials and Methods

Source of sera

A) Experimental infections

Two white rabbits, weighing 2.6 and 3.1 kg were used. They were each fixed on the backs and the shaved inguinal regions were exposed to the cercariae of *Trichobilharzia brevis*, three times in a week in total dose of 4,800 cercariae. They were bled just prior to infection and at weekly intervals for 11 weeks after infection.

Experimental infections with *T. brevis*, cercariae were also carried out on four vol-

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unteers in our laboratory. The cercariae were applied to the skin of the forearm by the "glass cylinder" method (Oliver, 1949). Two persons out of four were exposed to the cercariae three times in a week in total dose of 1,135 cercariae each and bled once a week for 21 weeks. Remaining two volunteers were exposed twice at a dose of 100 cercariae each, at an interval of 5 weeks, and bled once a week for 16 weeks after the primary exposure.

B) Sera from various groups of persons

Group 1: 10 persons with a paddy-field dermatitis in Gifu Prefecture. They acquired the dermatitis in June, 1976 while working in paddy-fields. Kobayashi *et al.* (1976) investigated those cases and suggested from the epidemiological and clinical findings that the invasion of *Trichobilharzia* cercariae was the cause. The sera were collected about a week after the symptom began to manifest.

Group 2: 13 persons with a paddy-field dermatitis of unknown etiology in Ishikawa Prefecture. The symptoms onset during the period from May to July, 1977. The sera obtained 1 or 3 weeks after the symptom onset.

Group 3: 11 persons with other dermatitis who had been diagnosed as atopic dermatitis or urticaria by the dermatologist at Hiroo Hospital, Tokyo.

Group 4: 40 health blood deners who live in the city of Tokyo.

Antigen

The cercariae of *T. brevis* were used as antigen in the fluorescent antibody test. The strain of *T. brevis* used in this study was originally isolated and identified by Suzuki *et al.* (1973, 1977) and has been maintained in our laboratory for 10 years. The maintenance has been carried on through domestic ducks as the definitive host and the *Lymnaea* snails (*Austropeplea ollula*) as the intermediate host. The cercariae used were those shed from the infected snails. The preparation of cercarial antigen was made by the following procedure.

A) The infected snails were placed in beakers containing dechlorinated tap water and exposed to an artificial light for a few hours.

B) After removing the snails the cercarial suspension was allowed to stand undisturbed until snail feces had settled to the bottom. The supernate containing the living cercariae was gently transferred to a siliconized glass tube and chilled in an ice cold bath for 30 min.

C) An appropriate quantitiy of fixative of 1% acetic acid in 95% ethylalcohol was added to the suspension, it was then centrifuged for 10 min. at 1,500 rev./min. at 4–5C and the supernate was discarded.

D) The sediment was re-suspended in the fixative to obtain a concentration of 10 to 15 cercariae per one drop with a micropipette.

E) A dorp of suspension was pipetted into a small circle specially prepared on a microscope slide.

F) The specimen was dried at room temperature to ensure the attachment of cercariae to the slide during the following manipulation in the test.

Fluorescein conjugated (FITC) antisera

A commercial preparation by Behringwerke Institute was used. The prelimnary tests indicated that a dilution of 1 : 150 of anti-rabbit-γ-globulin-serum and 1 : 100 of anti-human-IgG-serum were the most suitable concentrations.

Test procedure

Indirect fluorescent antibody staining

The diluted test serum was dropped on the cercarial antigen. After the preparation was kept in a moist chamber at 37C for 45 min. It was rinsed in PBS 3 times for 5 minutes each with gentle continuous vibration to wash non-reacted globulins. The preparation was dried in air and the diluted fluorescent antibody was applied at 37C for 45 min. After washing and drying by the same procedure as mentioned above it was mounted in buffered glycerol and covered with a cover slip.

Microscopic examination was conducted by the use of a fluorescence microscope (Olympus-FLM, Tokyo) using UV exiting filter system. The reactions were graded into five

classes : − : no fluorescence, ± : faint fluorescence, + : moderate fluorescence, ++ : intense fluorescence, +++ : most intense fluorescence. The titer of the test serum was expressed by a reciprocal of the highest dilution which gave the reaction +.

Results

IFA tests with sera from experimentally infected rabbits and persons

A series of experiments was designed to determine the applicability of IFA test using *T. brevis* cercarial antigen. The homologous antisera obtained from the experimentally infected rabbits and persons were first tested. The changes in the titer that occurred during the course of infection were shown in Figures 1, 2 and 3.

As seen in Figure 1, the titer in both of two rabbits increased rapidly and reached a

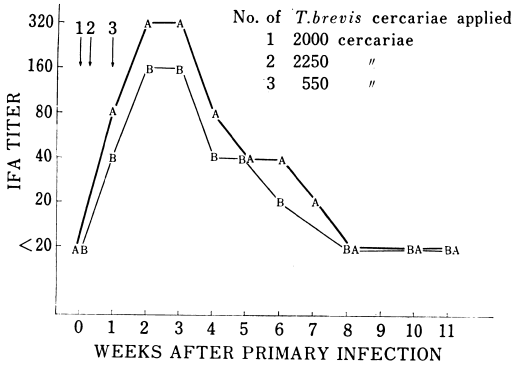


Fig. 1 IFA titers of sera from the experimentally infected rabbits.

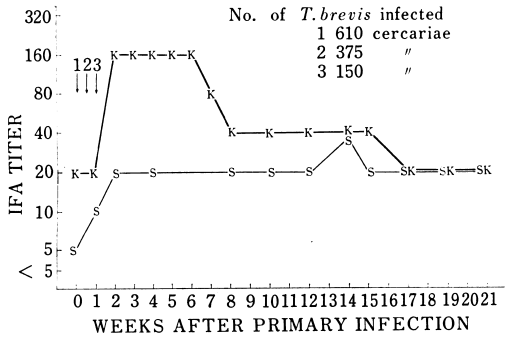


Fig. 2 IFA titers of human sera taken from the volunteers experimentally infected (I).

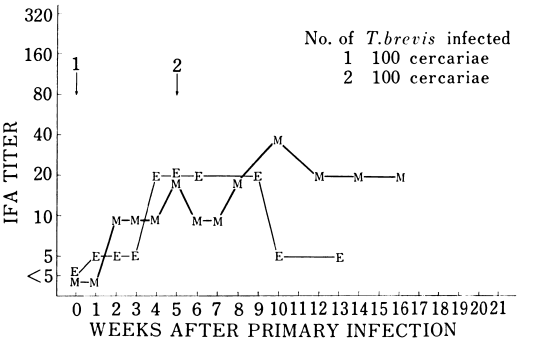


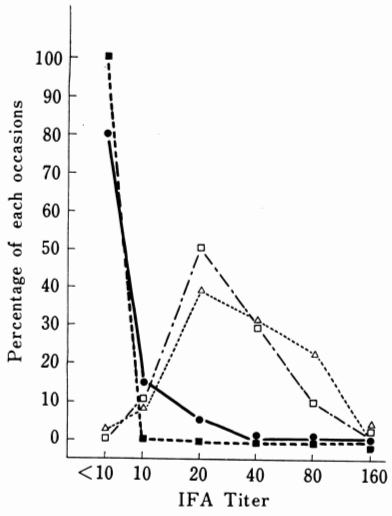
Fig. 3 IFA titers of human sera taken from the volunteers experimentally infected (II).

peak at 2–3 weeks after the primary exposure. It was considered appropriate to put the criterion of positivity as a titer of 20 or greater. Then in these two rabbits the positive reactions were observed during the period of 1–7 weeks following the primary exposure.

The sera of the two persons who had received heavy exposures to cercariae three times in a week and developed a typical intense schistosome dermatitis became reactive 2 weeks after the primary exposure. The positive reaction was maintained up to 21st week when the observations were terminated. (Fig. 2) The maximum titers in 2 persons were 160 and 40, respectively.

The serologic responses of other two persons of light exposure also became positive 4 or 5 weeks after the primary exposure. (Fig. 3) In these cases the primary exposure to cercariae resulted in skin reactions but they were mild and inconspicuous. When the secondary exposure was applied 5 weeks later, the reactions to cercariae became progressively stronger and resulted in manifestations of a typical schistosome dermatitis. The titer of the sera apparently rose more slowly and the maximum level was lower comparing with those of heavy exposures.

In the positive reactions obtained with cercariae of *T. brevis* which were fixed with 1% acetic acid in 95% ethylalchohol the most intense specific fluorescence was observed in the cuticle of the cercariae. Occasionally a similar fluorescent mass was



IFA titer \ Sera	□ Gifu Pref.	△ Ishikawa Pref.	■ Other dermatitse	● Control
160	0	0	0	0
80	1(10%)	3(23.1%)	0	0
40	3(30%)	4(30.8%)	0	0
20	5(50%)	5(38.5%)	0	0(5%)
10	1(10%)	1(7.7%)	0	0(15%)
<10	0	0	11(100%)	32(80%)
Total	10	13	11	40

Fig. 4 Distributions of IFA titers in human cases of normal and cercarial dermatitis.

observed at the oral and/or at the tips of the tail. However, this occurred even with the normal sera especially when the undilted sera were used or when the preparation was not thoroughly washed prior to the observation. Therefore only the fluorescence on the cuticle was observed as a specifically positive reaction in the present study.

IFA tests with sera from various groups of persons

The test results of 4 groups of persons are summarized in Figure 4. With the sera from 10 patiants suspected to be of schistosome dermatitis caused by *Trichobilharzia* cercariae from Gifu Prefecture the titers ranged from 10 to 80 and 90% of them showed 20 or over. Of 13 sera from Ishikawa Prefecture with paddy-field dermatitis of unknown etiology 12 or 92% sera reacted at a dilution of 1 : 20 or above. The distribution pattern of titer was quite similar between Gifu cases and Ishikawa cases. None of the sera from persons with other kind of dermatitis reacted even at a dilution of 1 : 10. Among 40 control sera only 2 or 5% reacted at a dilution of 1 : 20. And no sera reacted positively at dilutions of 1 : 40 or higher.

Discussion

There have been some attempts made for developing immunological diagnosis techniques for schistosome dermatitis. Several researchers have studied on whether schistosome dermatitis caused by nonhuman or human schistosomes can produce cutaneous sensitivity to the antigen of human schistosomes, *S. mansoni* or *S. japonicum* (Cort, 1936; Augustine *et al.*, 1949; Hsü *et al.*, 1956; Moore *et al.*, 1968). However, they have not succeed completely in determination of nonhuman schistosome infections in man with the cross reaction of both human schistosome skintest antigen. It was reported that there were no positive results with the complement-fixation test obrained, and the bentonite-fiocculation and cholesterol-lecithin tests showed no consistent correlation with a history of schistosome dermatitis. In contrast, the fiuorescent antibody technique using cercariae of *S. mansoni* as antigen gave positive results in about 22% of the persons with a history of schistosome dermatitis.

In the present study, the sera of rabbits and persons experimentally infected with *T. brevis* were tested against the homologous cercariae of *T. brevis*. By the use of indirect fluorescent antibody technique the antibodies could be detected in the heavily infected rabbits during the period of 1–7 weeks following exposure to cercariae. In the cases of experimentally infected persons the ap-

pearance of detectable antibodies and their maximum levels seemed to correlate with the number of cercariae used for the infection. The antibody response appeared sooner and reached higher titers in the heavy infections than in the light ones. The results of this technique applied to various groups of human sera showed a high positive rate of more than 90% among the paddy-field dermatitis patients and a very low rate of false positive reactions; less than 5%, among the control groups. It was suggested that this procedure may be usuful in the serological diagnosis of avian schistosome dermatitis, although the cross reactions should be further investigated with the cases of human schistosomiasis and also with the cases of dermatitis due to other species of avian schistosomes.

Summary

A technique for the diagnosis of avian schistosome dermatitis was described. Cercariae of *Trichobilharzia brevis* fixed with 1% acetic acid in 95% ethylalchohol were used as antigen in the indirect fluorescent antibody test. The applicability of the test was demonstrated with the sera of 2 rabbits and 4 volunteers experimentally infected with *Trichobilharzia brevis* carcariae.

In the sera of heavily infected rabbits, positive reactions with specific fluorescence were observed during the period of 1–7 weeks following the primary exposure. The titer of the sera from lightly exposed volunteers rose more slowly and the maximum level was lower comparing with those of heavily exposed.

Sera from 4 groups of persons were examined by this technique. None of the sera from persons with other kind of dermatitis and from healthy donors reacted positively at a higher dilution than 1 : 20. With the sera from persons of paddy-field dermatitis from Gifu Pref. and from Ishikawa Pref. more than 90% of them reacted at a dilution of 1 : 20 or above.

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鳥類住血吸虫セルカリアを原因とする水田皮膚炎の間接螢光抗体法による診断

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鳥類住血吸虫セルカリアの皮膚侵入に起因する水田皮膚炎の診断は現在のところ，症状・発症部位・発症時期の調査と，中間宿主貝採集による病原セルカリアの証明とによつてなされている．この場合，水田作業中に起きうる皮膚炎としては，今日では本症の他に農薬その他未知の化学物質による刺激性またはアレルギー性皮膚炎が考えられるが，両者を確実に区別する診断法は確立されていない．

そこで，本症の免疫血清学的な診断法として，皮膚炎の原因となるセルカリアを抗原とした間接螢光抗体法を検討した．まず家兎2羽および有志4名につき *Trichobilharzia brevis* セルカリアを実験的に皮膚に侵入させ，その後経週的に採血を行なつて抗体の消長を追求した．抗原としては同種セルカリアを1％酢酸加95％エタノールで固定した後スライドグラスに付着させたものを用いた．その結果，家兎ではセルカリア感染後1～7週にわたつて血清稀釈20倍以上で陽性反応を維持し，ピークは感染後2～3週に現われ血清稀釈320倍で陽性を示した．また，実験感染を行なつたヒトの例では，軽度感染例は重度感染例に比較して抗体価の立ち上がりが遅く，ピーク時の抗体価も低い傾向を示した．

これらの実験成績に基づき，岐阜県および石川県から送付された水田皮膚炎患者の血清，他の原因によることが明らかな皮膚炎（アレルギー性皮膚炎，ジンマシン等）患者の血清，および正常人血清に本法を応用した．*Trichobilharzia* 属のセルカリアを原因とする皮膚炎と推定されている岐阜県の例では血清稀釈20倍以上で陽性反応を呈するものが90％であつた．また，原因不明の皮膚炎とされている石川県の例でも血清稀釈20倍以上のものが90％以上を占めた．一方，他の原因による皮膚炎患者の血清はすべて血清稀釈10倍以下であり，正常人血清では血清稀釈20倍で陽性反応を呈するものは5％にすぎなかつた．

以上のことから，原因セルカリアを抗原とする間接螢光抗体法は，血清稀釈20倍を限界とした場合，特異性，鋭敏性ともに優れ，本症診新には有用な方法と考えられた．