Electron Microscopic Study on the Amoeboid Microglial Cells in the Roof Plate of the Early Chick Embryo Brain

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Summary. The amoeboid microglial cells (AM cells), which were acid phosphatase-positive histochemically, present in the rhombencephalon coated with the leptomeninges of the 8-day incubation chick embryos were investigated by electron microscopy. It was found that the AM cells occurred simultaneously in the leptomeninges, in the roof plate, and in the ventricle. Considering the distribution of the cell in these three sites along with the present author's earlier findings of ACPase reactions for the AM cells, it is highly probable that in the rhombencephalon the AM cells originate from the leptomeninges and then enter the ventricle through the roof plate.

The round or amoeboid cells temporarily appearing in the brain during the developmental stage of the mammalian embryo were first demonstrated by Horteña (1919) using the silver carbonate method. He thought that these cells immigrated from the leptomeninges into the brain and changed there into microglial cells, and implied that these were embryonic forms, i.e., microglioblasts, of the microglial cells.

The electron micrographs revealed that the microglioblast in the fetal and neonatal brains of various kinds of animals were generally characterized by many vacuoles, dense bodies and lipid droplets in the cytoplasm and a lot of filopodial processes (Stensaa and Reichert, 1971; Stensaa, 1975; Booz and Felsing, 1973; Schmitt, 1973; Matsuyama, Komatsu and Senda, 1973, 1975; Ling and Tan, 1974; Ling, 1976, 1977; Kawaguchi, 1978). Thus, these cells are also named amoeboid microglial cells (AM cells) after their processes. However, there is as yet little agreement among investigators concerning the origin of these cells. Recently, the present author (1978), in her study of the brain of chick embryos using histochemical techniques for acid phosphatase (ACPase), assumed that the AM cells were derived from mesenchymal elements constituting the leptomeninges and made their way through the thin roof plate of the rhombencephalon or myelencephalon, metencephalon, diencephalon and telencephalon into their ventricles.

The present study was undertaken in the hope of gaining ultrastructural evidence for the process in which the AM cells in the leptomeninges might take a route to the ventricle through the roof plate of the brain. The work described here is an extension of the author's earlier study.
MATERIAL AND METHOD

Chick embryos of the incubation period of 8 days were used. Small pieces of the rhombencephalon coated with the leptomeninges were fixed for 1/2 hr in glutaraldehyde solution (25% glutaraldehyde 1.6 ml, 0.12 M cacodylate buffer 5 ml, 10% CaCl₂ 2 drops, H₂O₂ 0.2 ml and distilled water). Subsequently, the blocks were postfixed for 1 hr in 1% osmium tetroxide, dehydrated in graded ethanol series and embedded in araldite. Thin sections cut from the blocks were stained with uranyl acetate followed by lead citrate according to the technique of REYNOLDS (1963) and examined under a JEOL 100B electron microscope (NEC).

OBSERVATIONS

The leptomeninges closely adherent to the rhombencephalon of the 8-day incubation chick embryos were membranous tissues, in which many mesenchymal cells were reticulated (Fig. 1). The mesenchymal cells usually had large nuclei, and they were

![Fig. 1](image1.png)

Fig. 1. Amoeboid microglial cells (AMC) present in the leptomeninges (LM) of the rhombencephalon of the 8-day embryo. VEN ventricle, RP roof plate. ×2,400

![Fig. 2](image2.png)

Fig. 2. Amoeboid microglial cells (AMC) present in the leptomeninges (LM) of the rhombencephalon of the 8-day embryo. VEN ventricle, RP roof plate. ×2,100
relatively electron lucent. A large number of free ribosomes and slender, granular endoplasmic reticulum were observed in the cytoplasm. Mitochondria were small.

Singular, large cells (about 150 μ in diameter) were found here and there among the mesenchymal cells (Fig. 1, 2). These cells were characterized by irregular somata, round or oval in shape, and by long filopodial processes. The nuclei of these cells were small and indented, and the chromatin particles beneath the nuclear envelope coarsely clumped. The cytoplasm was large and contained many vacuoles, dense bodies, lipid droplets and occasional phagosomes. The Golgi complex was well-developed. Many small coated vesicles were often present in the peri-Golgi zone. Taking such a structural feature of these cells into account, these cells were considered the AM cells, as previously shown by the present author (1978).

The roof plate of the rhombencephalon of the 8-day incubation chick embryos consisted of a simple epithelium (Fig. 1, 2). The cells of the roof plate (from here on, we shall call them roof plate cells) seemed to change their morphology according to their position. This might be related to the developmental state of the cells. Among these roof plate cells junctional complexes were always observed facing the ventricle cavity. At the bases of these cells facing the leptomeninges, on the other hand, adjacent cells were in contact with each other by means of infolded cell membranes and the junctional complex was not present. The outer surface of the roof plate cells was coated closely with the basement membrane. The large cells, of which the nuclei were small and contained many chromatin particles beneath the nuclear envelope, were observed in the layer of the roof plate cells (Fig. 3–5) and in the ventricle (Fig. 6). The electron density of the cytoplasm of these cells was higher

![Fig. 3. Amoeboid microglial cell (AMC) present in the roof plate (RP) of the rhombencephalon of the 8-day embryo. Notice the very thin basement membrane coating the filopodial process of the AMC and the enlarged intercellular space in which the process are located (arrow). LM leptomeninges, VEN ventricle. ×5,600](image-url)
Fig. 4. Amoeboid microglial cell (AMC) present in the roof plate (RP) of the rhombencephalon of the 8-day embryo. In the cytoplasm prominent Golgi-complex, vacuoles and dense bodies are seen. Notice the loosened interdigitation of the mutual cytoplasm of the roof plate cells due to the invasion of the filopodial process (arrow) of the AMC. VEN ventricle, LM leptomeninges. ×6,100

Fig. 5. Amoeboid microglial cell (AMC) present in the roof plate (RP) of the rhombencephalon of the 8-day embryo. Abundant vacuoles (V) are seen in the cytoplasm. VEN ventricle, LM leptomeninges. ×3,000
Fig. 6. Amoeboid microglial cell (AMC) present in the ventricle (VEN) of the 8-day embryo. Arrow filopodia. V vacuole, E ependymal cell. ×4,200

Fig. 7. Amoeboid microglial cell (AMC) present in the roof plate (RP) of the rhombencephalon of the 8-day embryo. A greater part of the AMC is seen located in the ventricle (VEN). Notice filopodial processes (arrows) of the cell. LM leptomeninges. ×5,400
than that of the roof plate cells. The cytoplasm was characterized by a well-developed Golgi complex located near the nucleus, small coated vesicles, dense bodies, vacuoles and large mitochondria in the peri-Golgi zone. These cytoplasmic features of the cells were closely similar to those of the AM cells found in the leptomeninges. Some of these cells present in the layer of the roof plate cells projected their filopodial processes into the intercellular spaces of the latter cells (Fig. 3, 4). Others were located among the roof plate cells (Fig. 5). The most interesting situation of the large cells was the presence of the greater part of the cytoplasm in the lumen of the ventricle (Fig. 7). Such a feature made us think the cells were pushing the roof plate cells aside, protruding the cytoplasm into the lumen and stretching their filopodia in it.

**DISCUSSION**

At the electron microscopic level, the basic characteristics of the AM cells which appear during normal embryonic development of the chick brain (SCHMITT, 1973; KAWAGUCHI, 1978) are identical with those already described in the rat brain by LING and TAN (1974) and the rabbit brain by STENSAAS and REICHERT (1971). Namely, the AM cells have round or amoeboid somata with filopodial processes and small indented nuclei with chromatin particles beneath the nuclear envelope. The cytoplasm contained vacuoles, lysosome-like dense bodies and lipid droplets. These features of the cells are identical to those which are seen in the leptomeninges as well as in the layer of the roof plate cells of the 8-day incubation chick embryo brain. Regarding the site of origin of the AM cells, there are several hypotheses: ectodermal origin (LING), mesenchymal cell origin (SCHMITT) and blood cell origin (STENSAAS). As stated in the introduction, the present author (1978) claimed that the AM cells might originate from the mesenchymal leptomeninges and immigrate into the brain by way of the ventricle. It was based on the evidence that the ACPase-positive cells appeared in the leptomeninges of the chick embryo brain at the 5-day incubation stage and then in the roof plate of the rhombencephalon and its ventricle on the 7th day of incubation. LING (1977) also stated that the ACPase-positive cells in the neonatal rat brain were the AM cells. SCHMITT (1973) also arrived at the same conclusion on the basis of his periodic acid-bisulfit-aldehyde-thionin method on the 5- and 10-day incubation chick embryo brains.

In the present study, it was found that the AM cells were present in the leptomeninges, the layer of the roof plate cells, and the ventricle. Considering these findings along with the previous results obtained by the present author, it is highly probable that the AM cells originate first from the leptomeninges and then enter the ventricle via the roof plate. Thereupon, the question arose why the roof plate lies in the route of the AM cells into the ventricle. As the roof plate cells consist of a thin, simple layer and in their function of choroid plexus are concerned in the production of cerebrospinal fluid, the AM cells may be carried away through this layer as the cerebrospinal fluid moves into the ventricle. Further studies by the present author are now in progress to elucidate the function of the AM cells and, also, to make clear whether these cells are transformed into microglial cells in the mature brain.
初期鶏胚脳の蓋板領域におけるアメーバ様小膠細胞の電子顕微鏡的研究

川口 美智子

酸性フッフラートァーゼ染色法によって示唆された孵卵8日目の脳腔の蓋板領域におけるアメーバ様小膠細胞を顕微的に検索した。孵卵8日目の脳腔壁組織内や蓋板、脳室腔内にアメーバ様小膠細胞と電顕的形態の同一の細胞が多数散在する。著者の先のACPase反応の所見と今回の細胞の位置に関する三つの様式を考えてあわせると、アメーバ様小膠細胞は脳腔壁組織に発生したものであり、蓋板を通過して脳室腔内に遊走したものと思われる。

REFERENCES


