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Nature of Macrophages in Rat Brain

A Histochemical Study

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Abstract. The nature of phagocytes appearing in lesions of the central nervous system is strongly debated with a tendency to assess an exclusively hematogenous origin. We studied the origin of phagocytes appearing in a stab wound in the rat brain. Histochemical stains for acid phosphatase and peroxidase, and silver impregnation techniques were used for our study. The results obtained showed the existence of two macrophage types: endogenous microgliocytes and exogenous monocytes.

Introduction

The nature of phagocytic cells appearing in injured areas of the central nervous system is being debated at present. One of the main problems, as previously pointed out by Schultz and Pease [1959], is the impossibility to differentiate accurately among macrophage types by their light- or electron-microscopical appearance. According to Rio-Hortega [1932], brain macrophages are derived from microglia. This author described transitional forms between ramified or 'resting' microglia located away from the lesional area and compound granular corpuscles or 'gitter' cells which appeared in injured regions. This theory, which was widely accepted initially, was later challenged with the introduction of newer methods, mostly electron microscopy and autoradiography. For some authors, brain macrophages are entirely of hematogenous origin [Konigsmark and Sidman, 1963; Huntington and Terry, 1966; Sato, 1968; Olsson and Sjöstrand, 1969; Ticer and Tietz, 1969; Adrian and Smothermon, 1970; Kitamura, 1973; Fujita and Kitamura, 1975; Kitamura et al., 1977; Young, 1977; Imamoto and Leblond, 1977; Blinzinger et al., 1978; Adrian et al., 1978; Ling, 1978, 1979; Del Cerro and Monjan, 1979]. Other studies instead suggest that brain macrophages may derive from endogenous elements of the brain [Maxwell and Kruger, 1965; Hager, 1968; Mori and Leblond, 1969; Vaughn and Pease, 1970; Vaughn et al., 1970; Torvik and Skjörten, 1971; Baron and Gallego, 1972; Torvik, 1975; Boya, 1976].

There are many thorough studies regarding the content in hydrolytic enzymes of other body macrophages [Dannenberg et al., 1963; Enomoto and Kitani, 1966; North, 1966a, b; Seeman and Palade, 1967; Van Furth et al., 1970; Nichols et al., 1971; Nichols and Bainton, 1973]. However, histochemical studies dealing with central nervous system macrophages are both scare and inconclusive. Also, they are mainly oriented toward the study of ameboid microglia in perinatal stages [Ling, 1976, 1977; Boya et al., 1979].

Material and Methods

Twenty-four Wistar albino rats of an age of 3 months were used for our study. Under ether anesthesia, bilateral stab wounds were inflicted on both cerebral hemispheres through a hole located 3 mm behind the frontoparietal suture. A sterile candent needle was introduced 4 mm deep vertically to the skull. After 3, 5, 7 and 10 days, the animals were sacrificed by decapitation under ether anesthesia. The brain was removed and fixed by immersion in Bouin's fluid for routine hematoxylin-eosin (HE) staining or 3% glutaraldehyde in 0.1 *M* phosphate buffer for silver impregnation and histochemistry. The silver method of Weil and Davenport [1933] was used to impregnate microglial cells. The Miller and Palade [1964] and the Fahimi [1969] techniques were used for the demonstration of acid phosphatase and peroxidase activities, respectively. All sections were cut perpendicular to the needle path.

Results

Of the 4 postoperative intervals studied, the 5-day group showed the best macrophagic reaction around the wound, and thus the following description will be mostly

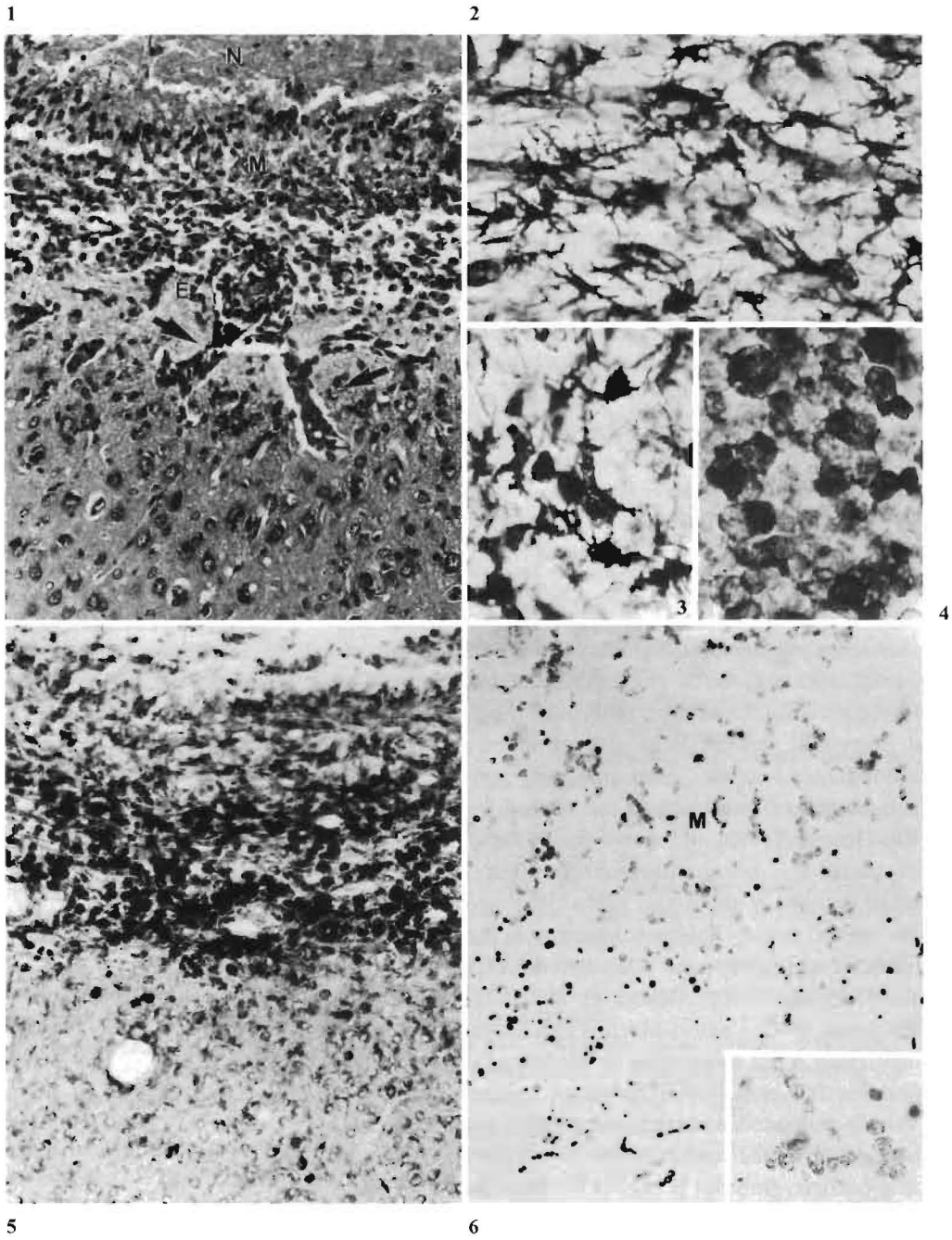

Fig. 1. A transversal section of a 5-day-old wound. The central necrotic zone (N), located on the top of the figure, is surrounded by a thick macrophagic layer (M) and an edematous layer (E) with neoformed blood vessels (arrows). The nervous parenchyma near the wound shows hypercellularity. HE. × 100.

Fig. 2–4. Microglial activation as seen in silver-impregnated sections. 5th postoperative day. × 200. **2** Nervous parenchyma near the wound; hypertrophic microglia. **3** Edematous layer; pseudopodial microglia. **4** Macrophagic layer; compound granular corpuscles.

Fig. 5. Acid phosphatase activity. Same field and magnification as figure 1. The phagocytic cells of the macrophagic layer show a strong positivity. There are also numerous acid-phosphatase-positive cells in the nervous parenchyma near the wound. 5th postoperative day. × 100.

Fig. 6. Peroxidase activity. Same field and magnification as figures 1 and 5. In the macrophagic layer (M), few phagocytic cells show peroxidase-positive granules. In the nervous parenchyma near the wound, positive cells are virtually absent. Darkly stained globules in this region are red blood cells. × 100. Inset: higher magnification of macrophages with positive reaction to peroxidase. × 200.

based on this group. With HE, 4 concentric zones can be differentiated in the sections (fig. 1). From the center of the wound to the periphery these zones were as follows: (1) a necrotic central zone; (2) a cellular layer rich in macrophages, with numerous globular and ameboid cells, which frequently contained phagocytosed material inside them. Scarce polymorphonuclear leukocytes were found in this layer; (3) an edematous layer with many neoformed blood vessels; and (4) an external region in which the nervous parenchyma showed only minimal alterations, having an increased cell count, however. This increase was due to the appearance of small dark nuclei, often of elongated shape (fig. 1). Mitotic figures were constantly found in this region.

The silver method of Weil and Davenport [1933] showed typical ramified microglia in regions distant from the wound. Approaching the wound [fig. 2], microglial cells became hypertrophic (with shorter and thicker processes which were less ramified), eventually becoming ameboid and pseudopodial (fig. 3), to be finally round-shaped near the necrotic central region (fig. 4).

The histochemical technique of Miller and Palade [1964] showed numerous rounded cells full of acid-phosphatase-positive granules. These cells were located in the macrophagic layer described above (fig. 5). Higher acid phosphatase activity was found in regions near the wound, corresponding to the hypercellular area described with HE (fig. 5). There was also a strong pericytic positivity in all regions around the wound, as well as in normal brain tissues.

With the Fahimi [1969] technique for peroxidase activity, the macrophagic layer contained few rounded cells with peroxidase-positive granules in their cytoplasms (fig. 6). Red blood cells were darkly stained due to the pseudo-peroxidase activity of their hemoglobin. Cells with peroxidase-positive granules were practically absent outside the macrophagic layer (fig. 6).

Discussion

According to our results, 5 days after a cerebral wound, a thick layer of globular phagocytic cells was already well-formed around the wound. Moreover, the development of a microglial reaction was also observed in the silver-impregnated sections, including evidence of microglial activation and migration to the wound, as described by Rio-Hortega [1932]. Practically all of these macrophages contained acid-phosphatase-positive granules, but only a small amount of them had peroxidase-positive granules. Peroxidase activity has been described in the monocytes of different animal species [Dannenberg et al., 1963; Van Furth et al., 1970; Nichols et al., 1971; Daems and Brederoo, 1973; Caxton-Martins and Daimon, 1976], and confirmed in rat monocytes by Daems et al. [1975, 1976] and Bentfeld et al. [1977]. However, this enzyme is absent in both endogenous brain macrophages and their precursors, which on the contrary both do present acid-phosphatase-positive granules [Ling, 1977; Boya et al., 1979]. At the edge of the wound, we found few cells with peroxidase-positive granules which would correspond to extravasated leukocytes. However, as most macrophages present do not contain this type of granule they should not be considered to be of hematogenous origin.

Our results suggest a double source for the phagocytes which appear in a brain stab wound: numerous acid-phosphatase-positive and peroxidase-negative macrophages (endogenous phagocytes of microglial origin) and scarce acid-phosphatase- and peroxidase-positive macrophages (exogenous phagocytes of hematogenous origin).

According to Blinzinger et al. [1978], migrating monocytes rapidly lose their peroxidase-positive granules, mainly when engaged in active phagocytosis. Thus, some of the acid-phosphatase-positive and peroxidase-negative macrophages may in fact be monocytes lacking peroxidase activity. However, if this were the case, peroxidase-positive cells should be observed in brain tissues more distant from the wound, where they have not yet initiated phagocytosis, and not exclusively close to the necrotic area, where they were really found. It should be emphasized that these regions near the wound showed increased numbers of small dark nuclei in anilin-stained sections, and numerous activated microgliocytes in silver-impregnated sections. Higher acid phosphatase activity was clearly observed in these regions as compared with normal brain tissue.

Regarding autoradiographic studies, Adrian and Schelper [1981] have demonstrated a more prolonged systemic availability of ^{3}H-thymidine than traditionally admitted. Therefore, some of the labeled macrophages would take the tracer up 'in situ', and thus may in fact not be of hematogenous origin. This finding raises the need for a careful reexamination of the conclusions obtained by means of this technique.

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