Japanese Journal of Ichthyology
Vol. 33, No. 2 1986

魚類学雑誌
33巻2号 1986年

Fine Structure of the Granulocytes Occurring in the Hypothalamic-hypophyseal Ventricle and Neurohypophysis of the Hagfish, *Paramyxine atami*

Akira Chiba and Yoshiharu Honma

(Received December 16, 1985)

Abstract Strange granulated cells in the hypothalamic-hypophyseal ventricle and neurohypophysis of the hagfish, *Paramyxine atami*, were examined under light and electron microscope. Based on pleomorphic electron dense granules scattered in the entire cytoplasm and eccentrically shifted nucleus, the cells in question were diagnosed as the extravascular granulocytes.

Living hagfish, considered as the most primitive vertebrates, form good material in the study of comparative anatomy including hematology and hemopoiesis from a phylogenetic point of view. Light and electron microscopic characterization of the peripheral blood cells of the hagfish has already been documented by several investigators (Jordan and Speidel, 1930; Tomonaga *et al.*, 1973a, b; Mattison and Fänge, 1977), and extravascular leucocytes emigrating into some organs and tissues were also reported (Tomonaga *et al.*, 1973a; Östberg *et al.*, 1976; Tsuneki and Gorbman, 1977).

During the course of our study on the comparative anatomy of the brain-ventricular system of the Japanese cyclostomes (Chiba and Honma, 1986), several granulated cells obviously different from neuroendocrine cells existing in this region were seen in the hypothalamus of the hagfish. Ultrastructurally, these cells were closely similar to the granulocytes described in several species of hagfishes (Tomonaga *et al.*, 1973b; Östberg *et al.*, 1976; Mattison and Fänge, 1977; Tanaka *et al.*, 1981).

Therefore, it is considered that the present information may contribute to studies on the cyclostome leucocytes in relation to the evolutionary aspect of immune system of vertebrates.

Materials and methods

Adult hagfish, *Paramyxine atami*, 30–40 cm long, were caught by eelpots containing dead sardine off Awashima Island in Niigata Prefecture facing the Japan Sea at a depth of 80–100 m. The collection was carried out on August 10, 1983. Five selected specimens were killed by decapitation. Then the brains were removed and immersed in various fixatives.

For light microscopy, the brains were fixed with Bouin or Bouin-Holland-sublimate solution, dehydrated in ethanol series, embedded in paraffin, cut serially at 8 μm thickness in sagittal and transverse directions and stained with the following: hematoxylin-eosin, azan trichrome, aldehyde fuchsin (AF)-fast green-orange G and periodic acid Schiff (PAS)-fast green-orange G.

For transmission electron microscopy (TEM), the tissue blocks were immersed in Karnovsky's solution for two days and postfixed with 1% OsO_4 for two hours, dehydrated in alcohol series and embedded in Epon 812. Thick sections stained with toluidine blue were used to search for wandering cells. Ultrathin sections cut with LKB ultratome were mounted on copper grids and double-stained with uranyl acetate and lead nitrate, and examined under a Hitachi H-500 and a Jeol 1200 EX electron microscopes.

For scanning electron microscopy, the tissue pieces fixed with the same solution adopted for TEM were dehydrated, critical point-dried, sputter-coated with gold and observed with a Hitachi S-500 scanning electron microscope.

Results and discussion

In light microscopic preparations, the wandering cells of various shapes and sizes, i.e. small round, fusiform, large round and ovoid cells, were detected in the hypothalamic-hypophyseal ventricle (Fig. 1a). The large cells were occasionally seen within the dorsal and ventral walls of the neurohypophysis (Fig. 1b). In the present study, attention was focused on the large wandering

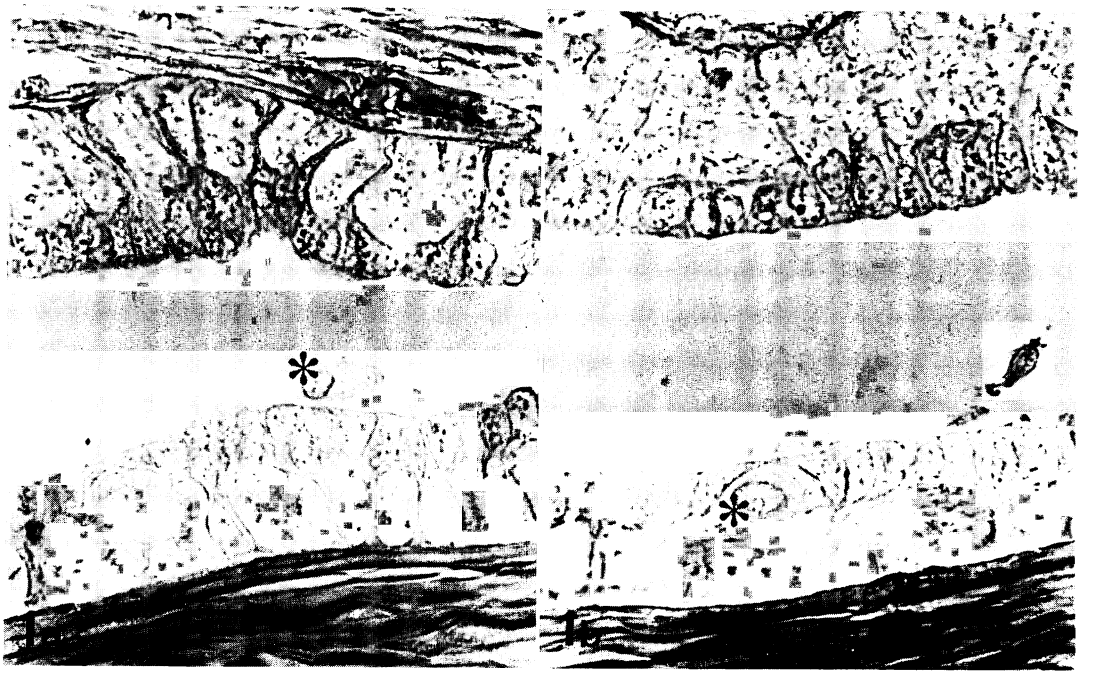

Fig. 1. Light microscopic section of the hagfish infundibular sac (=neurohypophysis) showing the wandering cells. a, the supraependymal cell (asterisk) regarded as possible granulocyte. PAS-fast green-orange G, ×800. b, the large wadnering cell (asterisk) in the ventral wall of the neurohypophysis. PAS-fast green-orange G, ×800.

cells. The cells, ranging 10–15 μm in longer axis, were round or reniform in shape, with eccentrical nucleus, and considerably rich cytoplasm in the nature of weak acidophil. At higher magnification, the cytoplasm revealed finely granular condition together with occasional occurrence of AF and also PAS positive coarse granules. Östberg *et al.* (1976) described the occurrence of extravascular heterophils in the intestinal wall and islet parenchyma of the Atlantic hagfish. These heterophils were pseudoeosinophilic, i.e. the cytoplasm was positive to PAS and contained a bean-shaped or bilobated nucleus. Therefore, the large wandering cells in question showed a resemblance to the heterophils of the Atlantic hagfish (Östberg *et al.*, 1976; Mattison and Fänge, 1977), and some of which might be diagnosed as the macrophages.

Scanning electron microscopy on the walls of the infundibular sac, so-called neurohypophysis, revealed that the wandering cells diagnosed as the supraependymal cells were scattered here and there. Most of these cells were round or ovoid with many nodules and a few processes regarded as microvilli (Fig. 2a). Cells with very slender processes were rarely detected (Fig. 2b). Under stereofine structural observation, the cells showed characteristics of the supraependymal cells or intraventricular macrophages described in the brains of tetrapods (Coates, 1973; McKenna and Chairetakis, 1980; Siever *et al.*, 1981). However, it was difficult to differentiate precisely the granulocytes from the macrophages by surface morphology only. Mattison and Fänge (1977) have reported that when a suspension of heat-killed yeast cells was injected into the blood of the Atlantic hagfish, the granulocytes extended their long slender pseudopodia like those of macrophages, and phagocytose the yeast cells. With the aid of scanning electron microscopy, Tokunaga *et al.* (1981) have found a similar ameboid behavior in the human leucocytes during *in vitro* phagocytosis.

Transmission electron microscopy clearly demonstrated the existence of the granulated cells in the hypothalamic-hypophyseal ventricle (Fig. 3) and neurohypophysis (Fig. 4). In ultrathin sections, the cells showed an oval or irregular

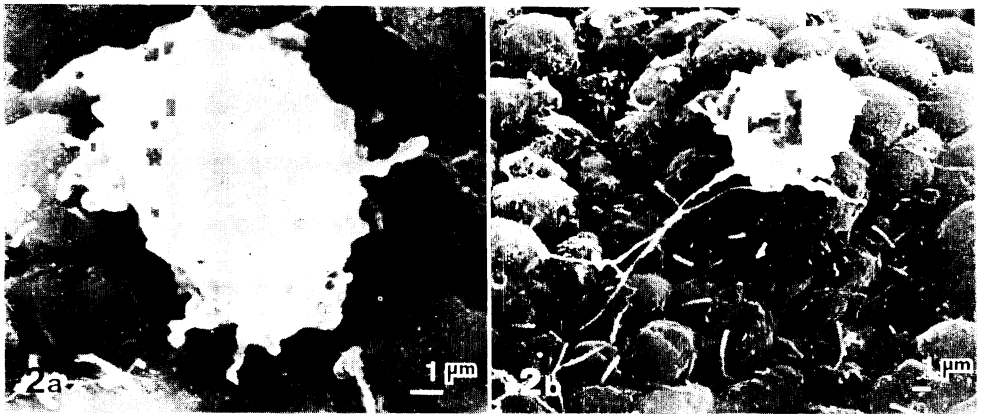

Fig. 2. Scanning electron microscopy of the wandering cells in the infundibular recess. a, the cell with a few cytoplasmic processes. b, the cell with very slender processes.

configuration. The cytoplasm contained a large number of pleomorphic granules, most of which are rod or elliptic-shaped and measured 0.1–0.5 μm in longer axis and 0.1–0.3 μm in shorter axis (Fig. 4). The granules with limiting membrane were homogeneous and electron dense. Apart from these specific granules, round ones, 0.2–0.4 μm in diameter and possibly the primary lysosomes, were also detected (Fig. 4). Vacuoles and vesicles were distributed evenly in the cytoplasm. Golgi bodies were poorly developed and usually located near the nucleus. Granular endoplasmic reticula were indistinct and mitochondria were sparsely seen. Free ribosomes and glycogen particles were demonstrated throughout the cytoplasm, although centrioles and microtubules were seldom encountered. Myelinated bodies and secondary lysosomes were rarely seen (Fig. 5). The cell nucleus, round (rarely reniform) in shape, was eccentrically placed and contained clumps of heterochromatins at the peripheral portions. Neither junctional complex nor synaptic apparatus was detected between the granulated cells and surrounding tanycytes and nerve fibers.

Ultrastructure of the present granulated cells was essentially identical with that of the granulocytes described in previous reports (Tomonaga *et al.*, 1973b; Östberg *et al.*, 1976; Mattison and Fänge, 1977; Tsuneki and Gorbman, 1977; Tanaka *et al.*, 1981). However the shape of the nucleus in the granulocytes of *Paramyxine* was not so irregular as that of the circulating granulocytes in the Atlantic hagfish, *Myxine* (Mattison and Fänge, 1977), i.e. the cells of the former were in an immature or developing stage. General appearance of the present granulocytes was morphologically similar to those of the heterophils or neutrophils in other fish and fish-like animals such as the lampreys (Potter *et al.*, 1982), elasmobranchs (Morrow and Pulsford, 1980; Mattison and Fänge, 1982; Fänge and Pulsford, 1983; Honma *et al.*, 1984) and teleosts (Ferguson, 1976; Cannon *et al.*, 1980). However, the terms used for the granulocytes in the hagfish differ according to the investigators: Östberg *et al.* (1976) adopt the name heterophils, while Jordan (1938) and Mattison and Fänge (1977) prefer the name neutrophils. According to Mattison and Fänge (1977), the granulocytes in the peripheral blood of the Atlantic hagfish occupy about half of the leucocytes. Nearly the same result was obtained by Page and Rowley (1983) in the river lamprey. Though there is no direct evidence, the granulocytes found in the present material may have originated from the vascular system as suspected by Östberg *et al.* (1976) and Tsuneki and Gorbman (1977). Morphological evidence showing the transendothelial migration of the granulocytes has recently been given by Tanaka *et al.* (1981) in the hemopoietic tissue of the intestinal submucosa of *Eptatretus burgeri*.

The present extravascular granulocytes may probably participate in phagocytosis of foreign and disintegrating materials in the ventricular cavity and neurohypophysis together with the macrophages. Previous studies by Fänge and Gidholm (1968) and Mattison and Fänge (1977)

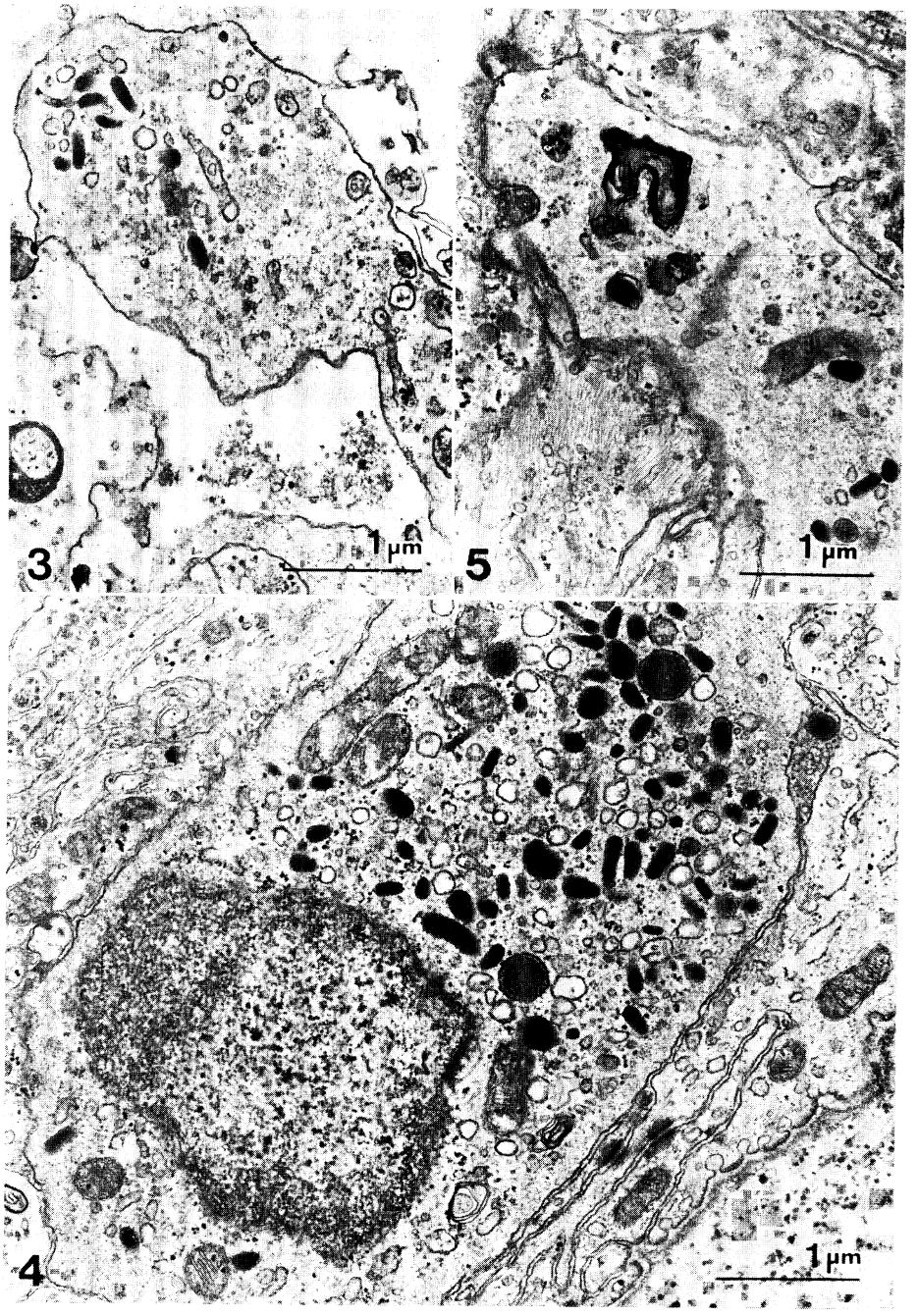

Fig. 3. A part of the granulocyte in the hypothalamic-hypophyseal ventricle. A few electron dense granules are seen in the cytoplasmic process.

Fig. 4. Extravascular granulocyte migrating into the neurohypophysis. The cell has eccentrical nucleus and cytoplasm with specific vacuoles and numerous pleomorphic granules.

Fig. 5. A part of the extravascular granulocyte in the neurohypophysis showing the myelinated bodies diagnosed as secondary lysosomes.

evidently showed that the granulocytes of the Atlantic hagfish phagocytose the foreign materials injected into the blood or peritoneal cavity. It is important to elucidate the cytochemical nature of the granules in the hagfish leucocytes to demonstrate the phagocytotic function and/or bactericidal role. Johansson (1973) showed no peroxidase activity in the granulocyte of *Myxine*. Kelényi and Olesen Larsen (1976) have reported weak or no peroxidase activity in the granulocytes of the river lamprey. Page and Rowley (1983), who identified only one type of granulocyte in the river lamprey, have demonstrated that the granulocytes exhibit no positive peroxidase activity, but strong acidophosphatase and β-glucuronidase activities. Since it is generally known that the granulocytes in higher vertebrates contain a bactericidal system based on peroxidase, hydrogen peroxide and halogen ions, the absence or weakness of peroxidase activity in the granulocytes of cyclostomes is remarkable. Mattison and Fänge (1977) have stated that in cyclostomes the systems active against microorganisms presumably differ from those of the leucocytes of higher vertebrates. Further cytochemical and experimental analyses are necessary to elucidate the role of the extravascular granulocytes in the brain-ventricular system of the hagfish.

Literature cited

Cannon, M. S., H. H. Mollenhauer, T. E. Eurell, D. H. Lewis, A. M. Cannon and C. Tompkins. 1980. An ultrastructural study of the leucocytes of the channel catfish *Ictalurus punctatus*. J. Morphol., 16: 1–23.

Chiba, A. and Y. Honma. 1986. Comparative anatomy of the brainventricular system of the lamprey and hagfish. Pages 76–85 *in* T. Uyeno, R. Arai, T. Taniuchi and K. Matsuura, eds. Indo-Pacific fish biology: Proceedings of the Second International Conference on Indo-Pacific Fishes. Ichthyological Society of Japan, Tokyo.

Coates, P. W. 1973. Supraependymal cells in recesses of the monkey third ventricle (1). Am. J. Anat., 136: 533–539.

Fänge, R. and L. Gidholm. 1968. A macrophage system in *Myxine glutinosa* L. Naturwiss., 55: 44.

Fänge, R. and A. Pulsford. 1983. Structural studies on lymphomyeloid tissues of the dogfish, *Scyliorhinus canicula* L. Cell Tissue Res., 230: 337–351.

Ferguson, H. W. 1976. The ultrastructure of the plaice (*Pleuronectes platessa*) leucocytes. J. Fish Biol., 8: 139–142.

Honma, Y., K. Okabe and A. Chiba. 1984. Comparative histology of the Leydig and epigonal organs in some elasmobranchs. Japan. J. Ichthyol., 31: 47–54.

Johansson, M. L. 1973. Peroxidase in blood cells of fish and cyclostomes. Acta Reg. Soc. Sci. Litt. Gothogurg. Zool., 8: 53–56.

Jordan, H. E. 1938. Comparative hematology. Pages 715–721 *in* H. Downey, ed. Handbook of hematology, Vol. 2.

Jordan, H. E. and C. C. Speidel. 1930. Blood formation in cyclostomes. Am. J. Anat., 46: 355–392.

Kelényi, G. and Olesen Larsen, L. 1976. The hematopoietic supraneural organ of adult, sexually immature river lamprey (*Lampetra fluviatilis* (L.) Gray) with particular reference to azurophil leucocytes. Acta Biol. Hung., 27: 45–56.

Mattison, A. and R. Fänge. 1977. Light- and electron microscopic observations on the blood cells of the Atlantic hagfish, *Myxine glutinosa* (L.). Acta Zool. (Stockh.), 58: 205–221.

Mattison, A. and R. Fänge. 1982. The cellular structure of the Leydig organ in the shark, *Etmopterus spinax* (L.). Biol. Bull., 162: 182–194.

McKenna, O. C. and J. S. Chairetakis. 1980. Scanning and transmission electron microscopic study of the supraependymal macrophages in the lateral ventricles of the toad brain. Cell Tissue Res., 207: 321–329.

Morrow, W. J. W. and A. Pulsford. 1980. Identification of peripheral blood leucocytes of the dogfish (*Scyliorhinus cancicula* L.). J. Fish Biol., 17: 461–475.

Östberg, Y., R. Fänge, A. Mattison and N. W. Thomas. 1976. Light and electron microscopical characterization of heterophilic granulocytes in the intestinal wall and islet parenchyma of the hagfish, *Myxine glutinosa* (Cyclostomata). Acta Zool. (Stockh.), 57: 89–102.

Page, M. and A. F. Rowley. 1983. A cytochemical, light and electron microscopical study of the leucocytes of the adult river lamprey, *Lampetra fluviatilis* (L.) Gray. J. Fish Biol., 22: 503–517.

Potter, I. C., L. R. Percy, D. L. Barber and D. J. Macey 1982. The morphology, development and physiology of blood cells. Pages 233–242 *in* M. W. Hardisty and I. C. Potter, eds. The Biology of lampreys. Vol. 4A. Academic Press, London.

Siever, J., D. Abele and U. Mangold. 1981. Transitory subependymal cysts in the developing rat rhombencephalon. Anat. Embryol., 161: 433–451.

Tanaka, Y., Y. Saito and H. Gotoh. 1981. Vascular architecture and intestinal hematopoietic nests of two cyclostomes, *Eptatretus burgeri* and ammocoetes of *Entosphenus reissneri*: a comparative morphological study. J. Morphol., 170: 71–93.

Tokunaga, M., J. Tokunaga and M. Niimi. 1981. Leucocyte and macrophage movements under phagocytosis. Pages 13–22 *in* K. Tanaka and T. Fujita, eds. Scanning electron microscopy in cell biology and medicine. Excerpta Medica, Amsterdam.

Tomonaga, S., T. Hirokane and K. Awaya. 1973a. Lymphoid cells in the hagfish. Zool. Mag., 82: 133–135.

Tomonaga, S., H. Shinohara and K. Awaya. 1973b. Fine structure of the peripheral blood cells of the hagfish. Zool. Mag., 82: 211–214.

Tsuneki, K. and A. Gorbman. 1977. Ultrastructure of the testicular tissue of the hagfish, *Eptatretus stouti*. Acta Zool. (Stockh.), 58: 17–25.

(AC: Biological Laboratory, Nippon Dental University Niigata Faculty, 1–8 Hamaura-cho, Niigata 951, Japan; YH: Faculty of Science, Niigata University, 8050 Igarashi-ninocho, Niigata 950–21, Japan)

クロメクラウナギの 視床下部・下垂体室ならびに神経性下垂体中に出現した顆粒球の微細構造

千葉　晃・本間義治

本邦産クロメクラウナギの視床下部-下垂体域を顕微鏡観察中，脳室壁面や神経性下垂体中に，しばしば遊走細胞を認めた．この細胞は，細胞質内に暗調の多形性顆粒を多数含み，核が偏在していることなどの特徴から顆粒球と同定された．

(千葉: 951 新潟市浜浦町 1–8 日本歯科大学新潟歯学部; 本間: 950–21 新潟市五十嵐２ノ町新潟大学理学部)