Acta neuropath. (Berl.) 32, 341—346 (1975)

Hematogenous Cells in Experimental Japanese Encephalitis

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Received May 14, 1975; Accepted June 24, 1975

Summary. Four adult mice were injected with ^{3}H-thymidine repeatedly so that in their brains only circulating blood cells were labelled with ^{3}H-thymidine. They then received an intracerebral injection of Japanese encephalitis virus, were sacrified on the 3rd, 4th and 5th day after inoculation: the brains were examined by light and electron microscopic autoradiography. Inflammatory cells appearing in the brain parenchyma and perivascularly in the acute stage of the experimental Japanese encephalitis are derived from circulating mononuclear leukocytes. They assume the shape of "rod cells" and are the main constituents of the "glial nodule" in the brain parenchyma. Their fine structural characteristics are discussed.

Key words: Japanese Encephalitis — Hematogenous Cells — Rod Cell — Glial Nodule — EM-Autoradiography.

Introduction

It is important to dicide precisely the participation of hematogenous cells in the various pathological changes in nervous tissue. We reported that in stab wounds of the brain, almost all the macrophages appearing in the wounds were derived from circulating blood mononuclear cells, especially monocytes, and that autochthonous cells of the brain, including pericytes and neuroglia do not participate in macrophage formation (Kitamura *et al.*, 1972a and b; Kitamura, 1973). In the present experiment we applied the same method to study the acute stage of virus encephalitis in which many inflammatory cells occur in the brain parenchyma and perivascularly. The cells in perivascular cuffings are believed to be of hematogenous origin. The origin of intraparenchymal inflammatory cells is still controversial and we have attempted to decide their origin. Changes in the perivascular areas of the same material reported elsewhere (Kitamura *et al.*, 1972c).

Materials and Methods

Virus. The JaGAr-01 strain of Japanese encephalitis virus was used. Several infected brains of mice, stored in a freezer, were mashed and centrifuged at 10000 rpm for 30 min. The supernatant was diluted with 1/100 M phosphate buffer solution to a concentration of 10^{-4} of original solution, and this suspension was used for inoculation.

Procedure. Four adult mice of ddN strain, weighing approximately 20 g, received 16 injections of ^{3}H-thymidine at intervals of 6 hrs for a total duration of 90 hrs. Each injection contained 80—150 μCi of ^{3}H-thymidine. Twelve hours after the last injection one mouse was sacrificed as a control and the remaining three mice were inoculated intracerebrally with the virus suspension. Each mouse was sacrificed by perfusion fixation with 2% glutaraldehyde, buffered at pH 7.6 with 1/20 M phosphate buffer solution, 3, 4 and 5 days after the inoculation, respectively. The cerebral cortex was obtained from each brain. After osmium fixation, the material was dehydrated and embedded in epoxy resin. Smear preparations of peripheral blood were made from the control mouse just before sacrifice. One micron sections and ultrathin sections were cut from the epon embedded material for light and electron microscopy. These

sections and smears of the peripheral blood were processed for autoradiography. The autoradiographic technique was reported in previous papers (Kitamura *et al.*, 1972a and b). The labelling indices of cells in the control cerebral cortex were counted on light microscopic autoradiographs and those of the infected brains on EM autoradiographs. The labelling indices of circulating leukocytes in the control mouse were obtained from autoradiographs on the smear preparations.

Results

Control Mouse. The labelling indices of each cell population in the brain are shown in Table 1 left. Although the injections of ^{3}H-thymidine were performed intensively and over a long period, only a few labelled cells were detected in each cell population in the normal brain. The labelling indices of the peripheral blood are shown in Table 1 right. Almost all neutrophils and a large portion of monocytes were labelled. The labelling indices of the lymphocytes were rather small.

Infected Brains. On the 4th day after inoculation many labelled mononuclear cells were observed in the infected brain on EM autoradiograph. They were detected mainly around small veins and some of them were seen penetrating the vascular wall. Labelled leukocytes were often found in the lumen of the vessels attached to the endothelial surface. Many labelled cells were detected also in the brain parenchyma, usually occurring singly, but sometimes forming a small cluster. Labelling indices of leukocytes in the vascular lumina of the infected brain was 71% and of cells forming perivascular cuffings 72%. The labelling index of the inflammatory cells in the brain parenchyma also reached 84% (Tab. 2).

In electron micrographs the labelled cells in the brain could be arranged in two groups according to their morphological characteristics. The cells of the first group had relatively much heterochromatin in the nucleus and a large amount of cytoplasm containing long segments of rough endoplasmic reticulum

Table 1. Labelling indices (LI) of cell populations in the brain and of the circulating blood (control mouse)

	LI %		LI %
Neuroglial cells	0.6	Neutrophils	97
Pericytes	0.3	Monocytes	77
Endothelial cells	0.2	Large lymphocytes	38
		Small lymphocytes	4

Table 2. Labelling indices (LI) of cell populations in the infected brain at the 4th day after the inoculation, counted on the electron microscopic autoradiographs

	LI %	cell number (labelled cell/total cell)
Inflammatory cells in the brain parenchyma	84	47/56
Inflammatory cells forming perivascular cuffings	72	64/89
Mononuclear leukocytes in the vascular lumina	71	22/31
Pericytes	0	0/10
Endothelial cells	0	0/44

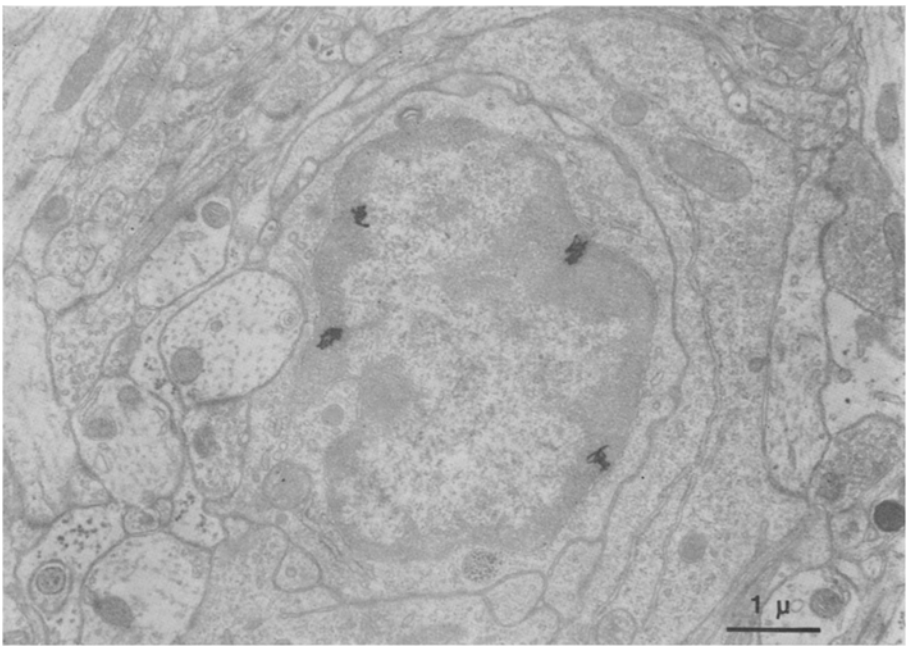

Fig. 1. Electron microscopic autoradiograph from mouse sacrificed on the 4th day after inoculation. A labelled monocyte in the brain parenchyma has a large cell body with long arrays of rough endoplasmic reticulum, a few lysosomal dense bodies and many polyribosomes. ×12800

and several lysosomal dense bodies. A number of polyribosomes and a well-developed Golgi-apparatus were also encountered. These cells had thick peripheral processes (Fig. 1). Occasionally the cytoplasmic organelles were observed only at one pole. The cells of the second group had more condensed chromatin in the nucleus and sometimes showed nuclear sphaeridium (nuclear body). The cytoplasm was rather electron-dense and relatively large but contained only few organelles having a few polyribosomes, few mitochondria and short arrays of rough endoplasmic reticulum. The Golgi apparatus was poorly developed. Lysosomal dense bodies were occasionally detected. The cells had cytoplasmic processes which were smaller than those of the other cell group (Fig. 2). The labelling index of this type of cell was 72%.

Plasma cells were rarely detected in our experiments.

Discussion

It is generally accepted that in the acute stage of virus encephalitis hematogenous cells are the main constituent of the perivascular cell-accumulations. Many inflammatory cells can be detected in the brain parenchyma. They have an elongated nucleus, and are called "rod cells" and are scattered in the parenchyma or form clusters or "glial nodules". These cells have been considered by many authors to

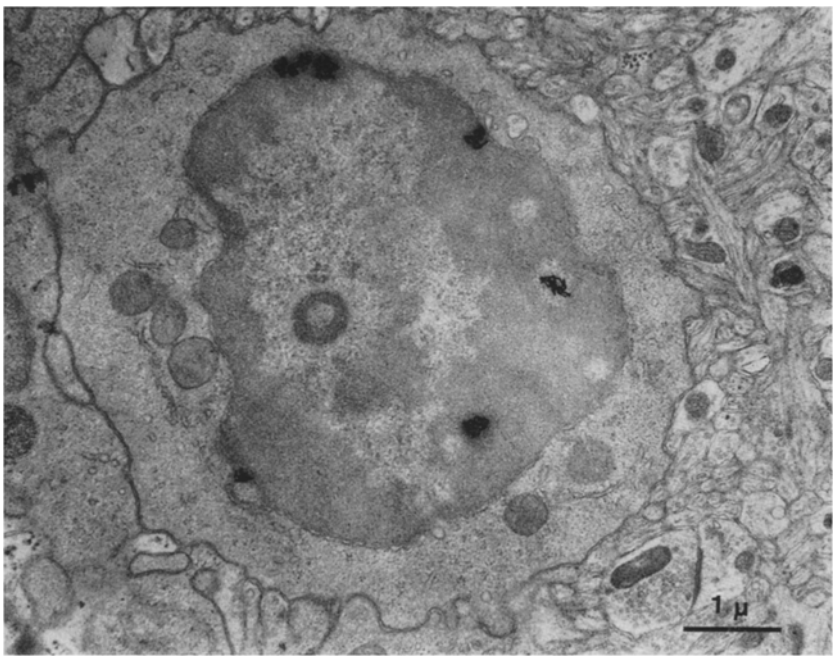

Fig. 2. Electron microscopic autoradiograph from mouse sacrificed on the 4th day after inoculation. A labelled mononuclear cell in the brain parenchyma, showing dense chromatin and a nuclear sphaeridium (nuclear body). The cytoplasm contains few mitochondria, several short arrays of rough endoplasmic reticulum and a small number of polyribosomes. The cytoplasm is relatively electron dense. ×12800

be derived from cells that pre-exist in the normal brain; the "resting microglia" and perivascular mesenchyme cells are thought to be their precursors.

We detected a peculiar cell population in the infected brain parenchyma: these cells had a characteristic ultrastructure and similar cells were seldom seen in normal brain parenchyma. They are similar to the cells in the perivascular cuffings. By autoradiography on the 4th day after inoculation, a large portion of them was tagged with silver grains, and their labelling index reached 84%. This value is almost on the same level with that of cells forming "perivascular cuffings" (72%) and of mononuclear leukocytes in the vascular lumina of the same brain (71%), whereas at the time of inoculation only a few labelled cells were detected in the brain (labelling indices of neuroglia, pericytes and endothelial cells were 0.6, 0.3 and 0.2%, respectively). Therefore, these high and comparable value of the labelling indices indicate that almost all of these inflammatory cells in the brain parenchyma and the cells forming the perivascular cuffings are derived from circulating mononuclear leukocytes.

The nucleus of labelled haematogenous cells was generally rich in heterochromatin and often elongated in shape. These nuclear characteristics were prominent in light microscopic preparations (Sato). It is reasonable to consider that a large portion of the so-called "rod cells" observed in the acute stage of

virus encephalitis may be of hematogenous origin. In the brain parenchyma we also observed clusters of inflammatory cells similar to those reported by Anzil *et al.* as an electron microscopic counterpart of the "glial nodule". They were not surrounded by basement membrane. On EM-autoradiographs a large portion of these inflammatory cells were tagged with silver grains and their fine structural characteristics were similar to hematogenous cells scattered in the parenchyma. Although a few ectodermal glial cells were detected in the cluster, they did not seem to be an active component but to be pre-existing elements included in the massive infiltration by hematogenous cells. We think, therefore, that hematogenous cells are also the main constituents of the so-called "glial nodule".

Electron micrographs of labelled hematogenous cells in the brain had a plump shape and their configuration was easily outlined. Generally their cytoplasm being filled with fine granules was more electron-dense than the surrounding nervous tissue. These migrating hematogenous cells could be divided into two groups. One group have a relatively large amount of cytoplasm containing a well-developed Golgi-apparatus, several lysosomal dense bodies and a large number of polyribosomes. They had often many thick, pseudopodia-like processes. Although the number of cytoplasmic organelles was smaller than that of "transformed monocytes" in stab wounds (Kitamura *et al.*, 1972b), their fine structure as well as their high labelling index suggest that these cells are derived from circulating monocytes. The second group of labelled cells contains only a small number of organelles in their dense cytoplasm. They had few mitochondria and short arrays of rough endoplasmic reticulum. The Golgi apparatus was poorly developed. Lysosomal dense bodies could be detected only occasionally. Their nucleus had more condensed chromatin than the former group and sometimes showed a nuclear sphaeridium. Their cytoplasmic processes were poorly developed. Cells having these morphological characteristics have been regarded generally as lymphocytes (Veerman). However, the labelling index of this type of cells was 72%. This value was much higher than that of circulating lymphocytes of the control mouse. The explanation of this discrepancy between their morphology and their labelling index and the precise nature of this type of cell remain to be solved in further investigations.

Acknowledgement. The author thanks Dr. Toshiro Karaki (Public Health Research Institute of Kyoto City) for his kind cooperation in the preparation of the virus suspension.

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