TABLE 3. *The mean (S.E.) levels of expression (as proportions of the level of expression of the β-actin used as a standard), of interleukin-5 (IL-5), interferon-γ (IFN-γ), Charcot–Leyden-crystal protein (CLC), mast-cell tryptase β1 (McTr), IgE receptor type I (FcεRI), glucose transporter 1 (GLUT-1) and glucose transporter 5 (GLUT-5) in the duodenal mucosa, and some mean (S.E.) ratios between them*

	Non-eosinophilics			Eosinophilics		
	Total ($N=21$)	Uninfected ($N=16$)	Infected ($N=5$)*	Total ($N=10$)	Uninfected ($N=6$)	Infected ($N=4$)†
IL-5	0.21 (0.09)	0.14 (0.08)	0.45 (0.33)	0.36 (0.10)	0.33 (0.11)	0.39 (0.24)
IFN-γ	0.65 (0.11)	0.66 (0.14)	0.62 (0.17)	1.20 (0.22)‡	1.32 (0.28)‡	1.02 (0.42)
IL-5/IFN-γ	0.27 (0.09)	0.15 (0.06)	0.65 (0.29)§	0.41 (0.17)	0.39 (0.24)	0.45 (0.25)
CLC	0.69 (0.10)	0.66 (0.11)	0.78 (0.31)	0.74 (0.19)	0.92 (0.27)	0.48 (0.21)
FcεRI	0.52 (0.11)	0.42 (0.09)	0.82 (0.32)	0.58 (0.09)	0.56 (0.12)	0.60 (0.14)
McTr	2.16 (0.19)	1.98 (0.18)	2.76 (0.52)	2.79 (0.44)	3.41 (0.57)	1.87 (0.13)
FcεRI/McTr	0.21 (0.04)	0.20 (0.04)	0.26 (0.06)	0.23 (0.05)	0.16 (0.03)	0.33 (0.09)¶
GLUT-1	1.17 (0.15)	1.13 (0.20)	1.23 (0.17)	0.90 (0.23)	1.01 (0.32)	0.74 (0.32)
GLUT-5	1.17 (0.09)	1.12 (0.10)	1.32 (0.25)	1.25 (0.13)	1.41 (0.17)	1.00 (0.12)

*Two with *Strongyloides* and three with *Taenia*, on presentation or within previous 3 months.
†Three with *Taenia* and one with *Ascaris*, on presentation or within previous 3 months.
‡Significantly higher than the corresponding values for the non-eosinophilics ($P<0.05$).
§Significantly higher than the value for the uninfected non-eosinophilics ($P<0.05$).
¶Significantly higher than the value for the uninfected eosinophilics ($P<0.05$).

TABLE 4. *The results of the principal-components analysis*

Parameter	Value
Eigenvalue (and percentage of variation explained)	2.13966 (42.8)
UNROTATED FACTOR LOADINGS	
Eosinophils	−0.55202
Interleukin-5	−0.43733
Interferon-γ	−0.72827
IgE receptor type I (FcεRI)	−0.61319
Mast-cell tryptase β1 (McTr)	−0.85866
MEAN (S.E.) COMPONENT SCORES	
Others (uninfected patients without eosinophilia; $N=16$)	0.761 (0.283)
Helminth-infected* ($N=9$)	−0.327 (0.375)†
Eosinophilia (uninfected patients with eosinophilia; $N=6$)	−1.538 (0.733)‡

*With intestinal helminth infection on presentation or within previous 3 months.
†Significantly different to other component scores ($P<0.05$).
‡Significantly different to other component scores ($P<0.01$).

including Taiwan, Korea, Indonesia and the Philippines (Fan *et al.*, 1990; Eom and Rim, 2001). Compared with those on intestinal nematode infections such as ascariasis, hookworm disease and strongyloidiasis, there have been relatively few studies to determine whether intestinal tapeworms could induce Th2-type responses. In murine models, however, a predominantly Th2 response has been reported to occur during the lumenal phase of both *Hymenolepis nana* and *H. diminuta* infection (Conchedda *et al.*, 1997; Palmas *et al.*, 1997). Experimental infection of gerbils or hamsters with adult *Ta. solium* induced goblet-cell hyperplasia and mastocytosis, indicating that Th2-related responses occurred in the intestine (Avila *et al.*, 2002). Peripheral-blood eosinophilia was induced

by the experimental infection of human volunteers with *Ta. saginata* or *Ta. saginata asiatica* (Chao *et al.*, 1988; Tesfa-Yohannes, 1990). Thus, it seems likely that intestinal infection with *Taenia* shares similar immunological features with intestinal infection with other common helminths.

In the present study, the Th2 cytokines IL-13 and IL-5 were expressed in the duodenal mucosa of 25.8% and 64.5% of the patients, respectively, whereas the Th1 cytokine IFN-γ was expressed in all of the patients. The biopsied tissues examined in the present study had been preserved in an RNA-preservation fluid, in a refrigerator, for varying lengths of time (4–50 days), before total RNA was extracted. No correlation was observed, however, between the frequencies or intensities of IL-13, IL-5 or IFN-γ expression and the duration of the tissue preservation, indicating that the frequencies and intensities observed reflected the immunological status in the mucosa. Although IL-5 is an important factor in eosinophil differentiation, proliferation, survival and migration, healthy individuals appear not to express IL-5 in the duodenal mucosa (Wallaert *et al.*, 1995; Vandezande *et al.*, 1999), indicating that there are few, if any, Th2-cytokine-producing cells in the non-lymphoid tissues of healthy individuals. In the present study, IL-5 expression in the duodenal mucosa was associated with peripheral-blood eosinophilia but CLC expression did not show a clear association with either IL-5 expression or peripheral-blood eosinophilia. Thus, locally produced, chemotactic or chemoattractant factors might play more important roles than IL-5 in the mucosal infiltration of eosinophils.

Patients with peripheral-blood eosinophilia showed significantly higher levels of IFN-γ expression in the duodenum than patients with lower numbers of eosinophils, indicating that Th1 and Th2 responses might occur concomitantly in the duodenal mucosa of these patients. Alternatively, since IFN-γ is produced not only by $CD4^+$ cells but also by $CD8^+$ and NK cells, the enhanced IFN-γ expression might reflect the abundance of cytotoxic cells in the mucosa. The IL-5/IFN-γ ratio is considered to reflect, at least in part, the Th1/Th2 balance. In the present study, the helminth-infected non-eosinophilic patients showed significantly higher IL-5/IFN-γ ratios than the uninfected non-eosinophilic patients, although the effect of helminth infection was not clear in patients with peripheral eosinophilia. It seems that, in the helminth-infected patients with peripheral eosinophilia, a more mixed Th1/Th2 status had developed rather than a shift to a simple, Th2-dominant status. Interestingly, however, the FcεRI/McTr ratios in the eosinophilic patients were significantly higher in the helminth-infected than in the uninfected. Since nematode infection or exposure of mast cells to a large amount of IgE antibodies are reported to result in striking up-regulation of FcεRI expression on mast cells (Chen and Enerback, 1996; Shaikh *et al.*, 1997; Yamaguchi *et al.*, 1997), the FcεRI/McTr ratio might reflect, at least in part, the functional status of the mast cells. Thus, in patients with eosinophilia, helminth infection seems to exert a unique Th2-related effect on the mucosal immunology. Principal-components analysis of variables including eosinophils, IL-5, IFN-γ, FcεRI and McTr could identify a component that reflects one aspect of Th2-type immune responses. This component could discriminate helminth-infected patients from the non-eosinophilic, uninfected patients, but not from the uninfected patients who had eosinophilia. All the uninfected patients with eosinophilia were clinically diagnosed as having non-ulcer dyspepsia. At least some of these patients might have had eosinophilic gastro-enteritis with an unknown allergic basis. On the other hand, it is also possible that some of the patients were infected with some kinds of parasites that migrate in the viscera, although none of them showed clinical signs of hepatitis, pneumonitis or cutaneous creeping disease.

Taken together, the present findings indicate that infections with common intestinal helminths, including taeniasis, are likely to

modify mucosal immunology so that it shifts to a Th2-predominant status. In rodents, expression of the apical-surface monosaccharide transporters SGLT-1 and GLUT-5 was recently found to be significantly down-regulated following infection with the intestinal nematode *N. brasiliensis* (Sekikawa *et al.*, 2003). In the present study, however, no decrease of monosaccharide-transporter expression was observed in patients with helminth infection. The impact of intestinal helminths on human nutrition remains to be clarified.

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VILLUS EPITHELIAL INJURY INDUCED BY INFECTION WITH THE NEMATODE *NIPPOSTRONGYLUS BRASILIENSIS* IS ASSOCIATED WITH UPREGULATION OF GRANZYME B

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ABSTRACT: Intestinal parasite infections induce thymus-dependent villus atrophy, but the effector mechanisms directly responsible for the development of villus atrophy are not thoroughly understood. In this study, we analyzed the expression of cytotoxic factors or ligands in athymic nude rnu/rnu rats and their littermate euthymic rnu/+ rats infected with the nematode *Nippostrongylus brasiliensis*. Morphometric analyses showed that partial villus atrophy developed 10 days after infection in euthymic but not in athymic rats, whereas crypt hyperplasia occurred in both types of animal. Reverse transcription–polymerase chain reaction analyses of the isolated jejunal epithelial fraction showed that the development of villus atrophy in euthymic rats was positively correlated with an increase of granzyme B transcript levels but not with Fas ligand or tumor necrosis factor-α expression. In addition, the number of granzyme B–immunoreactive cells was increased significantly in euthymic rat villus epithelium and the propria mucosa after infection. The $CD8^+$ cell number did not change significantly. Collectively, these findings showed that significant increases in the number of cells that express the cytotoxic factor granzyme B occur in the nematode-infected small intestine of immunocompetent hosts. The type of cells that express granzyme B and their role in the progression of enteropathy remain to be elucidated.

Intestinal villus atrophy with crypt hyperplasia has been reported in a number of intestinal diseases, such as celiac disease, giardiasis, intestinal helminth infection, autoimmune enteropathy, graft-versus-host disease, and allograft rejection of transplanted small intestine (MacDonald, 1992). Infection of rodents with the intestinal nematode *Nippostrongylus brasiliensis* induces partial villus atrophy and crypt hyperplasia in the jejunum together with various cytological alterations, such as decreases in the activities of the brush border enzymes sucrase and alkaline phosphatase, and causes deterioration of the epithelial permeability and barrier function (Symons, 1965; Nolla et al., 1985; Lunn et al., 1986; Perdue et al., 1989; Hyoh et al., 1999). The partial villus atrophy develops at the climax of infection, 7–10 days after infection, and disappears after T cell–dependent nematode clearance 12–14 days after infection. These histological alterations have been attributed largely, if not exclusively, to activation of mast cells in the intestinal mucosa accompanied by the release of mediators such as rat mast cell protease II (RMCP II) and the generation of leukotrienes (Woodbury et al., 1984; Perdue et al., 1989; D'Inca et al., 1990). On the other hand, anaphylactic release of RMCP II induces no significant change in mucosal histology (Scudamore et al., 1995), and mast cell–deficient *Ws/Ws* rats develop intense villus atrophy, as do wild-type +/+ rats, after nematode infection, suggesting that mast cell activation is not relevant to the progression of villus atrophy (Hyoh et al., 1999).

Ferguson and Jarrett (1975) reported that thymus-deprived (B) rats did not develop villus atrophy despite the fact that *N. brasiliensis* infection was prolonged. Similarly, in athymic rnu/rnu rats, villus atrophy does not develop until at least 10 days after infection, although it does develop 21 days after infection together with the occurrence of mucosal damage involving impaired barrier and digestive functions (D'Inca et al., 1992; McKay et al., 1995). These results indicate that the development of villus atrophy is thymus dependent, at least in the early period of infection, despite the fact that the innate immunity in athymic rats could induce villus injury and even villus atrophy over time. However, the mechanisms whereby T cells regulate enteropathy or the development of villus atrophy are not thoroughly understood. In this study, we analyzed the gene expression of granzyme B, Fas ligand (FasL), and tumor necrosis factor-α (TNF-α), which are expressed mainly in cytotoxic T lymphocytes (CTL), natural killer (NK) cells, or macrophages, as well as the tissue distribution of granzyme B–immunoreactive cells in athymic rats and their littermate euthymic rats infected with *N. brasiliensis*.

MATERIALS AND METHODS

Animals, nematode infection, and autopsy

Specific pathogen-free 8-wk-old female rnu/rnu (F344/N Jcl-rnu) rats and their littermate rnu/+ (F344/N Jcl-rnu/+) rats were purchased from Clea Japan, Inc. (Tokyo, Japan). The animals were inoculated subcutaneously with 1,000 *N. brasiliensis* L3 larvae as described previously (Hyoh et al., 1999). Uninfected animals and animals that had been infected 10 or 20 days previously (n = 4 in each group) were killed by inhalation of ether. The whole small intestine was removed and divided into the following segments (expressed as the distance from the pyloric ring): 0–18 cm, 18–22 cm, 22–26 cm, and 26 cm to the end of the ileum. The 0- to 18-cm and 26-cm to ileum end segments were used for the worm count, the 18- to 22-cm segment for paraffin-embedded tissue sections, and the 22- to 26-cm segment for separation of the epithelium, as described in the following sections.

Tissue preparation for histology

The segment of the jejunum 18–22 cm distal to the pyloric ring was opened longitudinally, fixed in 4% buffered formalin overnight, and embedded in paraffin in such a position that histological sections could be cut perpendicular to the luminar surface.

Morphometry

Measurements were carried out on hematoxylin and eosin–stained sections cut at 5 μm. Twenty villus–crypt units (VCU), which were cut as nearly perpendicularly as possible, were selected per animal, and the lengths of the villi and crypts were measured directly under a microscope using an ocular lens with a micrometer. The average length of 20 VCU was used as the representative length in a given animal, and the

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means and standard errors of 4 animals were calculated. The surface to volume ratio was measured as described by Dunnill and Whitehead (1972). In brief, sections were projected onto a white board, on which were drawn 15 lines of equal length, as described by Weibel (1963). The magnification was such that the length of each line corresponded to a length (L) of 1.4×10^{-2} cm. The section was projected at random onto the template lines, and the number of times the lines cut the mucosal surface (c) and the number of times the endpoints of the lines fell on mucosal tissue (h) were counted from 20 randomly selected fields per animal. The ratio c/Lh gives an index of the volume to surface ratio.

Worm counts

The numbers of worms in the intestinal segments of 0–18 cm and 26 cm to ileum end were determined by the saline incubation method. The numbers of worm profiles on the mucosal surface in the tissue sections 18–22 cm from the pyloric ring were also counted under a microscope.

Immunohistochemistry and cell count

Paraffin-embedded tissue sections were prepared as described above. The dewaxed sections were treated with 0.3% H_2O_2 for 20 min, immersed in 0.01 M sodium citrate buffer, pH 6.0, and then autoclaved at 121 C for 10 min for antigen retrieval according to the method described by Bankfalvi et al. (1994). The sections were incubated for 1 hr with anti–granzyme B or anti-CD3 goat IgG (Santa Cruz Biotechnology, Santa Cruz, California) or with anti-CD8 monoclonal antibody (OX-8) (Cymbus Biotechnology, Chandlers Ford, U.K.). For a negative control, species-matched normal IgG was used. After the sections were washed, they were incubated with horseradish peroxidase–conjugated anti-goat IgG (Nichirei Corp., Tokyo, Japan) or with anti-mouse IgG conjugated with peroxidase-labeled dextran polymer (Envision+, Dako, Carpinteria, California). The final reaction was carried out in 0.05 M Tris–HCl buffer (pH 7.6) containing 0.2 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (Dojin Lab., Kumamoto, Japan) and 0.005% hydrogen peroxide. The numbers of immunoreactive cells in 10 VCU were counted under a microscope, and the number per VCU was calculated.

Separation of intestinal epithelium

The separation of epithelium from the jejunum was carried out at 4 C in ethylenediaminetetraacetic acid (EDTA)–Hanks solution (Ca^{2+}-Mg^{2+}–free Hanks balanced salt solution supplemented with 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.3, and 1.0 mM EDTA), as described elsewhere (Hyoh et al., 2002) with a slight modification. In brief, a piece of jejunum 22–26 cm from the pyloric ring was removed, opened longitudinally, and cut into segments 1 cm in length. After a brief wash in phosphate-buffered saline, 4 pieces of tissue were put into a 15-ml tube containing 4 ml of 1.0 mM EDTA-Hanks solution, and debris attached to the mucosal surface was removed by vigorously shaking the tubes 15 times by hand. The tissues were then transferred into another tube containing 1.0 mM EDTA-Hanks solution. After 75 min on ice with occasional agitation of the tissues by inversion of the tubes, the epithelial cells were separated by vigorously shaking the tube by hand 60 times. After the tissue was discarded, the detached epithelia were collected by centrifugation at 600 *g* for 3 min at 4 C, and the pellets were kept at −80 C until use. Giemsa staining of the separated epithelial fractions showed not only epithelial cells but also mononuclear cells, the majority of which we considered might have been intraepithelial lymphocytes (IEL). Histological examination of the tissue after collection of the epithelia showed that villus epithelia were separated completely, whereas epithelial lining cells in the lower part of crypts were still attached to the tissue in approximately half the crypts. In uninfected rats, the basal lamina of the epithelium was intact, and lamina propria cells were retained in the tissue, but in animals after 10 days of infection, the basal lamina was partly obscured, indicating that some propria mucosal cells contaminated the epithelial fractions.

Extraction of total RNA, complementary DNA synthesis, and polymerase chain reaction

Total RNA was extracted using TRIZOL Reagent (Life Technologies, Rockville, Maryland). Two-microgram aliquots of RNA were reverse transcribed in 20 μl of reverse transcription (RT) buffer containing 5 mM $MgCl_2$, 1 mM deoxynucleoside triphosphate (dNTP) mixture, 1 U/μl ribonuclease inhibitor, 0.25 U/μl AMV reverse transcriptase, and 0.125 μM oligodT-adaptor primer (Takara RNA LA PCR kit, Takara Biomedicals, Osaka, Japan) at 42 C for 50 min. One-microliter aliquots of synthesized complementary DNA were added to polymerase chain reaction (PCR) buffer containing 2.5 mM $MgCl_2$, 0.2 μM dNTP mixture, 0.025 U/μl LA *Taq* DNA polymerase (Takara RNA LA PCR kit), and 0.2 μM sense and antisense primers in a final volume of 10 μl. PCR was carried out with cycles of 30 sec at 94 C, 30 sec at 62 C, and 30 sec at 72 C. The sense and antisense primers used were: 5′-AGAAGAGCTATGAGCTGCCTGACG-3′ and 5′-CTTCTGCATCCTGTCAGCGATGC-3′ for β-actin with a 236-bp product; 5′-GACTTTGTGCTGACTGCTGCTCAC-3′ and 5′-TTGTCCATAGGAGACGATGCCCGC-3′ for granzyme B with a 495-bp product; 5′-ATAGAGCTGTGGCTACCGGTG-3′ and 5′-CTCCAGAGATCAAAGCAGTTCC-3′ for FasL with a 286-bp product; and 5′-GAGTGACAAGCCCGTAGCCC-3′ and 5′-GCAATGACTCCAAAGTAGACC-3′ for TNF-α with a 441-bp product.

Density analyses of PCR products

Six microliters of the amplified product was electrophoresed on agar and stained with ethidium bromide. The fluorescence images were saved with an image saver (ATTO Incorporation, Tokyo, Japan), and the density of each band was analyzed with NIH Image software. To determine the optimal numbers of PCR cycles, the densities of the electrophoresed PCR product were analyzed after different numbers of PCR cycles. For semiquantitative analyses, band densities were normalized relative to those of β-actin as described elsewhere (Kuroda et al., 2002).

Statistical analysis

Student's *t*-test (2 tailed) was used for statistical analysis; a *P* value less than 0.05 was considered significant.

RESULTS

Comparison of intestinal epithelial injuries induced in euthymic and athymic rats after infection with the nematode *Nippostrongylus brasiliensis*

Villus atrophy, which is accompanied by compensatory crypt hyperplasia, is a hallmark of intestinal epithelial injury induced by intestinal parasite infections. To determine whether a thymus-dependent mechanism is involved in the development of intestinal epithelial injuries, athymic F-344 rnu/rnu rats and their littermate euthymic rnu/+ rats were infected with 1,000 *N. brasiliensis* L3 larvae. Gross inspection of the small intestinal lumen 10 days after infection showed that the worm population density was the highest in the upper part of the jejunum of euthymic as well as athymic rats, whereas few worms were found in the distal part of the small intestine. After the highly populated intestinal segment 18–26 cm from the pyloric ring was removed for tissue section preparation and separation of the epithelium, other parts of the small intestine were used to examine the worm burdens. The number of worms that emerged in the saline was significantly more in athymic than in euthymic rats 10 days after infection (Fig. 1a). Twenty days after infection, worms had been totally rejected from the euthymic rat intestine, whereas sustained infection was observed in athymic rats. Because worm counts were performed in intestinal segments where the worm population is low, we also counted the numbers of worm profiles observed in paraffin-embedded tissue sections that were obtained from the highly populated segment 18–22 cm from the pyloric ring. The number of worm profiles in tissue sections was also significantly more in athymic rats than in euthymic rats (Fig. 1b).

Histological examination of the proximal jejunum showed

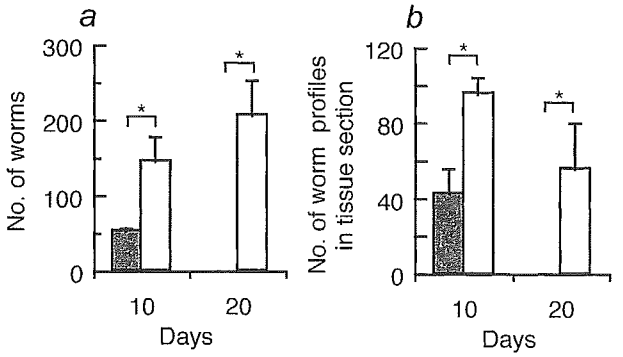

FIGURE 1. Worm burdens in the small intestine of rnu/+ (closed columns) and rnu/rnu rats (open columns) infected with 1,000 *Nippostrongylus brasiliensis* L3 larvae. (**a**) Number of worms in intestinal segments (expressed as the distance from the pyloric ring) 0–18 cm and 26 cm to the ileum end determined by the saline incubation method. (**b**) Number of worm profiles in 4-cm-long longitudinal sections of the proximal jejunum (18–22 cm from the pyloric ring) stained with hematoxylin–eosin. Columns and bars represent means + SE of 4 animals. * indicates $P < 0.05$.

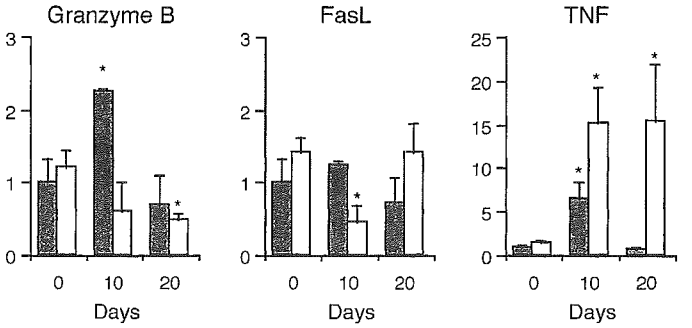

FIGURE 2. Expression of granzyme B, FasL, and TNF-α in the jejunum of rnu/+ (closed columns) and rnu/rnu rats (open columns) after *Nippostrongylus brasiliensis* infection. Total RNA was extracted from the jejunal epithelial fraction containing both epithelial and mononuclear cells, and RT-PCR was performed. The density of each PCR product was normalized with respect to that of β-actin. The data are means + SE of 4 animals. The vertical axis shows the expression levels, with the day-0 average levels in rnu/+ rats expressed as 1.0. * indicates those significantly different from the day-0 level ($P < 0.05$).

marked cytopathic alterations in the villus epithelium 10 days after infection. In euthymic rats, villi were blunt in shape and epithelial cells were occasionally desquamated in a sheet from the basal lamina, leaving an eroded mucosa. Athymic animals rarely showed blunted villi, although at the villus tips small clusters of rounded epithelial cells with pyknotic nuclei were frequently found. In some villi of euthymic and athymic rats, the propria mucosa was markedly edematous with dilated lymphatic vessels.

Morphometric analyses revealed that the villus length in euthymic rats was significantly reduced 10 days after infection and returned to the preinfection level after 20 days, whereas villus atrophy did not develop in athymic animals despite the fact that the nematode infection persisted until 20 days after infection (Table I). The surface to volume ratio, an index of villus surface area determined by the method described by Dunnill and Whitehead (1972), also showed a significant reduction in euthymic rats 10 days after infection, but not in athymic rats. Crypt length reflects the cell growth activity in the crypt growth zone, and it increases significantly in response to the villus epithelial cell loss. Although there was no significant alteration of villus length or surface to volume ratio in athymic rats, crypt length was increased in athymic rats as markedly as in euthymic animals. These results suggest that epithelial injuries and cell loss occurred in both euthymic and athymic rats, but that the severity of the injury in athymic rats might not have been sufficiently intense to cause the villus atrophy that developed in euthymic rats.

Upregulation of cytotoxic factors in euthymic and athymic rats after infection with the nematode *Nippostrongylus brasiliensis*

Granzyme B, FasL, and TNF-α, which are expressed mainly in CTL, NK cells, or macrophages, are important mediators or ligands that can induce target cell injury (Green, 1998). We examined granzyme B, FasL, and TNF-α messenger RNA expression in the isolated jejunal epithelial fraction, which is composed of epithelial cells and IEL, by RT-PCR. The granzyme B expression level in euthymic rats was increased significantly 10 days after infection and decreased to the preinfection level 20 days after infection, whereas that in athymic animals showed no significant increase after infection (Fig. 2). The FasL expression level in euthymic animals did not change significantly after infection, whereas that in athymic animals was decreased after 10 days. The TNF-α expression level in euthymic rats was increased after 10 days and decreased to the preinfection level after 20 days, whereas that in athymic rats was increased after 10 days and maintained at a high level through 20 days after infection.

TABLE I. Morphometric analyses of jejunal mucosa of euthymic rnu/+ and athymic rnu/rnu rats infected with *Nippostrongylus brasiliensis*.*

	Villus length (μm)		Crypt length (μm)		Surface–volume ratio (c/Lh)	
Day	rnu/+	rnu/rnu	rnu/+	rnu/rnu	rnu/+	rnu/rnu
0†	447.9 ± 7.4	444.6 ± 9.1	163.8 ± 10.5	166.3 ± 10.7	50.8 ± 2.2	42.7 ± 2.3
10	332.9 ± 29.4‡§	474.0 ± 16.0	368.0 ± 7.0‡	360.7 ± 8.4‡	24.3 ± 2.1‡§	45.9 ± 2.7
20	433.0 ± 19.8	417.0 ± 10.5	235.5 ± 12.4‡	261.3 ± 16.5‡	32.1 ± 1.7‡	39.5 ± 5.4

* Each measurement was performed on paraffin-embedded tissue sections. Surface–volume ratio was determined as described in the Materials and Methods. All data are mean ± SE of 4 rats.
† Uninfected control.
‡ Significantly different from rnu/rnu rats ($P < 0.05$).
§ Significantly different from day 0 ($P < 0.05$).

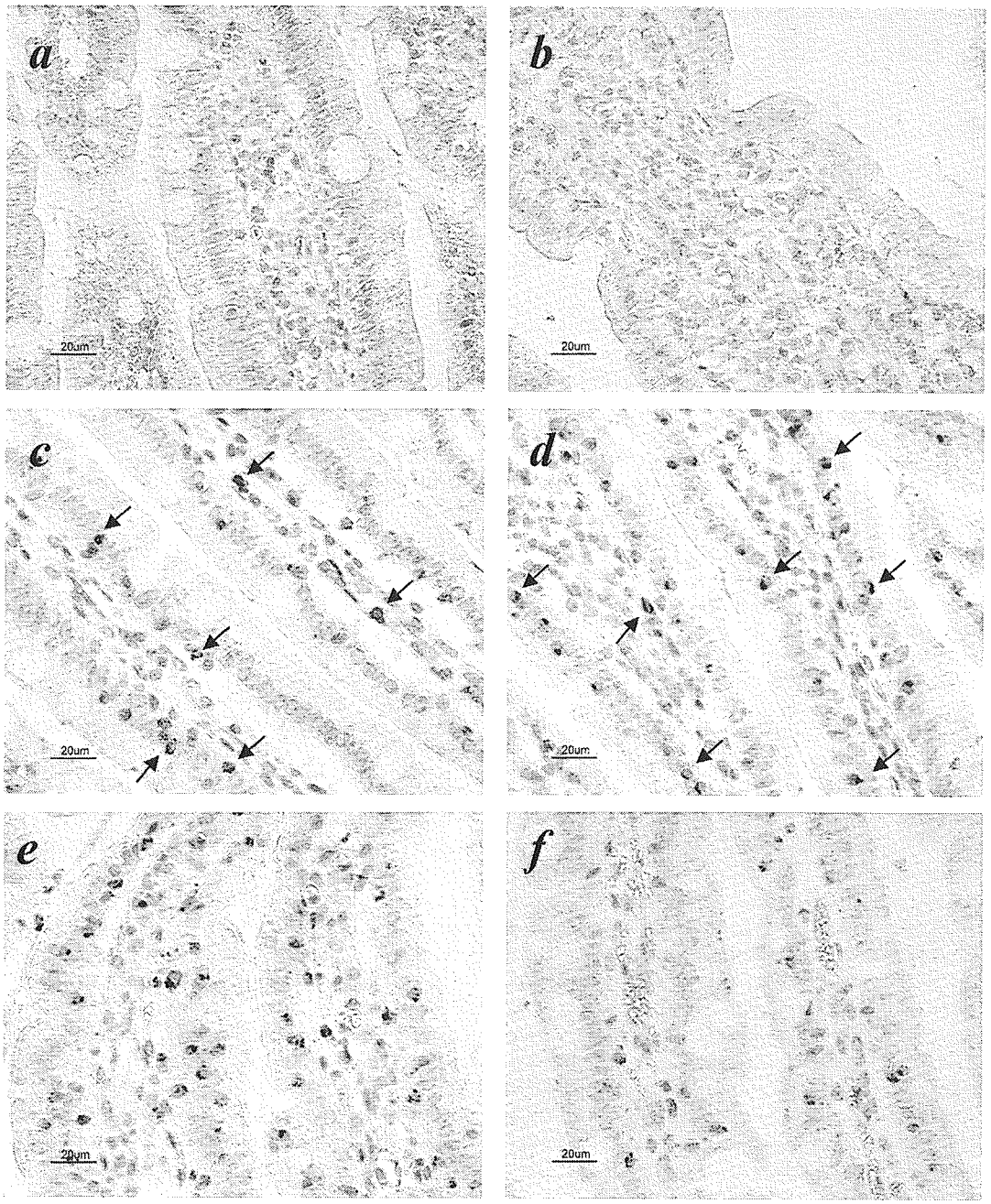

FIGURE 3. Immunohistochemical localization of granzyme B in the jejunum of rnu/+ (**a–c, e**) and rnu/rnu rats (**d, f**) performed on formalin-fixed paraffin-embedded tissue sections. (**a–b**) Negative control sections of rnu/+ rats, uninfected and 10 days after infection, respectively. Sections were incubated with normal IgG instead of granzyme B–specific IgG. (**c–d**) Granzyme B localization in uninfected rnu/+ and rnu/rnu rats, respectively. (**e–f**) Granzyme B localization in rnu/+ and rnu/rnu rats 10 days after *Nippostrongylus brasiliensis* infection, respectively. Granzyme B immunoreactivity (indicated by arrows in **c** and **d** but not indicated in **e** and **f**) shows granular staining localized in the perinuclear cytoplasmic region of small-sized mononuclear cells. Nuclei are lightly counterstained with hematoxylin.

Because the granzyme B expression level was increased in euthymic rats in association with the progression of villus atrophy, the granzyme B^+ cell distribution in the jejunal mucosa was examined by immunohistochemistry. Granzyme B immunoreactivity showed granular staining mainly in the perinuclear cytoplasmic region and was confined mostly to mononuclear cells (Fig. 3). The majority of granzyme B^+ cells were observed in the villus epithelium of euthymic as well as athymic rats, although some granzyme B^+ cells were also found in the propria mucosa. Unexpectedly, the granzyme B^+ cell numbers in the epithelium and propria mucosa of uninfected athymic rats were significantly more than those in euthymic rats (Table II). However, intraepithelial and propria mucosal granzyme B^+ cells showed a remarkable increase in euthymic rats 10 days after infection, the numbers being significantly more than those in corresponding athymic rats. Twenty days after infection, gran-

TABLE II. Numbers of granzyme B$^+$ cells in the jejunum of euthymic rnu/+ and athymic rnu/rnu rats infected with *Nippostrongylus brasiliensis*.*

	Granzyme B$^+$ cells (no./VCU)			
	rnu/+		rnu/rnu	
Day	IE	PM	IE	PM
0†	9.3 ± 0.8‡	4.1 ± 0.6‡	18.9 ± 1.3	6.8 ± 0.7
10	29.3 ± 1.0‡§	10.2 ± 0.1‡§	11.2 ± 2.0§	6.5 ± 0.3
20	8.5 ± 1.8‡	5.9 ± 0.7	20.2 ± 1.3	7.6 ± 1.3

* Granzyme B immunohistochemistry was performed on formalin-fixed paraffin–embedded tissue sections. Positive cells in the epithelium (IE) and the propria mucosa (PM) were counted in 10 villus-crypt units (VCU) and then the cell number per VCU was calculated. All data are means ± SE of 4 rats.
† Uninfected control.
‡ Significantly different from rnu/rnu rats ($P < 0.05$).
§ Significantly different from day 0 ($P < 0.05$).

zyme B$^+$ cell numbers returned to preinfection levels. In athymic rats, granzyme B$^+$ cell numbers were decreased 10 days after infection.

Granzyme B expression is reportedly found mainly in CTL and NK cells (Jenne and Tschopp, 1988). To examine the distribution of T cells in the jejunal mucosa, immunohistochemical studies were performed using antibodies to pan-T-cell marker CD3. In athymic animals, substantial numbers of CD3$^+$ cells were observed in the villus epithelium, whereas few cells were found in the lamina propria mucosa (Table III), consistent with previous reports that IEL are not depleted in athymic rats (Vaage et al., 1990; McKay et al., 1995). In euthymic rats, nematode infection induced significant increases of propria mucosal CD3$^+$ cell numbers, whereas intraepithelial CD3$^+$ cell numbers decreased after infection (Table III). CD8 is expressed on large proportions of mature CTL and some populations of NK cells. Despite the fact that athymic rats had large numbers of granzyme B$^+$ cells, only small numbers of CD8$^+$ cells were found in the epithelium as well as the propria mucosa (Table IV). In euthymic rats, intraepithelial CD8$^+$ cell numbers decreased significantly after infection, whereas propria mucosal CD8$^+$ cell numbers did not change significantly. We tried double immunostaining of CD8 and granzyme B on formalin-fixed paraffin-embedded tissue sections, but it was unsuccessful.

DISCUSSION

It is known that T cell–associated damage to the small intestine, such as that which occurs in graft-versus-host disease, produces villus atrophy (Felstein and Mowat, 1990; Lionetti et al., 1993). Ferguson and Jarrett (1975) reported that thymus-deprived (B) rats did not develop villus atrophy after *N. brasiliensis* infection, suggesting that T cell–dependent mechanisms have an important role in the progression of villus atrophy in nematode infection as well. These results showed that villus atrophy did not develop in nematode-infected athymic rats at least until 20 days after infection. However, as reported previously (D'Inca et al., 1992), crypt hyperplasia developed in athymic rats at a level comparable with that in euthymic rats, suggesting that certain levels of epithelial damage occurred even in athymic rats. In fact, nematode-infected athymic rats showed morphological alterations such as mucosal edema and sloughing off of some enterocytes from the villus tips, although these alterations were less intense than those in euthymic rats. Collectively, these results suggest that epithelial injuries and cell loss occurred in both euthymic and athymic rats but that the enteropathy in athymic rats was not sufficiently severe to cause villus atrophy and could have been compensated for by the crypt stem cell replication. D'Inca et al. (1992) reported that partial villus atrophy developed 21 days after infection in athymic rats together with decreases in disaccharide enzyme activities and increases in epithelial permeability, although 10 days after infection there was no villus atrophy. In their study, the number of L3 larvae inoculated in athymic rats was 3 times more than that in this study. It seems that T cell–dependent mechanisms play a major role in the development of villus atrophy, although under conditions of high worm burdens, T cell–independent mechanisms might also play a certain role.

TABLE III. Numbers of CD3$^+$ cells in the jejunum of euthymic rnu/+ and athymic rnu/rnu rats infected with *Nippostrongylus brasiliensis*.*

	CD3$^+$ cells (no./VCU)			
	rnu/+		rnu/rnu	
Day	IE	PM	IE	PM
0†	9.1 ± 1.0‡	5.3 ± 0.3‡	4.3 ± 0.7	0.02 ± 0.02
10	7.0 ± 2.4	24.5 ± 1.8‡§	4.2 ± 0.1	0.03 ± 0.02
20	4.0 ± 0.6§	8.1 ± 0.2‡§	4.0 ± 0.1	0.05 ± 0.01

* CD3 immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections. Positive cells in the epithelium (IE) and the propria mucosa (PM) were counted in 10 villus-crypt units (VCU) and then the cell number per VCU was calculated. All data are means ± SE of 4 rats.
† Uninfected control.
‡ Significantly different from rnu/rnu rats ($P < 0.05$).
§ Significantly different from day 0 ($P < 0.05$).

TABLE IV. Numbers of CD8$^+$ cells in the jejunum of euthymic rnu/+ and athymic rnu/rnu rats infected with *Nippostrongylus brasiliensis*.*

	CD8$^+$ cells (no./VCU)			
	rnu/+		rnu/rnu	
Day	IE	PM	IE	PM
0†	5.2 ± 0.6‡	8.5 ± 0.6‡	0.3 ± 0.2	1.1 ± 0.2
10	3.0 ± 0.7‡§	6.0 ± 1.2‡	0.5 ± 0.4	1.7 ± 0.2
20	1.9 ± 0.5‡§	8.0 ± 0.3‡	0.2 ± 0.1	1.3 ± 0.2

* CD8 immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue sections. Positive cells in the epithelium (IE) and the propria mucosa (PM) were counted in 10 villus-crypt units (VCU) and then the cell number per VCU was calculated. All data are means ± SE of 4 rats.
† Uninfected control.
‡ Significantly different from rnu/rnu rats ($P < 0.05$).
§ Significantly different from day 0 ($P < 0.05$).

The mechanisms whereby T cells regulate enteropathy or the development of villus atrophy are not thoroughly understood. Granzyme B is a cytotoxic cell granule protease that plays a critical role in mediating cytotoxicity and is expressed mainly in CD8$^+$ T cells and NK cells, as well as in some populations of CD4$^+$ T cells. Immunohistochemical studies showed that granzyme B$^+$ cells were found not only in euthymic but also in athymic rat intestinal mucosa, consistent with previous reports that IEL and NK cells are not depleted in athymic rats (Sfaksi et al., 1985; Vaage et al., 1990; Hougen, 1991; McKay et al., 1995). After infection, granzyme B transcript levels and granzyme B$^+$ cell numbers increased significantly in euthymic rats in association with the development of villus atrophy. In contrast, athymic rat granzyme B$^+$ cells in the epithelial compartment decreased in number after infection, whereas those in the propria mucosa did not change significantly. These results clearly indicate that granzyme B$^+$ cytotoxic cells did not proliferate in athymic rats, probably for lack of growth factors, such as interleukin (IL)-2, derived from activated T cells. The precise mechanisms causing the significant reduction in granzyme B–immunoreactive cells 10 days after infection in athymic rats were not clarified in this study. Athymic rats developed crypt hyperplasia, which peaked 10 days after infection, suggesting that epithelial turnover was accelerated during that period. Given the acceleration of epithelial turnover, a large proportion of intraepithelial granzyme B$^+$ cells may also have been lost while repopulation of the cells was delayed until 20 days after infection. Alternatively, it is also possible that the reduction of granzyme B$^+$ cells was partly due to reduced staining caused by secretion of the enzyme in response to the worm.

FasL is also expressed on some populations of CD4$^+$ T cells, CD8$^+$ T cells, and NK cells and mediates target cell killing through FasL–Fas (CD95) interaction. Unlike granzyme B expression, FasL expression was not increased significantly after infection in either athymic or euthymic rats, suggesting that FasL–Fas interaction might not play important roles in mediating villus epithelial injury or apoptosis after nematode infection (or both). The discrepancy in the expression of granzyme B and FasL after infection suggests that granzyme B and FasL are not necessarily regulated in a coordinated fashion.

TNF-α is produced by a wide variety of cells, including T cells, NK cells, macrophages, and mast cells. In macrophages, TNF-α synthesis can be induced by various pathogens, including viruses, bacteria, and parasites, as well as by cytokines (IL-1, IL-2, interferon-γ, granulocyte-macrophage–colony stimulating factor fluid, and TNF-α itself) (Herbein and O'Brien, 2000). In the euthymic rats in this study, the TNF-α transcript levels increased significantly in association with villus atrophy. Surprisingly, infected athymic rats showed higher TNF-α transcript levels than euthymic rats, and the high levels were sustained even 20 days after infection. The high and sustained TNF-α upregulation in infected athymic rats and the transient TNF-α upregulation in euthymic rats seemed to be positively correlated with the worm burden in the small intestine in each animal, suggesting the possibility that TNF-α upregulation was induced by nematode-related factors. TNF-α is a key cytokine in the inflammatory processes of various diseases such as inflammatory bowel disease and rheumatoid arthritis. It has been reported that neutralization of TNF-α by administration of a monoclonal antibody induced a detectable decrease in the inflammatory parameters of colitis (Van Deventer, 1999), and TNF-α antagonist ameliorated joint inflammation, although the antagonist had little effect on tissue destruction (Joosten et al., 1999). Thus, TNF-α upregulation after nematode infection might be associated with the development of some aspects of inflammatory responses in the intestinal mucosa that could occur without T cell regulation. It has been reported that villus atrophy did not occur in *Trichinella spiralis*–infected TNFR$^{-/-}$ or iNOS$^{-/-}$ mice, indicating that TNF-α and nitric oxide are involved in intestinal pathology in nematode infection (Lawrence et al., 1998, 2000). In this study, no association was observed between TNF-α upregulation and villus atrophy, suggesting that TNF-α–induced inflammation was not sufficiently intense to cause tissue damage. The mechanisms involved in the intestinal pathology might differ among parasite species as well as among host species. Collectively, the present RT-PCR and immunohistochemical studies showed that development of villus atrophy was associated most significantly with the proliferation of granzyme B$^+$ cells in the epithelium and propria mucosa and was not associated with the transcript levels of FasL or TNF-α.

In euthymic rats, CD3$^+$ T cells increased significantly in the intestinal propria mucosa, whereas intraepithelial CD3$^+$ T cell numbers were not increased but rather decreased 20 days after infection. CD8 is expressed on large proportions of mature CTL and some populations of NK cells. The CD8$^+$ cell numbers also decreased in the epithelium after infection, whereas those in the propria mucosa showed no significant change. The reduction of IEL numbers after *N. brasiliensis* infection is partly comparable with the effects on IEL kinetics in *T. spiralis*–infected mice, in which IEL numbers increased 4–7 days after infection but displayed a significant reduction to below normal levels from 14–29 days after infection (Bozic et al., 1998). Athymic rats, as reported previously, had substantial numbers of CD3$^+$ T cells in the epithelium but virtually none in the propria mucosa (Vaage et al., 1990; Hougen, 1991; McKay et al., 1995). Because our attempt to perform double immunostaining of CD8 and granzyme B on formalin-fixed paraffin-embedded tissue sections was unsuccessful in this study, it was not clarified whether some granzyme B$^+$ cells express CD8. However, our finding of the presence of large numbers of granzyme B$^+$ cells in athymic rats, which had few CD8$^+$ cells, as well as the dissociated kinetics of granzyme B$^+$ cells and CD8$^+$ cells in the propria mu-

cosa of euthymic rats after infection, suggests that the majority of granzyme B^+ cells might not express CD8.

It has been suggested that mucosal mast cell activation has an important role in *N. brasiliensis*–induced enteropathy in both immunocompetent and athymic rats (D'Inca et al., 1990, 1992; McKay et al., 1995). We reported previously that in mast cell–deficient *Ws/Ws* rats, villus atrophy developed as early as in wild-type +/+ rats after *N. brasiliensis* infection (Hyoh et al., 1999). Thus, despite the fact that mast cell activation aggravates the nematode-induced enteropathy, other effector mechanisms are more likely to be involved in the induction of villus atrophy.

Granzyme B–containing cells have the potential to induce apoptosis in closely contacted target cells after exposure to granzyme B together with perforin (Jenne and Tschopp, 1988). Thus, large numbers of activated granzyme B^+ cells might play a significant part in inducing enhanced cellular damage or apoptosis in epithelial cells, which could result in villus atrophy. The failure of villus atrophy to develop in athymic rats despite the presence of relatively large numbers of granzyme B^+ cells before infection might be due to the lack of proliferation or invasion of the cells from the propria mucosa into the epithelium during infection. It has been suggested that IL-4 and IL-13 are critical for *N. brasiliensis* worm expulsion, and mucin secretion or muscle contraction (or both) has been proposed as being directly involved in expelling worms from the intestine (Nawa et al., 1994; Urban et al., 1998; Zhao et al., 2003). It would be of interest to determine whether granzyme B^+ cells could also act as effector cells involved in worm expulsion. Although granzyme B reportedly has an antibacterial property (Shafer et al., 1991), it is not clear whether it has an antihelminthic effect as well or whether granzyme B^+ cell proliferation is dependent on IL-4 or IL-13 (or both).

Taken together, these results show for the first time that cells expressing the cytotoxic protease granzyme B infiltrate the villus epithelium and propria mucosa of the small intestine in association with the occurrence of villus atrophy after nematode infection. The types of granzyme B–expressing cells that infiltrate after nematode infection still remain to be identified, and their role in the progression of enteropathy should be further clarified.

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Case Report

Case of acute ileus caused by a spirurina larva

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A growing body of clinical cases suggests that a kind of nematode larva, type X larva of the suborder Spirurina that inhabits firefly squids (*Watasenia scintillans*, or 'Hotaru-ika' in Japanese), can cause acute ileus in humans. However, the larva itself has rarely been found in the wall of the obstructed intestine. We describe here a case of acute ileus, in which a type X spirurina larva was found histologically. A 60-year-old Japanese man suffered from acute abdomen, and an emergency laparotomy revealed a marked stenosis of the ileum. Histological study of the surgically resected ileum showed severe eosinophilic enteritis and a nematode larva. The morphological features of this larva were identical to those of the type X spirurina larva. Interestingly, the larva that was found existed within a small blood vessel, suggesting that the larva migrans of type X spirurina can take place via vasculature. The patient in the present case did not recall ingesting raw squids prior to the onset of his disease. Hence, this indicates that even if the ingestion of raw firefly squids is uncertain, spirurina infection should be included in the differential diagnosis of acute ileus or eosinophilic enteritis.

Key words: eosinophilic enteritis, ileus, larva migrans, raw fish, spirurina larva

Obstruction of the small intestine, or ileus, is caused by various kinds of disorders. Parasitic infections can cause acute ileus; anisakiasis is one such infection and is well known in countries where raw fish is one of the national culinary staples.[1,2]

Recently in Japan there has been an increasing number of clinical cases of acute ileus, which were believed to be caused by a parasite other than anisakis.[3–9] These cases arose from eating raw 'firefly' squids (*Watasenia scintillans*, or 'Hotaru-ika' in Japanese),[4–9] and the patients' peripheral blood showed eosinophilia,[6,7,9] compatible with a parasite infection. *W. scintillans* is known to be a host of type X larva of the suborder Spirurina,[10,11] a kind of nematode larva that was originally characterized by Hasegawa.[12,13] Many of the patients were shown to have serum antibodies against this larval nematode.[6–9] Thus, type X spirurina larva is suspected of infecting humans and causing acute ileus. However, cases in which the body of the spirurina larva was found in the obstructed intestine of patients are extremely rare.[3–5]

In contrast, there are a number of cases of cutaneous creeping eruption that have occurred after the eating of raw *W. scintillans* and histologically they have shown a type X spirurina larva in the skin eruption.[14,15] It is likely that type X spirurina larvae migrate from the intestine through the skin, but little is known about the route(s) of the spirurina larva migrans.

In the current paper, we describe a case of acute ileus, which showed a severe stenosis with eosinophilic enteritis of the ileum. A type X larva of the suborder Spirurina was shown histologically in the ileal wall, giving eloquent evidence that type X spirurina larva is one of the causative agents of acute ileus and eosinophilic enteritis in humans. A brief discussion about the possible vascular route of the spirurina larva migrans is also provided.

CLINICAL SUMMARY

A 60-year-old Japanese man complained of abdominal pain several hours after he had eaten several kinds of raw fish. He did not recall eating any squids. The pain became more severe the next day and he was admitted to Shakaihoken Kobe Central Hospital. On admission, he presented with symptoms of acute abdomen and the laboratory data showed that the serum C-reactive protein (2.3 mg/dL) and peripheral

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white blood cell count (12 900/mm^3) were significantly elevated over the normal ranges. Peripheral blood eosinophils were not counted. Emergency laparotomy revealed a severe stenosis of the ileum, and the ileum just oral to the stenosis was markedly dilated with ischemic changes. Partial resection of the ileum was performed. The patient's postoperative course was unremarkable.

The serum antibody against anisakis was examined 5 days after the operation, but was negative.

PATHOLOGICAL FINDINGS

The wall of the obstructed ileum was markedly thickened and the mucosa showed a small low prominence, approximately 4 mm in diameter, whose surface was eroded (Fig. 1).

Microscopically, there was a severe and extensive phlegmonous inflammation in the ileal wall (Fig. 2). Many of the inflammatory cells were eosinophils (Fig. 2). In the subserosa just below the small mucosal prominence, the body of a nematode larva was discovered (Fig. 3). It was found within a dilated venule (Fig. 4). The body of the larva was approximately 100 μm in diameter. The cuticle had no spines, but it had surface transverse annulations approximately 3 μm in width (Fig. 5). The muscle layer was of the polymyarian (24–27 muscle bundles in a quadrant) coelomyarian type. The lateral chords projected bilaterally and occupied the body cavity. Cross-section of the glandular esophagus measured approximately 38 × 45 μm. These morphological features are summarized in Table 1 and were identical to those of type X larva of the suborder Spirurina.

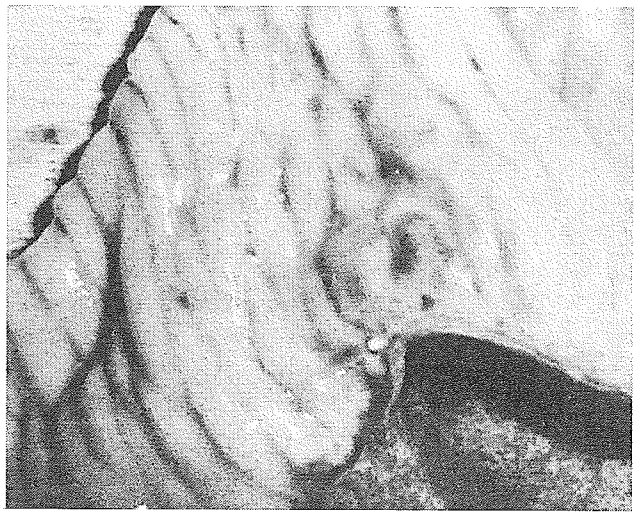

Figure 1 Macroscopic view of the ileal mucosa at the site of stenosis. The mucosa is edematous, and there is a small low prominence of the mucosa whose surface is eroded.

DISCUSSION

Acute ileus with eosinophilic enteritis can be caused after the ingestion of raw fish that are the normal hosts of parasites, and anisakiasis is the most popular of the parasite-induced acute ileus.[1,2] Freezing of raw fish prior to market delivery is effective in destroying the anisakis larvae and has succeeded in preventing anisakiasis in the Netherlands.[16]

Over the previous 20 years in Japan, it has been noted that acute ileus was brought on after the ingestion of a kind of raw squid.[4–9] This squid, *W. scintillans*, is not the host of anisakis, but recent parasitological investigations have found that the visceral organs of *W. scintillans* are inhabited by a kind of larval nematode, type X larva of the suborder Spirurina.[10,11] Serological examination of patients has often revealed the presence of antibodies against the type X spirurina larva, indicating infection with this parasite.[6–9] Thus,

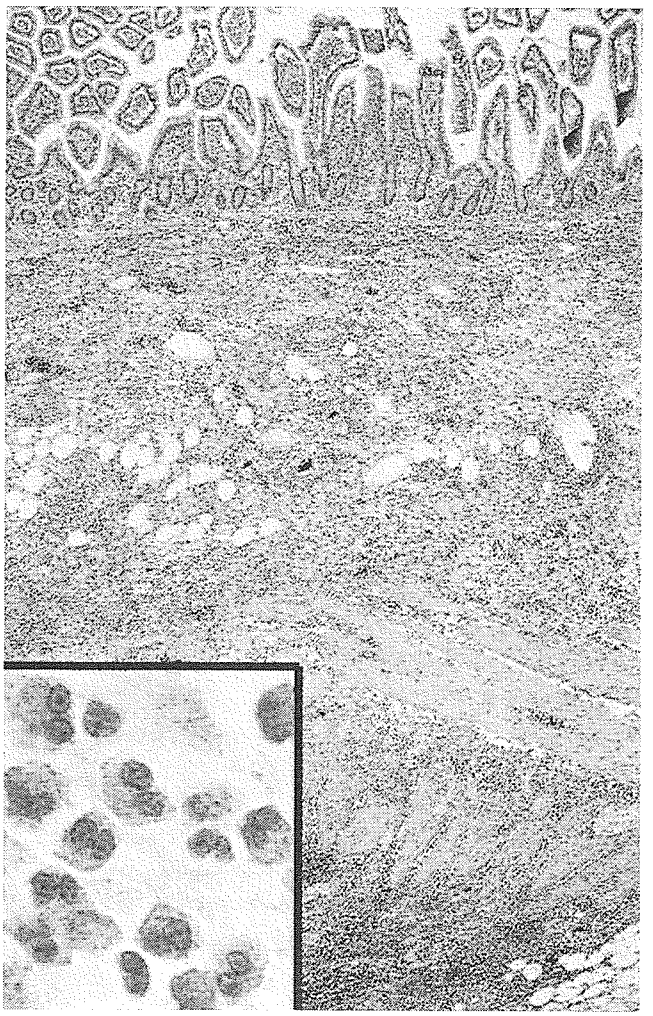

Figure 2 The ileal wall at the site of stenosis (HE). The ileal submucosa is markedly thickened, and there is a severe phlegmonous inflammation. Many of the inflammatory cells are eosinophils (inset).

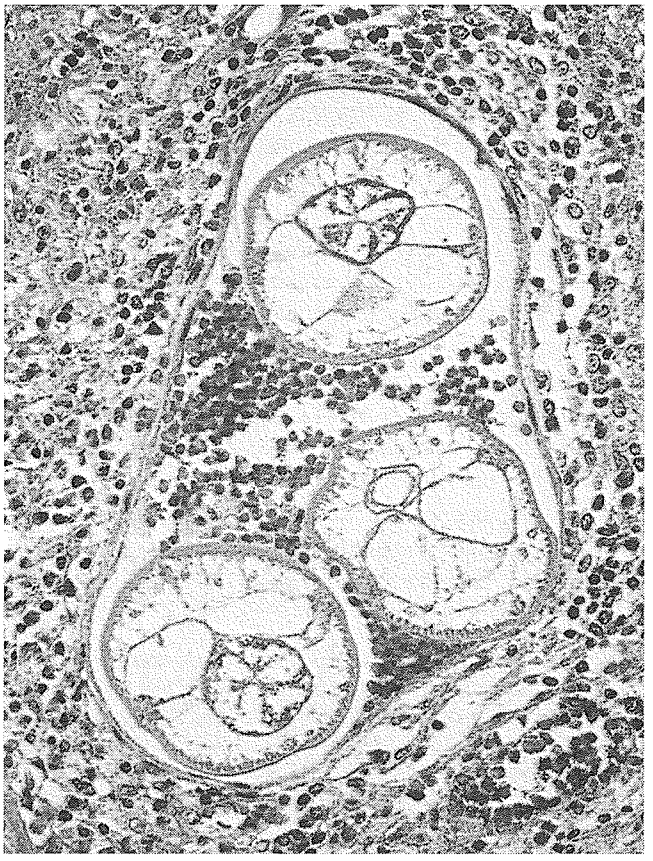

Figure 3 Three cross-sections of the body of a larval nematode found in the ileal subserosa (HE).

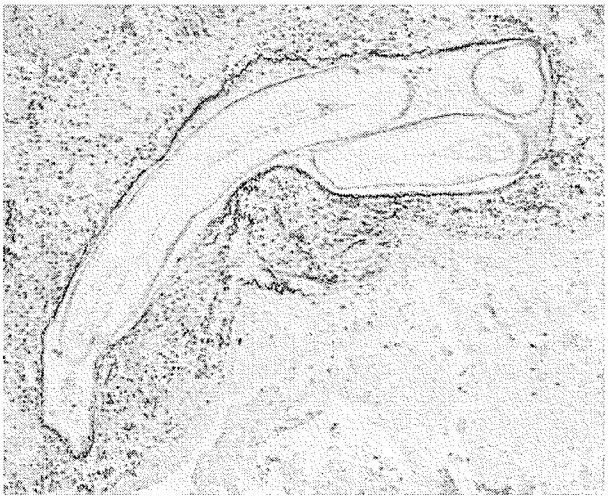

Figure 4 The larva is present within the lumina of a dilated venule, which is outlined by a thin layer of reticulin (elastica van Gieson).

acute ileus following ingestion of *W. scintillans* is believed to be an infectious disease caused by the type X spirurina larva. Although there was a large decrease in the number of clinical cases in 1995 when the squids were frozen to destroy any

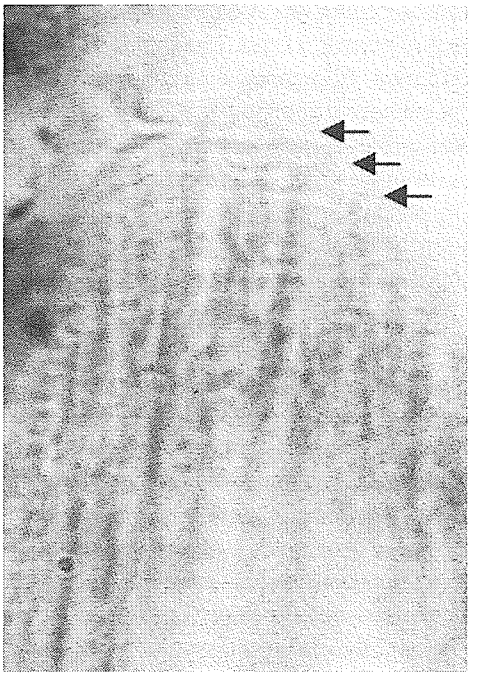

Figure 5 Transverse annulations (arrows) of the surface of the larval cuticle, which are characteristic to spirurina larvae.

Table 1 Morphological features of the larva

Body width	78–92 × 97–101 μm
Cuticle	Thin (1 μm)
Annulation	2–3 μm
Somatic musculature	Polymyarian coelomyarian type
Muscle cells in a quadrant	24–27
Dorsal and ventral chords	Small, clear
Lateral chords	Small, clear
Glandular esophagus	34–42 × 43–47 μm
No. branches	9
Intestine	18–24 × 25–29 μm
No. cells	6

larvae before they came into the market, a number of cases were still reported every year after 1996, probably because of inappropriate freezing procedures.[17] However, cases in which the body of the spirurina larva was found in the obstructed intestine of the patients are extremely rare; there are only four such cases reported in the literature.[3–5]

In the present case of acute ileus, we successfully showed the body of a larval nematode in the wall of the obstructed ileum, which showed severe eosinophilic enteritis. The structure of this larva was different from that of anisakis larvae;[18] for example, its body was much smaller than an anisakis larval body (approximately 200–600 μm in diameter), its muscle cells were much fewer than those of anisakis (60–90 muscle cells per quadrant), and its lateral chords expanded to occupy the body cavity (the lateral chords of anisakis larvae locally project into but do not occupy the body cavity).

We found that the surface of the larva had no spines, unlike Gnathostoma larvae, and its cuticle had annulations, unlike filariae.[12,13] The nematode larva was found to be morphologically identical to type X larva of the suborder Spirurina. The present case proves that this larva is one of the causative agents of acute ileus in humans.

The patient in the present case remembered eating raw fish but no squids before the onset of his disease. Previous parasitological studies have made it evident that type X spirurina larvae can inhabit several kinds of fish and squids other than *W. scintillans*.[3,10,11] Therefore, it is important to be aware of the possibility of spirurina infection in cases of acute ileus following ingestion of raw fish, even if the history of ingesting raw *W. scintillans* is uncertain.

Recent dermatological case studies have documented that type X spirurina larvae causes cutaneous larva migrans in humans; the body of this larva was shown histologically in the skin of a creeping eruption that occurred after a patient ate raw *W. scintillans*.[14,15] It is reasonable that type X spirurina larvae migrate from the intestine through the skin, but little is known about the route(s) of the spirurina larva migrans. A vascular route of larva migrans for some other nematodes is proposed from experimental studies; in mice, visceral larva migrans of *Baylisascaris transfuga* can take place by way of blood vessels.[19] The human case presented in the current paper showed, for the first time, the presence of type X spirurina larva within a blood vessel of the intestinal wall, suggesting that a vascular route is (one of) the way(s) of the spirurina larva migrans.

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特集

幼虫移行症の薬物治療

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SUMMARY

幼虫移行症は動物を本来の終宿主とする寄生虫がヒトに感染し，幼虫が体内をさまよう（移行する）ことによって生じる寄生虫症である．決してまれなものではなく，現在日本で遭遇する寄生虫症の中でも，重要な疾患群の１つと位置づけられている．幼虫移行症は診断が必ずしも容易でなく有効な抗寄生虫薬が知られていないものも多いが，原因不明の好酸球増多症，特有の症状，特有の食歴などを認めたときはこれを疑い，早期に診断し適切な治療を行うことが必要である．

はじめに

幼虫移行症とは，ヒトを固有宿主としない寄生虫，例えばイヌ回虫や顎口虫がヒトに感染した時，幼虫がヒト体内で成虫にまで発育することなく体内諸臓器や皮膚を移行することによって生じる寄生虫症である．中には皮膚爬行疹のように独特の症状を示すものもある．幼虫移行症の多くは線虫が原因となっている．末梢血好酸球増多症が見られる例が多いが，病型によっては好酸球増多症は見られない．主要な幼虫移行症とその病型を**表１**に示した．

表１　主要な幼虫移行症の病型

	病　型	寄生虫
内臓，眼，神経系などの幼虫移行症	内臓幼虫移行症	各種動物の回虫*
	眼幼虫移行症	各種動物の回虫*，広東住虫線虫**，顎口虫**
	神経系幼虫移行症	各種動物の回虫*，広東住虫線虫，顎口虫**，マンソン孤虫**
	その他	イヌ糸状虫（肺や軟部組織の腫瘤）
皮膚の幼虫移行症	遊走性限局性皮膚腫脹	顎口虫，マンソン孤虫
	皮膚爬行疹	顎口虫，ブラジル鉤虫，旋尾線虫
消化管の幼虫移行症	急性炎症型	アニサキス，旋尾線虫
	肉芽腫形成型	アニサキス

＊イヌ回虫，ネコ回虫，ブタ回虫，アライグマ回虫など
＊＊まれに生じるもの

Ⅰ 幼虫移行症の治療の原則

幼虫移行症には薬物治療の対象になるものと，必ずしも必要でないものがある．抗寄生虫薬による治療の対象となるのは，幼虫が一定期間以上人体内で生存しつづけ，種々の病態を惹起し，かつ幼虫の外科的摘出が困難な場合である．一方，イヌ糸状虫による肺腫瘤やアニサキスによる肉芽性腫瘤のように，発見されたときには多くの場合虫体がすでに死亡・変性している場合は，原則的に薬物治療は必要でない．

寄生虫症の治療に用いられる化学療法薬の多くは成虫を対象とした薬物であり，とくに腸管寄生虫を対象とした抗寄生虫薬の多くは，幼虫移行症に対しては全く無効である．幼虫移行症の薬物治療には，**ベンズイミダゾール誘導体**のチアベンダゾール（ミンテゾール®）やアルベンダゾール（エスカゾール®）が多く用いられている．これら薬剤はベータチュブリンに結合し，ミクロチュブルスの形成を阻害することが知られている．一方，土壌放線菌が産生する大環状ラクトン類 avermectin の誘導体，**イベルメクチン**（ストロメクトール®）も一部の幼虫移行症に有効なことが明らかになっており，今後期待できる薬剤である．

しかし，すべての幼虫移行症に対して薬物治療が確立しているわけではなく，とくに眼や中枢神経系に侵入した幼虫移行症の治療は困難なことが少なくない，薬剤選択にあたっては専門家に相談することが望ましい．なお，寄生虫学会ホームページ（http://jsp.tm.nagasaki-u.ac.jp/~parasite/welcome-2.html）でも相談を受け付けている．また「寄生虫症薬物治療の手引き」を同サイトからダウンロードできる．

Ⅱ 各種幼虫移行症に対する薬物治療

① 動物由来の回虫症

土壌中に排出された**イヌ回虫，ネコ回虫，ブタ回虫，アライグマ回虫**などの虫卵が，偶発的にヒトに経口的に摂取された場合に感染が生じる．これら回虫の幼虫の人体内における生存期間はきわめて長く，薬物治療が必要である．動物由来の回虫症は基本的に次の3病型に分類できる．(1) 肝，肺病変を中心とする**内臓幼虫移行症**，(2) 眼球内に侵入する**眼幼虫移行症**，(3) 神経系に侵入する**神経系幼虫移行症**．これらの病型が混在する場合もある．

a．動物由来の回虫による内臓幼虫移行症

動物由来の回虫症の中で最も一般的な病型である．発熱，咳嗽，全身倦怠などで発症し，肝腫大や末梢血好酸球増多症が見られ画像にて肝または肺に多発性小結節性病変が見出されることが多い．生検で幼虫が発見されることはまれで，通常血清診断が行われる．

治療にはベンズイミダゾール誘導体のエスカゾール®が最もよく使用されている．

処方例：エスカゾール®200mg 錠，10～15mg/kg/日，分3，2～4週間．5日間の投与を一定期間毎に2～3回繰り返すことも欧米では行われている．治療開始1週間以内に一過性に好酸球数が増加することがある．白血球減少，肝機能低下などの副作用に対する注意が必要である．

b．動物由来の回虫による眼幼虫移行症

イヌ回虫（トキソカラ）によるものが最も多く，**眼トキソカラ症**と呼ばれている．小児や思春期で

の発症が多く，大半が片眼性である．眼内炎型，後極部肉芽種型，周辺部腫瘤型に病型分類されている．好酸球増多症や肺・肝病変などの内臓幼虫移行症の症状を伴うことは少なく，眼に症状が限局して見られることが多い．血清診断のほか，眼房水中の抗体価の測定が診断に役立つ．

眼トキソカラ症に対する薬物治療の有効性はかならずしも確立していない．薬物治療の場合，ステロイド剤の単独使用または抗寄生虫薬とステロイド剤の併用が試みられている．抗寄生虫薬の使用にあたっては，死亡虫体によるアレルギー性炎症の発生を考慮する必要がある．内臓幼虫移行症と同じくエスカゾール®が使用されているほか，クエン酸ジエチルカルバマジン（スパトニン®）の使用例もある．

c．動物由来の回虫による神経系幼虫移行症

動物由来の回虫の感染により，髄膜脳炎，脊髄炎，脳症などを生じる症例が存在する．イヌ回虫で本病型が生じることはまれだが，ブタ回虫の感染では脊髄炎を生じた例が報告されている．一方，アライグマ回虫では重症の神経系幼虫移行症が高頻度に発現することが北米から報告されている[1]．

アライグマ回虫による神経系幼虫移行症の予後はきわめて不良で，死をまぬがれても重度の後遺症を残す例が多い．エスカゾール®とステロイド剤の併用による薬物治療が試みられているが，その有効性は低いと考えられている．日本国内における人体感染例はまだ報告されていないが，今後注意を払う必要のある幼虫移行症の1つである．

② 広東住虫線虫症

広東住虫線虫はネズミを終宿主，陸産・淡水産の貝類やナメクジを中間宿主，淡水産のエビなどを待機宿主とする寄生虫である．ヒトが貝やエビを摂食することによって広東住血線虫に感染すると，幼虫はクモ膜下腔に侵入し**好酸球性髄膜脳炎**を惹起する．幼虫はクモ膜下腔で幼若成虫にまでは発育するが，それ以上は発育せずいずれ死滅する．頭痛のほか，各種の髄膜炎症状を発現し，好酸球増多症が末梢血や脳脊髄液に見られる．診断は血清診断による．

重症度は感染数に依存する．一般的にはステロイド剤の投与や，脳圧降下剤による治療などで対処されるが，多数感染の場合は死の転帰をとることも少なくない．抗寄生虫薬の効果は不明だが，中国の症例ではエスカゾール®（200mg/日，5日間，その後400mg/日，5日間）とステロイド剤の併用[2]，台湾の症例ではメベンダゾールとステロイド剤の併用により症状が緩和されたと報告されている．

③ 顎口虫症

顎口虫は世界で約10種類存在するが，日本では有棘顎口虫，剛棘顎口虫，ドロレス顎口虫および日本顎口虫の4種による幼虫移行症が報告されている．自然界における終宿主は各種哺乳類，第1中間宿主はケンミジンコ，第2中間宿主および待機宿主はドジョウ，カエル，マムシ，渓流魚，雷魚などである．第2中間宿主または待機宿主の摂食によりヒトに感染する．多くの例で**皮膚幼虫移行症**を生じる．好酸球増多症は認められる例と認められない例がある．有棘顎口虫は主に皮下を遊走するために**遊走性限局性皮膚腫脹**を生じるが，その他3種の顎口虫は皮内を移動するため線状の**皮膚爬行疹**を生じる．このほか，内臓移行や**中枢神経系への侵入**が生じることもあり，とくに後者の場合予後は不良である．

虫体摘出が可能なら実施する．皮膚腫脹部や爬行疹の先端とそのやや前方の皮膚を切除し，連続組織切片やその他の方法で虫体を検索する．薬物治療も行われている．

処方例：エスカゾール®200mg錠，10～15mg/kg/日，分3，3週間．再発した場合は治療を繰り返す．このほか，ストロメクトール3mg錠，200μg/kg，単回服用が有効との報告もある．

④ その他の皮膚幼虫移行症

ブラジル鉤虫，イヌ鉤虫，旋尾線虫などの感染によっても顎口虫と同様の線状の皮膚爬行疹が生じる．基本的には診断をかねて皮膚切除し虫体摘出を試みるべきだが，薬物治療も行われている．ブラジル鉤虫，イヌ鉤虫の場合，エスカゾール®4日間連用やストロメクトール®単回投与が有効との報告がある．

条虫類の**マンソン孤虫**は皮下を遊走することにより遊走性限局性皮膚腫脹を生じる．体長10～20cmで，乳白色紐状のため肉眼で識別でき，外科的摘出が容易なことが多い．内臓移行したマンソン孤虫症に対してプラジカンテル（ビルトリシド®）の投与が有効であったとの報告もあるが，無効との報告もあり薬物療法は確立していない．

⑤ 消化管の幼虫移行症

海産魚類から感染する**アニサキス**幼虫は胃壁または腸壁に侵入する．胃アニサキス症に対しては内視鏡的虫体摘出が行われる．腸アニサキス症は，腹膜炎症状を伴い小腸部分切除によって発見される例が多いが，対症療法のみで回復した例もある．アニサキス症に対して抗寄生虫薬が使用された例はほとんど見られないが，動物実験ではイベルメクチンとアルベンダゾールの有効性が報告されている．

ホタルイカから感染する**旋尾線虫症**の多くはイレウスや腹膜炎症状を示す．旋尾線虫幼虫は人体内における生存期間が比較的長く，後に皮膚爬行症を生じることがあり，抗寄生虫薬による治療の対象とならないわけではない．しかし，一部の症例を除いては保存的療法で改善を見ており，薬物療法は試みられていない．

(参考文献)

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