Well Developed Splenic Ellipsoids in Japanese Conger *Conger myriaster*

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The histological examination of the spleen of Japanese conger *Conger myriaster* revealed that there were many eosinophilic island-like structures in splenic hematopoietic tissues, which consisted of large square cells and fibrous structure, when stained with hematoxylin and eosin. Silver stain showed these fibrous structures were composed of abundant reticular fibers that occasionally formed lattice structures. Electron microscopically, these structures consisted of cuboidal endothelial cells, reticular cells, and reticular fibers. In neighboring tissues, there were abundant thrombocytes, lymphoid cells, and macrophages (or monocytes). Basement membranes were poorly developed. When carbon suspensions were injected into the aortic bulb, carbon particles were ingested by macrophages and/or reticular cells in the islands, and were also observed on the bundles of reticular fibers. These data indicate that the eosinophilic island-like structures observed in Japanese conger spleens are well developed splenic ellipsoids, and suggest that such tissues provide a good model for the investigation of trapping foreign antigenic and non-antigenic substances in fish.

Key words: anguilliformes, macrophage, reticular cell, reticular fiber, phagocytosis, fish, teleost

Japanese conger *Conger myriaster* is a relatively important species in Japanese fisheries and distributed mostly in the coastal waters of Japan islands. The larvae of this species belonging to anguilliformes are “leptocephalus”, and are caught in the Pacific coastal sea nearby Japan islands. As Suzuki reported that blood cells were scarcely observed in Japanese eel *Anguilla japonica* leptocephalus tissues,¹⁾ the development of hematopoietic and immune systems in anguilliformes leptocephalus is one of the subjects we are interested in. Therefore, we thought the species was a good model for the investigation of leptocephalus biology, though there is little knowledge concerning this species.

Under such backgrounds, we started the study on the morphology of Japanese conger hematopoietic tissues and found well-developed ellipsoids in the spleens of this species, compared to Japanese eel spleen. This paper describes the histological and fine structural features of the tissue, and the carbon ingestion by splenic ellipsoidal macrophages and/or reticular cells.

Materials and Methods

Fish

Japanese conger *Conger myriaster* (100–250 g body weight (B.W.) and 45–50 cm body length (B.L.)) caught in October 1997 in Ryori bay, Iwate, and maintained in seawater (14–15°C) for one month without feeding, were

used for light (five fish) and electron microscopical (three fish) examinations. More than twenty Japanese Conger (22–50 g B.W. and 25–30 cm B.L.) caught in January 1998 in Hamanako lake, Shizuoka, and stocked in seawater (10–12°C) without feeding were used for carbon ingestion experiments. Japanese eel *Anguilla japonica* (about 200 g B.W.) obtained from a commercial source in Iwate were used for histological (three fish) examinations.

Light and Electron Microscopy

Fish were anesthetized in iced-cold sea water. Spleens were fixed with Bouin solution for light microscopy and 2.5% glutaraldehyde in 0.15 M cacodylate buffer for electron microscopy, immediately after autopsy. The procedures for the histological and examinations of spleens were described previously.^{2,3)} The paraffin sections were stained with hematoxylin and eosin (H-E), Azan, Berlin blue, and silver impregnation (Watanabe's method). Some preparations were bleached with 0.25% KMnO₄ prior to H-E stain and Berlin blue stain.

Carbon Ingestion

Fish were thoracolaparotomized after anesthetizing with 800 ppm 2-phenoxyethanol (Wako, Tokyo, Japan) in seawater, and injected with 0.2 ml of Indian ink (Pelican, Hannover, Germany) in 1.3% NaCl (1:100 dilution) into the aortic bulb. After bonded the operating region with Aronalpha® (Toagosei Co. Ltd., Tokyo, Japan), fish were

Fig. 1. Low magnification of Japanese conger spleen, showing abundant eosinophilic island-like structures (arrows) and melanomacrophage centers (arrow heads) in hematopoietic tissues.

Hematoxylin-eosin stain. Bar = 100 μ m.

Fig. 2. The spleen of Japanese eel showing diffuse hematopoietic tissues without clear ellipsoids, compared to Japanese conger spleen.

Hematoxylin-eosin stain. Bar = 100 μ m.

Fig. 3. High magnification of Fig. 1, showing reticular cell-like cells (R) with eosinophilic cytoplasm and fibrous structures (F) and a melanomacrophage center (arrow head).

Hematoxylin-eosin stain. Bar = 5 μ m.

Fig. 4. Silver stain of Japanese conger spleen.

Note the abundant darkly stained reticular fibers surrounding the arterioles. Bar = 10 μ m.

Fig. 5. Berlin blue stain of Japanese conger spleen after bleaching with 0.25% KMnO_4 , showing the presence of hemosiderin (arrow head) in a melanomacrophage center.

Bar = 100 μ m.

Fig. 6. Electron microphotograph of a Japanese conger splenic ellipsoid.

Note several cuboidal endothelial cells (E), reticular cells (R) with well-developed endoplasmic reticulum (arrows), and well-developed reticular fibers (arrow heads). Bar = 1 μ m.

Fig. 7. Electron microphotograph of interstitial tissues neighboring a Japanese conger splenic ellipsoid.

Note thrombocytes (T), a lymphoid cell (L), a macrophage or monocyte (M), and a reticular cell (R). Bar = 2 μ m.

Fig. 8. Electron microphotograph of a melanomacrophage center nearby a Japanese conger splenic ellipsoid, showing a macrophage with abundant melanin granules (left) and amelanotic macrophage with many phagosome-like structures (right).

Bar = 2 μ m.

maintained in seawater at 10°C. At each time point (1 h, 3 h, and 24 h), 3 fish were anesthetized and laparotomized. Spleens were fixed with Bouin solution, dehydrated, and embedded in paraffin.

Results

Histology

The spleens of Japanese conger consisted of basophilic hematopoietic tissues and abundant eosinophilic island-like structures (Fig. 1), differing from those of Japanese

eel (Fig. 2), consisting of abundant erythrocytes and a few basophilic hematopoietic cells, when stained with H-E. The high magnification of Fig. 1 showed these island-like structures were composed of large reticular cell-like cells with a polymorphic nucleus and eosinophilic cytoplasm, surrounded by fibrous structures (Fig. 3). Azan stain and silver integration revealed that these fibrous structures consisted of abundant reticular fibers that occasionally formed lattice structures (Fig. 4). There were a few melanomacrophage centers neighboring the island-like structures (Fig. 3). Dark granules in the centers were

Fig. 9. Carbon ingestion by reticular cells and/or macrophages in Japanese conger splenic ellipsoids.

Note many carbon particles on reticular fibers (Arrows). Arrow heads: melanomacrophage centers. H-E stain. Bar = 5 μ m.

Fig. 10. Berlin blue stain of Japanese conger spleen preparation after bleaching with 0.25% KMnO_4 in carbon incorporation experiment.

Note the numerous carbon particles in hematopoietic tissues (Arrow heads). Bar = 10 μ m.

bleached with KMnO_4 treatment. The Berlin blue stain of bleached preparations showed there were much hemosiderin in the centers (Fig. 5).

Fine Structure

Electron microscopically, the island-like structures observed in the spleens were typical splenic ellipsoids. Briefly, these structures consisted of cuboidal endothelial cells surrounded by poorly developed or sporadic basement membranes (Fig. 6). Bordering on arterioles, there were many reticular cells with a polymorphic nucleus and well developed rough surfaced endoplasmic reticulum (rER) (Fig. 6) and thick layers of reticular fibers (Fig. 6). Nearby the splenic ellipsoids, there were many thrombocytes with an oval or spindle-shaped chromatin-densed nucleus and a little cytoplasm, lymphoid cells with a large, round or indented heterochromatic nucleus and abundant free ribosomes, and macrophages or monocytes with a bilobed nucleus, abundant mitochondria and rER (Fig. 7). Melanomacrophage centers consisted of a few melanomacrophages and abundant amelanotic macrophages with numerous phagosomes (Fig. 8).

Carbon Ingestion

Reticular cells and/or macrophages in the splenic ellipsoids ingested carbon particles three hours after the carbon injection (Fig. 9), whereas many free carbon particles were observed in blood vessels one hour after the administration. Many free carbon particles were observed on the reticular fibers of the ellipsoids (Fig. 9) in 3 h samples. After 24 h, there were a few ingested carbon particles in reticular cells and/or macrophages. Even when bleached melanin granules with KMnO_4 , there were a few black particles or granules in the hematopoietic tissues (Fig. 10). Berlin blue stain showed that there were much hemosiderin in melanomacrophage centers.

Discussion

Splenic ellipsoids in vertebrates are known to consist of

stellate reticular cells and macrophages within a framework of reticular fibers which surround arterial capillaries.⁴⁾ The ellipsoids have been reported to have several functions such as supporting the blood vessels mechanically and acting as valves or filters removing aged blood cells.⁵⁾ Splenic ellipsoids in mammals and birds were interposed between the end of an artery and the beginning of vein and formed by terminal arterioles that run through a sheath of stellate reticular cells and macrophages supported in a matrix containing reticular fibers. This open vasculature with the interposition of reticular cell-reticular fiber filtration beds is present between terminal arterial vessels and proximal venules.⁶⁾ Therefore, the central function of the splenic ellipsoids is thought to be the selective clearance of cells, microbes, and other foreign particles from blood, depending upon these filtration beds. Admittedly, intravenously injected carbon particles were trapped by ellipsoidal macrophages.^{6,7)} Human serum albumin (HSA)-anti-HSA antibody complex and monoclonal antibody to chicken spleen were also trapped in ellipsoidal macrophages and/or reticular cells.^{8,9)} Although such reports described well developed splenic ellipsoids in mammals and birds, splenic ellipsoids are considered to be absent in some fishes, including rainbow trout *Oncorhynchus mykiss* and carp *Cyprinus carpio*.⁴⁾ The data on the Japanese conger spleen obtained in this study reveal that the species has well-developed splenic ellipsoids, whereas those of the Japanese ell are absent or poorly developed.

Espenes *et al.* reported that the ultrastructure of splenic ellipsoids of the rainbow trout, and that immune-complex was trapped in the splenic ellipsoids.^{10,11)} Lamers and Parmentier reported that the splenic ellipsoids were poorly developed in the carp and the rosy barb *Barbus conchoni*.¹²⁾ Secombes *et al.* reported that rapid localization of antigen occurred in the splenic ellipsoids in the carp preimmunized with protein antigen.¹³⁾ Secombes and Manning described that the ellipsoids of the carp consisted of a thin endothelial layer surrounded by a cuff of cells, usually a single cell thick and containing macrophages.¹⁴⁾ Ellis described the process of the localization of bovine serum al-

bumin within the ellipsoids of the plaice *Pleuronectes platessa*.¹⁵⁾ Although such reports described the antigen trapping in the splenic ellipsoids of fish, there are a few reports about the comparative morphology of fish splenic ellipsoids.^{4,5)} Tischendorf reported that ellipsoids were absent or poorly developed in cyclostomes and more evolved in teleost.¹⁶⁾ Therefore, our works on Japanese conger spleen indicate that this species has the extraordinary well-developed splenic ellipsoids in fish and provide a good model for the investigation of trapping antigenic or non-antigenic substances in fish, including pathogens and degenerated blood cells.

Although teleost spleen macrophages are thought to transport materials from the ellipsoids to melanomacrophage centers,⁹⁾ we failed to show that the carbon particles moved into the melanomacrophage center. The carbon particles were distributed diffusely in splenic hematopoietic tissues in 24 hour samples. A longer-term experiment than 24 h is required to resolve the question of where ingested carbon particles will be transported, as hemosiderin observed in melanomacrophage centers suggests that the aged erythrocytes trapped in the ellipsoids might be transported to the centers. We have plans on studying the histology and the immunology of Japanese conger *leptocephalus* and fry to investigate from where such well-developed ellipsoids come.

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