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Parasitic forms of a myxosporean in the kidney of the arctic lamprey, *Lampetra japonica* : An ultrastructural study

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Abstract

We found frequent and unique parasitism by an unidentified myxosporean in the kidney of the arctic lamprey, *Lampetra japonica*, living in Japan. Trophozoites (pseudoplasmodia with or without sporoblasts) existed predominantly in the lumina of proximal urinary tubules, but were rarely found in any other regions of the kidney. Since no mature spores were produced in the trophozoites, exact identification of the species was impossible. Two parasitic forms were recognized in proximal urinary tubules : one adhering to the epithelial cells of renal tubules, and the other free-floating in the lumina of tubules. Ultrastructurally, the attaching trophozoites developed microvilli-like projections towards the apical surface of epithelial cells and vigorously interdigitated with microvilli of the brush border. In contrast, the whole surface of the floating trophozoite was smooth without any cell projections. The developed projections in the former type of trophozoite may contribute to their firm attachment to the epithelial cells and/or to absorption of nutrients via the epithelial cells. Against the myxosporean infection, the lamprey as the host exhibited a local immune reaction by disposition of numerous lymphocytes and macrophages into the epithelium of urinary tubules.

Key words : Electron microscopy, Kidney, Lamprey, Myxosporea

Introduction

Myxosporeans are well-known as fish parasites and many of them live in the kidney⁸⁾. Although numerous studies have docu-

mented infection of myxosporea in the fish kidney, most of them focused on taxonomy ; few studies have dealt with host-parasite interaction and pathogenicity. Moreover, no information is available on the myxosporean in

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cyclostomata.

Myxosporea are divided into two types according to their parasitic location in the host: histozoic species, which are parasitic on various parenchymal tissues inter- or intracellularly, and coelozoic species, which live in body cavities such as the gall bladder, bile ducts, and the urinary tract. Some coelozoic species have been reported to adhere to the apical surfaces of host cells by anchoring cell projections¹⁹⁾. Nevertheless, the morphology of the cell projections and their topographical relationship with host cells vary among species^{5, 17, 18)}, and their functional significance remains unclear. On the other hand, although it is generally accepted that myxosporea cause no significant host reactions⁷⁾, recent papers have provided evidence that some of them are capable of inducing defensive responses^{12, 20)}.

The present morphological study deals with a myxosporean from the kidney of the arctic lamprey (*Lampetra japonica*), a cyclostome. Here, we show detailed parasitic forms of the myxosporea and host responses in the kidney by transmission and scanning electron microscopy.

Materials and methods

Thirteen adult female and male arctic lampreys, *Lampetra japonica*, (40–50 cm in length) were captured in the Agano River, Niigata (2/13) and Ebetsu River, Hokkaido (11/13), Japan in March and May 1998, respectively. Three ammocoetes were obtained from the Makomanai River, Hokkaido, in July 1998. Lampreys and ammocoetes were killed by over-exposure to the anesthetic MS-222 (Sigma, St. Louis, Mo., USA). The kidney, intestine, liver and gills were quickly removed and fixed in 10% phosphate-buffered formalin (pH 7.4) at 4 °C for 24 h. Seven or eight different parts were dissected out from the head (rostral) to the tail (caudal) of each kidney.

After fixation, the tissues were embedded in paraffin according to a conventional method. Thin sections, 3–4 µm in thickness, were prepared and stained with hematoxylin-eosin.

For the detection of acid phosphatase, which is a predominant lysosomal enzyme, the formalin-fixed tissues were also dipped in 30% sucrose dissolved in 0.1 M phosphate buffer (pH 7.4) overnight at 4 °C, and rapidly frozen in liquid nitrogen. Frozen sections, 16 µm in thickness, were cut in a cryostat, mounted on poly-L-lysine-coated glass slides and stained according to Burnstone⁴⁾. A control experiment for acid phosphatase reactions was simultaneously carried out by incubation with medium containing 10 mM NaF, a potent inhibitor of this enzyme.

For transmission electron microscopy (TEM), fresh tissues of the kidney were dissected into small pieces and fixed with 2.5% glutaraldehyde in the phosphate buffer for 2 h at 4 °C. The specimens were rinsed in the phosphate buffer and postfixed with 1% aqueous solution of OsO₄ for 1.5 h at room temperature. They were dehydrated through a graded series of ethanol and embedded in Epon 812 according to standard procedures. Semi-thin sections, 1 µm in thickness, were stained with toluidine blue for observation under a light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a JOEL JEM-1210 transmission electron microscope.

For scanning electron microscopy (SEM), small pieces of renal tissue were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer at 4 °C for 4 h. The specimens were conductive-stained by the tannin-osmium method according to Murakami¹⁶⁾, dehydrated through a graded series of ethanol and freeze-cracked in liquid nitrogen. The tissue pieces were dipped in isoamyl acetate and critical-point dried using liquid carbon dioxide. The dried

specimens were evaporation-coated with platinum-palladium and examined under a HITACHI S-4100 scanning electron microscope.

In order to isolate trophozoites adhering to apical surfaces of urinary tubules, some pieces of the fixed renal tissue were processed by a modification of the NaOH maceration method²¹ before the conductive stain. The fixed specimens were rinsed in 0.1M phosphate buffer (pH7.4) and subsequently placed in 6 N NaOH at 60°C for about 15min. After the alkaline maceration, the tissue blocks were degraded into small fragments with a rigorous stream of 0.01M phosphate buffer (pH7.2) ejaculated through a fine pipette.

Results

Light microscopic observation of the lamprey kidney demonstrated numerous round cells, which were 10-30µm in diameter and corresponded to myxozoan trophozoites, in the lumina of urinary tubules (Fig. 1A, B). The trophozoites were found in lampreys captured in both Niigata (50%) and Hokkaido (82%). They were distributed in almost all regions of proximal tubules but rare in Bowman's space and the lumina of distal and collecting tubules. Histochemistry for acid phosphatase showed that these cells stained positively (Fig. 1B). The trophozoites in the urinary tubules were classified into two types: those adhering to the brush border of proximal tubules (Fig. 1A) and those aggregating in the luminal space (Fig. 1B). Although less numerous, similar trophozoites of both adhering and aggregating types were recognized in the urinary tubules of two ammocoetes among three examined. No trophozoites were found in the intestine, liver and gills from either adult lampreys or ammocoetes.

Under TEM, the trophozoites (primary cells) of both adhering and aggregating types

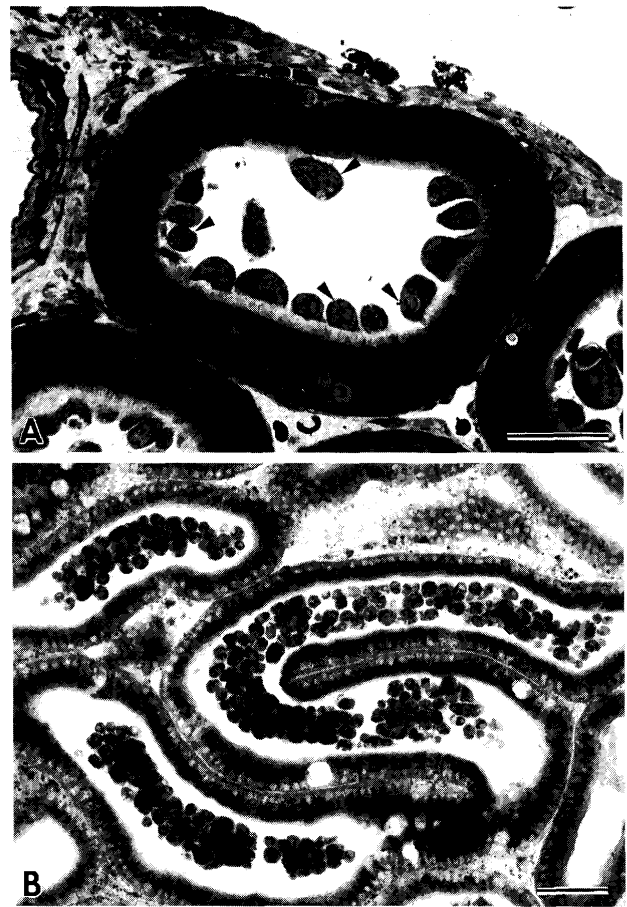


Fig. 1. Adhering-type (A, toluidine blue stain) and aggregating-type trophozoites (B, acid phosphatase stain) in the proximal urinary tubules of the lamprey.

Numerous adhering-type trophozoites containing generative cells (arrowheads) adhere to the brush border (A), while huge clusters of aggregating-type trophozoites that are strongly positive for acid phosphatase occupy the lumen (B). Scale bars: A=50µm; B=100µm.

possessed an oval nucleus and broad cytoplasm, containing lysosome-like electron-dense granules, clear vesicles, mitochondria, lipid droplets and glycogen particles (Fig. 2A, B). The central area of their cytoplasm was frequently occupied by various numbers (from one to ten) of generative cells with a prominent nucleolus and less-developed cell organelles (Fig. 2B). In trophozoites with more advanced sporogony, generative cells developed

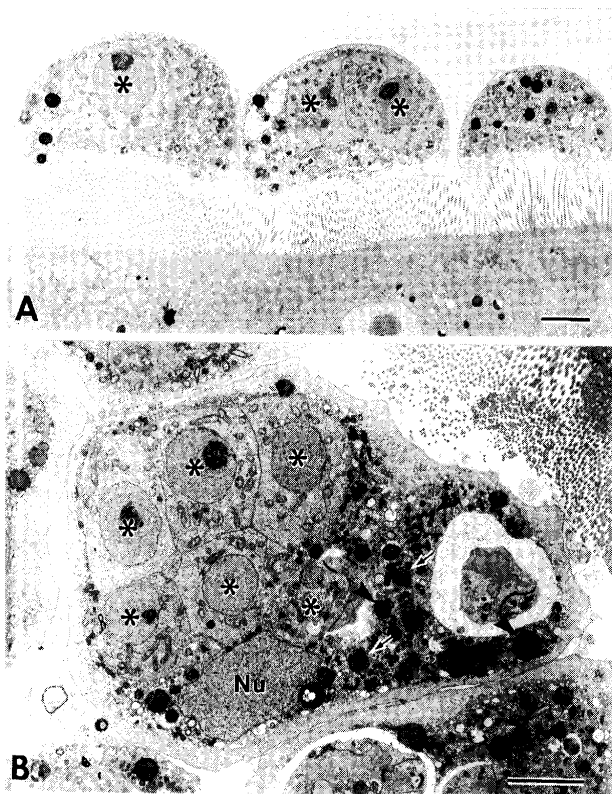


Fig. 2. TEM images of adhering- and aggregating-type trophozoites.

A: Adhering-type trophozoites directly contact the brush border of the proximal tubule; some contain generative cells (asterisks).

B: An aggregating-type trophozoite (primary cells) possesses an oval nucleus (Nu), lysosome-like granules (arrows), lipid droplets (large arrowheads), mitochondria (small arrowheads), and five or six generative cells (asterisks). Scale bars = 5 μ m.

to sporoblasts with sutures of valvogenic cells (Fig. 3A) and formed polar capsules and polar filaments (Fig. 3B). Immature trophozoites occasionally appeared within the epithelium of proximal tubules and were characterized by small electron-dense bodies, possibly sporoplasmosomes, lining the cell surfaces (Fig. 3C). Nevertheless, no mature spores were produced in the trophozoites.

Trophozoites of the adhering type directly contacted the brush border in proximal urinary tubules (Fig. 2A). The trophozoites extended numerous cell projections which inter-

digitated with microvilli of epithelial cells and were not easily distinguished from them in ultrastructure. Their tips tapered among tufts of the brush border; there was no penetration of cell projections into the cytoplasm of epithelial cells and there were no specialized structures at the point of attachment. Very thick, rhizoid-like projections were also observed between the cell projections.

SEM observation of trophozoites adhering to the brush border clearly demonstrated interdigitation of their cell projections with the brush border of the urinary tubules (Fig. 4B, C). Except for the attaching face, the cell surfaces of trophozoites were smooth without any projections (Fig. 4A, B, C). In contrast, the whole surfaces of trophozoites aggregating in the lumen were smooth (Fig. 4D). No structures suggesting a positive interaction with the epithelial cells were observed there. Trophozoites in both Bowman's space and lumen of distal or collecting tubules issued no projections and also displayed smooth surfaces.

Numerous immune cells invaded the epithelium of proximal tubules where many trophozoites occurred in the lumen (Fig. 5A, B). Ultrastructurally, most of them showed characteristics of lymphocytes (Fig. 5A, B). Occasionally, mitotic figures of lymphocytes were detected in the epithelium of proximal tubules. Macrophages possessing a large nucleus and several lysosomes (Fig. 5B), and plasma cells characterized by well-developed rough endoplasmic reticulum were also observed in the epithelium. These immune cells appeared rarely in the epithelia of distal and collecting tubules where few trophozoites existed and so in those of lampreys that were not infected by the myxosporea (data not shown).

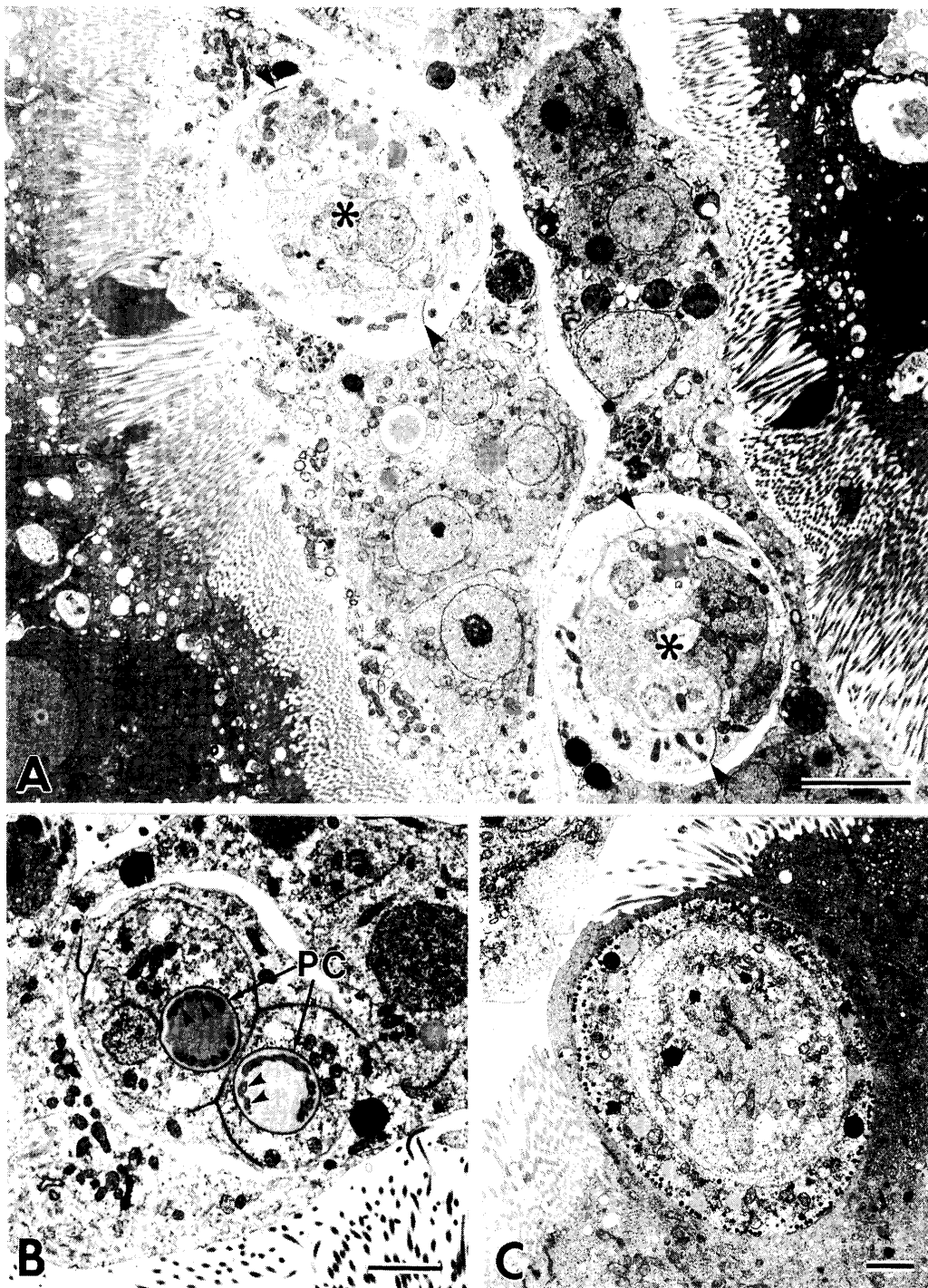


Fig. 3. TEM images of trophozoites.

A: Sporoblasts (asterisks) with sutures of valvogenic cells (arrowheads) are developed in trophozoites. B: Polar capsules (PC) and polar filaments (arrowheads) are seen in the immature spore. C: The epithelium of the proximal tubule contains an early stage of trophozoite with sporoplasmosomes lining the cell surface. Scale bars: A = 5 μm ; B and C = 2 μm .

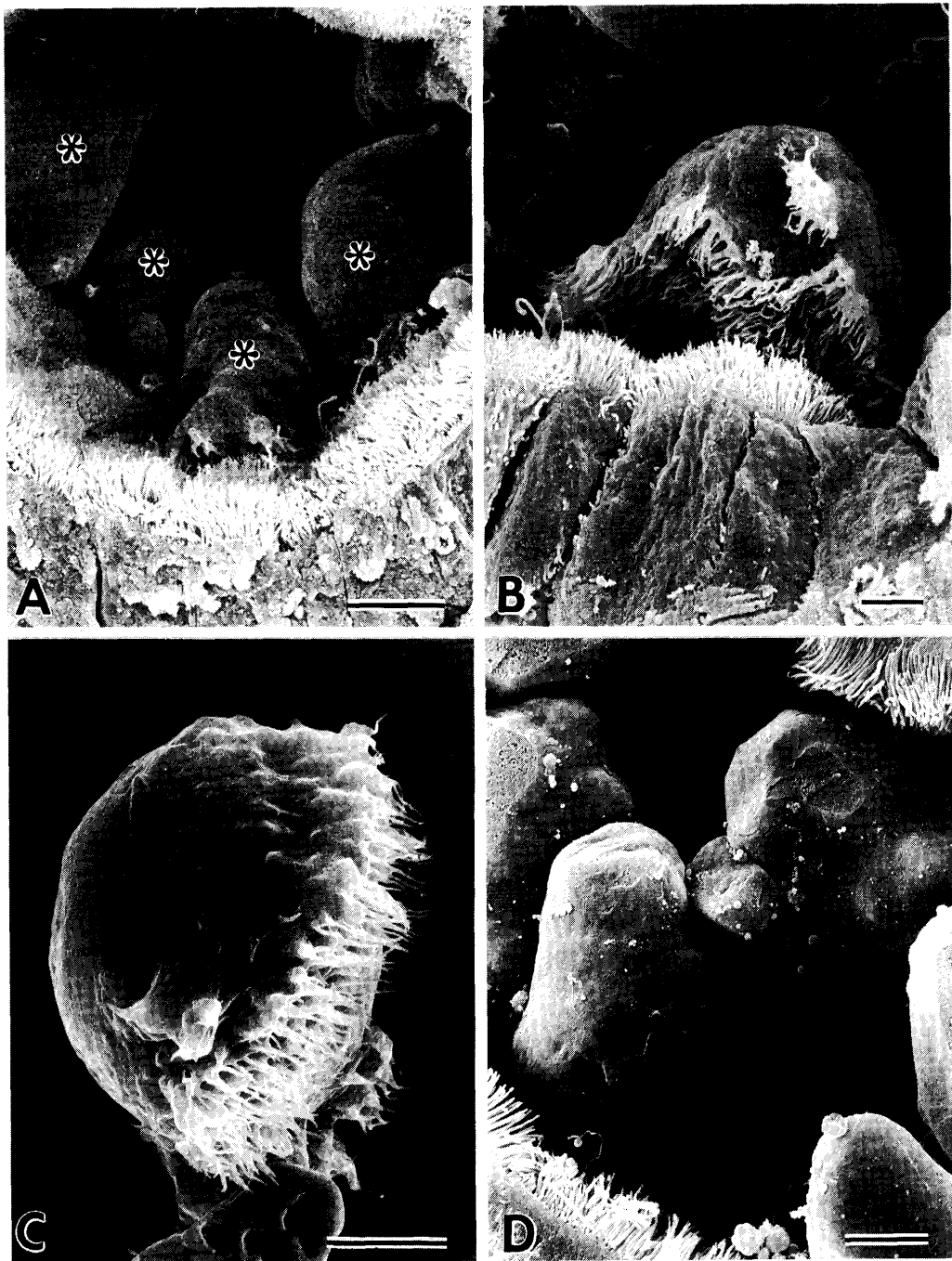


Fig. 4 . SEM images of trophozoites.

A : Trophozoites (asterisks) adhering to the brush border of a proximal tubule.
 B, C : Microvilli-like projections of trophozoites are developed only toward the brush border of the proximal tubule. D : In contrast to A, B and C, the whole surfaces of aggregating-type trophozoites in the lumen are smooth. Scale bars : A = 10 μ m ; B, C and D = 5 μ m.

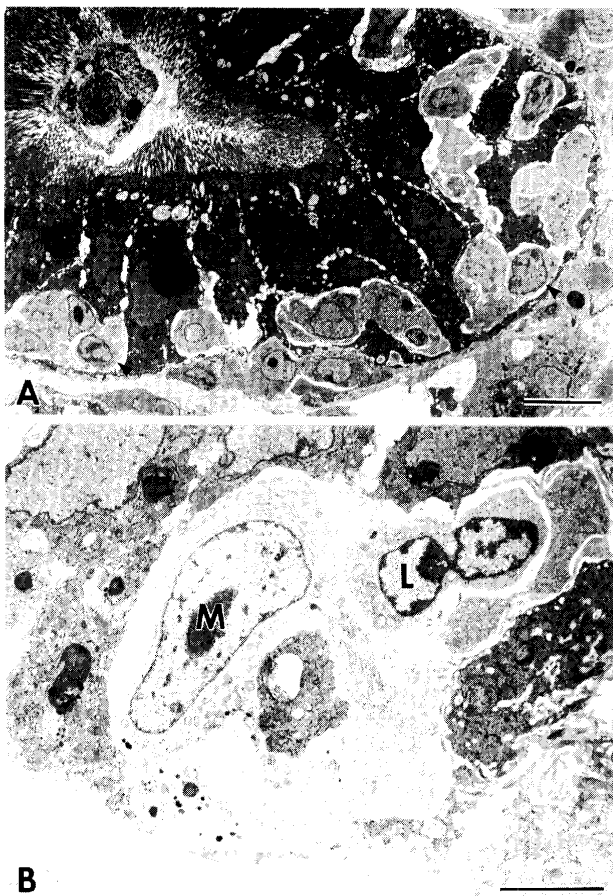


Fig. 5. TEM images of host reactions against the myxosporea.

A: Numerous inflammatory cells including lymphocytes (arrowheads) infiltrate into the proximal tubules that are main targets of myxosporean trophozoites. B: A lymphocyte (L) and a macrophage (M) invade the epithelium of the proximal tubule. Scale bars: A=10 μ m; B= 5 μ m.

Discussion

Yanagi and Miyoshi who observed the lamprey kidney first reported that numerous wandering cells were present in the lumina of urinary tubules and that their ultrastructural characteristics were similar to those of macrophages²⁴⁾. The original purpose of the present study was to clarify the origin and functional significance of the “macrophage-like cells” showing unique distribution. Actually, we con-

firmed that these cells in the lamprey kidney displayed intense reactivity for acid phosphatase and possessed numerous lysosomes and phagosomes in the cytoplasm, just like macrophages.

However, our further ultrastructural analysis, presented here, demonstrated a cluster of immature cells in the cytoplasm of the “macrophage-like cells”, suggesting their myxozoan nature. The cells observed in the lumina of proximal tubules are considered to be myxosporean trophozoites judging from the morphology of generative cells in the cytoplasm and of polar capsules or polar filaments in the immature spores^{8,11)}. Numerous species of myxosporeans are known to parasitize fish, and some of them such as *Myxobolus cerebralis*, an agent of whirling disease in salmonid fry⁶⁾, are important in pathogenicity. Although numerous studies have dealt with myxosporeans in the fish kidney, no information has been available on infection in cyclostomes, including the arctic lamprey¹⁾. The lamprey has a kidney histologically similar to that of chondrichthyes and teleosts except for a huge, elongated renal glomerulus and neck segment composed of ciliated cells^{13,14,25)}. Therefore, it is not strange that myxosporeans are parasitic in the kidney of the arctic lamprey. There is a possibility that this myxosporean displays a new species, but the exact identification of the species was impossible because no mature spores were detected throughout the study.

The infection of myxosporeans in the lamprey kidney may be established in rivers, since the trophozoites were also found in the kidney of ammocoetes living in fresh water. Infection with high prevalences and in different rivers indicates broad contamination by this myxosporean in Japan. The kidney may be a favorite site for infection of this myxosporean species, because no myxosporeans were

found in other organs, including the intestine, liver and gills.

For sporulation, it appears necessary for trophozoites to adhere to epithelial cells of the proximal urinary tubules. The attachment is characterized by vigorous interdigitation of the cell projections with the brush border of epithelial cells in the lamprey kidney. *Sphaerospora renicola* is a myxosporean living in the carp kidney and forms pseudoplasmodia in the lumina of urinary tubules. These pseudoplasmodia occasionally project "thin pseudopodia wedged between the microvilli"⁹⁾, but most of them lack cell projections and only loosely contact the tips of microvilli of the epithelial cells. Myxosporeans of the coelozoic type living in cavities and lumina other than the urinary tubule lumen are known to develop cell projections or invaginations toward the epithelial lining cells. The cell projections have been described as "villousities" of *Myxidium rhodei*⁵⁾ living in Bowman's space, "invaginations" of *Hoferellus gilsoni*¹⁰⁾ living in the urinary bladder and "branching rootlets or rhizoids" of *Myxidium giardi*¹⁸⁾. Moreover, myxosporeans living in the gall bladder project "finger-like projections" of *Zschokkella mugilis*¹⁹⁾, "flattened pseudopodia or cylindrical papillae" of *Ceratomyxa drepanopsettae*¹⁵⁾, and "filopodial projections" of *Ceratomyxa sparusaurati*¹⁷⁾. The projections of trophozoites demonstrated by the present observations, especially SEM observation, exhibited much more developed morphology, comparable to the brush border of urinary epithelial cells.

It is obvious that the cell projections of trophozoites play some important roles, since they developed only in the attaching face of the trophozoite. The vigorous interdigitation may make the attachment to epithelial cells firm. In the lamprey kidney, only the epithelial cells of proximal tubules are equipped

with developed microvilli, while in the distal and collecting tubules sparse, short microvilli are dispersed on the apical surfaces of the epithelial cells¹³⁾. Another possibility for their functional significance is absorption of nutrients. Trophozoites, which need much energy for sporulation, are considered to obtain necessary nutrients by pinocytosis or endocytosis²³⁾. The developed microvilli-like projections might enlarge the surface area and be effective for absorption of nutrients. Considering that the trophozoites uptake substances via the epithelial cells, proximal tubules that reabsorb most of the glucose and amino acids in urine^{2,22)} seem to be the best location for proliferation. Different from the trophozoites described above, trophozoites of the aggregating type developed no projections on the surface. Since sporulation was more advanced in the aggregating trophozoites, they might not need contact with the epithelium of proximal tubules any longer, as indicated by Palenzuela et al.¹⁷⁾.

Invasion of lymphocytes and macrophages into the epithelium of proximal tubules is a quite unique phenomenon. Such invasion of immune cells into the epithelium of urinary tubules has not been reported under normal conditions, including in mammals³⁾. The fact that the intraepithelial invasion of cells was restricted to parasitic location of the myxosporean suggests local immune responses against the infection. Massive organic reactions, including proliferation of immune cells might be provoked, since mitotic figures of lymphocyte-like cells were occasionally detected.

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