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Cerebellar ataxia due to *Toxocara* infection in Mongolian gerbils, *Meriones unguiculatus*

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Abstract

We assessed the usefulness of gerbils as an experimental model for neurologic toxocarosis. Mongolian gerbils, *Meriones unguiculatus*, infected with *Toxocara canis* or *Toxocara cati* (1000 eggs/gerbil) showed progressive neurologic disorders from 50 days after infection in *T. canis*-infected gerbils or from 120 days after infection in *T. cati*-infected gerbils. The incidence of the onset was 6 of the 13 gerbils (49%) in the *T. canis*-gerbils and 5 of the 7 gerbils (71%) in the *T. cati*-gerbils. Histopathologically, the cerebellum was the most affected in both groups. We observed loss of Purkinje cells, glial nerve fibers, and nerve sheaths. We also found foci consisting of aggregated macrophages scattered in the white matter of the cerebellum. The affected gerbils showed ataxia and ultimately died of cachexia. Our findings suggest that irreversible neurologic toxocarosis in gerbils can be induced by infection with either *T. canis* or *T. cati*.

Keywords: *Toxocara canis*; *Toxocara cati*; Nuerogic toxocarosis; Mongolian gerbil; Ataxia

1. Introduction

Central nervous system impairment is an uncommon feature of human toxocarosis. However, neurological problems, such as epilepsy, neuropsychologic deficits, and ataxia have been observed clinically. These features have been referred to as neurologic toxocarosis

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(Glickman et al., 1979; Magnaval et al., 1997), a significant medical condition caused by *Toxocara canis* larvae. However, there is no direct evidence in humans on the relation between the migrating larvae in the brain and neuropsychologic dysfunction. To clarify this issue, experimental animal models of neurologic toxocarosis have been intensively studied in mice (Summers et al., 1983; Epe et al., 1994; Skerrett and Holland, 1997; Cox and Holland, 1998; Holland and Cox, 2001), rabbits (Church et al., 1975), guinea pigs (Favati and Marconcini, 1966), baboons (Aljeboori et al., 1970), pigs (Done et al., 1960; Helwigh et al., 1999) and monkeys (Glickman and Summers, 1983). In mice, although the infection apparently affects murine behavioral changes, no neurologic abnormalities associated with motor ataxia have been observed histologically. By contrast, migrating larvae have been reported to accumulate in the brain and induce encephalomyelitis in rabbits and monkeys, although no neurologic disorders have been observed. These findings suggest that neurologic toxocarosis occurs in a species-dependent manner.

In addition, little is known about the pathophysiology of neurologic toxocarosis in humans due to the absence of a suitable animal model of this disease. The present study showed that the Mongolian gerbil, *Meriones unguiculatus*, develops irreversible brain damage after chronic infection of *T. canis* or *T. cati*, resulting in progressive ataxia. We believed that the *T. canis*-infected gerbil is an alternative animal model for human neurologic toxocarosis.

2. Materials and methods

2.1. Gerbils

Female Mongolian gerbils, *M. unguiculatus*, weighing 55–60 g, were infected with embryonated eggs of *T. canis* or *T. cati* (1000 eggs/gerbil) orally under light anesthesia as described previously (Takayanagi et al., 1999; Akao et al., 2000). A total of 65 gerbils (57 gerbils for *T. canis* infection and 8 gerbils for *T. cati*) were observed by examination of their fundi to identify ocular lesions induced by the migrating larvae. All experiments were conducted over a 2-year period. Although most of them were sacrificed for pathologic examinations of their eyes within 1 month, the gerbils that survived beyond that point showed progressive ataxia. In the present study, we compiled data on 20 gerbils (13 *T. canis*-infected gerbils and 7 *T. cati*-infected gerbils) that survived more than 50 days after infection. Behavioral abnormalities of the gerbils were assessed by careful observations of the animals in their cages.

2.2. Pathology

Two *T. canis-infected* gerbils that exhibited severe neurologic disturbance were sacrificed on days 100 and 144 by administration of pentobarbital sodium. Neither was able to maintain normal posture at the time of the necropsy. One *T. cati*-infected gerbil was sacrificed on day 182. Two uninfected gerbils were sacrificed as a control. The skulls including brains were removed and fixed in 2.5% glutaraldehyde and 4% formaldehyde in 0.15% phosphate buffer (pH 7.2) overnight. On the next day, the skull was carefully removed and soaked in the same

fixative for 3 days. The brains were then cut transversely into five blocks and fixed again for 12 h. The serial paraffin sections were then taken and stained with hematoxylin–eosin, Kluver-Barrera's, and Bodian staining.

For the immunohistological examination, we used monoclonal anti-glial fibrillary acidic protein (GFAP) antibody (code 422261, Nichirei, Japan) and anti-lysozyme/muramidase polyclonal antibody (code 422491, Nichirei, Japan). Working dilutions of the antibodies were made according to the manufacturer's instructions. We also employed a monoclonal antibody against larval excretory–secretory products of second-stage larvae of *T. canis* (courtesy of Dr. K. Yokoi, Tokyo Medical University). The localizations of the antigens were visualized using a streptavidin–biotin kit (code 03AM0770, Histofine SAB-PO kit, Nichirei, Japan).

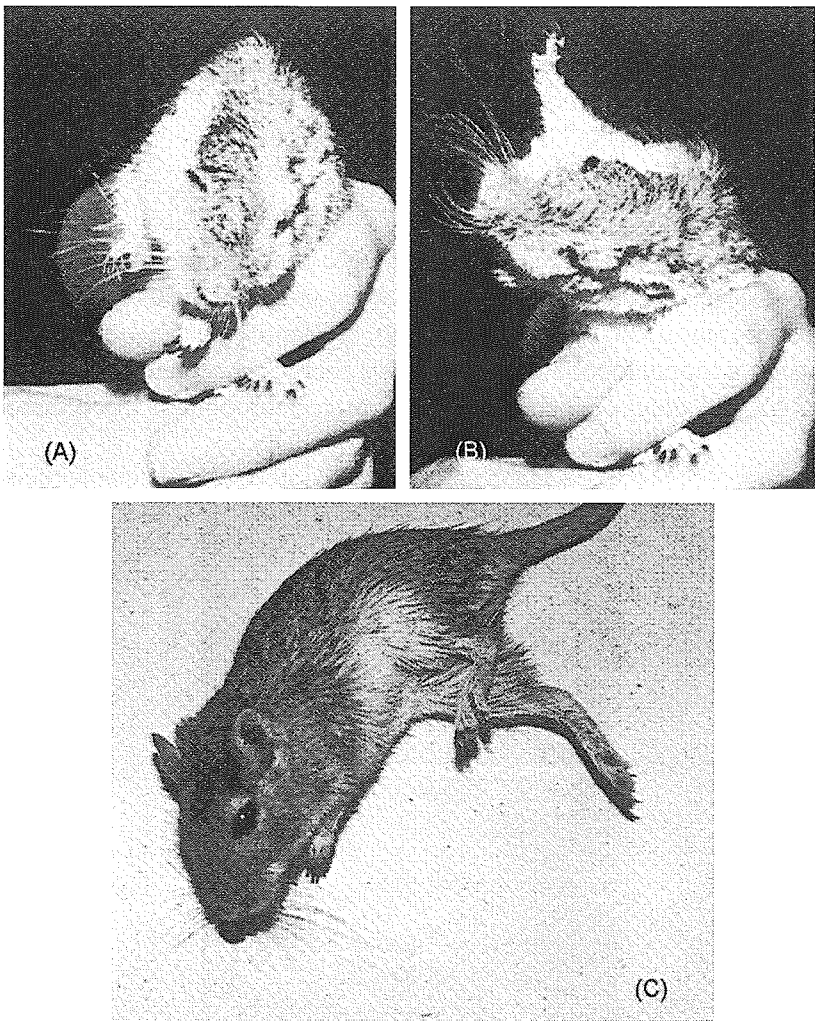

Fig. 1. Abnormal posture of a *T. canis*-infected gerbil 97 days after infection. The gerbil rotated her head clockwise around the body axis (B) and was just releasing after the normal position (A). The affected gerbil showed paraplegia of the hind limbs (C).

3. Results

3.1. Clinical findings

We observed the behavioral changes in 20 gerbils that had survived for more than 50 days after infection. Six out of the 13 gerbils (46%) infected with *T. canis*, and 5 of the 7 gerbils infected with *T. cati* (71%), developed neurological abnormalities. The affected gerbils first showed a swinging gait while attempting to stand on their hind legs. The gait difficulty persisted and worsened slowly over time. Other abnormalities included circulating movement in the same direction, difficulty in normal positioning of the head (Fig. 1A and B), paraplegia of the hind limbs, and urinary incontinence (Fig. 1C).

In the *T. canis*-infected gerbils, behavioral disturbance began to develop 50 days after infection. By contrast, gerbils infected with *T. cati* first showed these disturbances at about 120 days after infection. The gerbils ultimately developed astasia, although they retained a good appetite until they died, despite the severe illness.

None of these abnormalities were observed in the control colonies of gerbils feeding in the same room of our animal laboratory.

3.2. Pathological findings

Despite the existence of the larvae, no pathologic changes, including inflammatory cell infiltrations and degeneration of nerve fibers, were observed around the migrating larvae or in the parenchyma of the cerebrum (Fig. 2). Migrating larvae were clearly identified by staining using a monoclonal antibody against excretory–secretory products of *T. canis* larvae (Fig. 3A), and they were found to be scattered throughout the olfactory and pons

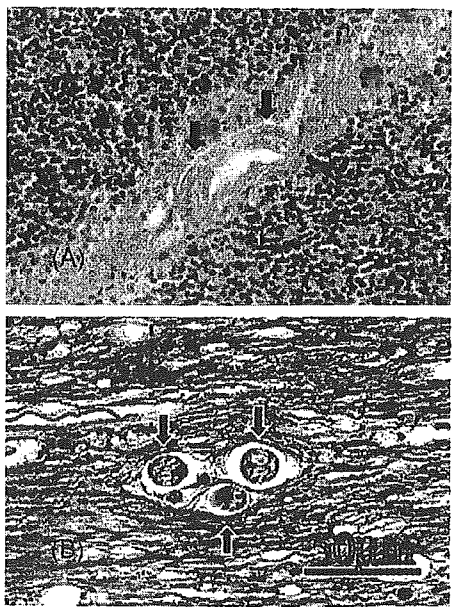

Fig. 2. Migrating larva in the cerebellum (A, HE stain) and pons (B, Bodian stain) of a gerbil 100 days after infection. Note the absence of an inflammatory cell infiltration or granulomatous response around the larva (arrows).

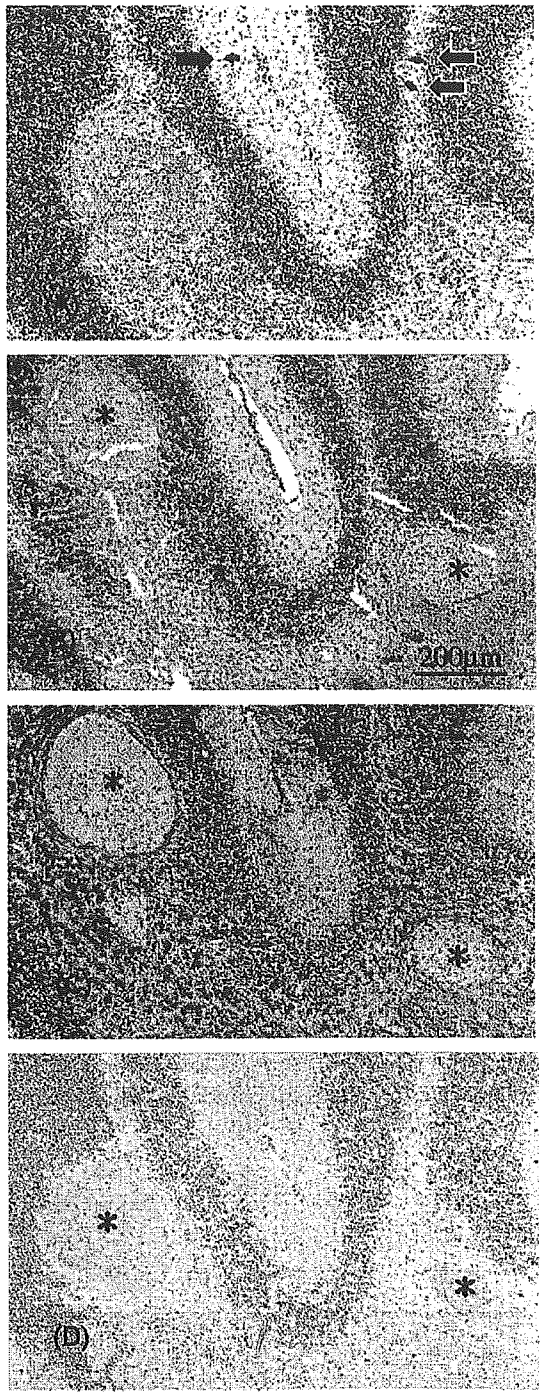

Fig. 3. Characteristic lesions of the cerebellum in a *T. canis*-infected gerbil. (A) Migratory larvae were clearly observed by staining with a monoclonal antibody against larval excretory products both in the gray matter and white matter of the cerebellum (arrows). (B) Aggregations of macrophages (*) were scattered in the white matter of the cerebellum (HE stain). The regions of macrophage aggregation (*) were negative for the monoclonal anti-GFAP antibody (C), but positive for the anti-lysozyme/muramidase polyclonal antibody (D).

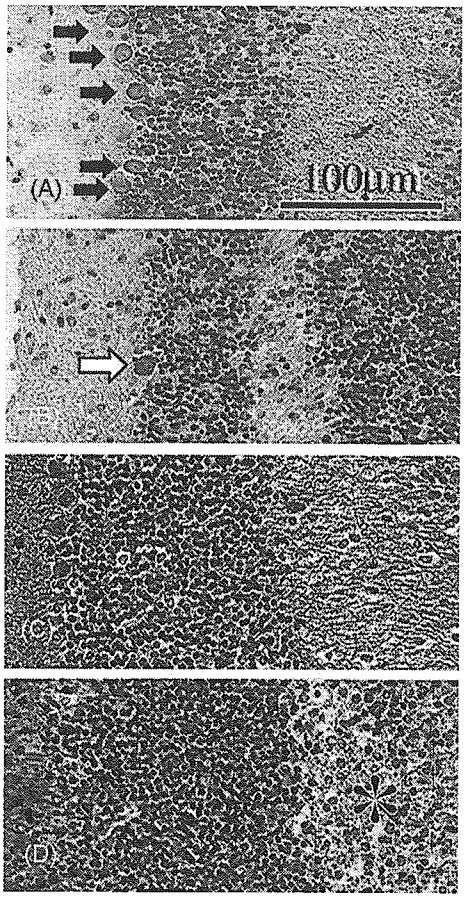

Fig. 4. Cerebellum of an uninfected gerbil (A, C) and that of an *T. canis*-infected gerbil (B, D). Atrophy and loss of Purkinje cells were found (B, Kluver-Barrera's stain), as was a degenerative change of the nerve fibers (*) of the white matter in the infected gerbil (D, Bodian stain). Closed arrows indicate normal Purkinje cells in the uninfected gerbil, and open arrow indicates necrosis of the Purkinje cell in the infected gerbil.

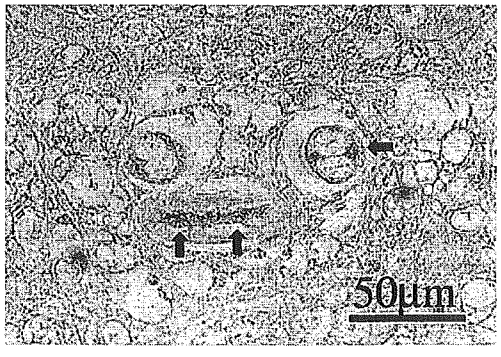

Fig. 5. Localization of lysozyme/muramidase in a migratory larva in the brain. Lysozyme/muramidase activity was strongly positive in a portion of the intestine and pseudocoecum (arrows).

areas of the brain. By contrast, we found severe degenerative changes in the cerebellum of the infected gerbils. These changes included loss of Purkinje cells, nerve fibers, and nerve sheaths (Fig. 4). Necrosis of the Purkinje cells was frequently observed in some areas. These lesions occurred frequently in either lobe of the cerebellum, and spread to some extent to the cerebellar folia or cerebellar fissures. No noticeable changes, such as atrophy of the molecular layer and a decrease in granular cells, were observed in the cerebellar cortex.

The foci were frequently found in the medulla of the cerebellum, and appeared to be round and pale under hematoxylin–eosin staining (Fig. 3B). These foci were negative for anti-GFAP antibody and positive for anti-lysozyme antibody (Fig. 3C and D), indicating that the focus comprised of an aggregation of macrophages instead of glial cells. Immunohistochemistry revealed that lysozyme activities were also localized in the intestine and pseudocoelom of the larvae (Fig. 5). No pathological changes were found in the brains of uninfected control gerbils. Pathological findings in *T. cati*-infected gerbil were the same as those in *T. canis*-infected gerbils.

4. Discussion

In a series of studies on ocular toxocarosis (Takayanagi et al., 1998, 1999; Akao et al., 2000), we noticed that some gerbils infected with *T. canis* or *T. cati* showed characteristic gait difficulty, and showed astasia after surviving the infection. To better understand these clinical manifestations, we examined the affected brains histopathologically. The gerbils showing neurologic disturbances had severe and irreversible degeneration of the cerebellum. We also observed atrophy and loss of Purkinje cells, as well as loss of glial fibers. However, there were no apparent pathologic changes in the brain except in the cerebellum. These results suggested that pathologic changes of the cerebellum were responsible for the neurologic disorders in the infected gerbils. We could not explain the significance of the aggregation of macrophages in the medulla. However, since the lesions were seen only in the affected gerbils, we suspected that those changes might also be responsible for the neurologic disturbance. The migrating larvae were seen both in the cortical layer and in the white matter, but the larvae and the lesions existed separately. Therefore, we assumed that the degenerative changes of the cerebellum might be the result of indirect effects of the larvae.

It is well known that gerbils show cerebral infarction after unilateral ligation of the common carotid artery because they exhibit frequent anomalies of the circle of Willis. In addition, gerbils have been used as a spontaneous epilepsy model. In our experiments, gross morphological observation revealed no infarct lesions in the brains of infected gerbils, and the onset was progressive and irreversible. Thus, we assumed that the neurologic disturbance in gerbils infected with *Toxocara* larvae is not due to genetic anomalies or cerebral infarction after obstruction of the brain capillaries by the larvae.

Previous studies on *Toxocara*-infected mice showed that behavior alterations, such as immobility, ambulation, rearing, digging, and climbing, were more common in outbred mice than in inbred mice (Holland and Cox, 2001). Summers et al. (1983) also described behavior alterations in Binghamton heterogeneous mice after infection with *T. canis*, although the mice showed only mild neurologic abnormalities and few lesions in the cerebellar cortices.

These data indicated that mice are an unsuitable animal for studies of neurologic toxocarosis. Glickman and Summers (1983) reported that 3 out of the 16 cynomolgus macaques developed nystagmus and ataxia, and that the lesions were most severe in the cerebellum, although the severity of ataxia gradually diminished in these monkeys. However, they did not discuss any pathologic changes of the Purkinje cells. In addition, little brain damage was reported in other animal models, including rats, guinea pigs, rabbits, and baboons that were given this infection. Therefore, we concluded that the gerbil might be the best animal model for progressive neurologic toxocarosis.

In our gerbil model, the onset of symptoms in *T. cati* infection appeared later than that in *T. canis* infection. While it may be that this diversity is attributable to the less migratory larvae of *T. cati* (Akao et al., 2000), gerbils infected with either parasite exhibited astasia, indicating that the pathogenesis of *T. cati* larvae is comparable to that of *T. canis* larvae.

We were also surprised to find that lysozyme, a protease inhibitor, was localized in the intestine and pseudocoecum of the larvae. Antigenic products of the larvae were found in the excretory cells (Kondo et al., 1987) and on the outer surface of *T. canis* larvae (Smith et al., 1981). While endopeptidase genes have been identified in *T. canis* larvae (Tetteh et al., 1999), this is the first study to report lysozyme/muramidase activity in the larvae. Lysozyme/muramidase has a widespread distribution in animals and plants, and acts as a natural antibiotic against microorganisms (Edgerton and Koshlukova, 2000; Ibrahim et al., 2001). We supposed that lysozyme/muramidase in the larvae might play significant roles in the pathogenesis of migrating larvae. Further investigations are needed to better understand this phenomenon.

Human neurologic toxocarosis is an unusual feature of visceral larva migrans due to *T. canis* (Glickman and Magnaval, 1993). Therefore, little information is available on its clinical manifestation or treatment. The present findings suggest that gerbils could provide us with a new animal model for understanding this disease.

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References

Akao, N., Takayanagi, T.H., Suzuki, R., Tsukidate, S., Fujita, K., 2000. Ocular larva migrans caused by *Toxocara cati* in Mongolian gerbils and a comparison of ophthalmologic findings with those produced by *T. canis*. J. Parasitol. 86, 1133–1135.

Aljeboori, T.I., Stout, C., Ivey, M.H., 1970. *Toxocara canis* infections in baboons. II. Distribution of larvae and histopathologic responses. Am. J. Trop. Med. Hyg. 19, 815–820.

Church, E.M., Wyand, D.S., Lein, D.H., 1975. Experimentally induced cerebrospinal nematodiasis in rabbits (*Oryctolagus cuniculus*). Am. J. Vet. Res. 36, 331–335.

Cox, D.M., Holland, C.V., 1998. The relationship between numbers of larvae recovered from the brain of *Toxocara canis*-infected mice and social behaviour and anxiety in the host. Parasitology 116, 579–594.

Done, J.T., Richardson, M.D., Gibson, T.E., 1960. Experimental visceral larva migrans in the pig. Res. Vet. Sci. 1, 133–151.

Edgerton, M., Koshlukova, S.E., 2000. Salivary histatin 5 and its similarities to the other antimicrobial proteins in human saliva. Adv. Dent. Res. 14, 16–21.

Epe, C., Sabel, T., Schnieder, T., Stoye, M., 1994. The behavior and pathogenicity of *Toxocara canis* larvae in mice of different strains. Parasitol. Res. 80, 691–695.

Favati, V., Marconcini, A., 1966. Localization in the brain of the guinea pig of 2nd stage larvae of *Toxocara canis* (Werner, 1782). Electroencephalographic, parasitological and anatomo-histopathological documentation. Rivista di Parassitologia 27, 169–184 (Italian with English abstract).

Glickman, L.T., Magnaval, J.F., 1993. Zoonotic roundworm infections. Infect. Dis. Clin. North Am. 7, 717–732.

Glickman, L.T., Summers, B.A., 1983. Experimental *Toxocara canis* infection in cynomolgus macaques (*Macaca fascicularis*). Am. J. Vet. Res. 44, 2347–2354.

Glickman, L.T., Cypess, R.H., Crumrine, P.K., Gitlin, D.A., 1979. *Toxocara* infection and epilepsy in children. J. Pediatr. 94, 75–78.

Helwigh, A.B., Lind, P., Nansen, P., 1999. Visceral larva migrans: migratory pattern of *Toxocara canis* in pigs. Int. J. Parasitol. 29, 559–565.

Holland, C.V., Cox, D.M., 2001. *Toxocara* in the mouse: a model for parasite-altered host behaviour? J. Helminthol. 75, 125–135.

Ibrahim, H.R., Thomas, U., Pellegrini, A., 2001. A helix–loop–helix peptide at the upper lip of the active site cleft of lysozyme confers potent antimicrobial activity with membrane permeabilization action. J. Biol. Chem. 276, 43767–43774.

Kondo, K., Akao, N., Konishi, Y., Yoshimura, H., Hirose, H., 1987. Immuno-electron microscopic observation of excretory cell of *Toxocara canis* larva. Jpn. J. Parasitol. 36, 187–189.

Magnaval, J.F., Galindo, V., Glickman, L.T., Clanet, M., 1997. Human *Toxocara* infection of the central nervous system and neurological disorders: a case–control study. Parasitology 115, 537–543.

Skerrett, H., Holland, C.V., 1997. Variation in the larval recovery of *Toxocara canis* from the murine brain: implications for behavioural studies. J. Helminthol. 71, 253–255.

Smith, H.V., Quinn, R., Kusel, J.R., Girdwood, R.W., 1981. The effect of temperature and antimetabolites on antibody binding to the outer surface of second stage *Toxocara canis* larvae. Mol. Biochem. Parasitol. 4, 183–193.

Summers, B., Cypess, R.H., Dolinsky, Z.S., Burright, R.G., Donovick, P.J., 1983. Neuropathological studies of experimental toxocariasis in lead exposed mice. Brain Res. Bull. 10, 547–550.

Takayanagi, T.H., Akao, N., Tsukidate, S., Tomoda, M., Fujita, K., 1998. Experimental ocular toxocariasis in *Meriones unguiculatus*: a new animal model. In: Tada, I., Kojima, S., Tsuji, M. (Eds.), Proceedings of the Ninth International Congress on Parasitology, Monduzzi Editore, Italy, pp. 627–630.

Takayanagi, T., Akao, N., Tomoda, M., Tsukidate, S., Fujita, K., 1999. New animal model for human ocular toxocariasis: ophthalmoscopic observation. Brit. J. Ophthalmol. 83, 967–972.

Tetteh, K.K., Loukas, A., Tripp, C., Maizels, R.M., 1999. Identification of abundantly expressed novel and conserved genes from the infective larval stage of *Toxocara canis* by an expressed sequence tag strategy. Infect. Immun. 67, 4771–4779.

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Evidence for the involvement of the optic nerve as a migration route for larvae in ocular toxocariasis of Mongolian gerbils

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Abstract

Although *Toxocara canis*, an important pathogen of ocular disease, tends to migrate to the eye, the precise migratory route has yet to be determined experimentally. Mongolian gerbils, *Meriones unguiculatus*, known as a useful animal model for human toxocariasis, were used to investigate the migration route toward the eyes. Infective larvae of *T. canis* were directly inoculated into the intracranial region. Haemorrhagic lesions or larvae were observed in 56.3% of cases. Histopathologically, a larva was observed in the optic nerve of gerbils 6 days after inoculation, and two larvae were found in the optic chiasma in the gerbils having a haemorrhage in the retina 9 days after inoculation. These results indicate that *T. canis* migrates from the brain to the eye through the optic nerve. Considering these data and previous studies showing that the ocular changes appear as early as 3 days of infection in the oral-administrated gerbils, there are two phases in the migration to the retina: a haematogenous early phase and an optic nerve route late phase.

Introduction

Toxocara canis and *T. cati* are ubiquitous gastro-intestinal parasites in dogs and cats, respectively. Humans, especially young children, can be infected with these parasites following accidental ingestion of eggs containing infective-stage larvae. The migration of larvae in human tissues results in either ocular, visceral or covert disease syndromes. Ingested infective eggs of *T. canis* or *T. cati* hatch in the upper alimentary tract. After penetrating the intestinal wall, larvae disseminate through the systemic circulation, and subsequently spread to the muscles and central nervous system (CNS) (Glickman & Magnaval, 1993). Although the ocular form of this disease can cause a severe vision defect, there are few reports on the precise migratory route of larvae to the retina in orally infected hosts. Parke & Shaver (1996) suggested that larvae initially passed into the retina haematogenously. Maguire *et al.* (1990) speculated that larvae migrated to the retina through either the choroidal, central retinal, or ciliary vasculature. In contrast, Takayanagi *et al.* (1999) suspected direct migration of larvae from the brain to the retina along the optic nerve, since *Toxocara* larvae tend to accumulate in the brain. We demonstrated that some of the larvae that were inoculated directly into the brain migrated in the retina of gerbils, which are highly susceptible to *T. canis* (Takayanagi *et al.*, 1999) and *T. cati* infection (Akao *et al.*, 2000). The aims of the present study are therefore to show the evidence for the involvement of the optic nerve as an alternative migration route for larvae in ocular toxocariasis.

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Materials and methods

Animals

Seventeen 4-week-old male Mongolian gerbils, *Meriones unguiculatus*, were used in all experiments, and all were raised in our laboratory and kept under specific pathogen-free conditions in the Animal Centre of Tokyo Medical and Dental University.

Parasite collection and infection procedures

Adult females of *T. canis* were collected from puppies after administration of parbendazole (Sankyo Pharmaceutical Co., Japan). Eggs were obtained from the uteri of gravid females and cultured *in vitro* as described by Oshima (1961). Second-stage larvae were then collected aseptically and maintained in a culture medium until use. A narrow parietal region of skin in each gerbil was cut open under anesthesia with pentobarbital sodium (30 mg kg^{-1}, Pittoman-Moore, New Jersey, USA), and 300 second-stage larvae were directly inoculated intracranially through the cranial bone using a 23-gauge needle. Prior to inoculation, larvae were suspended in physiological saline after repeated washings with culture medium. The wounds were then closed with sutures. Initially, five gerbils were used for the longitudinal observation of the ocular changes after inoculation, and 12 gerbils were used for the histopathological investigation as well as for ocular observations.

After the operation, one of the gerbils died within 24 h. A small number of air bubbles may have been injected together with the *T. canis* larvae and might account for the death of this gerbil. Alternatively, the number of larvae we applied may have been excessive for the gerbil. In addition, six gerbils showed severe convulsion, with progressive emaciation and gerbils finally died from cachexia with neurological symptoms 2 weeks after inoculation. No visible changes in behaviour or appetite were noticed in the remaining gerbils. These experiments were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University, and have been properly carried out under the Guidelines for Animal Experimentation in the Tokyo Medical and Dental University.

Ocular observations

Under anesthesia with pentobarbital sodium, the ocular fundi of inoculated gerbils were carefully observed using an ophthalmoscope (Scalar VMS-170M, Abbe Science, Japan) after the pupils were dilated with tropicamide (Takayanagi *et al.*, 1999). The images of the fundi were transferred to a video monitor for viewing, and were recorded on videotape every 3 days from days 0 to 60.

Pathological examination and counting of larvae in the brain

The experimental design is shown in table 1. On days 6, 9 and 30, one of the gerbils with ocular changes in the second group was killed on each day for histopathological investigation. On days 12 and 15, gerbils which became weak due to cachexia with neurological symptoms, and died during the ocular observation under anesthesia, were also histopathologically examined. A total of five gerbils were examined and the eyeballs, together with the optic nerve and brain, were removed from the skull and immediately fixed in 2.5% glutaraldehyde and 4% formaldehyde in 0.15% phosphate buffer (pH 7.2). Serial sections embedded in JB-4 plastic resin were stained with haematoxylin and eosin, and examined as described by Takayanagi *et al.* (1999). One of the gerbil brains without ocular changes 6 days after inoculation was cut into small pieces and each piece was pressed between two glass slides so that the larvae could be counted under a microscope (Kondo, 1970).

Table 1. Experimental design for observing pathological changes in gerbils infected with *Toxocara canis* larvae.

	Mongolian gerbils	
	Group 1	Group 2
Number examined	5	12
Ocular observations	Every 3 days	Every 3 days
Histopathological observations	ND*	Day 6†, 9†, 12, 15, 30†
Counting larvae in the brain	ND*	Day 6

*Not done.
†On each day one of the gerbils with ophthalmoscopic changes was sacrificed.

Results

Haemorrhagic lesions and larvae

Either vitreous or choroidal haemorrhages were observed in the gerbils from 6 days after the intracranial inoculation. Choroidal and vitreous haemorrhages were observed at the peripheral region of the retina, and the optic papilla, respectively (fig. 1). Larvae were also observed simultaneously with the emergence of the haemorrhages. Haemorrhagic lesions or larvae were observed in nine of 16 gerbils (56.3%), two of which showed vitreous and choroidal haemorrhages, six had only choroidal haemorrhages, and one had larvae without haemorrhaging. Most of the ocular changes appeared on day 12. Abnormal ocular findings were observed in 12 of 32 eyeballs (37.5%) examined. A total number of 15 haemorrhagic legions occurred in eight gerbils, in which nine lesions were observed on day 12 for the first time. Once these ocular changes occurred, there were no new haemorrhages or larvae in the eye (table 2). Haemorrhagic legions were absorbed gradually within 1 month after they appeared. No granulomatous lesions were detected in any of the gerbils examined.

Pathological findings

Six of 12 gerbils of the second group were sacrificed for pathological studies or for counting larvae. No abscesses or haemorrhages were observed at the inoculation site. Microscopically, 329 larvae were counted in one gerbil brain 6 days after inoculation. Histopathological sections revealed a larva in the optic nerve of the gerbil 6 days after inoculation (fig. 2). After 9 days, two larvae were found in the optic chiasma plus a haemorrhagic legion and a larva in the retina (fig. 3). No inflammatory changes were observed around the larvae, although eosinophil infiltrations were scattered beneath the sheath of the optic nerve.

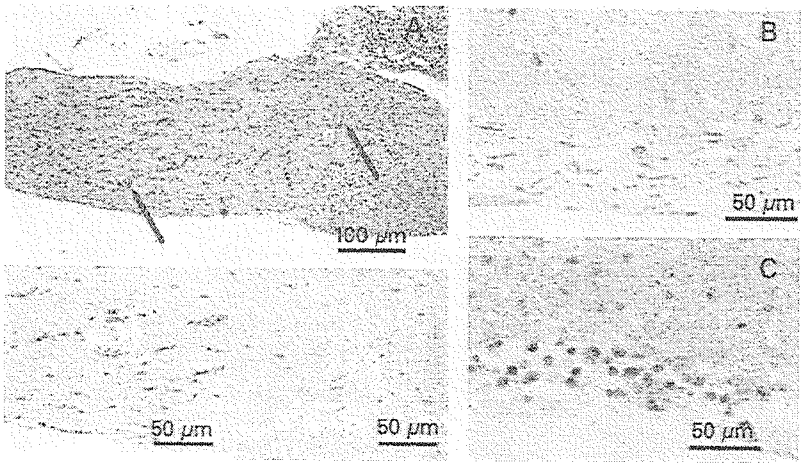

Fig. 3. Cross-sections of the optic chiasma of the Mongolian gerbil at day 9 following intracranial inoculation with *Toxocara canis* larvae: A, two migrating larvae, in the optic chiasma, and higher magnification. B, eosinophil infiltration and C, mononuclear cell infiltration beneath the optic nerve sheath.

In the present study, *T. canis* larvae were shown to migrate from the brain to the retina of gerbils through the optic nerve. Choroidal and vitreous haemorrhages were observed in the retina of gerbils inoculated with larvae, although no vascular changes were noted. Previously, Takayanagi *et al.* (1999) reported that ocular changes occurred 3 days after oral administration, which was clearly earlier than those in the present study, indicating that larvae move into the retina not only along the optic nerve but also via a haematogenous route. Larvae may migrate directly through the arteries from the internal carotid artery to the ophthalmic, retinal central or ciliary arteries. Takayanagi *et al.* (1999) showed that choroidal haemorrhages with or without larvae re-emerged after oral infections of gerbils. The present data, therefore, suggest that a re-emerged lesion could be attributed to a larva arriving late. In addition, the incidence of ocular changes was low (56.3%) compared with the oral inoculation (95%) (Takayanagi *et al.*, 1999). These data also strongly indicate that following oral inoculation, larvae accumulate in the eye both haematogenously and neurotropically in gerbils.

There have been several studies on experimental ocular toxocariasis. Among these, mice (Kunishige, 1964; Olson, 1976; Ghafoor *et al.*, 1984), rabbits (Kunishige, 1964), guinea pigs (Miyamoto, 1972; John *et al.*, 1983) and monkeys (Luxenberg, 1979; Watzke *et al.*, 1984) were evaluated as animal models for this disease. However, none of these animal models exhibited a high incidence of ocular lesions following a single oral inoculation. Additionally, there are no reports on the migration route of the larvae involved in an infection of the eye. In the present study, when larvae were injected into the iliac vein of gerbils to determine whether larvae had migrated through the blood vessels, no ocular change was observed (data not shown).

Larvae injected directly into the brain of NIH mice were capable of migrating into the viscera and musculature (Abo-Shehada & Harbert, 1984). In contrast, we found that larvae that had migrated to the brain remained there, although some migrated to the eyes. These data suggested that larvae have an affinity for the CNS and eyes in gerbils. However, further studies are needed to clarify the migratory route of larvae following arrival in the brain.

Histopathologically, we found a larva in the optic nerve and optic chiasma. No pathological changes were observed around the larva, neither in the optic nerve nor in the brain, including the optic chiasma. Interestingly, eosinophil infiltrations were present in the optic nerve sheath that were unrelated to the larva; although eosinophilic granulomata are frequently found in human ocular toxocariasis (Irvine & Irvine, 1959; Duguid, 1961; Harris, 1961; Rey, 1962). No granulomatous lesions were detected in any of the gerbils in the present study. As Takayanagi *et al.* (1999) suspected, motile larvae would not be able to induce a local immune response as long as they were alive. However, the present study suggests that cytokines, including an eosinophil chemotactic factor, might be produced in the optic nerve, but further studies are needed to confirm this.

Kira *et al.* (1997) reported that infiltrations of eosinophils in the CNS, especially in the spinal cord, associated with atopic diseases should be referred to as atopic myelitis. Although the aetiology of this disease is unknown, Kira *et al.* (1997) assumed that helminth infections might be associated with atopic myelitis. In this respect, the gerbil might also be a suitable animal model for neurological toxocariasis.

In the present study, some gerbils became weak and died due to cachexia with neurological symptoms and severe convulsion. Neither an abscess nor haemorrhaging in the brain was found macroscopically in these cases, suggesting that the symptoms were associated with a larval infection, either directly or indirectly. Gerbils orally inoculated with larvae showed neurological symptoms by 2 months after inoculation. The brains of these gerbils showed no inflammatory changes, but degenerative alterations in the axon of neurons in the cerebellum have previously been reported (Tomoda *et al.*, 2000).

The route of infection in ocular toxocariasis has been unclear, with the debate focusing on routes that are either

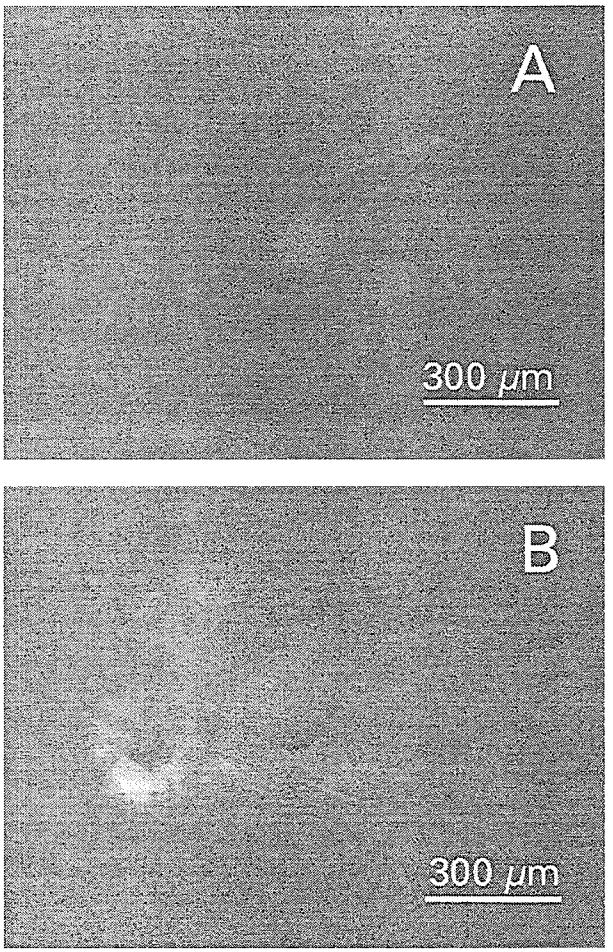

Fig. 1. Ocular fundi of the Mongolian gerbil following intracranial inoculation with *Toxocara canis* larvae: A, choroidal haemorrhage at peripheral region of retina. B, reddish vitreous haemorrhage around optic papilla.

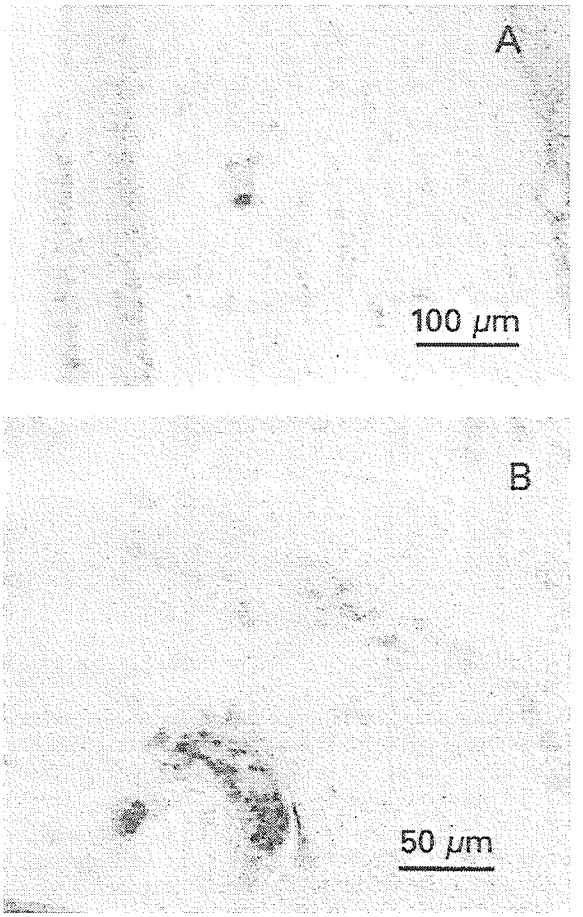

Fig. 2. Cross-section of the optic nerve of the Mongolian gerbil at day 6 following intracranial inoculation with *Toxocara canis* larvae: A, larvae migrating in the optic nerve. B, as A, but at a higher magnification.

Discussion

While there have been a number of clinical and experimental reports on ocular toxocariasis, it remains unclear just how larvae migrate to the retina. Maguire *et al.* (1990) suspected that larvae might enter the eye via the choroidal, central retinal and ciliary vasculature on the basis of their clinical cases. Shields (1984) stated that larvae reach the eye through ciliary vessels and the central retinal artery. Parke & Shaver (1996) suggested that most of the migration is initially haematogenous. On the other hand, Watzke *et al.* (1984) demonstrated that a larva appeared in the optic nerve of cynomolgus monkeys after intravitreal injection of live larvae, suggesting that *T. canis* larvae can make a retrograde migration from the eye to the central nervous system.

Table 2. Ophthalmoscopic changes in Mongolian gerbils after inoculation with *Toxocara canis* larvae intracranially. Approximately 300 larvae were injected into the brain with a 23-guage needle, and ocular changes were observed every 3 days from day 0 to day 60.

Haemorrhagic lesions with larva	Haemorrhagic lesions without larva	No haemorrhagic lesions with larva	No ophthalmological changes	Total
Day 6* (1)†	Day 6 (1)			
Day 12 (3)	Day 9 (1)	Day 15 (1)	(7)	(16)
Day 18 (1)	Day 12 (1)			

* Days after inoculation when the lesions were observed for the first time.
† The number of gerbils observed is shown in parentheses.

haematogenous or neurotropic. Sero-negative cases are not unknown in human cases (Glickman & Magnaval, 1993). Furthermore, optic neuritis caused by *T. canis* has been diagnosed in humans (Komiyama *et al.*, 1995). Considering the previous reports and the present findings, we suspect that larvae sequestered in the brain for a long period of time might begin to migrate to the eyes through the optic nerve in response to changes in the host's physiological status under some form of stimulation, such as hormone changes, aging, or an immunodeficiency.

References

Abo-Shehada, M.N. & Harbert, I.V. (1984) The migration of larval *Toxocara canis* in mice. II. Post-intestinal migration in primary infections. *Veterinary Parasitology* **17**, 75–83.

Akao, N., Takayanagi, H.T., Suzuki, R., Tsukidate, S. & Fujita, K. (2000) Ocular larva migrans caused by *Toxocara cati* in Mongolian gerbils and comparison of ophthalmologic findings with those produced by *T. canis*. *Journal of Parasitology* **86**, 1133–1135.

Duguid, I.M. (1961) Chronic endophthalmitis due to *Toxocara*. *British Journal of Ophthalmology* **45**, 787–796.

Ghafoor, S.Y., Smith, H.V. & Lee, W.R. (1984) Experimental ocular toxocariasis: a mouse model. *British Journal of Ophthalmology* **68**, 89–96.

Glickman, L.T. & Magnaval, J.F. (1993) Zoonotic roundworm infections. *Infectious Disease Clinics of North America* **3**, 717–732.

Harris, W. (1961) Pseudo-glioma due to larval choroid-retinal granulomatosis. *British Journal of Ophthalmology* **45**, 144–146.

Irvine, W.L. & Irvine, A.R. Jr (1959) Nematode endophthalmitis. *American Journal of Ophthalmology* **47**, 185–191.

John, T., Donnelly, J.J. & Rockey, J.H. (1983) Experimental ocular *Toxocara canis* and *Ascaris suum* infection: *in vivo* and *in vitro* study. *Transactions of the Pennsylvania Academy of Ophthalmology and Otolaryngology* **36**, 131–137.

Kira, J., Yamasaki, K., Kawano, Y. & Kobayashi, T. (1997) Acute myelitis associated with hyperIgEemia and atopic dermatitis. *Journal of the Neurological Sciences* **148**, 199–203.

Komiyama, A., Hasegawa, O., Nakamura, S., Ohono, S. & Kondo, K. (1995) Optic neuritis in cerebral toxocariasis. *Journal of Neurology, Neurosurgery and Psychiatry* **59**, 197–198.

Kondo, K. (1970) Experimental studies of "larva migrans". *Journal of Kyoto Prefectural University of Medicine* **79**, 32–56 (in Japanese with English abstract).

Kunishige, A. (1964) Histologic and histochemical studies on experimental "visceral larva migrans" in rabbits and mice. *Shikoku Acta Medica* **21**, 546–567 (in Japanese).

Luxenberg, M.N. (1979) An experimental approach to the study of intraocular *Toxocara canis*. *Transactions of the American Ophthalmological Society* **77**, 542–602.

Maguire, A.M., Green, W.R. & Michels, R.G. (1990) Recovery of intraocular *Toxocara canis* by pars plana vitrectomy. *Ophthalmology* **97**, 675–680.

Miyamoto, K. (1972) Experimental toxocariasis in abnormal hosts 2) Histophotological studies on mice and guinea pigs infected with *Toxocara canis*. *Japanese Journal of Parasitology* **21** (Suppl.), 54 (in Japanese).

Olson, L.J. (1976) Ocular toxocariasis in mice: distribution of larvae and lesions. *International Journal for Parasitology* **6**, 247–251.

Oshima, T. (1961) Standardization of techniques for infecting mice with *Toxocara canis* and observations on the normal migration routes of the larvae. *Journal of Parasitology* **47**, 652–656.

Parke, D.W. II. & Shaver, R.P. (1996) Toxocariasis. pp. 1225–1235 *in* Pepose, J.S., Holland, G.N. & Wilhelmus, K.R. (*Eds*) *Ocular infection and immunity*. St Lois, Mosby.

Rey, A. (1962) Chronic endophthalmitis due to *Toxocara*. *British Journal of Ophthalmology* **46**, 616–618.

Shields, J.A. (1984) Ocular toxocariasis. A review. *Survey of Ophthalmology* **28**, 361–381.

Takayanagi, H.T., Akao, N., Suzuki, R., Tomoda, M., Tsukidate, S. & Fujita, K. (1999) New animal model for human ocular toxocariasis: ophthalmoscopic observation. *British Journal of Ophthalmology* **83**, 967–972.

Tomoda, M., Suzuki, R., Takayanagi, T., Akao, N., Yokoi, K., Sakai, J. & Fujita, K. (2000) Neuro-histopathological study of gerbils infected with *Toxocara canis*. *Proceeding of the Regional Meeting of the Japanese Society of Parasitology the East Branch* 32 (in Japanese).

Watzke, R.C., Oaks, J.A. & Folk, J.C. (1984) *Toxocara canis* infection of the eye. Correlation of clinical observations with developing pathology in the primate model. *Archives of Ophthalmology* **102**, 282–291.

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Diffuse Retinochoroiditis due to *Baylisascaris procyonis* in Monglian Gerbils

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ABSTRACT: *Baylisascaris procyonis*, raccoon roundworm, causes a severe retinal lesion in humans. The lesion is termed as diffuse unilateral subacute neuroretinitis (DUSN). To understand the pathogenesis of *B. procyonis* in gerbils, we inoculated 17 embryonated eggs/g body weight of *B. procyonis* into 15 male Mongolian gerbils, *Meriones unguiculatus*, and monitored their fundi with an ophthalmoscope. Six of 15 gerbils (40%) showed severe retinitis with a sinuous track due to larval movement. The lesions extended across nearly half of the affected fundi. Histopathological examination revealed perivasculitis in the optic disk region, inflammatory proliferation of the pigment cells, and vitreitis in most cases. These findings were similar to those in human cases of DUSN, suggesting that gerbils might be a useful model for understanding the pathogenesis of *B. procyonis* infection in humans.

DUSN is defined as a posterior segment intraocular inflammation caused by infestation of 1 eye with a subretinal nematode and is characterized by a diffuse neuroretinitis and pigmentary retinopathy with loss of vision (Gass and Scelfo, 1978). Several different roundworms have been implicated in this condition, but there is extensive epidemiologic evidence suggesting that the raccoon roundworm *Baylisascaris procyonis* is the most common cause (Kazacos et al., 1985).

To understand the pathogenesis of this disease in humans, several animal models have been investigated. Nonhuman primates, such as cynomolgus monkeys and squirrel monkeys, have been shown to be highly susceptible to *B. procyonis* and DUSN (Kazacos et al., 1985). In contrast, the prevalence of DUSN is relatively low in experimentally infected mice, with only 16% of exposed hosts exhibiting this pathology in a low-dose infection (100 eggs/mouse) and 21% in a high-dose infection (1,000 eggs/mouse) (Kazacos et al., 1985). In addition, although the characteristic findings of DUSN in humans include optic disc edema and recurrent crops of evanescent gray-white lesions of the outer retina (Kazacos et al., 1985), the most common lesions in mice were vitreous hemorrhage and vitreitis, which suggests that mice might not be a suitable model to investigate ocular larva migrans due to *B. procyonis*. The present study describes DUSN-like lesions in Mongolian gerbils infected with *B. procyonis* and found that lesions closely resembled those seen in infected humans.

Mongolian gerbils, ranging between 4 and 5 mo of age, were raised in our laboratory and maintained under pathogen-free conditions. Eleven *B. procyonis* adult females were collected from the intestinal cavity of raccoons whose feces were responsible for the first outbreak of *B. procyonis* larva migrans in rabbits in Japan (Sato et al., 2002). Embryonated eggs of *B. procyonis* were treated with 50% sodium hypochlorite, and then 17 eggs/g body weight were inoculated into the stomachs of the gerbils, under light anesthesia, using a Teflon tube (Takayanagi et al., 1999). The count of eggs was performed using the method of Oshima (1961). Eight male gerbils were used in the first experiment, and they were observed daily to monitor the onset and development of clinical central nervous system disorders. Ophthalmoscopic observation was carried out twice a week, as previously described (Takayanagi et al., 1999). Measurement of larvae in the retinas was conducted by an image analysis system software (Image-Pro Plus, Media Cybernetics, Silver Spring, Maryland). In the second experiment, 7 male gerbils were given inoculations of the same size and under the same conditions as in the first experiment. For histopathological investigation, the eyes were removed at a predetermined time and were fixed with 2.5% glutaraldehyde and 4% formaldehyde in phosphate buffer (pH 7.2). Histopathological sections were then prepared as described previously (Akao et al., 2000).

The clinical signs of neurologic disturbance were first noted on the 11th day of infection. By the 15th day of infection, 8 of 15 gerbils (53%) showed severe ataxia, including a circulating movement along the body axis and falling-down behavior. By the 31st day of infection, 5 additional gerbils showed progressive ataxia. Two of 15 gerbils (13%) showed no neurologic disorder during the observation period.

Ophthalmologic lesions were first observed on the 8th day of infection, and retinal inflammations were consistently present on a fundus of 5 gerbils. One additional gerbil, however, had ocular lesions in both eyes and seemed to be blind. In all examinations of the gerbils, we found larvae on the fundi, measuring an average of 1,114 ± 190 μm (n = 5) in length and 83 ± 5.9 μm (n = 5) in width. During the observation period, we noticed no hemorrhagic lesions of the type reported in gerbils infected with *Toxocara canis* or *T. cati* (Takayanagi et al., 1999; Akao et al., 2000). Eventually, 6 of 15 gerbils (40%) showed retinal lesions. Ophthalmologically, the lesions were characterized by a sinuous track from the larval movement and widespread yellowish-gray retinal inflammation (Fig. 1A–D). They covered nearly half of the affected fundus in each case.

Histopathologically, 3 prominent and 1 faint transverse sections of a large *B. procyonis* larva were found in the subretinal layer (Fig. 2). Inflammatory proliferation of pigment cells was obvious not only around the larvae but also around their tracks. Perivascular infiltration of eosinophils was observed at the optic papilla in some cases, and inflammatory cells were also found in the vitreous cavity.

Kazacos et al. (1985) reported that hyphema, retinitis, and retinal hemorrhage are the most prominent features of infected mice. In the gerbils in the present study, however, hemorrhagic lesions were not common, although there was clear and extensive retinitis due to larval movement. These lesions were much more severe than those induced by *T. canis* larvae in gerbils reported by Takayanagi et al. (1999). We did not observe subretinal granuloma-containing larvae as described by Kazacos et al. (1985) in both the ophthalmologic and histologic examinations. In human DUSN, however, histopathology has demonstrated nongranulomatous vitreitis, retinitis, and retinal and optic nerve perivasculitis (Gass and Scelfo, 1978). In addition, human cases of DUSN have shown that retinal lesions are distinctly tracklike and are associated with the presence of intraretinal or subretinal larvae (Kazacos et al., 1985). These findings are consistent with the retinal pathology in gerbils seen in the present study and suggest that gerbils are a suitable experimental model for DUSN in humans.

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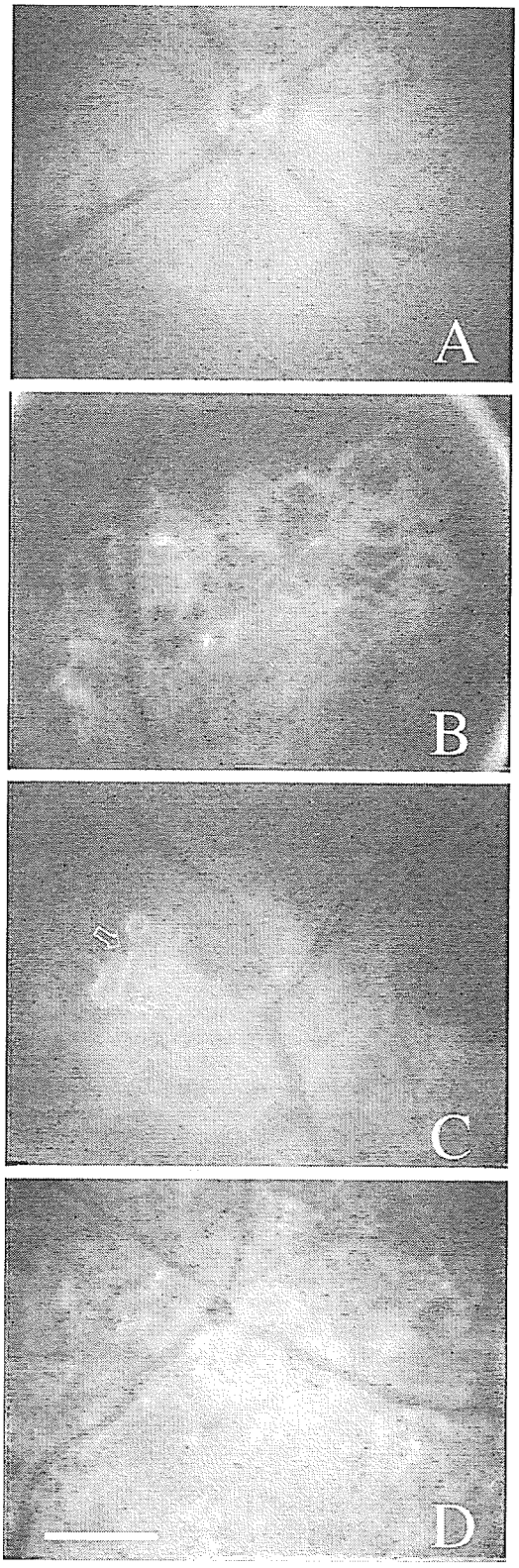

FIGURE 1. Ophthalmologic changes of the fundus in Mongolian gerbil. **A.** Normal fundus of Mongolian gerbils before infection. **B.** Sinuous track induced by *Baylisascaris procyonis* larva, 10 days after infection. **C.** A motile larva (arrows) appeared in the peripheral region of retinitis. **D.** Severe retinochoroiditis in gerbil, 18 days after infection (bar = 1 mm).

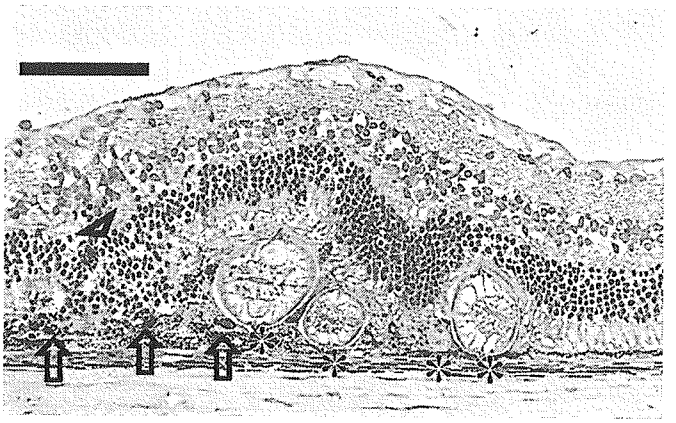

FIGURE 2. Histopathological observation of retina in gerbil, 20 days after infection. Three prominent and 1 faint transverse sections of a larva (*) were seen in the subretinal layer. Note the inflammatory reaction of the retinal pigment epithelium (arrows) and the disruption of the outer nuclear layer (arrow head) (Giemsa, bar = 100 μm).

LITERATURE CITED

AKAO, N., T. H. TAKAYANAGI, R. SUZUKI, S. TSUKIDATE, AND K. FUJITA. 2000. Ocular larva migrans caused by *Toxocara cati* in Mongolian gerbils and a comparison of ophthalmologic findings with those produced by *T. canis*. Journal of Parasitology **86:** 1133–1135.

GASS, J. D., AND R. SCELFO. 1978. Diffuse unilateral subacute neuroretinitis. Journal of the Royal Society of Medicine **71:** 95–111.

KAZACOS, K. R., L. A. RAYMOND, E. A. KAZACOS, AND W. A. VESTRE. 1985. The raccoon ascarid. A probable cause of human ocular larva migrans. Ophthalmology **92:** 1735–1744.

OSHIMA, T. 1961. Standardization of techniques for infecting mice with *T. canis* and observations on the normal migration routes of the larvae. Journal of Parasitology **47:** 652–656.

SATO, H., H. FURUOKA, AND H. KAMIYA. 2002. First outbreak of *Baylisascaris procyonis* larva migrans in rabbits in Japan. Parasitology International **51:** 105–108.

TAKAYANAGI, T. H., N. AKAO, M. TOMODA, S. TSUKIDATE, AND K. FUJITA. 1999. New animal model for human ocular toxocariasis: Ophthalmoscopic observation. British Journal of Ophthalmology **83:** 967–972.

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Recovery, Growth, and Development of *Acanthoparyphium tyosenense* (Digenea: Echinostomatidae) in Experimental Chicks

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ABSTRACT: Chicks were experimentally infected with *Acanthoparyphium tyosenense* (Digenea: Echinostomatidae) metacercariae per os, and the growth and development of worms in this host were observed from days 1 to 38 postinfection (PI). The worms grew rapidly and matured sexually in the small intestine (chiefly in the jejunum) of chicks by day 5 PI, and survived at least up to day 38 PI, although worm recovery decreased after day 5 PI. Both parenchymal and reproductive organs increased greatly in size from day 2 to day 10 PI and then continued to increase gradually in size up to day 38 PI. The number of uterine eggs reached a peak on days 10 and 15 PI and then decreased gradually. The results suggest that chicks are a fairly suitable definitive host for experimental infection with *A. tyosenense*.

Acanthoparyphium Dietz, 1909 (Digenea: Echinostomatidae) is a group of intestinal trematodes for which 14 species, including 1 subspecies, have been reported (Yamaguti, 1958; Chai et al., 2001). Adult flukes have been found in the small intestines of aquatic birds such as ducks, plovers, godwits, knots, and dotterels (Yamaguti, 1939; Chen, 1985). Brackish water gastropods, bivalves, and oysters play the role of first or second intermediate host, or both (Bearup, 1960; Martin and Adams, 1961; Velasquez, 1964; Little et al., 1966; Rybakov and Lukomskaya, 1988; Abdul-Salam and Sreelatha, 1999; Chai et al., 2001).

The only known species of *Acanthoparyphium* that infects humans is *A. tyosenense* Yamaguti, 1939; 10 cases of infection in humans have been reported from the Republic of Korea (Chai et al., 2001). The patients had eaten various kinds of brackish water mollusks from an estuary near their villages, and metacercariae of *A. tyosenense* were discovered in bivalves, including *Mactra veneriformis* and *Solen grandis*, and in the gastropod *Neverita bicolor* (Chai et al., 2001). The adult parasite was originally identified in naturally infected ducks, *Melanitta fusca stejnegeri* and *M. nigra americana*, from Korea (Yamaguti, 1939).

Experimental infection of chicks with metacercariae of *A. tyosenense*, isolated from infected mollusks, was successful (Chai et al., 2001). However, detailed information on the suitability of chicks as an experimental definitive host is lacking, especially in terms of the sexual maturation and survival of worms. In the present study, the recovery, growth, and development of worms were chronologically observed in chicks, thereby establishing the suitability of chicks as a definitive host for *A. tyosenense*.

Metacercariae were obtained from bivalves, *M. veneriformis*, purchased from a market near Kyehwa-myon, Puan-gun, Chollabuk-do, Republic of Korea, where the human infections were discovered (Chai et al., 2001). They were isolated from muscles and gills of the bivalves using a sharp, pointed pin and a stereoscope. After placing on glass slides, a cover slip was placed on the specimen and gentle pressure was applied. The parasites were then individually identified using a light microscope. Metacercariae, 0.42–0.46 mm in diameter, with 23 collar spines and dark and extensive excretory bladder (Chai et al., 2001) were selected as those of *A. tyosenense*. About 30 metacercariae were excysted under a cover slip, by quickly applying high pressure with the pin on the cover slip, and observed morphologically.

Thirty domestic chicks (3 days old), *Gallus domesticus*, were purchased from a hatchery in Kyonggi-do (Province), Republic of Korea. They were infected orally with 50–150 metacercariae per individual, killed by cervical dislocation on days 1, 2, 3, 4, 5, 10, 15, 20, and 38 postinfection (PI), and worms were recovered from their small intestines (Table I). Briefly, small intestines were resected, opened longitudinally in a petri dish containing saline, and examined for worms using a stereomicroscope. Excysted metacercariae, juveniles, and adults were fixed in 10% neutral formalin under cover slip pressure, stained with Semichon's acetocarmine, dehydrated with a graded series of ethanol, and mounted in Canada balsam. The degree of development and the maturity of worms were observed using a light microscope, and 10 specimens in each age group (including excysted metacercariae) were measured. Measurements (in millimeters) are expressed as mean and standard deviation values.

From day 1 to day 38 PI, a total of 152 worms, juveniles, or adults, was recovered from 30 chicks that had been infected with a total of 2,050 metacercariae (Table I). The average worm recovery rate per chick was 7.4%, and this varied from 3.5 to 15.5% according to the number of days PI. There was a tendency for the worm recovery to decrease after day 5 PI. Almost all the flukes were recovered from the jejunum, but some were also recovered from the ileum if the infection progressed to more than 10 days (data not shown). Fecal eggs were first observed on day 10 PI and detected continuously up to day 38 PI.

In excysted metacercariae (Fig. 1), the presence of the oral sucker, with a prominent head crown and collar spines, the ventral sucker, located near the equatorial line of the body, and 4 genital primordia was recognizable. The primordium of the cirrus pouch was seen near the anterior margin of the ventral sucker, and 3 other primordia were visible in the posterior part of the body.

Worms recovered from day 1 to day 4 PI were in juvenile stages of development. One-day-old juveniles (Fig. 2) were slightly larger than excysted metacercariae, and 2-day-old juveniles (Fig. 3) were twice the size of excysted metacercariae. Genital primordia increased in size but

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FIGURES 1–8. Photographs showing the growth and development of *Acanthoparyphium tyosenense* in experimental chicks up to day 15 PI. Scale bar = 0.2 mm. **1.** Excysted metacercaria, showing 4 genital primordia; 1 is seen anterior to the ventral sucker and 3 in the posterior part of the body. **2.** One-day-old juvenile worm. The primordia of the cirrus pouch, ovary, and 2 testes, from anterior to posterior direction, became more apparent. **3.** Two-day-old juvenile worm, showing further development of the primordia of reproductive organs. The development of vitellaria is recognizable at this time. **4.** Three-day-old juvenile worm showing the elongated cirrus pouch passing beyond the ventral sucker, the appearance of the Mehlis' gland and ovary, and the 2 greatly enlarged testes. **5.** Four-day-old juvenile worm. All male and female reproductive organs show their full maturity except for the uterus that contains no recognizable eggs. Vitelline follicles are extended from the posterior extremity to the level of posterior margin of the Mehlis' gland. **6.** Five-day-old adult showing full maturation of all reproductive organs. The seminal vesicle is filled with sperm, and the uterus contains many eggs. **7.** Ten-day-old adult showing the increased length and width of the seminal vesicle and an increased number of uterine eggs. **8.** Fifteen-day-old adult. Because of its remarkably enlarged posterior body, the anterior testis level became the equatorial portion of the worm. OS, oral sucker; CP, cirrus pouch; VS, ventral sucker; SV, seminal vesicle; U, uterus; OV, ovary; MG, Mehlis' gland; AT, anterior testis; PT, posterior testis; VT, vitellaria.

Optical Coherence Tomographic and Angiographic Findings of a Case With Subretinal *Toxocara* Granuloma

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PURPOSE: To study a case with a subretinal *Toxocara* granuloma.

DESIGN: Interventional case report.

METHODS: A patient with a exudative macular lesion, diagnosed as ocular toxocariasis, was examined with optical coherence tomography (OCT) and angiography before and after systemic corticosteroid and anthelminthic therapy.

RESULTS: In OCT images, the macular granuloma initially appeared as a highly reflective mass protruding above the retinal pigment epithelium and showed dye leakage by angiography. Posttherapy, the lesion was no longer exudative, was less elevated, and was covered by the retinal pigment epithelium. There was reticular hyperfluorescence surrounded by a hypofluorescent rim in the angiograms.

CONCLUSIONS: Subretinal *Toxocara* granuloma may have a presentation similar to idiopathic choroidal neovascularization (CNV) and should be included in the differential diagnosis of the idiopathic CNV. (Am J Ophthalmol 2003;136:188–190.

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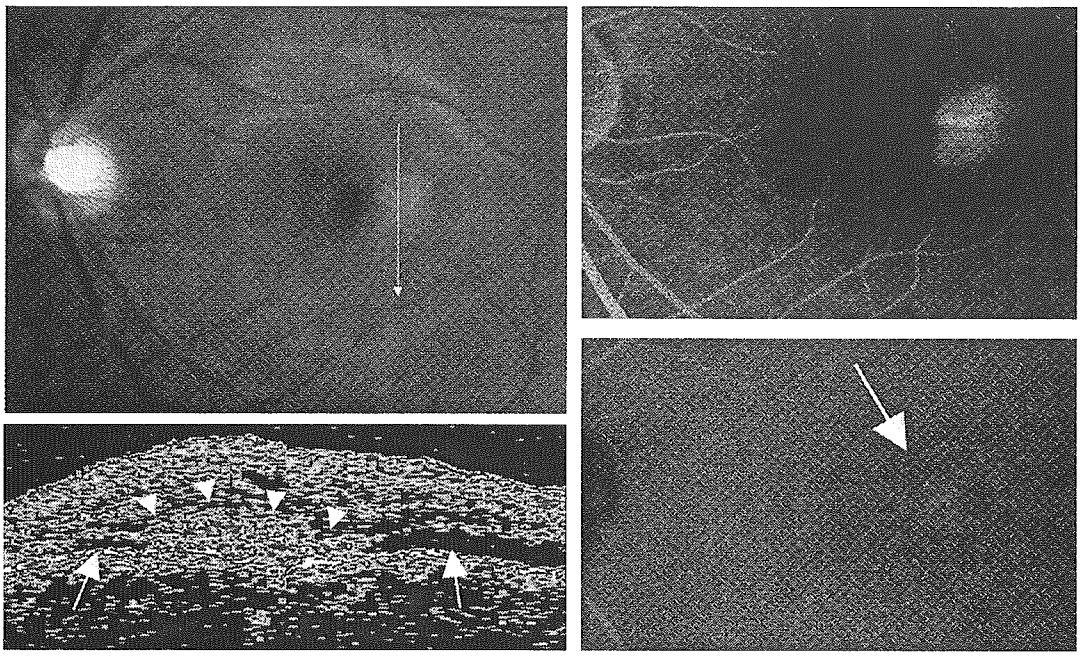

FIGURE 1. The *Toxocara* granuloma at the initial examination. (Top left) Fundus photograph shows an ill-defined yellowish elevated lesion with serous retinal detachment. The direction of the optical coherence tomographic scan is shown by the arrow. (Top right) Late-phase fluorescein angiogram shows dye leakage from the center of the macular lesion. (Bottom left) Optical coherence tomography shows a highly reflective mass protruding above the retinal pigment epithelium (arrowheads) and surrounding subretinal fluid (arrows). (Bottom right) Late-phase indocyanine green angiogram shows a relatively hyperfluorescent macular lesion due to dye leakage (arrow).

A POSTERIOR POLE GRANULOMA[1] IS A TYPICAL PRESENTATION of ocular toxocariasis. Early subretinal lesions without uveitis or tractional changes has not been well characterized, however. We studied the features of a subretinal *Toxocara* granuloma with optical coherence tomography (OCT) and fluorescein and indocyanine green angiography.

A 26-year-old woman presented with metamorphopsia and a paracentral scotoma in her left eye of two weeks' duration. Her best-corrected visual acuity was 20/100 in the left eye, and slit-lamp examination was normal. An ill-defined yellowish elevation with a serous retinal detachment was present in the macula of the left eye (Figure 1). No abnormalities were seen in the right eye.

Upon OCT examination, a highly reflective mass, surrounded by subretinal fluid, was seen protruding above the retinal pigment epithelium (Figure 1). Fluorescein angiography demonstrated central hyperfluorescence of this macular lesion with late-phase dye leakage (Figure 1). Indocyanine green angiography demonstrated an oval hypofluorescent lesion in the early phase that became relatively hyperfluorescent from dye leakage in the late phase (Figure 1).

A clinical diagnosis of ocular toxocariasis was made from a positive history of repeated ingestion of raw liver, which is regarded as a significant risk factor of *Toxocara* infestation[2]; by detection of serum antibody to antigens from second-stage larvae of *Toxocara canis* by enzyme-linked immunosorbent assay; and by exclusion of other ocular conditions causing exudative macular lesions.

She was started on oral prednisone (30 mg daily, tapered over 2 months) and diethylcarbamazine (100 mg daily for 3 days, 300 mg daily for 3 days, followed by 300 mg once a week for 8 weeks). The serous retinal detachment gradually decreased, and the macular lesion became smaller and more clearly bordered with pigment.

Three months after the first examination, her visual acuity had improved to 20/20. Upon OCT examination, the macular lesion had decreased in elevation and was covered by the retinal pigment epithelium without signs of retinal edema (Figure 2). Fluorescein and indocyanine green angiography disclosed an oval-shaped, reticular hyperfluorescent lesion with minimal dye leakage surrounded by a hypofluorescent rim (Figure 2). The macular lesion remained stable at 6 months' follow-up.

In our patient, *Toxocara* granuloma had a subretinal extension in OCT images and resembled an idiopathic choroidal neovascularization (CNV) in the active stage.[3,4] The granuloma responded to the systemic corticosteroid and anthelminthic therapy; it was no longer exudative and was confined below the retinal pigment epithelium in the OCT images. Angiography revealed a hypofluorescent rim surrounding the CNV-like reticular hyperfluorescence in

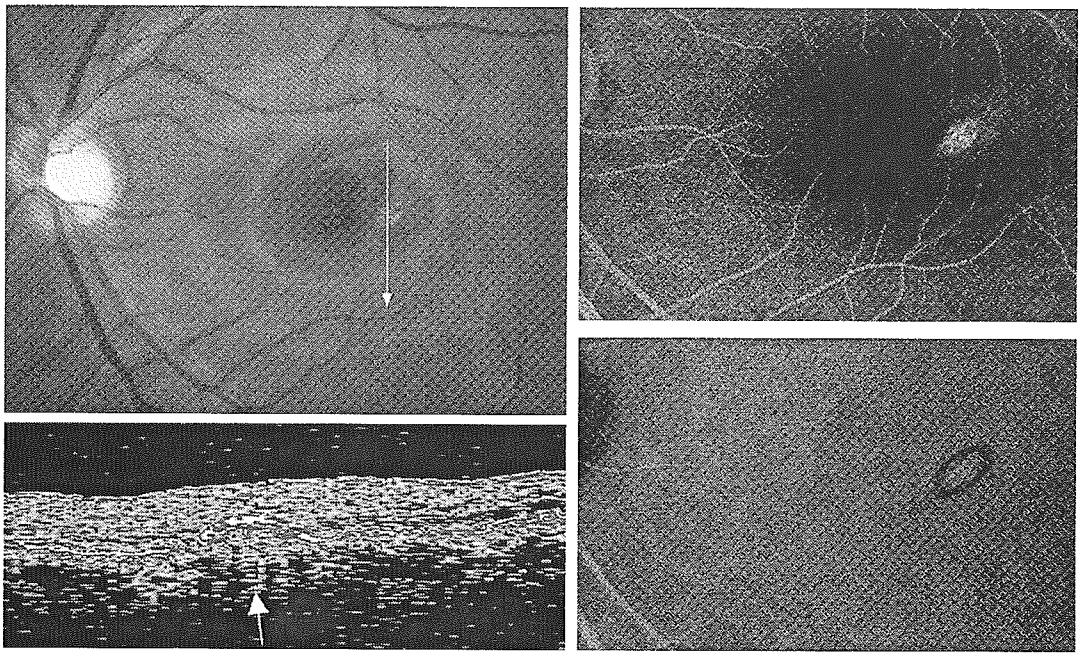

FIGURE 2. The *Toxocara* granuloma 3 months after onset. (Top left) Fundus photograph shows a pigmented lesion without exudation. The arrow indicates the location and direction of the scan by optical coherence tomography. (Top right) Late-phase fluorescein angiogram shows an oval-shaped reticular hyperfluorescent lesion surrounded by a hypofluorescent rim with minimal dye leakage. (Bottom left) Optical coherence tomography shows an elevated lesion confined below the retinal pigment epithelium (arrow). (Bottom right) Late-phase indocyanine green angiogram shows an oval-shaped, hyperfluorescent lesion surrounded by a hypofluorescent rim.

the lesion. The transition to a subretinal pigment epithelium location and the emergence of a hypofluorescent rim are, respectively, characteristic OCT[4] and indocyanine green angiographic[5] findings of idiopathic CNV in the involution stage, indicating the envelopment of the CNV by the retinal pigment epithelium. Thus, retinal pigment epithelium proliferation may play an important role in the regression of a granuloma and of CNV.

In conclusion, subretinal *Toxocara* granuloma can have a presentation similar to idiopathic CNV in OCT images and angiograms and therefore should be included in the differential diagnosis of the idiopathic CNV.

REFERENCES

1. Ashton N. Larval granulomatosis of the retina due to *Toxocara*. Br J Ophthalmol 1960;44:129–148.
2. Salem G, Schantz P. Toxocaral visceral larva migrans after ingestion of raw lamb liver. Clin Infect Dis 1992;15:743–744.
3. Iida T, Hagimura N, Sato T, Kishi S. Optical coherence tomographic features of idiopathic submacular choroidal neovascularization. Am J Ophthalmol 2000;130:763–768.
4. Fukuchi T, Takahashi K, Ida H, Sho K, Matsumura M. Staging of idiopathic choroidal neovascularization by optical coherence tomography. Graefes Arch Clin Exp Ophthalmol 2001;239:424–429.
5. Iida T, Hagimura N, Kishi S, Shimizu K. Indocyanine green angiographic features of idiopathic submacular neovascularization. Am J Ophthalmol 1998;126:70–76.

Bilateral Optic Disk Melanocytoma in a 10-month-old Infant

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PURPOSE: To report bilateral optic disk melanocytoma in a healthy infant,

METHOD: Case report.

RESULTS: A 10-month-old healthy boy presented with intermittent right exotropia. Dilated fundus examination showed a heavily pigmented mass within both optic disks. The optic disks were hypoplastic, and there was diffuse retinal pigment epithelial mottling around the optic disks. Systemic evaluation including thyroid function tests, pituitary gland hormone levels, adrenal gland hormone levels, and magnetic resonance imaging of the brain revealed normal findings. After 1 year, the lesions re-

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