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Regional adaptation of Müller cells in the chick retina. A Golgi and electron microscopical study

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Summary. We report the morphological differences of Müller cells in relation to their topography, using the Golgi method. Müller cells in the central retina are long and slender, with numerous inner prolongations. In the peripheral retina, the morphology of the Müller cells adapts to the reduced thickness of the retinal layers. In this zone, they are short and have thick inner prolongations which end in a large foot in the internal limiting membrane. In the optic disc margin, Müller cells have a particular morphology characterized by thick, arched prolongations that in general form a glial network between the retina and optic nerve. The ultrastructure of these cells is also described. The results are discussed with respect to the nature of Müller cells.

Key words: Müller cells, Golgi method, Optic disc, Chick

Introduction

Müller cells are the most prominent glial elements of the vertebrate retina (Cajal, 1892), and have been classified as modified astrocytes (Polyak, 1957), which extend perpendicularly between the internal and external limiting membrane providing mechanical support and physiological insulation for nerve cells. In addition, Müller cells, particularly those in the avascular retina, play an important role in relation to nutrition of the retina and in storage of energy sources (Kuwabara and Cogan, 1961; Magalhaes and Coimbra, 1972; Schabadasch and Schabadash, 1972; Loredana D'Este et al., 1983; Prada et al., 1988). On the other hand, the findings of Prada (1980), Smith (1982) and Prada et al., (1988) suggest that Müller cells of the chick retina may function in myelogenesis in a similar manner to the oligondendroglia in the central nervous system (C.N.S.). From the histogenetic point of view, Müller cells have been considered as typical glioependymal derivatives (Meller and Glees, 1965) or as derived from glioblast (Ikeda et al., 1980). More recently, Turner and Cepko (1987) have shown that a common progenitor for nerve and Müller glial cells persists in the rat retina late in development. Many fundamental aspects of Müller cells are not well understood, including their nature, functional capacity and structural behaviour. In this paper we report the morphological differences of Müller cells in relation to their topography. The particular morphology and structural behaviour of these cells in the optic disc zone, which has scarcely been studied, is also described. Only Marchesani's (1926) and Wolter's (1956) studies refer to this area of glial cells.

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Materials and methods

We used White Leghorn chick embryos and adult retinas. Chick embryos were incubated at 37.5° C. They were staged according to Hamburger and Hamilton (1951), at half-day intervals between the 6th and 20th days of incubation. The whole embryos were directly immersed in Stensaas solution (1967) and the fixation time was varied between two and four days. Fixed embryos were briefly rinsed in tap water, then washed in a 0.75% (w/v) silver nitrate solution, and finally impregnated in a large volume of the same solution for two days.

The eye globes from the adult chicks (one month after hatching) were immersed in Colonnier's solution (1964). The fixation time varied between 7 and 8 days. They were then washed prior to staining with silver nitrate 0.5%.

Microphotographs were made from the most representative fields using a Leitz Orthoplan microscope and Copex Pan film (35 mm).

For electron microscopy, the adult retinas were fixed by immersion in 1% glutaraldehyde, 1% paraformalde-

hyde in 0.1 M phosphate buffer, and postfixed in 1% osmium tetroxide solution. Pieces of retina of 1-3 mm were embedded in Epon 812.

Ultrathin sections were cut with a LKB-III ultramicrotome and stained with uranyl acetate and lead citrate. They were viewed in a transmission electron microscope JEOL 100-C.

Results

The Müller cells of the chicken show morphological differences depending on the retinal zone in which they lie (Fig. 13). Thus Figs. 1 to 4 show the morphological characteristics possesed by the Müller cells in the central retina (Fig. 1), in the equatorial retina (Figs. 2, 3) and in the peripheral retina (Fig. 4). The Müller cells of the central retina (Fig. 1) have a polygonal perikaryon, situated in the middle third of the inner nuclear layer (INL). Two poles are distinguishable in the perikaryon. One is apical and gives rise to the external prolongation of the cell, which travels in scleral direction. The other pole is internal and a network of prolongations rise from it in vitreal direction reaching, in the majority of cases, the internal limiting membrane (ILM). The external prolongation of the Müller cells is of considerable thickness and shows a very constant morphological pattern in comparison with the morphological characteristics of the internal prolongations. The internal network of the Müller cell prolongations of the central retina is composed of 4 to 6 principal trunks, which ramify inside the inner plexiform layer in an equal number of secondary branches. Overall the internal prolongations have a similar diameter to the external prolongation. This fact remains constant in all the Müller cells and in the various regions of the retina.

A comparison of Figs. 1-4 shows that the Müller cells are thicker and not so long when the retina decreases in thickness. The number of prolongations of the internal network of the Müller cells diminishes progressively from the central to the peripheral retina, and these prolongations become progressively shorter and thicker. Thus, in the peripheral retina (Fig. 4) the Müller cells adapt to the reduced thickness of the retinal layers. They show a thick cytoplasm with shorter and thicker prolongations than in the equatorial and central retina. On the other hand, it may frequently be observed in the peripheral retina that some internal prolongations adopt tangential and oblique paths through the IPL, before reaching the ILM (Fig. 4 arrow-heads). This fact makes the field occupied by the internal prolongations of these cells, at the level of the ILM, approximately double that occupied by the Müller cells lying in the central retina.

In the very centre of the retina near the optic disc the Müller cells show the following variations with respect to the morphological pattern of the Müller cells of the central retina: (a) increase in length of the internal prolongations at the same time as a decrease in length of the glial cytoplasm between the nucleus of the external limiting membrane (ELM) (Fig. 5); (b) it is frequently observed that one of the internal prolongations takes a path parallel to and in opposite direction to that of the fibres of the optic nerve, before reaching the ILM (Fig. 5 arrow-heads).

The morphological differentiation of the Müller cells lying in the juxta-optic disc region of the chick retina takes place between the 6th and 7th day of incubation. Fig. 6 shows one of these cells (outline arrow) on the 7th day of incubation. Note the similarity of this cell to that of the cell in Fig. 5 described earlier.

In the optic disc, the morphological modifications shown by the Müller cells are very important. The greater part of the body of these cells lies in the outer half of the retina (Fig. 9 horizontal arrows) at the same level as the Müller cells of the juxta-optic disc region. The outer pole of the perikaryon of these cells continues by means of filamentous bands (Fig. 8 white arrow) from which rise some prolongations that travel in oblique direction within the optic disc (Fig. 8 arrow-heads). The internal part of the cell ramifies into 5 to 8 thick arched prolongations, that distribute themselves randomly within the optic disc, by the optic disc margin and within the IPL of the retina, without reaching the ILM. The morphology of some of these cells evokes that of the typical protoplasmic astrocytes. In summary, the glia of the optic disc margin forms an arched network, which separates the retina from the optic nerve and which the axons of this nerve pass through (Figs. 8, 9). The cells described previously show the most significant morphological changes during the 12th to 14th day of incubation (Fig. 7). These changes consist of a condensation of the glial cytoplasm in the outer part of the cell and development of the arched prolongations at the inner level. Fig. 7 shows 3 cells of which the central one has a greater degree of development.

Figs. 10 and 11 show two photomounts of a vertical section of the retina, lying in the marked areas in the drawing of Fig. 12. In Fig. 10 the last ganglionar cells (LGC) lying near the optic disc may be seen. The last radial processes of the Müller cells of the juxta-optic disc region are seen crossing the IPL, the glangionar cell layer (GCL) and the optic nerve fibre layer (ONFL) (Fig. 10 M). Fig. 11 shows the ultrastructure of the optic disc margin. In this zone there is no ganglionar cell layer, and therefore the IPL of the retina would remain in contact with the ONFL, except for the barrier, formed at this level by the glial cells as shown by the light microscope in Figs. 8 and 9. Through the electron microscope this appears as a band of greater electron density that separates the aforementioned layers. From the structural point of view, the importance of these cells is that they constitute a real barrier between, on the one hand, the retina and the ONFL, and on the other hand, between the blood vessels (BV) and the retina. The ultrastructural characteristics of these cells are very similar to those of other Müller cells lying in different zones of the retina. Their nucleus has a polygonal shape, is elongated and the chromatin is uniformly distributed (Fig. 11 N) as in the Müller cells lying in the central retina. The cytoplasm, which lies in the part innermost from the perikaryon, contains the majority of the cell organelles. The very

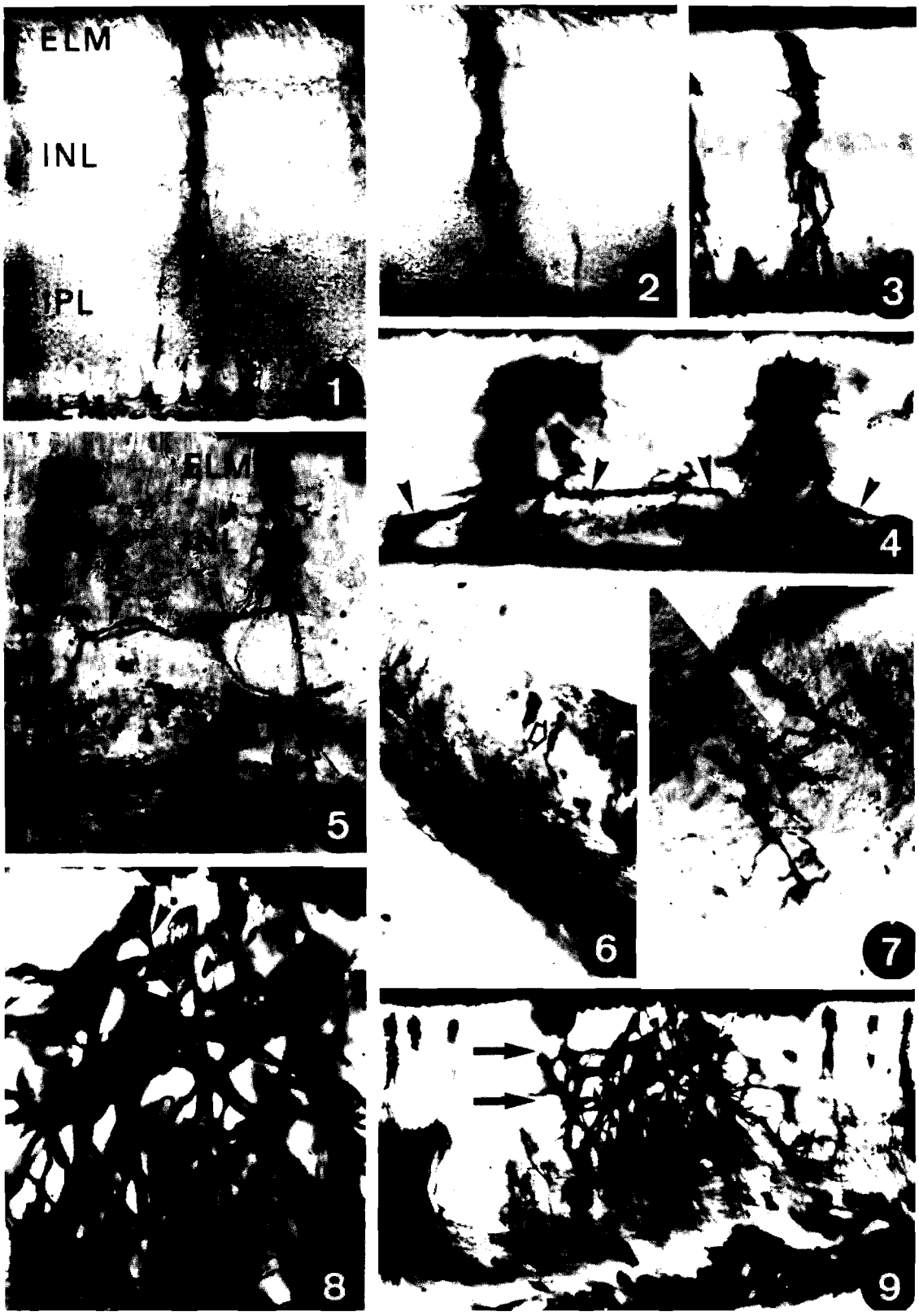

Fig. 1. Müller cell lying in the central retina; external limiting membrane (ELM); inner nuclear layer (INL); inner plexiform layer (IPL); internal limiting membrane (ILM) × 400

Figs. 2 and 3. Müller cell lying in the equatorial retina. × 400

Fig. 4. Müller cell lying in the peripheral retina. The arrowheads show the tangential trajectory of the inner prolongations through the inner plexiform layer. × 400

Fig. 5. Müller cell lying in the juxta-optic disc region; external limiting membrane (ELM); inner nuclear layer (INL); The arrowheads indicate a prolongation with tangential path through the inner plexiform layer. × 400

Fig. 6. The outline arrow shows a Müller cell of the juxta-optic disc region during the 7th day of incubation. × 200

Fig. 7. Müller cells lying in the optic disc margin during 12th to 14th days of incubation. × 400

Figs. 8 and 9. Müller cells lying in the optic disc margin. × 400 and 200 respectively. The white arrow shows a hair-like expansion that continues in arched prolongations (arrowheads). The horizontal arrow shows the zone of localization of the perikaryon of these cells.

Fig. 10. Electron micrograph of the inner portion of the juxta-optic disc region. Ganglionar cell layer (GCL). The arrow shows the last ganglionar cell of this layer. Inner plexiform layer (IPL). Optic nerve fibre layer (ONFL). Müller cell (M). × 2,600

Fig. 11. Electron micrograph of the optic disc margin. The glial cells of this zone (N) are situated between the inner plexiform layer (IPL) and the optic nerve fibre layer (ONFL). Blood vessel (BV). The arrow shows a glial expansion that continues wrapping around the ganglion cell axons. × 5,000

Fig. 12. Camera-lucida drawing of different glial cell types found in the juxta-optic disc region and the optic disc margin. The delineated zones correspond to the electron micrograph fields of figs. 10 and 11.

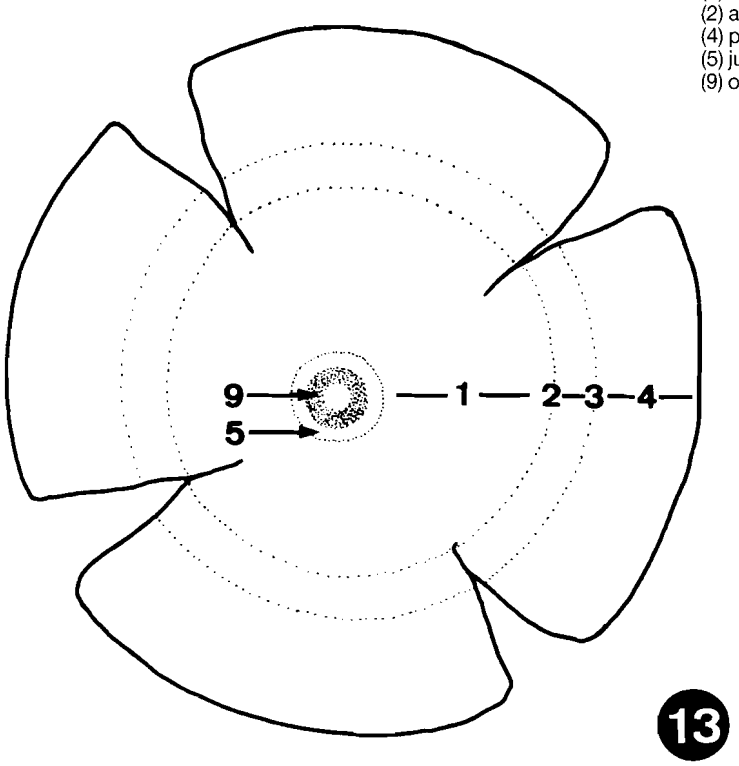

Fig. 13. Retina location map of Müller cells corresponding to Figs. 1, 2, 3, 4, 5 and 9.
(1) Central retina,
(2) and (3) equatorial retina,
(4) peripheral retina,
(5) juxta-optic disc zone,
(9) optic disc.

developed Golgi apparatus, the rough endoplasmic reticulum and numerous mitochondria may be seen at this level. The greatest accumulation of mitochondria lie in the limitrophic zones with the blood vessels and in the outermost part of the cell. The internal prolongations of these cells mostly have microfilaments, some mitochondria and numerous glycogen particles. In addition they give rise to the myelin that covers the large sized axons lying in this zone (Fig. 11 curved arrows).

Discussion

Müller cells have been considered to be astrocytes (Polyak, 1957), although completely transformed in the outer portion to a specialization for absorption and intracellular transport of nutrients (Magalhaes and Coimbra, 1972). In an earlier paper we suggested Müller cells could carry out all the functions that are ascribed to different types of glial cells (Prada et al., 1988). This would mean that Müller cells have to modify their morphology and functional behaviour depending on the zone where they lie. Reichenbach and Wohlrab (1986) and Reichenbach (1987), have made a study of topographical distribution, density and morphometry of the Müller cells of the rabbit retina. These authors find that the morphometric data of the Müller cells vary greatly with their situation in various parts of the retina. Thus, the long Müller cells of the central retina possess thinner vitrea and smaller terminal feet than the short cells lying in the peripheral retina. These data are related (Eberhardt and Reichenbach, 1987) to the extracellular falling and rising of potassium level after neuronal activity.

A complete comparative analysis of the morphology and ultrastructure of these cells in the different zones of the retina including the juxta-optic disc and the optic disc zones, has not previously been carried out in the retina of birds.

The retina functionally requires the same macroglial behaviour as the rest of the CNS tissue. In the retina of mammals such as man (Wolter, 1956) or the monkey (Ogden, 1978, 1983), the astrocytes and oligodendrocytes play an important neuroglial role. The chicken retina, however, which is avascular, has few astrocytes and olygodondendrocytes at the level of the IPL and ONFL (Prada, 1980), so, therefore, functions such as those of nutrition and myelogenesis have to be carried out by some type of neuroglial cell.

The results of our studies (Prada et al., 1979, 1988; Prada et al., 1980) and those of the present study, suggest that the Müller cell of the chick retina is a special class of neuroglial cell. The term modified astrocyte is not adequate for its functional and structural

behaviour. The following facts support our criterion:

A. The ultrastructural characteristics of the outermost portion of the Müller cells corresponding to the desmosomic junctions that they make at the level of the OLM, as well as microvilli that lie among the internal segments of the photoreceptors, allow them to be considered as being of ependymal nature (Meller et Glees, 1965; Prada et al., 1988).

B. The inner portion of the Müller cell shows ultrastructural characteristics considered to be typical of the brain astrocytes, i.e. abundance of microfilaments, numerous glycogen particles and presence of dense bodies (Mori and Leblond, 1969; Magalhaes and Coimbra, 1972; Prada et al., 1988).

C. At the level of the ONFL, the continuity of the myelin wrapping with the inner processes of the Müller cells is evident (Ladman and Soper, 1962; Hughes and La Velle, 1975; Prada, 1980; Smith, 1982; Prada et al., 1988). Our observations suggest that in the optic disc the Müller cells are the origin of the compact myelin that covers the large sized axons. In this retinal zone we have not observed a significant number of oligondendrocytes to justify the myelogenesis.

D. The morphological variations that we have described in the different regions of the retina analysed show that it is a cell of great plasticity, which transforms depending on the type of mechanical support and cell insulation that it has to carry out. The resistant and complicated glial network formed in the optic disc by the modified Müller cells classically described as spider cells (Marchesani, 1926) (Figs. 8, 9) suggests that they serve not only for protection and ordered conduction of the nerve fibres of the eye in its rest position, but also have to rise to all the requirements imposed by the normal movements of the eye, particularly in this zone, in the same way the modifications that these cells show in the more peripheral zones of the retina (Fig. 4). Further supporting the mechanical nature of the morphological modifications undergone by the Müller cell, there is the fact that in the chameleon retina these cells develop large accessory prolongations that support and insulate the photoreceptor axons on their passage through Henle's layer (Prada et al., 1979).

The ability of Müller cells to alter their expression was shown from the pathological point of view by Ersenfeld et al. (1984) after genetic and experimental degeneration of the photoreceptor cells of the rat retina. The glial modifications in this study were observed by means of a specific immunocytochemical marking of the glial fibrillary acidic protein.

E. The Müller cells play an important role in relation to nutrition of the retina. This role is more important in avascular retinas such as those of the chicken or the frog. In an earlier paper (Prada et al., 1988), we have suggested that the constant formation of vesicles at the level of the outer and inner limiting membranes represents an interesting way of explaining in part the metabolism and nutrition of retinas lacking blood vessels.

F. Our results support the idea that the modified Müller cells in the optic disc of the chick retina may act as a blood-retina barrier like Bruch's membrane, which separates the chorio capillaries from the retinal epithelium. In Fig. 11 the glial cytoplasm surrounds and insulates the blood vessel (BV) from the nerve tissue.

G. The data supplied by Bhattcharjee and Sabyal (1975), by Meller and Tetzlaff (1976) and recently by us (Prada et al., 1988), suggest that during development, the vertical cytoplasmic processes of the Müller cells may provide support for the migrating neuronal cells in the way that Bergman glial fibres act in the cerebellum (Rakic, 1972).

In conclusion our results and the data discussed earlier suggest that the Müller cell is not a transformed astrocyte. We believe that it is a special type of glial cell capable of carrying out all the functions described for the macroglia and showing in addition a great morphological plasticity in response to the different types of mechanical support and cell insulation that it has to carry out in the different zones and various layers of the retina.

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