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Immunohistochemical Localization of Antigenic Substances in *Trichinella spiralis* Muscle Larvae

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Abstract

We localized, in situ, the *Trichinella spiralis* antigen recognized by Wistar rat sera of 36-week post infection, employing immunoperoxidase and immunofluorescence techniques. The antigen was found in stichocyte granules, the banded structure, esophagus- and midgut-occupying substances. The nucleus, nucleolus, and muscle fibers were not antigenic. By the present method, consistent immunostaining results were not obtained as to the cuticle and glycogen aggregates within a wide variety of cells. The antigen often was in close proximity to PAS-positive substances.

Key words: *Trichinella spiralis*, antigen, PAS, immunocytochemistry

Introduction

Trichinella spiralis is known as one of parasites with the least host-specificity, and is noted for its ability to infect a wide range of vertebrate hosts (Despommier 1983). Soon after infection, *T. spiralis* presents the host with a strong antigenic stimulus thus evoking a powerful immune response which affords protection against reinfection (Despommier, 1977; 1981; 1983; Mota et al., 1969; Ottesen et al., 1975; Ljungström and Ruitenberg, 1976; Silberstein and Despommier, 1984; 1985; Wakelin and Denham, 1983). Attempts to identify and characterize the antigen(s) responsible for such protection-inducing activity have consisted in most cases of an immunochemical approach (Despommier 1981; Despommier and Lacetti 1981a, b; Herrera-Esparza et al., 1987). In addition to such biochemical information, topography of antigen localization is also critical to study both the target antigen of immune attack and the best antigen for immunodiagnosis. In this paper, taking advantage of recent progress in histochemistry, we will identify the tissue localizations and describe the histochemical staining profile of the antigen recognized by Wistar rats during the course of an experimental infection which, we hope, will contribute to a wide variety of studies relating immunity to *T. spiralis*.

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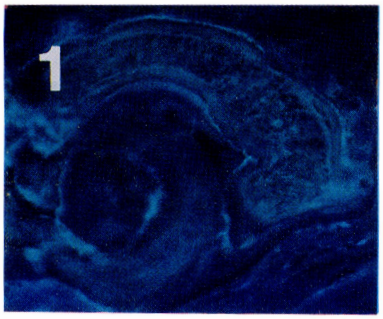
Materials and Methods

Immunoperoxidase staining procedure

Larvae of *T. spiralis* from host mouse muscle were isolated by pepsin-HCl digestion (Despommier, 1974), fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) for 30 minutes at 4°C, dehydrated via increasing concentrations of ethanol and embedded in Acrytron E (MITSUBISHI RAYON CO. LTD, Tokyo Japan). Semi-thin sections, 1-2μm, were made and incubated either with infected Wistar rat antisera (36-week post infection, diluted to 1:40) for 30 minutes at 4°C, or with normal rat sera for control purposes. Immunoperoxidase staining was completed with the VECTASTAIN ABC KIT system (Vector Laboratories, Inc., CA., USA) which is composed of biotinated second antibody and avidin conjugated peroxidase. Antigen-positive sites were identified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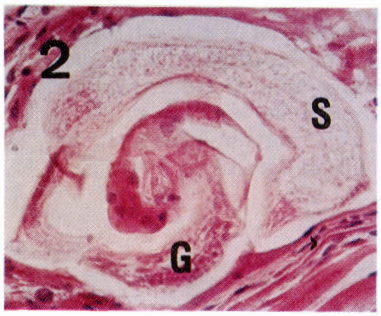
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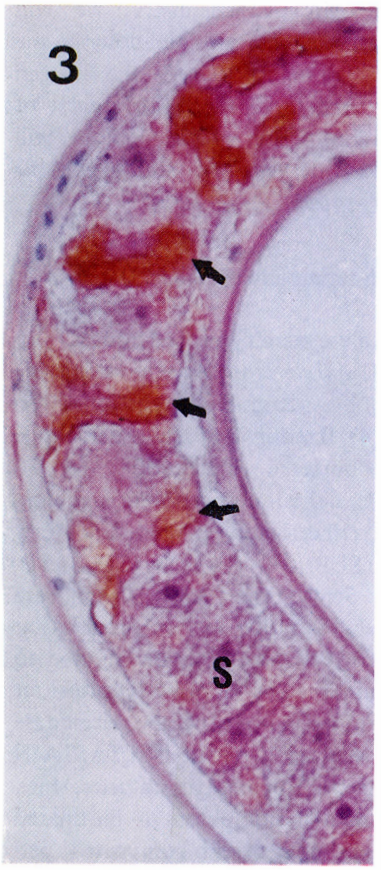
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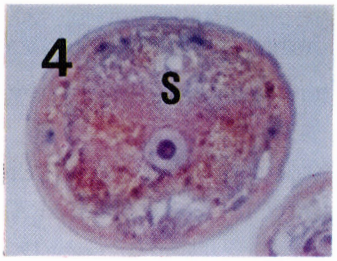
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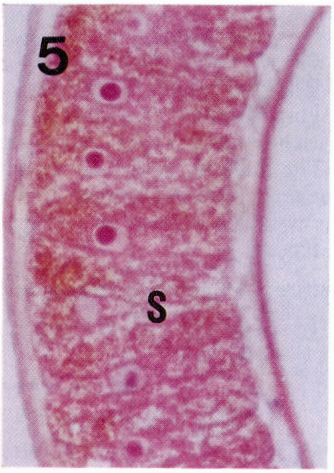
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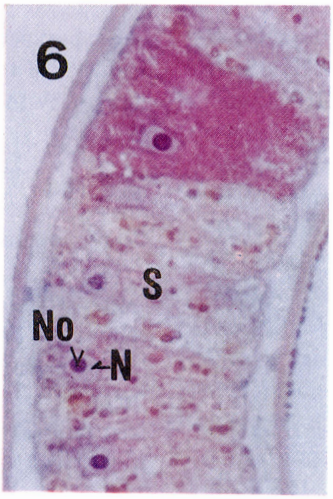
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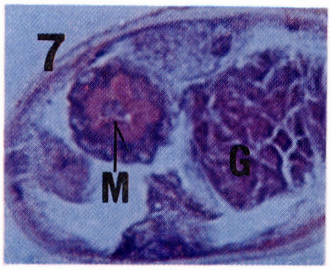
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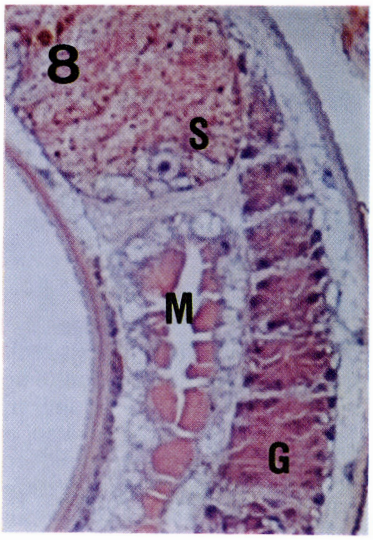
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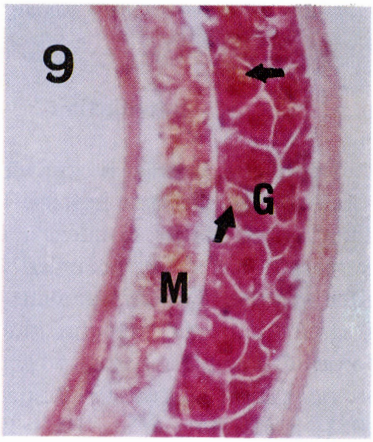
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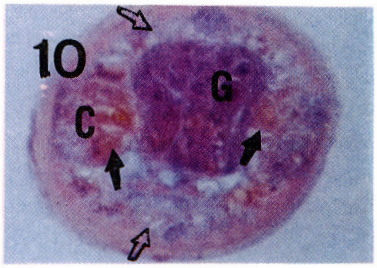
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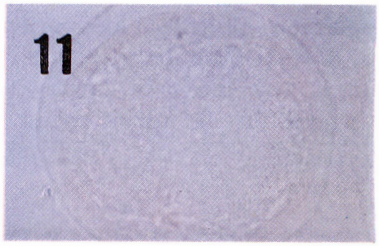
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by the end product of the peroxidase reaction, as indicated by brown deposits. Immunostained sections were counterstained with hematoxylin and eosin (H & E), and serial sections were stained with periodic acid schiff (PAS), according to established histochemical procedures.

Immunofluorescent staining procedure

The diaphragm of an infected mouse was fixed in 4% paraformaldehyde, cryo-sectioned, incubated with the primary antisera (1:20 dilution), and then treated with fluorescein-conjugated-second antibody (1:40 dilution, purchased from Miles Yeda Ltd.,Israel) for 30 minutes at 4°C. After taking photographs, the fluorescent-stained sections were fixed in 10% neutral buffered formalin and counterstained with H & E. Thus, a given encysted-larva was double stained, first with fluorescein and then with H & E.

Results

For orientation purposes the location of each organ is shown in Fig. 12.

The immunofluorescent antibody technique resulted in strong staining. By marking the sites of fluorescence (Fig. 1) with subsequent H & E (Fig. 2) on the same section, the identification of the immunofluorescent structures was possible. The outer surface of the worm stained in a linear fashion, whereas positive staining within the stichosome appeared diffuse and spotty.

Cells of bright appearance in the banded structure (B in Fig. 12) were peroxidase-positive (arrow in Fig. 3). The peroxidase-positive structures in the stichosome (ST in Fig. 12) were more or less granular in appearance (Figs. 3, 4, 5, 6 and 8). There were differences in staining intensity among stichocytes; eosinophilic stichocytes reacted less intensely (Fig. 6). The midgut-occupying substance (MOS) (M in Figs. 7 and 8) and the esophagus-occupying substance (EOS, data not shown) were both diffusely positive. The lumen of the esophagus was negative when it was devoid of EOS (data

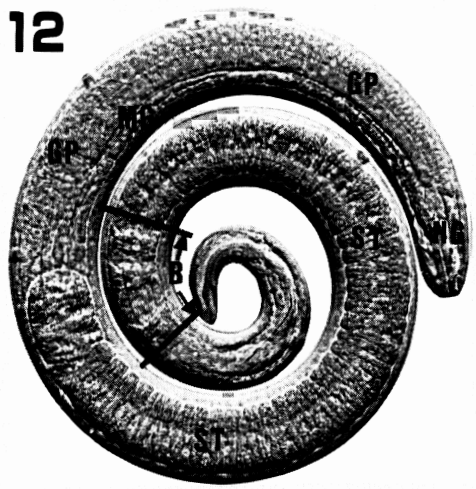

Fig. 12. A phase contrast micrograph showing the location of each organ including the stichosome (ST), the banded structure (B), the midgut (MG), the hindgut (HG) and the genital primordium (GP).

Fig. 1. An encysted larva stained by the immunofluorescent technique.
Fig. 2. The immunoflorescent-stained larva postfixed in formalin and counterstained with H & E. S: stichosome G: genital primordium
Fig. 3. The bright cells in the banded structure are strongly immunopositive. Peroxidase, H & E.
Fig. 4. A transverse section through the stichosome. Peroxidase, H & E.
Fig. 5. A longitudinal section through the upper stichosome. Peroxidase, H & E.
Fig. 6. A longitudinal section through the lower stichosome. Peroxidase, H & E. N: nucleus No: nucleolus.
Fig. 7. A transverse section through the midgut (M) and the genital primordium. Peroxidase, H & E.
Fig. 8. A longitudinal section through the lower stichosome, the midgut and the genital primordium. Peroxidase, H & E.
Fig. 9. A longitudinal section through the midgut and the genital primordium. Peroxidase, H & E.
Fig. 10. A transverse section through the genital primordium and the cords (C). The midgut is poorly seen because of its small size at the level of this section. Peroxidase, H & E.
Fig. 11. A control section incubated in normal rat serum. No counter staining.

no shown). Examination of PAS-stained serial sections provided direct and convincing evidence that both EOS and MOS are PAS-positive.

Small discrete areas in female genital primordial cells (GP in Fig. 12) were strongly immunostained (arrow in Fig. 9), while male genital primordial cells were negative (Fig. 8); a criteria for the differentiation of males from females in tissue sections has been described in our previous paper (Takahashi et al., 1987). The distribution pattern of the positively-stained areas in the female genital primordial cells resembled the distribution of PAS-positive substances as described by Takahashi et al. (1988b). A transverse section revealed that the lateral cords (comprising a part of the body wall) were strongly immunopositive in glycogen-rich areas (closed arrow in Fig. 10).

Inconsistent results were obtained with regard to the cuticle and glycogen aggregates in a variety of tissues. For instance, as shown in Fig. 10, glycogen aggregates in the cord were immunopositive but those in muscles of the body wall were immunonegative (open arrow in Fig. 10). Glycogen aggregates were PAS-positive, whereas the cuticle was PAS-negative (data not shown). As shown in Fig. 11, staining did not occur with control sera, confirming the specificity of our stainings. The nucleus, nucleolus (Figs. 5 and 6) and muscle fibers (Figs. 7 and 10) likewise were immunonegative and thus served as a good built-in control.

Discussion

In this study the tissue localization of an antigen(s) of *T. spiralis* muscle larva was demonstrated by means of immunoperoxidase and immunofluorescent staining at the light microscope level. Immunoperoxidase staining was so amplified with the aid of the avidin-biotin system that positively stained structures could be clearly identified on H & E stained sections. Immunofluorescent staining followed by H & E also enabled identification of antigenic structures, but with poor resolution.

Although no biological functions have yet been ascribed to the secretions of the stichosome (Despommier, 1977), its antigenicity had been recognized, the present study again confirmed the antigenicity of the stichosome. In the stichosome, positive staining was granular probably reflecting positive reactions on stichocyte granules and glycogen granules. Indeed, via immunoelectron microscopy, the areas of positive staining within the stichosome were seen to correlate exactly with the location of stichocyte granules and glycogen aggregates (Takahashi *et al.*, in press a; Uno *et al.*, 1988). There was some variability in the intensity of staining among stichocytes; the less-eosinophilic stichocytes were more antigenic. Since α-stichocytes are less eosinophilic (based on our preliminary observations comparing adjacent H & E and electron microscopic sections). The present observation supports previous reports that stichocyte α-granules are strongly antigenic (Despommier and Müller, 1976; Despommier and Laccetti, 1981a, b; Silberstein and Despommier, 1984; 1985; Takahashi *et al.*, in press a; Uno *et al.*, 1988). Stichocyte α-granules appear to contain PAS-positive substances (Niimura *et al.*, 1988; Takahashi *et al.*, 1988b).

Although the inner layers of the cuticle are antigenic (Takahashi and Araki, 1988; Takahashi *et al.*, 1988a; Uno *et al.*, 1988), our immunostaining results were not convincing, probably due to the cuticle's impervious nature (Takahashi and Araki, 1988). Early authors to localize cuticle antigen at the light microscopical level confronted the similar problem (Sadun *et al.*, 1962; Sadun 1963). Unlike other antigenic structures, the cuticle was PAS-negative.

The occurrence of EOS and MOS was reported by Takahashi *et al.* (1988c, d), and their antigenic properties were demonstrated by Uno *et al.* (1988) and Takahashi *et al.* (in press a, b). As yet, the origin of these substances is not known. Judging from their location, they likely are a part of the ES antigen which is recovered from nematode culture supernatants.

Jackson (1959) showed the presence of an antigen(s) in the gut by means of immuno-

fluorescence, and Silberstein and Despommier (1984) localized a 50/55 kilodalton antigen in the gut. It seems likely that MOS is at least partly responsible for the positive immunostaining observed by the earlier investigators.

Although glycogen granules were antigenic, as reported by Uno *et al.* (1988), they gave inconsistent results in immunostaining. This may be due to inconsistency in glycogen granule preservation; the chemical fixative used in this study, paraformaldehyde, is not thought to directly fix glycogen granules. Therefore, it may be that most of the glycogen granules were removed during the tissue preparation procedure.

The in-situ localization of antigen at the light microscope level was herein demonstrated. However, because of limitations in microscope resolution, the interpretation of the present observations would be difficult without first knowing the immunostaining results at the electron microscope level. Immunoelectron microscopy allows a much more detailed localization (Uno *et al.*, 1988; Takahashi *et al.*, 1988a) but needs highly sophisticated equipment and well-developed skills. The advantage of light microscope immunohistochemistry seems to reside in its less-tedious and quicker procedures in matching a variety of host antibodies with antigenic constituents of the worm, and also the combination of hydrophilic resin and advidin-biotin system offers the opportunity to elucidate a variety of histochemical profiles of antigenic constituents. For example, close association of antigen with PAS-positive substances was demonstrated, which provides compelling evidence that glycoprotein antigen may play a major role in immune response.

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