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Antibodies to Nurse Cell and Various Stages of *Trichinella spiralis* in Patient Sera

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Introduction

Many immunodiagnostic tests for trichinosis have been investigated but practically all these tests employed the infective muscle larvae of *Trichinella spiralis* as antigen (Kagan and Norman, 1970; Kagan, 1981). This is because of the ease in obtaining the infective muscle larvae in large quantity. Phillip *et al.* (1981) reported the presence of primary serum antibody response in rat serum to antigenic surface proteins of newborn and infective larvae in the muscle, and adults in the intestine.

We report herein the presence of antigens in the *Trichinella* infected muscle cells, which are known as nurse cells (Purkerson and Despommier, 1974; Despommier, 1976), and also in various stages of the parasite, to the antibodies in trichinosis patient sera by using indirect immunoperoxidase test (IPT). The IPT was done along with the indirect fluorescent antibody test (IFAT) and an attempt to observe a correlation between the antigenic site and periodic acid Schiff (PAS) positive area was also carried out.

Materials and Methods

The diaphragm and femoral muscles of a mouse infected with *T. spiralis* larvae eight months earlier, were fixed in Carnoy's fluid, Bouin's fluid or 10% formalin solution. This is to determine the most suitable type of fixative. The remaining carcass was digested in 0.5% HCl-pepsin solution to obtain the infective larvae, which were then inoculated orally to ddY mice at a dose of 400 larvae per mouse. The infected mice were killed on days 8, 10, 16, 24, 31 postinfection (PI) and, their intestine and muscle samples fixed in Carnoy's fluid. After dehydration, the samples were embedded in paraffin and sectioned at 5 μm. Adjoining sections were used for patient and control sera simultaneously.

Positive sera used in this study were obtained from 8 patients who had eaten *T. spiralis* infected bear meat about 1.5–2 months before in Sapporo, Japan (Ohbayashi and Yamaguchi, 1980). Negative control sera were obtained from 10 healthy persons.

For IPT, sections were deparaffinized and washed in phosphate buffered saline (PBS, pH 7.2). Serum samples, 0.05 ml of trichinosis patient and control sera, diluted 1:20 in PBS, were placed onto adjoining sections and incubated at 37°C for 30 min. The secitons were then washed in 5 changes of PBS followed by incubation with horseradish peroxidase conjugated goat anti-human IgG (H+L specific, Cappel Lab., U.S.A.), diluted 1:640 in PBS, at 37°C for 30 min. After washing in PBS,

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the sections were dipped into the substrate, diaminobenzidine tetrachloride-H_2O_2 (Wako Junyaku, Japan) for 4 min at room temperature. The sections were then washed in distilled water, dehydrated, mounted and examined under light microscope.

For IFAT, the procedure was practically the same as for IPT except that FITC conjugated goat anti-human IgG (H+L specific, Hyland, U.S.A.), diluted 1:40 in PBS, was used instead of horseradish peroxidase conjugated anti-human IgG. After the incubation, the sections were washed thoroughly and mounted in non-fluorescent buffered glycerine. The sections were then examined under an Olympus fluorescence microscope.

Sections adjoining to those used in IPT and IFAT were stained with Schiff reagent after oxidation with 0.5% periodic acid. Some of these sections were counterstained with haematoxylin.

Results

Among the three types of fixatives used, Carnoy's fluid was found to be the most suitable, giving a good contrast in positive reaction. Bouin's fluid and 10% formalin solution gave rise to strong non-specific background staining.

The results of the IPT, IFAT and PAS staining are summarized in Table 1.

Table 1 Staining results of various stages of *T. spiralis* and infected muscle cell

	IPT	IFAT	PAS
Infective muscle larvae	+++	+++	+++*
Adult worms	+++	+++	+++*
Newborn larvae	+++	+++	–
Nurse cells	+++	–	+
Capsule of larvae	–	–	+++

+++: Strongly positive, +: Slightly positive, –: negative, on an arbitrary scale.
IPT: Immunoperoxidase test
IFAT: Indirect fluorescent antibody test
PAS: Periodic acid Schiff staining
*: With exception to reproductive organs

In IPT, all stages of the parasite showed specific staining when patient sera were used (Figs. 1–3). Infected muscle cells, which are known as nurse cells (Purkerson and Despommier, 1974; Despommier, 1976), also showed specific staining on day 10 PI. On day 16 PI the nuclei and cytoplasm of the nurse cell were strongly stained (Fig. 4).

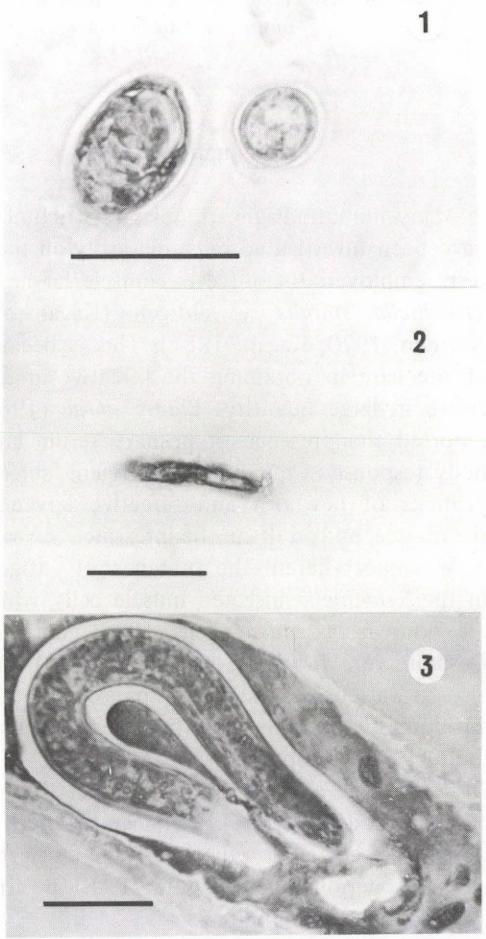

Fig. 1 Positive reaction by IPT on adult worm on day 8 PI. Bar = 0.05 mm

Fig. 2 Positive reaction by IPT on newborn larva on day 10 PI. Bar = 0.05 mm

Fig. 3 Positive reaction by IPT on infective muscle larva at 8 months PI. Bar = 0.05 mm

In IFAT, all stages of the parasite showed specific fluorescence when treated with patient sera (Figs. 5–6). Nurse cell and the capsule wall did not show any specific fluorescence.

Embryos in the uterus of the adult and day 8 PI newborn larvae were PAS negative. PAS positive areas began to appear sporadically on the muscular layer of the muscle larva on day 16 PI. On day 24 PI, PAS positive substances had accumulated as a continuous mass in the muscular layer of the larva. On day 31 PI and after, the whole body area, except the reproductive organ, was PAS positive. The capsule was strongly PAS positive but the nurse cell itself showed only slightly fine PAS positive granules.

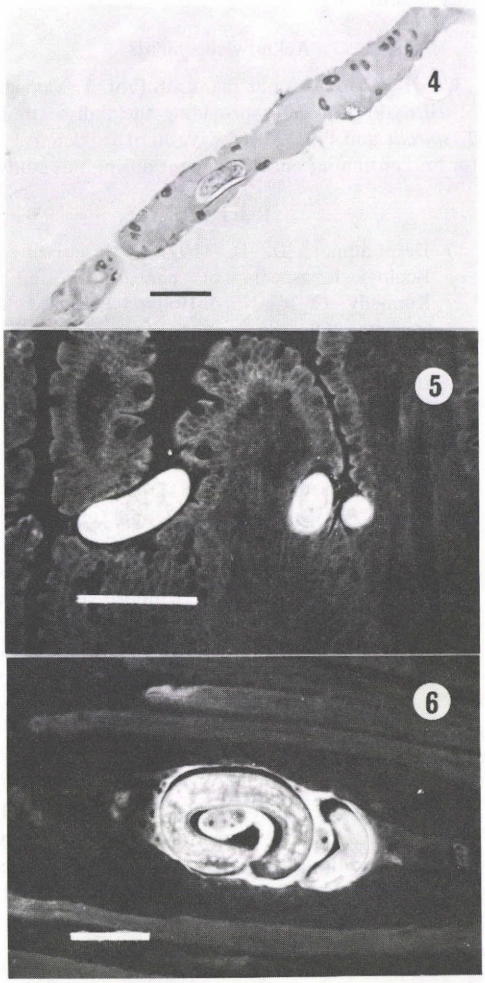

Fig. 4 Positive reaction by IPT on the infected muscle cell on day 16 PI. Bar = 0.05 mm

Fig. 5 Positive reaction by IFAT on adult worm on day 8 PI. Bar = 0.05 mm

Fig. 6 Positive reaction by IFAT on infective muscle larva at 8 months PI. Bar = 0.05 mm

Discussion

Our results showed that specific antibodies to all stages of *T. spiralis* developed in the patient sera. Zeromski and Jazbor (1969) failed to demonstrate the presence of larval antigens in the infected muscle cell by using the direct and indirect fluorescent antibody test. We confirmed their findings in our IFAT but parasite antigens were detected in the nurse cell by IPT. This may be due to the use of different conjugates and the different chemical reaction involved, thus resulting in a difference in sensitivity. The enzyme-linked immunosorbent assay (ELISA) was found to be more sensitive than the IFAT in human and porcine trichinosis (Ruitenberg *et al.*, 1974; Engvall and Ljungstrom, 1975; Knapen *et al.*, 1981, 1982). Some ELISA employed the same conjugate as that used in the present IPT. It is most interesting to note that the nurse cell showed specific staining by IPT.

Morphological alteration at microscopic and ultrastructural level, and the increase in enzyme activities such as succinic acid dehydrogenase, esterase and alkaline phosphatase, of the infected muscle cells had been reviewed by Stewart and Giannini (1982). However, we showed the possibility of the whole nurse cell being filled with larval antigens. This is clearly seen in the nurse cell on day 16 PI. It is suggested that the antigen found in the nurse cell may be of excretory/secretory type, which may be a by-product of the metabolism of the growing larva. This observation of a large quantity of larval antigens in the nurse cell on day 16 PI may help to explain for an active role of immunological mechanism in bringing about the symptoms such as myalgia

and skin eruptions during the early muscular phase of the disease.

Since the embryos in the uterus and the day 8 PI newborn larva were negative to PAS staining but showed a gradual increase in PAS positive area on their body on day 16 and after, we suggest that the muscle larva accumulated glycogen in its body only after the complete formation of the nurse cell. The newborn larvae were PAS negative but antigenic substance at this stage is not dependent upon PAS positive substances such as polysaccharide or glycoprotein.

Stage specific antibodies in the sera of rodents (Mackenzie *et al.*, 1978; Phillip *et al.*, 1981) and rabbit (Oliver-Gonzalez and Levine, 1962) have been reported. However, stage specific antibodies to *T. spiralis* in human sera have yet to be reported although specific IgG, IgM, IgA and IgE to infective muscle larvae antigen have been detected in the patient sera (Stumpf *et al.*, 1981). It was shown herein that the patient sera contained specific IgG against newborn and mature infective larvae in the muscle, and adults in the intestine. Unpublished data in our laboratory showed that newborn larvae were specifically stained by patient sera which had been adsorbed with infective muscle larvae antigen. The attempt to see if specific antibodies might develop against adult and newborn larva can lead to the use of these antigens in epidemiological monitoring of the chronology of trichinosis infection.

Retention of antigenicity in paraffin section indicated that it can replace cryo-section in future immunocytochemistry tests.

Summary

Specific antibodies to the somatic antigens of infective muscle larvae, intestinal adult worms and newborn larvae of *T. spiralis* were detected in trichinosis pateint sera by using the indirect immunoperoxidase test on paraffin sections. Carnoy's fluid was found to be a more suitable fixative for this test than either Bouin's fluid or 10% formalin. Detection of antigen in the nurse cell by this method led to the discussion of this cell playing a role in the growth of the larva in the muscle. Attempt to correlate PAS positive area with the antigenic site showed that in the newborn larva, the antigen does not correspond to PAS positive substance. Indirect fluorescent antibody test done along with the indirect immunoperoxidase test failed to detect the antigen in the nurse cell.

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患者血清中の各期旋毛虫および *nurse cell* に対する抗体の検出

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旋毛虫の感染筋肉幼虫，成虫および新生幼虫の抗原に対する患者血清中の抗体をパラフィン切片を応用した酵素抗体法によって検出した．虫体の固定法を比較するために，ブアン固定，カルノア固定および10%ホルマリン固定を行ったが，非特異的反応の少ないことでカルノア固定が最もすぐれていた．

新生幼虫は PAS 染色で陰性であるが酵素抗体法では陽性であった．間接螢光抗体法では幼虫が感染している筋肉細胞，すなわち“nurse cell”の細胞質に反応が認められなかったが酵素抗体法では nurse cell の細胞質に特異的反応が認められた．