Invasion of *Metagonimus yokogawai* into the submucosal layer of the small intestine of immunosuppressed mice

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**Abstract:** *Metagonimus yokogawai* was found deeply invaded into the submucosa of the small intestine of mice (ICR) when they were immunosuppressed by prednisolone injection. Experimental groups consisted of control, fluke infection (1,800 metacercariae per mouse), and fluke infection plus immunosuppression. In fluke infection group, many worms were found sectioned in the intussusception space of the jejunum and ileum at 6 hrs, 12 hrs, and 1 day after infection, and pathological changes characterized by villous atrophy and crypt hyperplasia were observed. After 3 days, only a few worms were found in intestinal sections, and after 7 days, the pathological changes became minimal. No worm was found penetrated beyond the mucosal layer. On the other hand, in immunosuppressed mice, numerous worms were found sectioned in the duodenum and jejunum, irrespective of the infection period up to 14 days. Pathological changes of the mucosa were minimal until 3 days after infection, but at 5 days marked destruction of the mucosal layer was observed. At this time many flukes were found invaded deeply into the submucosa facing the muscular layer. Despite continuous immunosuppression, the mucosal damage was gradually recovered at 7-21 days post-infection. The results showed that immunosuppression of ICR mice can induce, for a short period of time, severe mucosal damage, and allow deep invasion of *M. yokogawai* into the submucosa of the small intestine.

**Key words:** *Metagonimus yokogawai*, prednisolone, immunosuppression, intestinal pathology, worm invasion

**INTRODUCTION**

*Metagonimus yokogawai* (Heterophyidae) is well known as one of the most common trematodes infecting the intestinal tract of man in Korea (Chai and Lee, 1990). The most frequent symptoms complained by the infected patients are abdominal pain and diarrhea (Cho et al., 1984). This fluke has drawn medical attention because of such clinical symptoms, high prevalence, and wide geographical distribution in Korea (Song et al., 1985, Ahn and Ryang, 1988).

The major histopathological changes in the small intestine of animals and man infected with *M. yokogawai* are villous atrophy and crypt hyperplasia with inflammatory cell infiltration in the stroma of villi (Chai, 1979; Lee et al., 1981; Kang et al., 1983; Chi et al., 1988). Worms are most frequently found facing the crypt of Lieberkühn or between the villi, and invasion of worms is confined to the mucosal layer (Kang et al., 1983; Rho et al.,

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* Received Oct. 20 1995, accepted after revision Nov. 15 1995.
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Table 1. Experimental design for observation of intestinal histopathology due to *M. yokogawai* infection in immunocompetent and immunosuppressed ICR mice

<table>
<thead>
<tr>
<th>Interval from infection to sacrifice</th>
<th>Immunocompetent mice</th>
<th>Immunosuppressed mice</th>
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<tr>
<td></td>
<td>No. mice used</td>
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<td>6 hrs</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>24</strong></td>
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Number of uninfected control mice: 6

1984; Jang et al., 1985).

In other heterophyid flukes such as *Haplorchis* spp., *Procerovum* spp., and *Stellantchasmus falcatus*, however, extraintestinal migration of adult flukes or their eggs were reported, making granulomas in the heart, brain and spinal cord of man (Africa et al., 1940). Yokogawa (1940) tried to explain this phenomenon with an hypothesis that those patients might have suffered from severe malnutrition causing remarkable decrease of immune responses to the parasites. Without immune responses, worms could invade into the submucosal layer, and eggs and/or fluke themselves could be carried to other organs through the blood stream.

However, there has been no experimental proof to support this hypothesis. Moreover, in the case of *M. yokogawai*, extraintestinal metagonimiasis has never been reported in the literature, and it is unknown whether they can invade into the submucosa in immunocompromised hosts. This study was undertaken to know whether *M. yokogawai* could invade into the submucosal layer of the small intestine when ICR mice were immunosuppressed by prednisolone injection.

**MATERIALS AND METHODS**

Metacercariae of *M. yokogawai* were isolated from the muscle of the sweetfish, *Plecoglossus altivelis*, caught from Tamjin-gang[River], Chollanam-do, Korea. The mortar-ground fish flesh was mixed with 10-fold volume of artificial gastric juice, and the mixture was incubated at 37°C for longer than 12 hrs. The freed metacercariae were collected under dissecting microscope, and preserved at 4°C until use.

A total of 54 male mice (ICR strain) weighing about 20 g were divided into three groups: 6 uninfected control group, 24 fluke infection group, and 24 fluke infection plus immunosuppression group. The latter two groups of mice were orally infected each with 1,800 metacercariae through a polyethylene capillary tube, 1.2 mm in diameter, under slight anesthesia with ether. Three mice each were sacrificed by cervical dislocation at 6 hrs, 12 hrs, 1 day, 3 days, 7 days, 14 days, and 21 days post-infection (Table 1).

For fluke infection plus immunosuppression group, the mice were intramuscularly injected with 10 mg/kg prednisolone every other day to the inner thigh starting from 7 days prior to infection until sacrifice.

For microscopic examinations, segments of the duodenum, jejunum and ileum were resected, fixed in 10% formalin, and processed for routine hematoxylin and eosin stain. When more than two of three mice in each experimental group revealed the same histopathological findings, the results were interpreted as valid ones.
RESULTS

1. Uninfected control mice
Sections of the duodenum, jejunum and ileum revealed long and slender villi, and their villus/crypt (V/C) ratio was about 1.5-3.0:1. Villous epithelial layers were preserved well. But occasionally non-specific inflammatory cell infiltrations were observed in the villous stroma.

2. M. yokogawai-infected mice
From 6 hrs to 1 day post-infection, a lot of sectioned worms were found in the small intestine of mice infected with the metacercariae of *M. yokogawai*. Worms were located in relatively deep mucosal layer, that is, just above the proximal part of the Lieberkühn's crypt. Pathological changes appeared in all sections of the small intestine, however, the changes and sectioned worms were mainly observed in the jejunum and ileum. At 6 to 12 hrs, the villi adjacent to worms were damaged by compression. Especially, edematous change or lymphatic dilation was observed near the tip portions of the villi. However, there were no inflammatory cell infiltrations in the villous stroma. In 1 day group, the worms were somewhat enlarged in size. The neighboring villi showed flattening of epithelial cells and pressure atrophy of epithelial layers (Fig. 1). Marked crypt hyperplasia was also observed in the same sections (Fig. 1). Inflammatory cell infiltrations were noted in the villous stroma, and almost all of them were composed of neutrophils (Fig. 2).

At 3 days after infection, worms were not found easily except in a few sections, and pathological changes were gradually restored. At this time, the extent of pathological changes of the mucosa was generally similar to that of 1 day group. However, exceptionally, there was marked increase of inflammatory cell infiltrations, composed of many neutrophils and a few eosinophils, and V/C ratio was decreased to about 1:1 due to shortening of villi and hyperplasia of crypts.

At 7 days the pathological changes became minimal and at 21 days the mucosa became completely normalized.

3. M. yokogawai-infected immunosuppressed mice
Many sectioned worms of *M. yokogawai* were found throughout the experimental period in immunosuppressed mice (Figs. 3-8). Immunosuppression of mice brought about different results on the worm location and pathological changes in the small intestine. The sectioned worms and pathological changes were mainly observed in the duodenum and jejunum. The ileum was no more the main parasitic location, which is different from the infected immunocompetent mice. Inflammatory reactions were also remarkably reduced compared with the immunocompetent mice.

At 6 hrs, 12 hrs, and 1 day post-infection, the pathological changes were similar to one another. Juvenile worms were observed in the intervillous space of the jejunum and ileum. There was no atrophy of the villous epithelial layer and inflammatory cell infiltration in the villous stroma (Fig. 3). Edematous changes were occasionally observed near the tip of the villi.

At 3 days post-infection, the main parasitic location was changed into a more proximal portion, i.e., the duodenum and jejunum. Although some edematous changes of the villi were recognizable (Fig. 4), no other remarkable pathological changes were noted in the architecture of the villi and crypts, and cellular composition of the villous stroma.

At 5 days, however, two of three mice showed marked destruction of the mucosal layer especially in the duodenum (Figs. 5-8). Villi were degenerated and detached from the intestinal wall, and epithelial layers of the crypts were destroyed (Figs. 5-8). The intestinal lumen was filled with intestinal contents and degenerating mucosal debris (Figs. 5-7). The muscularis mucosa was also destroyed, so that the thickness of the intestinal wall became markedly thin, and many worms were found intruded into the submucosal and even muscular layers (Figs. 5-8). Their oral suckers were facing and compressing the thin intestinal wall (Figs. 7 & 8). The remaining basal portion of the villi adjacent to worms was compressed (Fig. 5). Degeneration and loss of all mucosal and/or submucosal layers were
Fig. 1. Jejunal villi of another immunocompetent mouse, 1-day post-infection. Two juvenile worms are seen between three or four villi, which show atrophy of the lining epithelial layer. Crypts show hyperplasia. H-E stain, × 45. Fig. 2. Jejunal villi of an immunocompetent mouse, 1-day post-infection. A juvenile worm is facing the crypt in a space between two villi and inflammatory cells are infiltrated in the stroma of villi. H-E stain, × 100. Fig. 3. Ileum of an immunosuppressed mouse, 1-day post-infection. A juvenile worm is seen in the intervillous space not intruding into the crypt. H-E stain, × 45. Fig. 4. Duodenum of an immunosuppressed mouse, 3-day post-infection. Villi show their normal contour with few inflammatory reaction in the stroma. However, dilatation of the lymphatic space at the tip portion of the villi is recognizable. H-E stain, × 45.

often noticed, and around these areas, worms were found deeply invaded into the submucosa facing the muscular layer (Figs. 6-8). Until this time, worms were not fully matured (Figs. 5-8).
Fig. 5. Duodenum of an immunosuppressed mouse, 5-day post-infection. The villi have lost their normal contour. Two worms are seen sectioned at the basal portions of villi and they are about to intrude into the crypt. H-E stain, × 45. Fig. 6. Duodenum of an immunosuppressed mouse, 5-day post-infection. Villi and crypts, i.e., mucosa, are completely destroyed, which allowed deep penetration of worms (arrows). H-E stain, × 100. Fig. 7. Duodenum of another immunosuppressed mouse, 5-day post-infection. An adolescent fluke is actively penetrating into the basal portion of the crypt and pressing the muscle layer (arrows). H-E stain, × 45. Fig. 8. Other portion of the duodenum of an immunosuppressed mouse, 5-day post-infection. An adolescent worm is facing the muscle layer of the intestinal wall, where the mucosa and submucosa have been completely destroyed. The oral sucker (OS) and pharynx (P) of the worm are visible. H-E stain, × 100.

At 7 days post-infection, the pathological changes began to restore, and eggs of M. yokogawai were found sectioned in worm uteri. Crypts showed nearly normal feature, and worms were observed in the intervillous space just above the crypt. At 14 and 21 days, pressure atrophy of villi was still recognizable, but the mucosa generally showed their normal features.
DISCUSSION

It is well known that susceptibility of animal hosts to certain parasitic infection is widely variable according to different species of host animals, and even among different strains of animals, due to different genetic backgrounds (Stirewalt et al., 1965; Colley, 1972; Murrell et al., 1979). In the case of *M. yokogawai*, dogs, cats, and hamsters are known to be highly susceptible (Takahashi, 1929; Koga, 1938; Yokogawa and Sano, 1968; Kang et al., 1983), whereas small laboratory animals such as mice, rats and guinea pigs are in general less susceptible (Koga, 1938; Gushima, 1939; Yokogawa and Sano, 1968; Kagei and Kihata, 1970; Chai, 1979).

Among several strains of mice studied, KK (diabetic) mice revealed the highest susceptibility and C57BL mice the next, whereas CBH, DBA, A or ICR mice showed very low susceptibility to *M. yokogawai* infection (Chai et al., 1984). Especially ICR mice, which was also used in the present study, showed less than 1% worm recovery rate at 3-7 days post-infection in immunocompetent mice. When ICR mice were immunosuppressed, however, the worm recovery rate was elevated up to 60-80% at the same post-infection days (Chai et al., 1984), which indicated that the genetic susceptibility is closely related with immune responses of the host.

In the present study, at early stages of infection (at 1-3 days), immunocompetent ICR mice showed remarkable pathological changes with many sectioned worms in the small intestine, although the changes and worm location were confined to the mucosal layer. At 3-7 days, spontaneous resolution of the mucosal pathology was observed, and only a few worms were found in sections of the small intestine. Such a rapid spontaneous resolution of the mucosal pathology is considered due greatly to expulsion of worms, which is a common and prominent feature in less susceptible animals. Hence, in this study, the susceptibility of ICR mice to *M. yokogawai* infection is estimated also very low.

It is well known that prednisolone has a pronounced anti-inflammatory action, and compromises both humoral (Fischel et al., 1952) and cellular immune responses (Gilman et al., 1991). The drug also weakens the mucosal layer of the gastrointestinal tract and makes ulcers (Gilman et al., 1991). Therefore, prednisolone injection into host animals could allow persistence, invasion, and destructive behavior of parasites. In the case of trichinosis, for example, administration of prednisolone improved clinical symptoms, however, it allowed long persistence of numerous adult worms in the intestine and migration of a lot of larvae to skeletal muscles due to deterioration of host protective mechanisms (Coker, 1955). Similarly, immunosuppressed mice infected with *Trichuris muris* showed increased severity of colitis probably due to persistence of worm infection (Jenkins and Wakelin, 1994). Grove et al. (1983) also reported that immunosuppressed dogs infected with *Strongyloides stercoralis* showed a disseminated infection occurring in ectopic sites, and increased pathological lesions in the small intestine and colon.

In the present study, persistence of *M. yokogawai* worms was remarkable and host responses including inflammatory reactions of the mucosa were very weak in immunosuppressed mice. These findings represent that immunosuppression of ICR mice by prednisolone injection was successful. The extent of intestinal pathology due to *M. yokogawai* infection in immunosuppressed mice was minimal until 3 days after infection. However, after 5 days, probably due to fragility of the mucosa and persistence of many worms, there was marked destruction of mucosal layers in the duodenum and jejunum, and complete obliteration of mucosal integrity was frequently observed. At this time, the flukes were found to have invaded deeply into the submucosa facing the muscular layer of the intestinal wall. It is strongly suggested, therefore, that if the mucosal immune responses were suppressed *M. yokogawai* could easily invade into the submucosal layer. In such cases it is further suggested that adult flukes themselves and/or their eggs might flow into the blood stream via exposed small vessels, and it would be possible to make fatal egg granulomas in the heart, brain, and spinal
cord like other kinds of heterophyid flukes (Africa et al., 1940).

Posterior migration of helminths in the intestinal tract has been reported in relation to the immunity or susceptibility of host animals (Hong and Seo, 1969; Wastling et al., 1990; Hong et al., 1990). When administered a specific T cell-suppressing drug to Hymenolepis diminuta-infected mice, it was shown that posterior migration of worms was restricted in a dose-dependent manner (Wastling et al., 1990). It was also reported that as infection period elapsed M. yokogawai worms shifted down to the lower parts of the small intestine or even to the large intestine (Hong and Seo, 1969). Posterior migration of worms in less susceptible hosts is also known in another intestinal fluke, Heterophyopsis continua (Hong et al., 1990).

In the present study, there was a significant difference in the location of M. yokogawai between immunocompetent and immunocompromised ICR mice. In immunocompetent mice, the main worm location was the jejunum and ileum, but it was changed into a proximal part, the duodenum and jejunum, in immunosuppressed mice. It is already known that in highly susceptible animals to M. yokogawai infection such as dogs and cats, the main worm location is the upper part of the small intestine (Ito, 1964; Lee et al., 1981). Therefore, we presume that the worms found in the duodenum of immunosuppressed mice would parasitize well, but those found in the ileum of immunocompetent mice would be expelled before long.

However, it was difficult to explain that, despite continuous immunosuppression, the number of worms sectioned was decreased and mucosal pathology was restored after 14 days post-infection in immunosuppressed mice. If immunosuppression is the sole responsible factor for the worm persistence and mucosal pathology, the worms should have survived longer than actually observed, and pathological changes of the mucosa should have continued or aggravated as infection progressed. In this respect, we suggest that there should be other factors affecting persistence of worms and restoration of mucosal pathology in M. yokogawai-infected mice.

In conclusion, immunosuppression of ICR mice by prednisolone injection can induce, for a short period of time, severe mucosal damage and deep invasion of M. yokogawai into the submucosal layer of the small intestine. In order to clarify the mechanisms of worm invasion, mucosal damage and restoration, further studies are required.

REFERENCES


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면역억제 마우스에 있어서 요코가와흡충의 소장 점막하 조직 침입

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요코가와흡충을 실험적으로 마우스(ICR)에 감염시키고 프레드니솔론을 주입하여 마우스의 면역 반응을 저하시킬 때 장의 병변과 충혈 관리부위의 변화를 알아보기 위하여 이 연구를 시행하였다. ICR계 마우스를 비특별 대조군, 요코가와흡충 감염군(마리냥 피남유충 1,800개 감염), 및 요코가와흡충 감염/면역억제군으로 나누고 감염 후 6시간부터 3주까지 관찰하였다. 요코가와흡충 감염군에 있어서는 감염 1일까지 많은 충혈과 장 점막내에서 발견되었고, 장 병변은 점막에 국한되어 있었다. 그러나 감염 3일 이후부터는 충혈과 점막이 완화되지 않았고, 1주 후에는 이미 장 병변이 외부화 되어 가는 양상을 보였다. 한편 면역억제 마우스에 있어서는 감염군과도 달리 점막내에서 전 기간동안 충혈이 발견되었고 감염이 오랫동안 유지되었다. 염증 반응은 전 기간을 통해 미미하였고, 감염 3일까지 장 병변도 염증의 부종이나 상피층의 가변은 위축 외에는 거의 관찰되지 않았다. 그러나 감염 5일째에는 마우스 십이지장 및 공장의 홍모 및 장선이 매우 심하게 파괴되고, 장벽으로부터 밖으로의 양상을 보였으며 이때 충혈은 점막하 조직을 통과하고 거의 근육층까지 침범하여 장벽을 박해하고 있었다. 이러한 소견은 계속된 면역억제에서도 불구하고 감염 7일부터 21일까지 점막 침범이 진행되며, 이상의 결과는 요코가와흡충을 면역억제 마우스에 감염시키면 빨간 가간 동반 장 점막의 심한 파괴가 일어나고, 점막하부까지 충혈이 침범할 수 있음을 나타내었다.