Transformation of Amoeboid Microglial Cells into Microglia in the Corpus Callosum of the Postnatal Rat Brain. An Electron Microscopical Study*

C. KAUR, E. A. LING and W. C. WONG

Department of Anatomy, Faculty of Medicine, National University of Singapore, Singapore

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Summary. An electron microscope study of the corpus callosum in postnatal rats of various ages was carried out to elucidate the fate of the amoeboid microglial cells. The cells present in the corpus callosum of younger rats (3-5 days) were round and showed an eccentric nucleus with marginal chromatin. They displayed numerous lysosomal granules and vacuoles in the cytoplasm. In older animals, i.e., from 7 days onwards some of the cells became oval so that by 15-20 days of age most of the cells were elongated and branched. In the latter, the cells showed a flattened or angular nucleus with dense chromatin clumps. The cytoplasm showed fewer lysosomal granules and vacuoles which were absent in cells of 20 day old animals. Quantitative measurements showed that there was a gradual diminution in the amount of cytoplasm at the cell body of amoeboid microglial cells with age, so that by the age of 20 days the cells were reduced to less than one-third of their original size as seen in 3 day old rats. The reduction of cytoplasm at the cell body is probably because it is channelled to the cytoplasmic processes which are evident in older rats. Some cytoplasm may have been extruded and phagocytosed by companion cell types.

The corpus callosum of the early postnatal (up to 5 day old) rat brain contains loosely organized nerve fibres with interspersed glial cells (LING and TAN, 1974). The small microglial cells described by MORI and LEBLOND (1969) in adult animals, however, are negligible in number or virtually absent during this period as they first appear about the 5th postnatal day (LING and TAN, 1974; IMAMOTO and LEBLOND, 1978). Soon after their appearance, their number increases steadily with age (LING and TAN, 1974) so that by weaning age, they form about 5% of the total glial population and their total number remains fairly constant thereafter (LING and LEBLOND, 1973). Since it appears that microglial cells are non-dividing under normal circumstances, (MORI and LEBLOND, 1969; IMAMOTO, 1981) it is reasoned that they must be derived developmentally from specific precursor cell types of which the macrophagic amoeboid microglial cell has been considered to be one possible candidate (LING, 1976, 1977; IMAMOTO and LEBLOND, 1978). In fact, the pioneer study by KERSHMAN (1939) had already noted the gradual morphological changes of amoeboid microglial cells into more branched adult microglia. However, recent work by KITAMURA and his colleagues (FUJITA, TSUCHIHASHI

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and KITAMURA, 1981; MIYAKE et al., 1984; KITAMURA, MIYAKE and FUJITA, 1984) denied the possibility of amoeboid microglial cells becoming adult microglia. Instead these authors maintained that microglial cells are derived from glioblasts. The present study was therefore undertaken to demonstrate the morphological differentiation of amoeboid microglial cells into microglia at the electron microscopical level, a process which was noted by KERSHMAN (1939) in his pioneer study but at the light microscopical level.

MATERIALS AND METHODS

Postnatal Wistar rats aged 3, 5, 7, 10, 12, 15 and 20 days were used in this study. Following ether anaesthesia, between 2 to 5 rats from each age group were perfused with a mixed aldehyde solution composed of 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.3. After perfusion, which lasted for about 15 min, coronal slices of approximately 1 mm thickness were cut out at the level of the optic chiasma. Blocks of corpus callosum about 1 mm $\times$ 1 mm immediately above the lateral ventricles were removed. They were rinsed overnight in 0.1 M cacodylate buffer containing sucrose. They were postfixed for 2 hr in 1% osmium tetroxide containing 1.5% potassium ferrocyanide. The blocks were then dehydrated in graded concentrations of ethanol and finally embedded in Araldite mixture. All ultrathin sections were cut parallel to the axons in the corpus callosum which run between the two cerebral hemispheres and stained with lead citrate and uranyl acetate and examined in an Hitachi HS-8 electron microscope. The whole area of the corpus callosum was thoroughly scanned for amoeboid microglial cells. Once identified, they were photographed at $\times 4,200$ and subsequently printed on photographic paper at $\times 10,500$. A minimum of 30 cells from each age group were examined. For each cell, the areas of the whole cell and its nucleus in cm^2 were carefully measured with a Kent planimeter; the difference between the two being the area of the cytoplasm. Only those cells in which the nucleus contained the nucleolus were used for the measurement. The total areas of the cytoplasm and nucleus were then averaged in each age group and a histogram was plotted.

OBSERVATIONS

The amoeboid microglial cells in the corpus callosum of postnatal rats were readily distinguished from other glial cells in the electron microscope. In early postnatal rats, i.e., 3–5 days old, the round cells usually lay in a wide interstitial space. They showed a copious cytoplasm loaded with abundant lysosomal granules and some large dense bodies (Fig. 1). Isolated or occasionally stacked profiles of rough endoplasmic reticulum were scattered throughout the cytoplasm (Fig. 1). Numerous vacuoles of various sizes were present in the periphery of the cytoplasm. The Golgi complex was well developed and this often encircled a centriole (Fig. 1). Some cells showed phagosomes in their cytoplasm. The round or oval nucleus was usually eccentrically placed and showed a margination of chromatin masses. A nucleolus was often seen (Fig. 1). The cell surface displayed a smooth contour (Fig. 1) or short stout processes. Figures 1–4 are intended to show the sequential changes of the cell type with age. Thus, in older animals, i.e., 7–10 days, the cells became oval with a concomitant reduction of cyto-

Fig. 1. An amoeboid microglial cell in the corpus callosum of a 3 day old rat with an eccentric round nucleus showing marginal chromatin. The cell shows a smooth contour. The cytoplasm shows numerous lysosomal granules (*Ly*) and some vacuoles (*V*). The rough endoplasmic reticulum is in isolated profiles. *G* Golgi complex. $\times 10,500$

plasm and the number of the lysosomal granules (Fig. 2). By 12 days of age, the cytoplasm was further attenuated. The sparse lysosomal granules clustered mainly in the perinuclear region and they appeared denser (Fig. 3). While most of the cells in this age group still displayed vacuolation, some contained only one to two vacuoles or even none (Fig. 3). The nucleus was oval or angular and contained extremely dense chromatin masses (Fig. 3). As the animals aged further, i.e., by 15 and 20 days, almost all the cells observed were elongated or flattened showing long processes at both ends (Fig. 4). They were intercalated in the narrow interstitial spaces, between the closely packed nerve fibres, some of which were myelinated. The flattened nucleus contained distinct chromatin masses. The cytoplasm was markedly reduced and was without vacuolation (Fig. 4). The Golgi apparatus was poorly developed and displayed small flattened saccules (Fig. 4). The lysosomal granules, though few, were highly electron dense. Cisternae of rough endoplasmic reticulum were rare and, if present, were in isolated profiles (Fig. 4).

Examination of the corpus callosum in the various age groups showed sporadic degenerating amoeboid microglial cells. Occasionally, a portion of the cytoplasm of an amoeboid microglial cell appeared to be extruded which was then partially or totally engulfed by a similar cell type (Fig. 5).

Measurements of cells of representative ages showed that in the course of differ-

Fig. 2. An oval amoeboid microglial cell in the corpus callosum of a 7 day old rat showing an eccentrically placed nucleus with some chromatin clumps. The cytoplasm shows some lysosomal granules (*Ly*), Golgi apparatus (*G*) as well as vacuoles (*v*). $\times 10,500$

entiation, amoeboid microglial cells suffered a gradual reduction in the amount of cytoplasm in the cell body. Initially, the cells remained unchanged up to the age of 5 days. However, as time elapsed, i.e., from 5th postnatal day onward, there was a steady decline in the amount of cytoplasm, so that by weaning age, i.e., around 20 days of age, only one-sixth of its original cytoplasm seen in 3 day old rats was retained (Fig. 6). Despite the alteration of its shape, the area of the nucleus remained relatively constant in the period studied (Fig. 6).

When the nucleus and cytoplasm were taken together into consideration as a cell in toto, there was a significant reduction in area of about 70% within the period studied, e.g., comparing the cells from 3 days to those of 20 days of age ($t=2.50$, $p<0.05$). In other words, the cell body of amoeboid microglial cells present in 20 day old rat was less than one-third of their original size as seen in early postnatal rats, i.e., 3 day old animals (compare the cells in Fig. 1 and Fig. 4).

DISCUSSION

Microglia, also referred to as the "third neuroglial type" (VAUGHN and PETERS, 1968), multipotential glia (SKOFF and VAUGHN, 1971) or resting microglia (LEWIS, 1968; MORI and LEBLOND, 1969; STENSAAS and REICHERT, 1971; PHILIPS, 1973; FUJITA et al., 1981) encompasses a group of small glial elements distinct from astrocytes and oligodendrocytes. The electron microscopic features of microglia were first detailed by MORI and LEBLOND (1969) who characterized the cell type by combining Rio-Hortega silver

Fig. 3. An amoeboid microglial cell in the corpus callosum of a 12 day old rat. The nucleus is angular with extremely dense chromatin masses. The cytoplasm shows a few lysosomal granules (*Ly*) and a few small vacuoles on the left (*v*). The Golgi apparatus (*G*) shows small and delicate saccules. The rough endoplasmic reticulum (arrows) is present in single isolated profiles. $\times 10,500$

staining with electron microscopy. According to them, the microglial cell had an elongated or angular nucleus containing dense chromatin masses in a light nucleoplasm. The cytoplasm formed a thin rim around the nucleus, but it often accumulated at one end. The cytoplasm contained cisternae of endoplasmic reticulum which were slightly distended and these appeared either short or long. There were one or several Golgi stacks composed of long, delicate flattened saccules with a few vesicles nearby. Dense bodies of various sizes were present. In contrast to the general consensus on their ultrastructure, there has been controversy regarding the origin of such cells. RIO-HORTEGA (1932) first hypothesized that microglial cells were derived from pial elements which invaded the brain tissues by amoeboidic movement, first as microglioblasts or amoeboid microglia and subsequently settled down as microglia. This hypothesis later received support from PENFIELD (1932), KERSHMAN (1939) and BOYA et al. (1979), although many authors until recently still hold a completely different view that these microglial cells are neuroectodermal (LEWIS, 1968; OEHMICHEN, 1978; OEHMICHEN et al., 1979; FUJITA et al., 1981; MIYAKE et al., 1984; KITAMURA et al., 1984).

With the electron microscope, the present study in the corpus callosum of post-natal rats has demonstrated a gradual morphological change of amoeboid microglia in 3 day old rat into cells which are indistinguishable from microglia in 20 days of age according to the diagnostic features listed by MORI and LEBLOND (1969). These metamorphic changes include a gradual reduction of its cytoplasm and vacuolations and a concurrent diminution of lysosomal granules at the cell body. Other changes included shape, size and cellular outline. This sequential changing process strongly supports

the hypothesis of the transformation of amoeboid microglia into microglia (RIO-HORTEGA, 1932; KERSHMAN, 1939; IMAMOTO and LEBLOND, 1978; LING, 1981; IMAMOTO et al., 1982). Further evidence is the fact that the population of amoeboid microglia steadily declines with age with a concomitant upsurge of microglial cells (LING and TAN, 1974; IMAMOTO, 1981).

Fig. 4. An elongated amoeboid microglial cell in the corpus callosum of a 20 day old rat lying between closely packed nerve fibres some of which are myelinated. The cytoplasm is accumulated at one pole of the cell and continues into its long slender process (arrows). The lysosomal granules (*Ly*) are few. The Golgi apparatus (*G*) is poorly developed showing small and flattened saccules. This particular cell has all the diagnostic features of a microglia in adult rats as described by MORI and LEBLOND (1969). $\times 9,100$

Fig. 5. An amoeboid microglial cell from the corpus callosum of a 5 day old rat engulfing a portion of the cytoplasm of another cell (*N*) of the same category. $\times 4,300$

Fig. 6. Histogram showing the surface area of nucleus and cytoplasm of amoeboid microglial cells in the corpus callosum in different age groups of rats. Each bar represents an average value of 30 cells $\pm$ standard error.

The reason for the transformation of the amoeboid microglial cells into microglia in developing brain is more likely a regressive than a passive phenomenon as postulated by KITAMURA *et al.* (1984). It is known that in the early postnatal rats they are active macrophages phagocytosing degenerating cells and processes (LING, 1976; INNOCENTI *et al.*, 1983a, b). It is at this early stage that the cells are rich in heterogeneous hydrolytic enzymes including acid phosphatase, arylsulphatase and non-specific esterase (LING, 1977; FERRER and SARMIENTO, 1980; VALENTINO and JONES, 1980; LING *et al.*, 1982), 5'-nucleotidase (KAUR *et al.*, 1984). The enzyme activities, however, gradually subside with age (LING *et al.*, 1982; KAUR *et al.*, 1984). This suggests that the amoeboid microglial cells gradually retract their phagocytic activity so that by weaning age the cells, which are now microglia-like, are in a resting form or dormant state. Indeed, the ultrastructural evidence in the present study also suggests that from 5th–7th postnatal days onwards, the phagocytic function of amoeboid microglia gradually diminishes. This is indicated by the gradual attenuation of their cytoplasm, lysosomes, small Golgi apparatus and scarcity of cisternae of rough endoplasmic reticulum during their transformation process at the cell body.

Our quantitative study shows that in the course of the transformation of amoeboid microglial cells into microglia, the cell body as represented by the area measurement, is reduced to less than one-third over a period of about 17 days, i.e. from 3 days to 20 days of age. The alteration in size is attributed largely to the reduction of cytoplasm and not the nucleus which remains fairly constant in surface area throughout the period studied. Much of the cytoplasm at the cell body may have been channelled into the cytoplasmic processes as suggested by KERSHMAN (1939).

Another possible explanation for the diminution of the cytoplasm may be that it is partially extruded and then is phagocytosed by their companion cells as has been observed in the present study. This would mean an actual loss of the cytoplasm in the course of differentiation of the cell.

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Dr. Charanjit KAUR
Department of Anatomy
Faculty of Medicine
National University of Singapore
Lower Kent Ridge Road
Singapore 0511