LIGHT AND ELECTRON MICROSCOPICAL STUDIES OF THE LOCATION OF STRONGYLOIDES STERCORALIS IN THE JEJUNUM OF THE IMMUNOSUPPRESSED DOG

D. I. Grove,* A. Warton,† L. L. Yu,† C. Northern* and J. M. Papadimitriou†

Departments of *Medicine and †Pathology, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, Western Australia, 6009

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Abstract—Grove D. I., Warton A., Yu L. L., Northern C. and Papadimitriou J. M. 1987. Light and electron microscopical studies of the location of Strongyloides stercoralis in the jejunum of the immuno-suppressed dog. International Journal for Parasitology 17: 1257-1265. This study was undertaken to define the precise anatomical location of S. stercoralis in the intestinal mucosa of the dog. In order to facilitate finding worms, hyperinfection was induced by immunosuppression of the host with prednisolone. Light microscopy showed adult worms in vacuoles in close relationship with the columnar epithelium although portions of worms were sometimes seen in the intestinal lumen. Electron microscopy demonstrated that adult worms were situated between the enterocytes. The enterocytes were compressed and distorted and appeared to form a tunnel through which the worm probably moved. Adult worms were never observed to penetrate the basement lamina and enter the lamina propria. Rhabditiform and filariform larvae were never seen in the mucosa. These data suggest that autoinfection does not occur by rhabditiform larvae being seeded directly into the tissues, but supports the hypothesis that they are deposited in the small bowel lumen, moult, then penetrate the mucosa of the lower gastrointestinal tract. Since adult worms dwell in the epithelial layer of the mucosa, they are susceptible to attack by cellular elements of the host's defences, although this was not observed in the present study because the host was immunosuppressed.

INDEX KEY WORDS: Strongyloides stercoralis; dog; immunosuppression; electron microscopy.

INTRODUCTION

Nine years after the discovery of Strongyloides stercoralis by Normand in 1876, Golgi and Monti reported that the adult worms were located in the crypts of Lieberkuhn in the small intestines (Golgi & Monti, 1885). Several years later, Sonsino reported finding eggs and larvae, not only in the depths of the crypts, but also in the mucosa (Sonsino, 1891). In 1900, Askanazy indicated that he had found Strongyloides infection during the course of a post-mortem examination of a patient who had died from metastatic cancer of the lung. He asked the rhetorical question:

“What relationship does A. intestinalis [Anguillula intestinalis = S. stercoralis] have to the intestinal wall?” (Askanazy, 1900)

Remarking that the muscularis mucosae usually checked the further penetration of the parasite, he answered his question thus:

“The A. intestinalis bores into the intestinal wall, primarily into the mucosa, and often into the epithelium.” (Askanazy, 1900)

Confusion as to the precise location of the parasite in the small intestine has led to a succession of reviewers merely stating that S. stercoralis invades the intestinal mucosa (Belding, 1942; Faust, Russell & Jung, 1970; Manson-Bahr & Apted, 1982; Markell, 1985). Similar uncertainty has surrounded the location of S. ratti in the intestinal mucosa of rodents. Some authors have considered that worms invaded and developed in the gut mucosa (Abadie, 1963; Wertheim & Lengy, 1965), whereas others were of the view that adult worms were located deep in the lumen of the crypts and did not penetrate the epithelium (Genta & Ward, 1980). We have shown recently by electron microscopical studies of mice that S. ratti adult worms are situated between the enterocytes and that neither they nor rhabditiform larvae penetrate deep into the basement lamina (Dawkins, Robertson, Papadimitriou & Grove, 1983).

Strongyloides stercoralis differs from S. ratti in that it has the capacity to autoinfect. The mechanisms of...
autoinfection are obscure and it is conceivable that the structural nature of the host–parasite interaction may play a part in the genesis of this phenomenon. We have therefore used electron microscopical techniques to examine the precise location of *S. stercoralis* in the small intestine of the dog. In order to facilitate the finding of worms, multiplication of the parasite was enhanced by immunosuppression of the host.

**MATERIALS AND METHODS**

*Strongyloides stercoralis* was derived originally from infected humans (Grove, 1980) and since that time has been passaged through dogs, as described previously (Grove & Northern, 1982). Furthermore, persistent and disseminated infections result in dogs immunosuppressed with prednisolone, as described earlier (Grove, Heenan & Northern, 1983).

A male, mongrel dog, 15 kg in weight, was obtained from a pound then was infected with 5000 infective larvae. Four weeks later, the administration of prednisolone 50 mg per day was begun. Larvae were excrated in the faeces over the next 9 months, with counts ranging between 50 and 300 larvae/g faeces. The immunosuppressive dose was then doubled, and 1 month later, the numbers of larvae excrated in the faeces has risen to 22,000/g. The dog was then prepared for examination. The method of *in vivo* fixation of the tissue was similar to that described by Thompson, Dunsmore & Hayton (1979). Briefly, the small intestine was brought to the exterior while the dog was anaesthetised with halothane. A segment of small bowel 10 cm in length and located 20 cm below the pyloric sphincter was ligated but the mesenteric blood supply was left intact. Paraformaldehyde–glutaraldehyde fixative (Graham & Karnovsky, 1966) was injected into the lumen of the ligated segment until it was distended fully. The dog was then killed, while still under anaesthesia, by intravenous injection of an overdose of pentobarbitone sodium.

The blood vessels were then ligated and the segment was excised. The removed small intestine was cut into rings several millimetres in width and immersed in 4% formaldehyde or in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2 at 4°C. Specimens fixed in formalin were embedded in araldite or in 2.5% glutaraldehyde in 0.1 M phosphate buffer. Specimens were then dehydrated in graded solutions of ethanol and propylene oxide and embedded in araldite. Sections 50 nm thick were cut on an LKB ultrotome (LKB, Sweden), stained with lead citrate, then examined in the lumen of the ligated segment until it was distended fully. The dog was then killed, while still under anaesthesia, by intravenous injection of an overdose of pentobarbitone sodium.

Histological examination of serial sections suggested that *S. stercoralis* wound its way through the mucosa. Adult worms appeared close proximity to the columnar epithelium. Occasionally, portions of adult worms were seen in the lumen of the intestine. Like *S. ratti*, *S. stercoralis* appears on the light microscopical level to occupy a niche very similar to that held by *Trichinella spiralis*. Gardiner (1976) concluded that the adult forms of the latter parasite, which were also found mostly at the bases of the villi and in the crypts, were embedded in the epithelial layer of the mucosa, while Desppommier, Sukhdeo & Meer-

**RESULTS**

Vast numbers of adult worms were present and were easily seen at both the light and electron microscopical levels. The adult worms were observed in close proximity to the epithelial cells. Histological sections disclosed worms deep in the crypts of Lieberkühn (Fig. 1). Many worms were located within vacuoles adjacent to the lumen and which appeared to be formed by a thin rim of distorted and distended epithelial cells (Fig. 2), although portions of some worms were noted free in the intestinal lumen. Serial sections disclosed that these vacuoles were really tunnels in which the worms lay.

Greater detail of the interface between worm and host tissue was provided by electron microscopical examination. Figures 3 and 4 show complete cross-sections of adult worms at two different levels along the length of the parasite. The worms lay in tunnels between a number of enterocytes which were compressed and distorted. There was no evidence of syncytial formation but occasionally the epithelial cells were necrotic (Figs. 5–6). The microvillous border of some of the enterocytes could be clearly seen (Figs. 3–6). Adult worms were never observed deep to the basement lamina. A sparse infiltrate of plasma cells and lymphocytes was noted in the vicinity of the worms (Figs. 6–7). Examination of the interface between the worms and the enterocytes revealed an electron-dense fibrillar granular deposit on the surface of the enterocytes forming the tunnels (Fig. 8). The deposit often had projections which coincided with grooves on the external cortical layer of the worm cuticle (Fig. 9). Furthermore, deposits of this material were also seen more distant from the parasite in the intercellular spaces between the enterocytes (Fig. 8). High power examination of the interface indicated that the integrity of the enterocyte plasma membrane adjacent to the worm cuticle was sometimes broken (Fig. 10). Eggs/larvae were never observed, either in the lamina propria or in the tunnels between the epithelial cells.

**DISCUSSION**

Knowledge of the anatomical location of *S. stercoralis* in the small bowel is essential for an understanding of the mechanisms of both immune expulsion of helminths from the gut or evasion of immune responses leading to persistence of worms in the gut. Furthermore, it may shed some light on the mechanism by which the unusual phenomenon of autoinfection occurs in this infection as compared with most other helminthiasis, including infections with other species of *Strongyloides* such as *S. ratti*. This study has clearly shown that *S. stercoralis* does penetrate the intestinal mucosa with female adult worms being located within the glandular epithelium, but we found no evidence of either adult worms or larvae in the tissues deep to the basement lamina.

Histological examination of serial sections suggested that *S. stercoralis* wound its way through the mucosa. Adult worms appeared to be in tunnels situated in close proximity to the columnar epithelium. Occasionally, portions of adult worms were seen in the lumen of the intestine. Like *S. ratti*, *S. stercoralis* appears on the light microscopical level to occupy a niche very similar to that held by *Trichinella spiralis*. Gardiner (1976) concluded that the adult forms of the latter parasite, which were also found mostly at the bases of the villi and in the crypts, were embedded in the epithelial layer of the mucosa, while Desppommier, Sukhdeo & Meer-
Intestinal location of *Strongyloides stercoralis*

**FIG. 1.** Low power light microscopical view of small intestine showing cross-sections of adult worms (w) in the crypts of Lieberkühn and at the bases of the villi. Scale bar = 100 μm.

**FIG. 2.** High power light microscopical view of cross-sections of adult worms in the crypts of Lieberkühn. Note the distended epithelial lining (e) and the vacuole (v) around the worm. Scale bar = 40 μm.

Vitch (1976) thought that adult *Trichinella spiralis* were located beneath the epithelium and above the lamina propria. Furthermore, both papers reported that larvae were released directly into the lamina propria by adult worms.

More recently, Wright (1979) examined the intestinal location of *T. spiralis* by electron microscopy. He believed that the adult worms were intracellular rather than extracellular in location and occupied both absorptive and goblet epithelial cells. Although our electron micrographs are very similar to those taken by Wright (1979) of *T. spiralis*, we believe that like *S. ratti*, *S. stercoralis* adult worms lie between distorted, compressed and displaced columnar epithelial cells, with several enterocytes surrounding each worm. Interdigitations were noted between the epithelial cells surrounding worms, thus confirming their separate identity. Moreover, it would seem unlikely that enterocytes could survive the continuous plasmalemmal disruption and direct continuity with the extracellular space that intracytoplasmic invasion would require. Nevertheless, the plasma membrane of enterocytes adjacent to a worm was sometimes disrupted, and occasionally cells became necrotic.
FIG. 3. Transmission electron micrograph of an adult worm (w) showing the surrounding vacuole (v) and the enterocytes (e) forming the tunnel. Note the microvilli (mv) of the enterocytes and the small bowel lumen (L). Scale bar = 6 μm.

FIG. 4. Transmission electron micrograph of adult worms (w) showing a goblet cell (g) in close proximity to the enterocytes (e). Scale bar = 6 μm.
Fig. 5. Transmission electron micrograph of an adult worm (w) showing necrotic cells (n) and amorphous material (a). Scale bar = 6 μm.

Fig. 6. Transmission electron micrograph of an adult worm showing the margin of a necrosed enterocyte (n) and a plasma cell (p) adjacent to it. w = worm. Scale bar = 6 μm.
Fig. 7. Transmission electron micrograph showing an adult worm surrounded by inflammatory cells. p = plasma cell, Ly = lymphocyte, w = worm. Scale bar = 5 μm.

Fig. 8. High power transmission electron micrograph showing an electron-dense deposit (d) on the inner margin of the vacuole and between the enterocytes (e), w = worm. Scale bar = 1.2 μm.
Fig. 9. High power transmission electron micrograph showing the electron-dense deposit (d) on the inner margin of the enterocytes (e). Note the projection (pr) opposite the annulations in the cuticle (c) of the worm. Scale bar = 0.7 μm.

Fig. 10. High power transmission electron micrograph showing disruption (arrow) of the cell membrane (cm) of the enterocyte (e) in close proximity to the cuticle (c) of the worm. Scale bar = 0.5 μm.
These changes may be a consequence of enzymes released by the worms. A fibrillar and granular electron-dense deposit was seen on the surface of the enterocytes opposed to the worms, and this material often infiltrated into the spaces between the enterocytes. The nature of this deposit is uncertain, but may be derived from helminth, host, or both. Possibilities include antigen–antibody complexes and the remnants of necrosed cells. Since the worms are located in the intestinal mucosa between epithelial cells, they are open to attack by inflammatory cells. Because of the profound immunosuppression of the host in this experiment, however, we noted only a sparse inflammatory infiltrate.

It seems probable that an invading worm is able to fracture junctional complexes between adjoining enterocytes and move actively between them, forming (in cross-section) an apparent vacuole around the worm. This vacuole could be either real or an artefact. Some evidence in favour of the latter is perhaps provided by Figure 9 which shows projections of the fibrillo-granular deposit on the surface of enterocytes opposite depressions in the external cortical layer of the worm cuticle, yet the two are no longer contiguous. On the other hand, observations with *S. ratti* have shown that the vacuole was not present at the cephalic end of the worm, but that the vacuole became obvious as sections were cut more caudally (Dawkins *et al.*, 1983). We did not observe such a phenomenon in this study, but that may simply represent a sampling error. It seems reasonable to assume that the tunnel represents a fluid-filled space bathing the worm, resulting from the movement of the parasite through the epithelium.

In contrast to *T. spiralis* in which newborn larvae are found in the lamina propria and deeper parts of the small intestinal wall, we never observed rhabditiform or filariform larval of *S. stercoralis* in this location. It seems unlikely that this is a sampling error as a large number of sections were examined and multiple adult worms were seen. Likewise, larvae were never observed in the tunnels and it seems probable that embryos are released directly into the small intestinal lumen either when adult worms wander through the lumen or when the vulvar region of the female worms is exposed to the small bowel lumen. Alternatively, rhabditiform larvae could be deposited in the tunnels then quickly move through them into the intestinal lumen. These findings suggest that it is unlikely that autoinfection occurs by rhabditiform larvae being deposited directly in the mucosa and migrating through the tissues. They provide indirect support for the hypothesis that rhabditiform larvae are released into the small intestinal contents, moult twice, then infective larvae penetrate the mucosa lower down in the intestinal tract.

In conclusion, it appears that *S. stercoralis* adult worms move through the epithelium creating tunnels lined by enterocytes. It seems probable that they exit into the lumen and re-enter the epithelial layer. Adult worms do not traverse the basement lamina and probably deposit eggs/larvae directly into the small intestine lumen. These observations are consistent with the view that autoinfection results from rhabditiform larvae moulting within the lumen of the gastrointestinal tract then filariform larvae penetrating the colonic mucosa.

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REFERENCES


Intestinal location of *Strongyloides stercoralis*


