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Summary. **The development of microglial cells in the postnatal rat retina is described using histochemical techniques for acid phosphatase and peroxidase as well as silver impregnations for microglia. On the second postnatal day, round acid phosphatase-positive macrophages appeared on the vitreal surface of retina, locating themselves close to developing blood vessels. Later, microglial precursors invaded retinal tissues, reaching the outer plexiform layer by the tenth postnatal day. In all stages studied, microglia or their precursors were peroxidase-negative. The transformation of round microglial precursors into adult ramified microglia is also described. Owing to the relation found between developing microglia and blood vessels, a vascular origin is proposed for the retinal microglial cells.**

RIO-HORTEGA (1919-1932) described microglia as mesodermal cells which invade nervous tissues during perinatal stages, ultimately spreading throughout the entire central nervous system where they act as macrophagic cells. The presence of microglia cells in the retina of mammals has been reported by several authors (LOPEZ ENRIQUEZ, 1926; VRABEC, 1968, 1975; BOYCOTT and HOPKINS, 1981; LING, 1981, 1982). Retinal microglia have the same histochemical (TERUBAYASHI et al., 1984) and immunological markers (HUME et al., 1983) as brain microglia.

From his findings on the origin of microglial cells, RIO-HORTEGA (1919) proposed a mesodermal nature for these cells. Using histochemical and ultrastructural methods we have found evidence supporting a double source, meningeal and vascular, for brain microglia cells (BOYA et al., 1979, 1986 b). The origin of retinal microglia has some special and interesting features. First of all, owing to the absence of a meningeal covering, blood vessels become the only possible source for microglial cells. Moreover, these blood vessels penetrate retinal tissues only from the vitreal surface of the retina. This orderly pattern of blood vessel ingrowth as well as the regular layering of the retina makes the invasion of the retina by microglial cells easy to follow.

The appearance of macrophagic cells in the retina of mammals during the first stages of postnatal life has been reported using light microscopy (LING, 1982; PERRY et al., 1983) histochemistry (SANYAL et al., 1980; LING, 1981; MURABE and SANO, 1981; SANYAL and DE RUITER, 1985; LINDEN et al., 1986), immunohistochemistry (HUME et al., 1983), and electron microscopy (LING, 1981). These cells were interpreted as monocytes (LING, 1981; LINDEN et al., 1986) or non-monocytic microglial precursors (SANYAL and DE RUITER, 1985).

In the present light microscope study, we describe the appearance and evolution of retinal microglia using histochemical and silver impregnation techniques.

MATERIALS AND METHODS

Albino rats were sacrified by an overdose of ether at intervals of two days after birth until the 18th postnatal day. The eyes were removed and fixed by immersion in 3% glutaraldehyde in 0.1M phosphate buffer. Sagittal frozen sections were stained with the MILLER and PALADE (1964) technique for acid phosphatase, the DEIMANN (1984) technique for peroxidase and the silver impregnation method by WEIL and DAVENPORT (COOK, 1974) for microglial cells. Since the vascular network located on the vitreal surface of the retina was usually detached from the frozen sections, the whole retina was dissected out from the eyes of one rat at each interval of age. The dissected retinas were incubated en bloc for the two enzymatic activities studied, and flat-mounted on gelatinized slides. In this way, the enzymatic activity of macrophages associated to vitreal blood vessels could be demonstrated.

RESULTS

In the first postnatal week, foamy rounded cells were seen in routinely stained paraffin sections (Fig. 1). These cells were strongly acid phosphatase-positive but negative for peroxidase activity. The appearance, location and mode of spreading of these cells were closely related with developing retinal blood vessels. Thus, in the 2 day old rat, acid phosphatase-positive cells were found near developing blood vessels located on the vitreal surface of retina (Fig. 2). Between 4 and 6 postnatal days, as blood vessels grew into the most internal layers of the retina, acid phosphatase-positive cells began to appear in these layers. These cells showed a deeper location with increasing age. Thus, acid phosphatase-positive cells were found in nerve fibre and ganglion cell layers 4 days after birth (Fig. 3). In 6 day-old rats, these cells penetrated to the inner plexiform layer, reaching the inner granular layer at 8 days (Fig. 4). In all of these stages the cells located in central regions of the retina, near the optic nerve, penetrated deeper than in peripheral retina.

Fig. 1. Foamy rounded cells (arrowheads) in the nerve fiber layer of the retina. 4-day-old rat. Hematoxylin-eosin. ×420

Fig. 2. Flat-mounted retina. 2-day-old rat. Acid phosphatase. Macrophagic cells close to blood vessels located on the vitreal surface of the retina. ×200

Fig. 3. Acid phosphatase-positive rounded cells in the innermost layers of the retina. 4-day-old rat. Acid phosphatase. ×290

Fig. 4. Acid phosphatase-positive cells in the inner granular layer. 8-day-old rat. Acid phosphatase. ×200

Fig. 5. Ameboid and pseudopodical microglia in the innermost layers of the retina. 4-day-old rat. Silver impregnation. ×520

Fig. 6. Ramified microglia (arrowheads) in inner layers of the retina. One of these is already located in the inner granular layer. 6-day-old rat. Silver impregnation. ×200

Fig. 7. Acid phosphatase-positive cells in the inner granular layer and outer plexiform layer (arrowhead). 10-day-old rat. Acid phosphatase. ×330

Fig. 8. Final distribution of microglial cells in the rat retina. 14-day-old rat. Silver impregnation. Microglial somata are mainly located in the outer plexiform layer, inner granular layer and ganglion cell layer. ×240

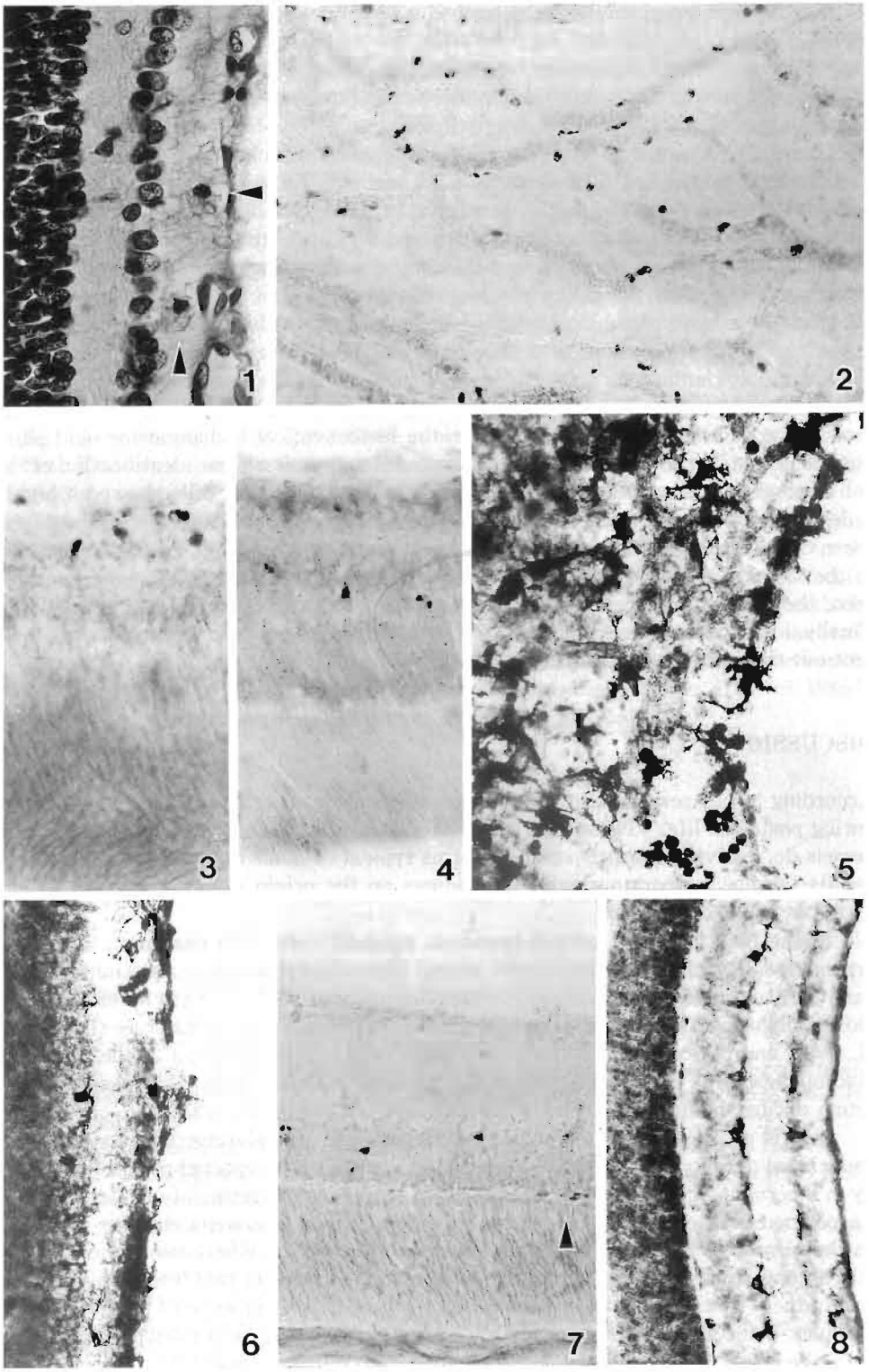

Fig. 1-6. Legends on the opposite page.

Results similar to the above described with an acid phosphatase technique were found in silver impregnated sections. On the earliest postnatal days, round and ameboid impregnated cells were observed in the innermost layers of the retina (Fig. 5). At the end of the first postnatal week, these cells branched out while penetrating into deeper layers (Fig. 6).

From day 8 onwards, the rat retina displayed a histological appearance and a vascular pattern similar to those of the adult rat. With the acid phosphatase method a gradual replacement was found, consisting of globular cells loaded with large acid phosphatase-positive granules for scattered granules of decreasing size. From day 10 onwards, acid phosphatase-positive cells were seen throughout the inner layers of the retina inwards from the outer plexiform layer (Fig. 7). Neither cells with acid phosphatase activity nor blood vessels were found outwards from the outer granular layer. Silver impregnations also showed a gradual transition of ameboid microglia towards adult ramified forms. Peroxidase negative results remained unchanged.

From the second postnatal week onwards, the retinal microglia cells reached their final shape and distribution. At this age, the histochemical technique for acid phosphatase showed granules too small and scattered to permit a clear identification of the cell contour. In silver impregnated sections, retinal microglia cells showed a highly ordered distribution with three typical locations (Fig. 8). In the outer plexiform layer, these cells were placed horizontally with processes branching out on only one plane. In the vitreal side of the inner granular layer, numerous microglial somata could be seen, their processes branching out extensively towards the inner plexiform layer. Finally, in ganglion cell and nerve fibre layers there were also microglial somata which sent out their processes towards the inner plexiform layer (Fig. 8).

DISCUSSION

According to our results, acid phosphatase-positive round cells invaded the rat retina during postnatal life. These cells come into retinal tissues at the same time as blood vessels do, evolving towards ramified forms typical of adult microglia. The present results thereby support our previous findings on the origin of brain microglia cells (BOYA et al., 1979, 1986 b).

In the first postnatal period, round or ameboid cells with enzymatic activities typical of macrophages appear in the retina. Thus, the following enzymatic activities have been reported for these cells: N-acetyl-β-glucosaminidase (SANYAL et al., 1980), inosine diphosphatase (SANYAL and DE RUITER, 1985), non-specific esterase (LINDEN et al., 1986) and peroxidase (LING, 1981; LINDEN et al., 1986). Invasion of the retina by macrophagic cells is probably related to the extensive cell death that occurs in the retina during the first five to ten postnatal days (PERRY et al., 1983).

In this study, globular cells showed strong acid phosphatase activity although peroxidase activity was absent. LINDEN et al. (1986) have reported peroxidase activity in macrophagic cells located on the vitreal surface of the retina of one day-old rats. According to these authors, however, peroxidase activity was weak and was seen only on the vitreal surface but not within the retina itself. LING (1981), using the electron microscope, described two macrophagic types in the retina of rats 5 to 6 days of age. One of these macrophage types showed peroxidase activity in some of its cytoplasmic granules. According to our results, microglial precursors lack peroxidase activity in both the brain (BOYA et al., 1979, 1986 b) and retina. Peroxidase activity was,

however, found in some of the macrophages appearing after a brain wound (BOYA et al., 1986 a).

A perfect parallelism was found between the results obtained with both acid phosphatase histochemical technique and silver impregnation for microglial cells. The disappearance of acid phosphatase-positive rounded cells occurs at the same time as microglia cells become progressively ramified as shown by the silver impregnation technique. This ramification, together with a decrease in phagocitic activity and, accordingly, of lysosomal number and size, accounts for the failure to visualize microglial cells through the acid phosphatase technique as the animal age increases. The transformation of round microglial precursors into final ramified forms is related to retinal maturity, and is probably a consequence of the decreasing extracellular spaces associated with the increasing maturity of nervous tissues. A similar differentiation has been found during the development of brain microglia (BOYA et al., 1986 b).

The differentiation of retinal microglia is closely related to the development of retinal blood vessels. The appearance as well as the penetration into retinal tissues by both blood vessels and microglial precursors occur simultaneously. Adult ramified microglia are present only in vascular retinal layers. Histochemical techniques for thiamine pyrophosphatase (TERUBAYASHI et al., 1984) and inosine diphosphatase (SANYAL and DE RUITER, 1985) stain both blood vessels and microglial cells in the retina of several species of mammals. All these findings seem to suggest a vascular origin for retinal microglia. Therefore, microglial cells could be mesodermal elements which derive from round or ameboid cells that become detached from the walls of developing blood vessels. SANYAL and DE RUITER (1985) came to the same conclusions for the origin of retinal microglia. In previous studies (BOYA et al., 1979, 1986 b) we also found evidence of a vascular origin for brain microglial cells.

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