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Pericytes and Macrophages in the Cat Cerebral Capillaries : Some Fine Structural Implications

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ABSTRACT. The cerebral capillaries were examined electron microscopically, in cats receiving horseradish peroxidase (HRP) either intraventricularly or intracerebrally to enhance the morphological and functional characteristics between pericytes and macrophages. Pericytes and macrophages were collectively referred to as perithelial cells, and these two cell types were distinguished by their morphological features. Pericytes were located only around capillaries or post-capillary venules and were completely enveloped and separated from the surrounding cerebral parenchyma by thick basal laminae. In the pericyte cytoplasm, microfilaments and plasmalemmal caveolae were found in the adluminal and abluminal sides, respectively. Macrophages were located around microvessels throughout from arterioles to venules. Macrophage lacked both basal laminae and plasmalemmal caveolae. The differences between pericytes and macrophages were clearly illustrated by their reaction to HRP. HRP was actively ingested into the cytoplasm of macrophages, whereas pericytes did not ingest HRP even after long exposure to the tracers. The pericytes of cerebral capillaries are distinct from other perithelial cells such as macrophages 'of scavenger cells.

Key words : pericyte — macrophage — HRP — central nervous system —
fine structure

Endothelial cells are the mainstay of the mammalian cardiovascular system. The cells overlapping the endothelial tube are generally known as perithelial cells. In the microcirculation, the perithelial cells are smooth muscle cell in arterioles and venules, and pericytes in the capillaries. The local metabolic activities of microvascular bed can be determined by the interactions between endothelial and perithelial cells. Pericytes have been assumed to play a significant role in the regulation of cerebral microcirculation and metabolism, although the nature of pericytes remains unknown. The term "pericyte" has been rather loosely applied since its introduction by Zimmermann in 1923. Few morphological studies have attempted to distinguish pericytes from other perithelial cells in the mammalian central nervous system (Roggendorf *et al*, 1981). An understanding of the functional roles of pericytes has been further hampered by confusion between pericytes and other perithelial cells (Jones, 1970 ; Lafarga and Palacious, 1975 ; Cancilla *et al*, 1972 ; van Deurs, 1976 ;

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Broadwell and Salcman, 1981). Two lasting hypotheses are still in conflict; namely, the one that the pericytes are contractile cells which modulate capillary blood flow (Dahl, 1973; Stensaas, 1975; LeBeaux and Willemot, 1978; Allsoop and Gamble, 1979) or the other in which they are phagocytic cells that contribute to the blood-brain barrier (Barðn and Gallego, 1972, Castejon, 1984). It is curious that the phagocytotic role of pericyte has been postulated only in the central nervous system. In the present study, horseradish peroxidase (HRP) was infused into either the cerebral parenchyma or ventricles of the cat to test the phagocytic role of capillary perithelial cells.

MATERIALS AND METHODS

Twelve adult cats of either sex were anesthetized with intraperitoneal injections of 30 mg/kg of sodium pentobarbital, and they were intubated and artificially ventilated with a mixture of gas of O_2 and N_2O (2:3) during the experimental procedure. All animals used in this study were housed and handled according to the guidelines established by the Animal Care and Use Committee of Kawasaki Medical School (No.93-166, 1993).

Ventricular infusion of HRP: HRP (40 mg, type II, Sigma Chemical Co., St. Louis) dissolved in 1.0 ml of artificial CSF was infused into the lateral ventricle in 10 minutes with Harvard pump. A cannula was inserted into the lumbar cistern to avoid increases in subarachnoid pressure during HRP infusion.

Intracerebral infusion of HRP: HRP (10 mg) dissolved in 0.25 ml of artificial CSF was infused through a 25-gauge needle inserted into the centrum semiovale of both hemispheres in five minutes following stereotactic coordinates (AP: +19.0, LAT: 9.0, V: +19.0) in five minutes.

Electron microscopy: Between 20 to 120 minutes following HRP infusion, the chest was opened and the vascular lumen was flushed with Hank's balanced salt solution (HBSS) through an aortic cannula. A mixture of 1% paraformaldehyde and 3% glutaraldehyde in phosphate buffer (pH 7.2-7.4) was then perfused at room temperature (30-40 min). Brains were removed and frontal sections (100 μm thickness) were prepared on a Microslicer™ (Doosaka, Kyoto) and incubated for HRP activity in a 0.1% solution of 3,3'-diaminobenzidine in 0.5 M Tris buffer. Sections were examined by light microscopy for distribution of HRP and selected areas of the basal forebrain were excised for electron microscopy. The sections were washed in the same buffer and post-fixed for one hour at 4℃ in OsO_4. Tissues were stained 'en block' in saturated aqueous uranyl acetate for 20-30 min., dehydrated in ascending concentrations of ethanol, rinsed in propylene oxide, and embedded in Epon 812. Thin sections were stained with lead citrate and examined with JEL-200EX or Hitachi H-7100 electron microscopes. Brains from two cats were prepared as a control for transmission electron microscopy without HRP infusion.

RESULTS

The term "microvessels" has been applied to blood vessels having luminal diameters of less than 100 μm (Rhodin, 1967). Thus, the arterioles, capillaries

and post-capillary venules were classified as microvessels. The capillaries in the central nervous system had a luminal diameter of 3 to 10 μm and were composed of a single continuous layer of endothelial cells. Two to three endothelial cells made up the wall of the capillaries in cross section, and they were connected with each other by tight junctions at their periphery. In cross-sectional profiles, the nucleated cell bodies or cytoplasmic processes of pericytes were found immediately adjacent to the abluminal endothelial surface (Fig 1-4). The surface of the pericytes was entirely covered with basal laminae, which was continuous with that of the endothelial cells. Endothelial cells, pericytes and basal laminae were the fundamental constituents of the capillaries. The capillaries were completely enveloped by the cytoplasmic processes of astroglial cells, forming perivascular space between the abluminal and adluminal surfaces of the capillary tube and astroglial endfeet, respectively. In the central nervous system, the perivascular space of the capillaries was tight or absent.

The pericytes had a smooth contour and were characterized by orderly arranged cytoplasmic processes. The abluminal surface of a pericyte cell body was marked by plasmalemmal caveolae. Small mitochondria, smooth and rough endoplasmic reticulum, free ribosomes, Golgi apparati and microfilaments were found in the pericyte cytoplasm. Occasionally, small
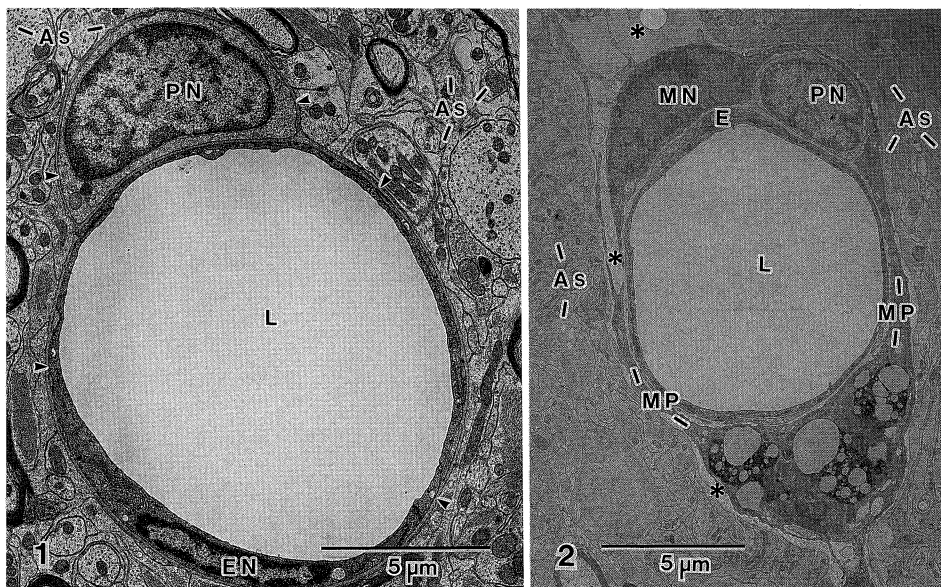

Fig 1. Capillary of the hypothalamus from a control animal. Cell body of a pericyte directly abutting the abluminal surface of an endothelial cell. PN, pericyte nucleus; EN, endothelial nucleus; L, lumen of capillary; As, astroglial processes; arrowheads, basal lamina

Fig 2. Capillary of the interhemispheric region from a control animal. A macrophage coexist with a pericyte in the perivascular space. The cytoplasmic processes of the macrophage (MP) overlay the cell body of the pericyte (PN). Lipid-laden bodies, and lysosomes are observed in the cytoplasm of the macrophage. MN, nucleus of macrophage; E, endothelial cell; L, lumen of capillary; As, astroglial processes; *perivascular space

lysosomes were present. The cell bodies which bulged out to the surrounding astroglial processes, were the most voluminous portion of the pericyte. The main cytoplasmic processes extended longitudinally, and side processes circumferentially either from the main processes or cell bodies. Pericytes were located only on the surface of capillaries and post capillary venules. Basal lamina was present in the space between the abluminal surface of endothelial cells and the adluminal surface of pericytes (Fig 1, 3). However, the tips of the cytoplasmic processes, in contrast, were closely apposed to the abluminal surface of the endothelial cells underneath without any intervention of basal lamina. Two hours following HRP intraventricular administration, the perivascular spaces were filled with HRP (Fig 3). In the pericytes, HRP were trapped only within the recesses of the caveolae of the abluminal plasmalemma. Neither the plasmalemmal caveolae nor lysosomes in the pericytes, however, increased in number or size after HRP administration. These fine structural characteristics of pericytes in response to HRP were consistent and were not affected by the difference in time sequence or procedure employed.

Macrophages also appeared in relation to microvessels and were located within the perivascular spaces (Fig 2). The macrophages lacked a coverage of basal lamina. The abluminal surface of the macrophages was corrugated, although the abluminal contour of the endothelial cells and pericytes followed the contour of the adluminal surface of perivascular macrophages. The nucleus of the macrophages was irregular in shape and eccentrically located in the cell body. Large numbers of membrane-bound lipid droplets and lysosomes were prominent features of cytoplasmic organelles of cerebral macrophages. Numerous elongated pseudopod-like cytoplasmic processes of the macrophages extended in variable dispositions along and around the vessel walls.
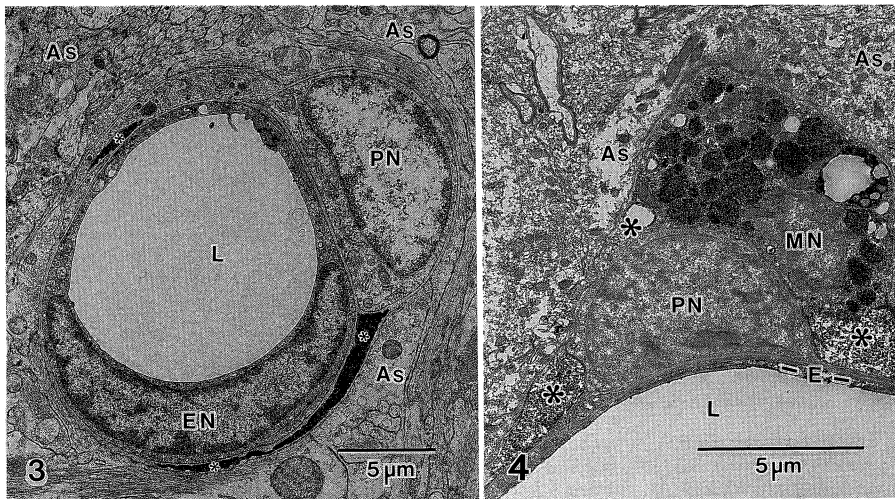

Fig 3   Two hours after intraventricular infusion of HRP. The narrow perivascular space (*) is outlined with HRP. PN, pericyte nucleus; EN, endothelial nucleus; L, lumen of capillary; As, astroglial process Occipital cortex

Fig 4   Post-capillary venule of the parietal cortex, two hours after intracerebral HRP infusion. A macrophage with its nucleus (MN) located in the perivascular space (*). PN, pericyte nucleus; E, endothelial cell; As, astroglial processes

Occasionally these processes encircled almost the entire circumference of the microvessels, and extended into the recesses between the astroglial endfeet. The most striking change after HRP administration was seen in the perivascular macrophages. The abluminal plasmalemma of the macrophages became more expanded than that of the control animals. Membrane-bound lipid droplets and dense bodies were replaced by HRP-positive large dense bodies (Fig 4). The cytoplasm of the macrophages was filled with HRP-reaction products to the tip of the smallest cytoplasmic processes. No differences were discerned in the morphology of macrophages, in specimens between intracerebral and intraventricular administration of HRP. Light microscopically, they were most clearly observed 24 hours after HRP administration, as perivascular macrophages prominently increased in density. Intense brown-stained macrophage were recognizable along the vasculature with a light microscope, so that nearly every branch of the microvascular tree contained irregularly distributed HRP-labelled macrophages within its perivascular space. Microglial cells were located in the parenchyma of the central nervous system. Occasionally their cytoplasmic surfaces were partly exposed to the perivascular space. These cells were devoid of a basal lamina and were characterized by a relatively large nucleus within a small sized cell body.

DISCUSSION

Based on the present state of the art, Rhodin's illustration (1967) is still tenable as the ubiquitous module of microvascular classification. Any attempt to define the consecutive segments of microvessels in a random collection of sectioned tissues is impaired by the extreme scatter of variations and the lack of additional criteria necessary for a distinct definition. With these inadequacies in mind, arterioles, capillaries, and post-capillary venules are identified by applying the following parameters: luminal diameter, and composition as well as completeness of the perithelial cells. In recent years, the cerebral capillaries have been extensively examined by both 'in vitro' and 'in vivo' protocols. Almost without exception, investigators have used the term "pericyte" to describe any ramified cells located on the surface of the capillary endothelial tube, regardless of their morphological appearance. In the present experiment, the structural and functional definitions of pericytes and macrophages were illustrated. It should be emphasized that pericytes are a different entity from other perithelial cells.

Pericytes were first described as Rouget's cell (1873), and the term "pericyte" was coined by Zimmermann (1923). They were recognized by their morphological features and their location around the capillary endothelial tube. It is apparent, however, that some perithelial cells are also characterized by the presence of organized cytoplasmic processes too delicate to be resolved by light microscopy. The discrepancies in the morphology of perithelial cells at the level of light and electron microscopy led a number of investigators to attempt to establish a new system of nomenclature (reviewed by Ashton and Oliveira, 1966). The microvessels of the central nervous system differ from vessels in other tissues in several ways. Most importantly, the endothelial cells form an effective barrier to foreign blood-born substances. Thus, the perithelial cells in the central nervous system have been examined thoroughly from the standpoint

of their relationship to vascular permeability.

Pericytes and macrophages have only a few morphological identities. Both cells possess fine, finger-like cytoplasmic processes. Macrophages differ from pericytes in being irregularly shaped and in lacking surrounding basal laminae. Furthermore, macrophages have no intimate membranous relationships with endothelial cells. Pericytes extend processes, which are oriented either longitudinally or circumferentially with respect to the long axis of a capillary or post-capillary venule. The location of these cells is also distinct. Pericytes directly abut on the abluminal surface of capillaries or post-capillary venules. On the other hand, macrophages overlap the outer surface of capillary pericytes, and are scattered within the perivascular space. Thus, occasionally, a single cross-sectional profile of capillary can contain both pericytes and macrophages. These spatial relationships between pericytes and macrophages are comparable, as suggested by Ookawara and his colleagues (1996). The structure, locations and cytoplasmic tropomyosins of these cells have led many to consider pericytes as miniature smooth muscle cells (Joyce *et al*, 1985). Although, the pericytes on capillaries in the central nervous system are easily identifiable by detailed electron microscopic observations, these cells have often been confused with perivascular macrophages.

The differences between pericytes and macrophages become more distinctive after the administration of HRP. Macrophages actively ingest HRP and distribute it throughout to the tip of its cytoplasmic processes. These cells were easily identified in the perivascular space of the central nervous system of cats receiving intraventricular injections of HRP, which were allowed to circulate from 20 minutes to 24 hours prior to fixation. These HRP-egested macrophages were densely distributed in the perivascular spaces of the circumventricular region where the blood-brain barrier is absent. Pericytes, on the other hand, never actively ingested HRP, and the reaction product in these cells was restricted to the recesses of the · plasmalemmal caveolae. The differences between pericytes and macrophages are not only identified at the electron microscopic level. Some authors have nominated the perivascular macrophages as fluorescent granular perithelial cells (FGP cells, Ookawara *et al*, 1996). There has also been some confusion in nomenclature regarding macrophage and microglia. Macrophages are located in the perivascular spaces, whereas microglia are found in the parenchyma of the central nervous system. Critically reviewing the descriptions of perithelial cells reported in the literature, granular pericytes (Commermeyer, 1970; Farrel *et al*, 1987), phagocytic pericytes (Cancilla *et al*, 1972; van Deurs, 1976), the neurolipomastoid cells of Ibrahim (1974), the pericytal microglia of Mori and Leblond (1969), and FGP cells (Ookawara *et al*, 1996) seem to be identical to the perivascular macrophages. Therefore, although "macrophage" is highly inaccurate, if not an inappropriate term from different points of view, we believe that its acceptance in neurocytological studies demands its continued use.

Although the results presented were based on experiments using HRP, it is clear that cerebral pericytes do not actively egest HRP. This is quite distinct from the macrophages of scavenger cells. The functional role of the pericyte, remains uncertain. The infrequent lysosomal elements in the pericyte would not suggest an important role in phagocytic activities under normal conditions,

but this may differ in pathological conditions. It may be speculated that the pericytes in different tissues vary in morphological characteristics as well as in functional roles (Fujimoto, 1995).

ACKNOWLEDGMENTS

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