Macrophagic Ameboid Cells in the Brain Ventricles of the Neonatal Rat

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Summary. Scanning electron microscopy revealed numerous macrophagic ameboid cells on the ependymal surface of all brain ventricles in neonatal rats. Macrophagic ameboid cells aggregated in the sulcus medianus of the fossa rhomboidea, the recessus of the cerebral aqueduct and the recessus infundibuli, i.e. the ventromedial floor of the ventricular cavity covered mainly with non-ciliated ependyma. Macrophagic ameboid cells were numerous in the first few days after birth, often intermingling with extravasated erythrocytes. Subsequently, these cells decreased in number until 10 days after birth. Thus, it was rather difficult to find such ameboid cells in the brain ventricles of 21-day-old rats. Intravenous injection of primuline, a fluorescence dye used as a cytoplasmic marker in the previous study, enhanced the appearance of the ameboid cells and caused them to remain longer on the ventricular surface.

In the previous report we investigated the distribution and fate of macrophagic ameboid cells following an intravenous injection of primuline in neonatal rats, and concluded that the primuline-labeled ameboid cells in the brain parenchyma were phagocytic cells derived from blood monocytes and destined to become microglia (IMAMOTO et al., 1982). In addition, we postulated that some of subependymal ameboid cells arose from the ventricular cavity, passing through the ependymal lining, on the basis of their morphological similarities to the primuline-labeled free cells in the ventricular cavity. There might be a precursor-product relationship among the blood monocyte, intraventricular and subependymal ameboid cell and microglia in the white matter. The present experiment aims to visualize by scanning electron microscopy the distinct existence of precursors of macrophagic ameboid cells in the brain ventricles.

MATERIALS AND METHODS

Fifty-two newborn rats were used in this study. Thirty-two rats were injected with 12 μl of 10% primuline either at birth or one day after birth in the same manner as in the previous study (IMAMOTO et al., 1982). The rest were non-injected controls. The animals were sacrificed by perfusion fixation at various times up to 21 days after birth. Each brain was coronally cut into four pieces and the ventricular cavity was exposed by sagittal and occasionally horizontal sections. The specimens were dehydrated in a
graded series of ethanol, dried by the CO₂ critical point method, coated with gold in a vacuum evaporator and observed by a scanning electron microscope (S-500AS, Hitachi) under 20 kV of accelerating voltage.

RESULTS

Scanning electron microscopy revealed numerous supraependymal cells on the ventricular floor and on the surface of the choroid plexus. Some of them were regarded as supraependymal neurons with an extended fiber network, but in this experiment we focused on the supraependymal macrophages.

At one day after birth, a surface view of the ventricular floor revealed a cluster of macrophagic ameboid cells both in the primuline-injected rats and the controls. Concentrations of the ameboid cells were recognized on the ependymal surface in the following regions: the sulcus medianus of the fossa rhomboidea (Fig. 1, 2), recessus retrocommissuralis and recessus isthmicus of the aqueductus cerebri (Fig. 3, 5), and recessus infundibuli (Fig. 4). These regions, located in the lower or ventromedial part of the ventricular floor, were non-ciliated but widely covered with bleb-like protrusions of ependymal cells. Ciliated regions located in the upper or dorsolateral part of the ventricular cavity usually revealed few macrophagic ameboid cells throughout the experimental period.

In the first few days after birth, the macrophagic ameboid cells were spherical in shape and often attached to extravasated erythrocytes (Fig. 3, 4). The bumpy surface of these cells, as well as the dull white spots indicating a richer secondary emission from rounded structures beneath the cell surface, implied inclusion bodies internalized by phagocytosis. Subsequently, the spherical ameboid cells seemed to transform into elongated cells with filmy pseudopodia and slender filopodia extending from a bumpy or ruffled perikaryal surface (Fig. 2). The ependymal surface of the choroid plexus also displayed the existence of similar ameboid cells (Fig. 6). These epichoroidal cells, as well as macrophagic ameboid cells on the supraependyma, soon decreased in number. Thus, it was rather difficult to find a cluster of the ameboid cells in the ventricular cavity at 21 days after birth. In those animals given an intravenous injection of primuline, the supraependymal ameboid cells were obviously more numerous than in the controls. Furthermore, they remained longer in the ventricles of primuline-injected rats than those of the controls. However, precise quantitative data were not obtained in this experiment.

Fig. 1. A row of macrophagic ameboid cells in the sulcus medianus of the fossa rhomboidea. A 3-day-old primuline injected rat. ×1,200
Fig. 2. A macrophagic ameboid cell showing slender filopodia and filmy pseudopodia on the bumpy perikaryal surface, observed in the sulcus medianus of the fossa rhomboidea. A 3-day-old primuline injected rat. × 4,800

Fig. 3. Macrophagic ameboid cells intermingling with erythrocytes in the recessus isthmicus. A 1-day-old non-injected rat. × 4,200
DISCUSSION

Previous investigators have examined the ependymal surface in various animals by scanning electron microscopy, recording the features of supraependymal cells. In fact, there have been two distinct categories of supraependymal cells: one group being the neuroepithelial derivatives such as neurons and glia (Coates, 1973; Scott et al., 1977; Card and Mitchell, 1978), and the other the non-neuronal cells termed Kolmer cells (Hosoya and Fujita, 1973; Schwarze, 1975), epiplexus cells (Persky, 1978; Merchant, 1979), and supraependymal macrophages (Scott et al., 1977; McKenna and Chairetakis, 1980; Bleier and Albrecht, 1980; Mestres, 1981). During this experiment we recognized both neuronal and non-neuronal elements, but turned our attention to the free cells wandering about on the ventricular cavity. These were the macrophagic ameboid cells described here. Ameboid cells probably constitute a single cell type together with the above-mentioned non-neuronal phagocytic cells, although they are morphologically depicted as showing subtly different reactions.

It is now evident that the existence of intraventricular phagocytic cells is common to prenatal and neonatal animals under normal conditions (Sturrock, 1979; Bleier et
al., 1982). Based on our present observation that ameboid cells closely adhere to the extravasated erythrocytes in the newborn rat, we suspect that erythrophagocytosis is one of the most important events in the ventricular cavity of the neonatal animal. Provided that the cerebral blood vessels possess incomplete junctional apparatuses between endothelial cells and no basement membrane in the early embryonic stage (Roy

Fig. 5. Macrophagic ameboid cells in the recessus retrocommissuralis. A 9-day-old primuline injected rat. × 640

Fig. 6. Two macrophagic ameboid cells on the ependymal surface of the choroid plexus in the fourth ventricle. A 2-day-old non-injected rat. × 3,000
et al., 1974), the extravasation of erythrocytes seems to occur rather easily through such fragile vessel walls during the fetal period. It is probable that macrophagic ameboid cells become rounded or full-blown in shape during active phagocytosis, while they transform into elongated cells with filmy pseudopodia and slender filopodia during migration by ameboid movement. In addition to the erythrophagocytosis, macrophagic ameboid cells may serve to remove disused materials, cellular debris and foreign substances in the cerebrospinal fluid as cell components of the defense system of the body (HOSOYA and FUJITA, 1973; BLEIER and ALBRECHT, 1980; MERCHANT and MERCHANT, 1980).

Recently the idea that the supraependymal macrophages belong to the macrophage-monocyte cell line has been put forward (BLEIER and ALBRECHT, 1980; MERCHANT and MERCHANT, 1980). In our previous report, we have also stressed that the ameboid cells appearing not only in the ventricular cavity but also in the brain parenchyma are derived from the circulating monocytes (IMAMOTO et al., 1982). McKENNA and CHAIRETAKIS (1980) have noted the fact that both the supraependymal and subependymal macrophages react to stains for microglial cells, and thus have insisted that the supra- and subependymal cells are probably an identical cell type capable of exchanging locations. Such opinions are relevant when considering the origin of microglia. However, further studies are required to clarify how such supraependymal ameboid cells can pass through the ependymal lining, as its cells are tightly connected with zonula occludens (BRIGHTMAN and REESE, 1969; MERCHANT, 1979).

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