GLIA 27:226–238 (1999)

Circumferential Migration of Ameboid Microglia in the Margin of the Developing Quail Retina

JOSÉ L. MARÍN-TEVA, RUTH CALVENTE, MIGUEL A. CUADROS, ANTONIO ALMENDROS, AND JULIO NAVASCUÉS*

Departamento de Biología Celular, Facultad de Ciencias, Universidad de Granada, Granada, Spain

KEY WORDS macrophages; ameboid microglia; cell migration; dendritic cells; ciliary body; immunocytochemistry

ABSTRACT Central-to-peripheral migration of QH1-positive microglial precursors occurs in the vitrealmost part of the developing quail retina. This study shows that some QH1-positive ameboid cells with morphological features of migrating cells are already present in the margin of the retina before microglial precursors migrating centrally to peripherally arrive in this zone. Because the earlier cells are oriented parallel to the ora serrata, we deduce that some microglial cells migrate circumferentially in the margin of the retina, whereas other microglial precursors migrate from central to peripheral zones. Microglial cells that migrate circumferentially are first seen on embryonic day 6 (E6) and advance in a temporal-to-dorsal-to-nasal direction from the temporoventral quadrant of the retina. When cells migrating centrally to peripherally reach the retinal margin, they meet those migrating circumferentially. From E6 on, some QH1-positive dendritic cells in the ciliary body bear processes that penetrate the retina, where they are oriented circumferentially. These observations suggest that microglial cells that migrate circumferentially in the retinal margin share a common origin with dendritic cells of the ciliary body. Therefore, microglial cells of the quail retina appear to make up a heterogeneous population, with some cells originating from the pecten/optic nerve head area and others from the ciliary body. *GLIA 27:226–238, 1999.*

INTRODUCTION

In the adult quail retina, microglial cells are located mainly in the inner and outer plexiform layers, although a few are also observed in the ganglion cell layer and the nerve fiber layer (Navascués et al., 1994). This pattern of distribution is similar to that in different species of adult mammals (Boycott and Hopkins, 1981; Hume et al., 1983; Sanyal and De Ruiter, 1985; Boya et al., 1987; Penfold et al., 1991; Provis et al., 1995; Humphrey and Moore, 1995; Thanos et al., 1996). The retinal microglia have an exogenous origin (Ashwell, 1989; Ashwell et al., 1989; Schnitzer, 1989; Diaz-Araya et al., 1995a,b; Navascués et al., 1995, 1996).

During development of the quail retina, microglial precursors migrate first tangentially and later radially (Navascués et al., 1995, 1996). Tangential migration takes place in the vitrealmost part of the retina and advances from the center to the periphery, until microglial precursors colonize the entire retinal surface. Subsequently, microglial precursors move radially to the inner and outer plexiform layers, where they differentiate to give rise to the adult pattern of microglial distribution.

Recent studies in the developing human retina (Diaz-Araya et al., 1995a,b) have shown that microglial precursors migrate tangentially from two different sources. Some precursors enter the retina from the optic nerve head (ONH) and others from the anlage of

Grant sponsor: DGICYT of the Spanish Ministry of Education and Culture; Grant number: PM97-0178.

*Correspondence to: Dr. Julio Navascués, Departamento de Biología Celular, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain. E-mail: navascue@goliat.ugr.es

Received 17 February 1999; Accepted 29 March 1999

the ciliary body and iris. The first cells from the ciliary body enter the retina before vasculogenesis begins, whereas microglial precursors from the ONH enter the retina as retinal vessels are developing. Microglial precursors from the ONH migrate within the retina from the center to the periphery, whereas those entering from the ciliary body migrate in the opposite direction. We have shown that microglial precursors in the quail retina migrate from the pecten/ONH (P/ONH) area toward the periphery (Navascués et al., 1995), but did not detect migration from the ciliary body. This difference in comparison to the process described in the developing human retina may reflect differences between birds and mammals. Alternatively, it might be due to technical difficulties in obtaining retinal wholemounts: Because the vitreous adheres tightly to the margin of the retina, a rim of retinal tissue is frequently removed together with the vitreous. We studied wholemounted retinas of quail embryos in which the retinal margin was preserved, as well as wholemounts that included both the ciliary body and the peripheral retina. In addition to the microglial precursors that migrate centrally to peripherally from the P/ONH area, we found other microglial precursors coming from the ciliary body anlage and migrating circumferentially in the margin of the embryonic quail retina.

MATERIALS AND METHODS

Animals

Eyes of embryonic and hatched quails (*Coturnix coturnix japonica*) were obtained from day 5 of incubation (E5) to hatching (E16) and from posthatching days 1 to 3 (P1–P3). These developmental stages include the entire period of entry and tangential migration of microglial precursors from the center to the periphery of the retina (Navascués et al., 1995). The animals were handled in accordance with European guidelines for animal care. When hatched animals were used, they were anesthetized with ether before the eye was enucleated.

Whole-mounted retinas and wholemounts including both the ciliary body and the peripheral retina (CB/PR wholemounts) were obtained from the isolated eyes. Two whole-mounted retinas of each developmental stage and four CB/PR wholemounts from E5 to E10 were used. Sheets containing the inner limiting membrane (basal lamina) covered by a carpet of Müller cell endfeet (ILM/MCEF sheets) were also obtained from E8 retinas. Tangentially migrating ameboid microglial cells remained adhered on these sheets (Marín-Teva et al., 1998).

Preparation of Wholemounts

Whole-mounted retinas were prepared in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4) as previously reported (Navascués et al., 1995; Marín-Teva et al., 1998). A different procedure was used to obtain CB/PR wholemounts. Briefly, the eye was rapidly dissected in 4% paraformaldehyde in 0.1 M PBS. The cornea was then removed and the eye divided in two halves (external and internal). Vitreous body remnants along with the sclera, choroid, and lens were carefully removed from the external half in which the retinal periphery, containing the pigment epithelium, remained together with the ciliary body and iris.

Finally, CB/PR wholemounts were flattened by making several radial incisions and postfixed for 6–12 h at 4°C in the same fixative as was used during dissection.

Immunostaining With QH1

Microglial precursors in the developing quail retina were immunolabeled with the monoclonal antibody QH1 (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), which recognizes quail cells of hemangioblastic lineage except mature erythrocytes (Pardanaud et al., 1987) and all phases of development of microglial cells (Cuadros et al., 1992). Immunocytochemical studies with QH1 in whole-mounted retinas were done by using a biotinylated antimouse IgG (Sigma, St. Louis, MO) as the secondary antibody, which was visualized with avidin-biotin-peroxidase complex (Extravidin; Sigma) and revealed with diaminobenzidine. The technical procedure has been reported in detail elsewhere (Marín-Teva et al., 1998).

CB/PR wholemounts were treated with QH1 immunofluorescence. To improve antibody penetration, the specimens were treated with 3% hydrogen peroxide in 0.1 M PBS for 5 min, washed in 0.1 M PBS for 15 min, and immersed in 1% Triton X-100 in 0.1 M PBS for 4 h, always at room temperature and with gentle shaking. CB/PR wholemounts were washed again in PBS and incubated in a blocking solution of normal goat serum (Sigma) diluted 1:30 in 1% bovine serum albumin in PBS (BSA-PBS) for 1 h at room temperature. They were then incubated in QH1 supernatant diluted 1:2 in BSA-PBS with 0.2% Triton X-100 for 18 h at 4°C with gentle shaking and were thoroughly washed in PBS. Finally the specimens were incubated in tetramethylrhodamine isothiocyanate-conjugated antimouse IgG (Sigma) diluted 1:60 in BSA-PBS for 3 h, washed in PBS, and coverslipped with glycerol-PBS (1:1) containing 0.1% *p*-phenylenediamine dihydrochloride (Sigma). Immunofluorescence observations were made either with a Zeiss Axiophot microscope or with a Zeiss 310 confocal laser scanning microscope.

Double Immunofluorescence of ILM/MCEF Sheets

The ILM/MCEF sheets were obtained from E8 retinas according to the method of Halfter et al. (1987),

with slight modifications as described previously (Marín-Teva et al., 1998, 1999). Double immunofluorescence with monoclonal antibodies C4 (Developmental Studies Hybridoma Bank) and QH1 was used with these sheets to label both migrating ameboid microglia and the substrate on which they migrate. This substrate consists of grooves flanked by rows of Müller cell radial processes and paved with Müller cell endfeet. Antibody C4 recognizes s-laminin in the radial processes of Müller cells (Libby et al., 1997). Therefore, it labeled rows of Müller cell radial processes, and double immunofluorescence with C4 and QH1 revealed the topographical relationship of migrating ameboid microglial cells with the substrate.

The ILM/MCEF sheets were fixed in 4% paraformaldehyde in 0.1 M PBS for 15 min at room temperature, washed in PBS, and immersed for 1 h in normal goat serum diluted 1:30 in BSA-PBS. The sheets were then incubated in C4 supernatant diluted 1:1 in BSA-PBS for 18 h, washed in PBS, and immersed in fluorescein isothiocyanate-conjugated antimouse IgG (Sigma) diluted 1:50 in BSA-PBS for 1 h. The specimens were then rinsed in PBS, incubated in QH1 supernatant diluted 1:2 in BSA-PBS for 1 h at room temperature, and washed in PBS. Finally they were incubated in tetramethylrhodamine isothiocyanate-conjugated antimouse IgG (Sigma) diluted 1:50 in BSA-PBS for 1 h, washed in PBS, and coverslipped with glycerol-PBS (1:1) containing 0.1% *p*-phenylenediamine dihydrochloride.

Distribution Maps of Microglial Precursors Migrating Tangentially

Maps showing the distribution of microglial precursors in the process of tangential migration were obtained from QH1-immunostained whole-mounted retinas. The areas occupied by microglial precursors in the vitreal surface were drawn on the outline of each whole-mounted retina magnified ×12. These areas were carefully identified under a light microscope using a 20× objective.

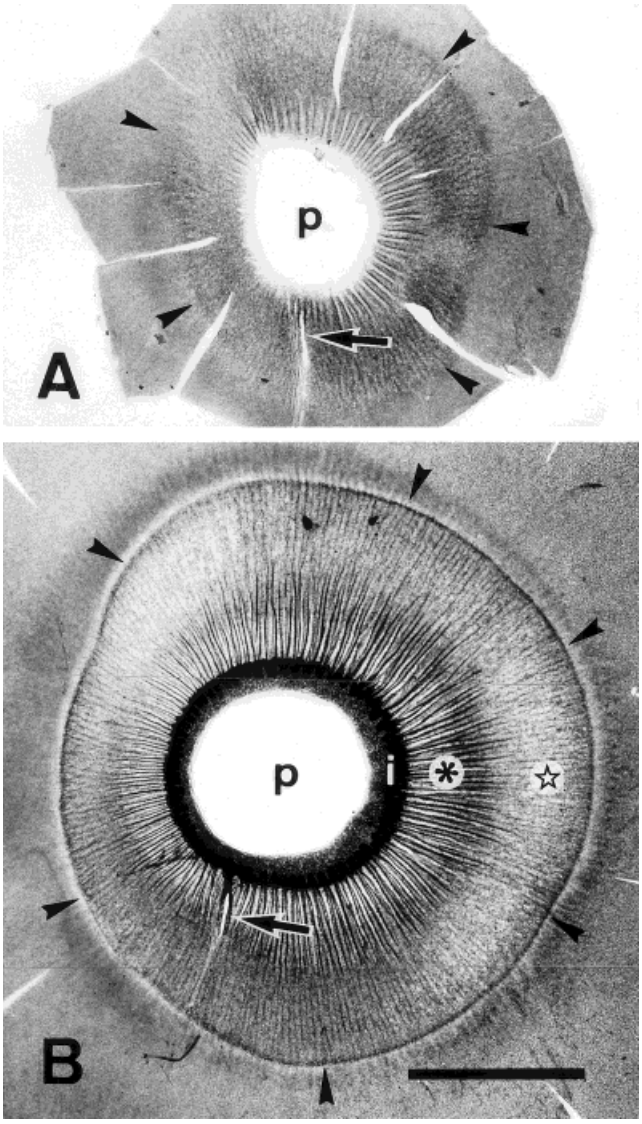

Fig. 1. CB/PR wholemounts from eyes of E6 (**A**) and E10 (**B**) quail embryos, showing the pupil (p), the iris anlage (i), the folded area (pars plicata; asterisk) and the smooth area (pars plana; star) of the ciliary body, and the ora serrata (arrowheads). A narrow unpigmented band (arrow) occupied by a large blood vessel parallel to the folds of the pars plicata is seen in the ventral part of the ciliary body and is useful in orienting the wholemount. At E6, this unpigmented band extends from the ora serrata to the iris anlage (A). In later stages, it is located only in the folded area of the ciliary body (B). In both micrographs dorsal is at the top and temporal on the right. Scale bar = 1.2 mm.

RESULTS

The study of tangential migration of microglial precursors in the retinal periphery was facilitated by using CB/PR wholemounts (Fig. 1). The center of these wholemounts was occupied by the pupil, surrounded by the iris anlage and the ciliary body, with a folded area (pars plicata), showing many radially oriented folds, and a smooth area (pars plana). The ora serrata was visible as a line around the ciliary body. This line was blurred in E5 and E6 (Fig. 1A) and was much sharper from E7 onward (Fig. 1B).

In each stage of development the temporal and dorsal halves of the ciliary body were more developed than the nasal and ventral halves. This meant that the dorsotemporal quadrant was always the most developed, whereas the ventronasal quadrant was the least developed. The ventral location of an unpigmented band and the differential development of each quadrant of the ciliary body (Fig. 1) helped to identify the topographical level of each area in CB/PR wholemounts. This identification was of great importance because the distribution of migrating microglial cells differed depending on the topographical zone of the retina.

Ameboid Microglia Oriented Circumferentially in the Retinal Margin

Observations of whole-mounted retinas confirmed the central-to-peripheral tangential migration of QH1-positive microglial precursors from E7 onward, as previously described (Navascués et al., 1995; Marín-Teva et al., 1998). These precursors appeared as long ameboid cells with their main axis oriented obliquely to the ora serrata in temporal and nasal parts of the retina (Figs. 2C, 3C,D).

In whole-mounted retinas and CB/PR wholemounts, QH1-positive ameboid microglial cells with a circumferential orientation, i.e., parallel to the ora serrata, were observed in the peripheralmost parts of the retina (Fig. 2B,E). Therefore, some microglial precursors appeared to migrate tangentially in the retinal margin along a circumferential course. On E7–E10, the area filled with microglial precursors migrating from the center to the periphery (area of central-to-peripheral migration) and the narrow area in the retinal margin filled with microglial precursors oriented circumferentially (area of circumferential migration) were separated by an area with no microglial cells (Fig. 2A–C). This suggested that microglial precursors migrating circumferentially were present in the peripheralmost part of the retina before microglial cells migrating centrally to peripherally had reached this region.

Bipolar microglial precursors with thick processes and lamellipodia emerging from both poles of the cell body (Fig. 2B,E) were frequently observed in the area of circumferential migration; monopolar and multipolar cells were also seen. Some microglial precursors in this area had threadlike processes similar to those observed in microglial precursors migrating centrally to peripherally (Marín-Teva et al., 1998). Pairs of bridged microglial precursors in the retinal margin were similar to the just-divided microglial cells in the area of central-to-peripheral migration (Marín-Teva et al., 1999). These observations suggested that the migratory behavior and proliferative activity of microglial cells migrating circumferentially in the retinal margin were similar to the features shown by cells in the area of central-to-peripheral migration (Marín-Teva et al., 1998, 1999).

The substrate on which ameboid microglial cells migrated circumferentially in the retinal margin was also oriented circumferentially. Thus, these cells were parallel to rows of Müller cell processes in this part of the retina and to the ora serrata (Fig. 4A). In contrast, the substrate in the area of central-to-peripheral migration was oriented oblique to the ora serrata, i.e., parallel to the microglial cells migrating from the center toward the periphery (Fig. 4B).

Spatiotemporal Pattern of Circumferential Migration of Microglial Precursors

A few circumferentially oriented microglial precursors were first seen in the margin of the temporoventral quadrant of the retina at E6 (Fig. 5A), before any microglial precursors entered the central retina from the P/ONH. At E7, larger numbers of QH1-positive ameboid microglial cells were seen in the area of circumferential migration, which had spread from the temporoventral quadrant toward the temporodorsal one (Fig. 5B). At this stage of development a few microglial precursors were observed in the area of central-to-peripheral migration around the P/ONH. In addition, some microglial precursors were located in a narrow band in the temporoventral quadrant of the retina, bridging the gap between the area of central-to-peripheral migration and the area of circumferential migration (Fig. 5B).

At E8, the number of microglial precursors increased in the area of circumferential migration, which continued to advance in a temporal-to-dorsal-to-nasal direction, reaching the nasodorsal quadrant of the retina (Fig. 5C). The number of microglial precursors in the area of central-to-peripheral migration also increased. In the nasal half, the leading edge of this area was equidistant between the P/ONH and the area of circumferential migration, whereas the two areas were nearer one another in the temporal half and had begun to merge in the temporoventral quadrant (Fig. 5C).

At E9, the area of circumferential migration reached the nasoventral quadrant of the retina (Fig. 5D). This area was slightly wider at several places in the nasal half (Fig. 5D, arrows) owing to the presence of microglial cells oriented obliquely to the ora serrata (Fig. 3E), which appeared to be changing course from circumferential to peripheral-to-central migration. The zone where central-to-peripheral migration merged with circumferential migration was still in the temporoventral quadrant (Fig. 3C), but it was slightly larger than in E8 (Fig. 5D).

At E10, the area of circumferential migration reached the ventralmost part of the retina, so that it occupied the entire perimeter of the retinal margin (Fig. 5E). The density of microglial precursors in the margin of the temporal half (Fig. 6A) was much higher than in the nasal half (Fig. 6B). The zone where central-to-peripheral migration merged with circumferential migration (Fig. 6A) extended around the perimeter of the entire temporal half of the retina (Fig. 5E). In the nasal half, a narrow band lacking microglial precursors was observed between the two areas of migration. Narrow bridges containing microglial precursors oriented obliquely to the ora serrata linked the two areas in the nasodorsal quadrant (Fig. 5E).

As development progressed, these bridges became wider as a result of the incorporation of additional microglial precursors. At E11, only two small bands devoid of microglial precursors were observed in the nasodorsal quadrant (Fig. 5F). The bands disappeared at E12, so that the entire vitreal surface of the retina was filled with microglial precursors (Fig. 5G).

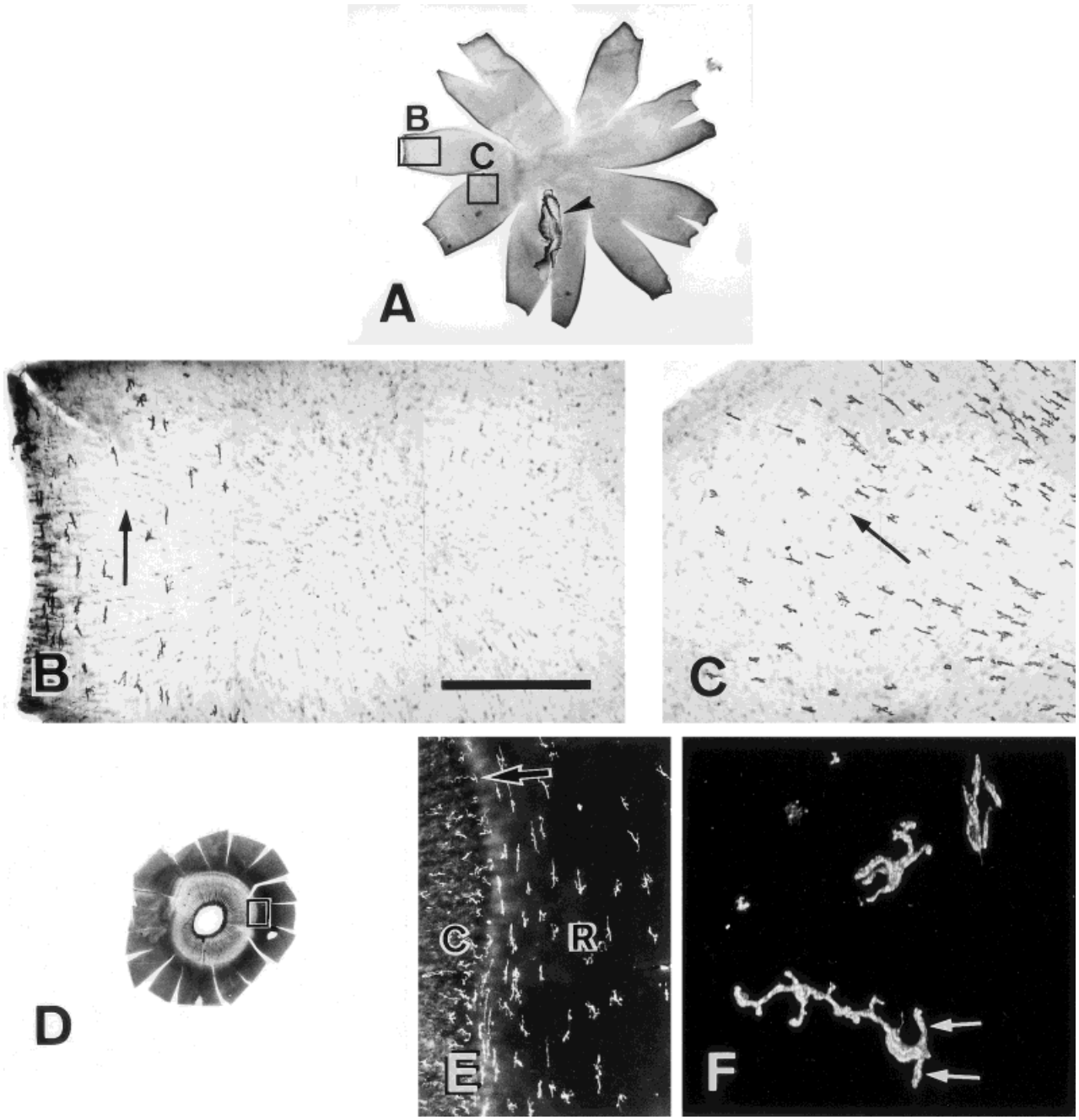

Fig. 2. Whole-mounted retina (A–C) and CB/PR wholemount (D–F) from E8, immunolabeled with the QH1 antibody. **A:** Low-power view of the whole-mounted retina. The boxed areas are enlarged in B and C. The P/ONH area (arrowhead) is located in the ventral half of the retina, slightly displaced toward the temporal side. Dorsal is at the top and temporal on the left. **B:** Magnification of the temporal periphery of the retina. QH1-positive microglial precursors oriented circumferentially (parallel to the arrow) are observed in the retinal margin, but not in nonmarginal peripheral areas. **C:** Magnification of an area in the temporal retina equidistant from the margin and the P/ONH. The arrow indicates the orientation of the QH1-positive cells oblique to the ora serrata. **D:** Low-power brightfield microscopic view of the CB/PR wholemount. The boxed area is enlarged in E. Dorsal is at the top and temporal on the right. **E:** Magnification of the retinal margin (R) and the periphery of the ciliary body (C) in the temporal side of the eye, as seen with fluorescence microscopy. QH1-positive microglial precursors with a circumferential orientation (parallel to the ora serrata) are observed in the retinal margin. QH1-positive cells with dendritic morphological features are seen within the ciliary body. The cell marked with an arrow is shown in **F** at higher magnification with confocal microscopy. This cell is oriented perpendicular to the ora serrata, and bears two circumferential processes (arrows) that enter the retina. Scale bar = 5 mm for A,D; 440 μm for B,C,E; 60 μm for F.

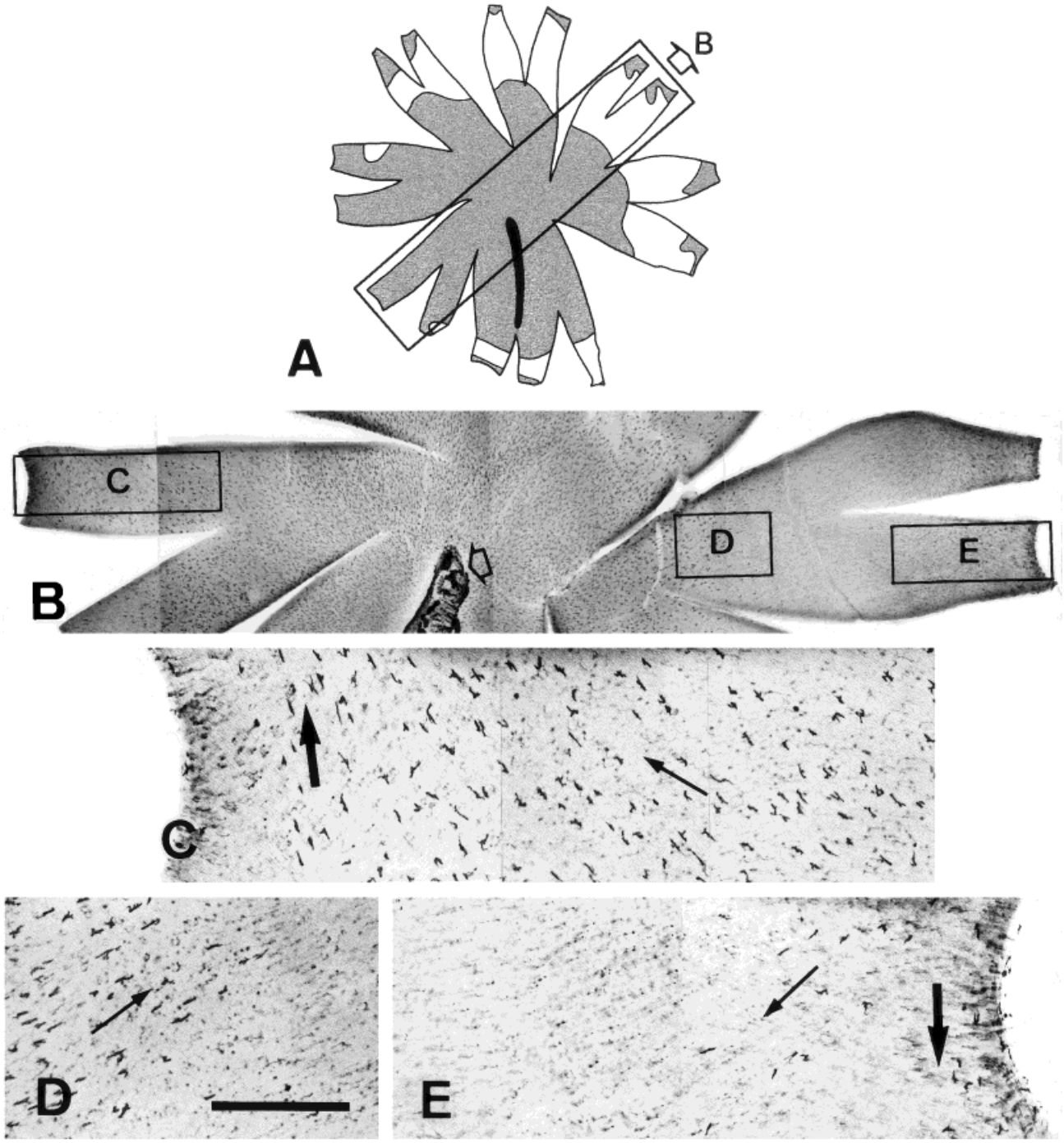

Fig. 3. Tangentially migrating microglial cells in a QH1-immunostained whole-mounted retina from an E9 quail embryo. **A:** Drawing of the profile of the whole-mounted retina in which gray areas represent both the central-to-peripheral and the circumferential areas of migration. The boxed area is enlarged in B. The narrow black band represents the P/ONH. Dorsal is at the top and temporal on the left. **B:** Low-power view of a retinal strip from the temporoventral quadrant (left) to the nasodorsal one (right), with the dorsalmost part of the P/ONH (arrow) centered at the botton. The boxed areas are magnified in C, D and E. **C:** Retinal periphery of the temporoventral quadrant in which the areas of central-to-peripheral and circumferential migration have merged. Microglial cells in the central-to-peripheral area are oriented oblique to the ora serrata (parallel to the thin arrow), in contrast to those oriented circumferentially (parallel to the thick arrow) in the retinal margin. **D:** Part of the retina equidistant from the P/ONH and the nasal margin showing the leading edge of the area of central-to-peripheral migration in the nasal half. Microglial precursors in this area are oriented oblique to the ora serrata (parallel to the arrow). **E:** Magnification of a wide zone in the area of circumferential migration in the nasodorsal quadrant of the retina. The position of some microglial precursors oriented oblique to the ora serrata (parallel to the thin arrow) suggests that they were moving toward the central retina. The thick arrow indicates the circumferential orientation of microglial precursors in the retinal margin. Scale bar = 1.6 mm for B; 400 μm for C–E.

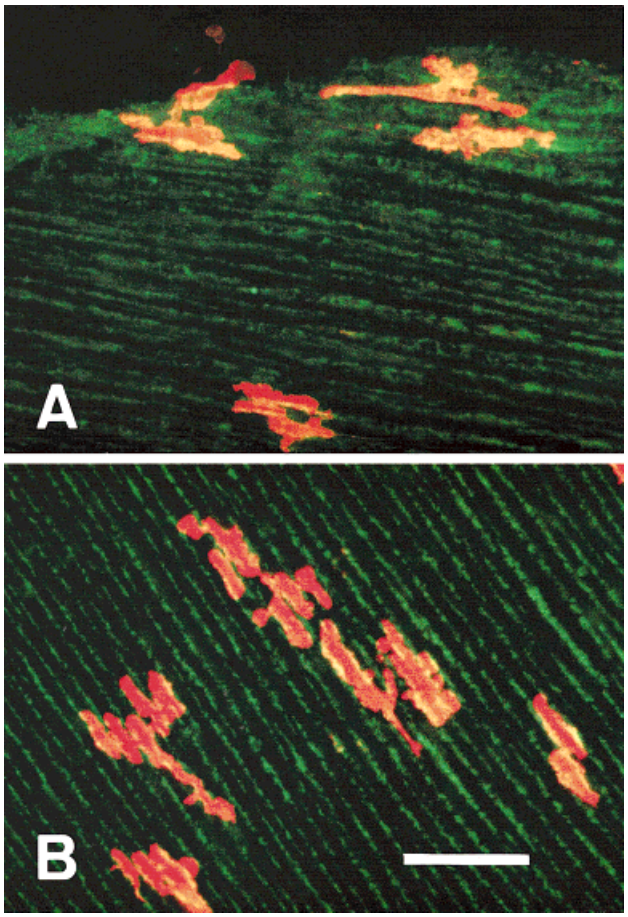

Fig. 4. Orientation of migrating ameboid microglial cells (red) along the substrate of grooves flanked by rows of Müller cell radial processes (green), as seen with double immunofluorescence with C4 and QH1 in an ILM/MCEF sheet from an E8 retina. Micrographs in A and B were obtained parallel to one another in the temporal half of the retina. **A:** In the retinal margin, the substrate, like migrating microglial cells, is oriented parallel to the ora serrata (at the top). **B:** In the area of central-to-peripheral migration, both the substrate and the migrating microglial cells are oriented oblique to the ora serrata. Scale bar = 50 μm.

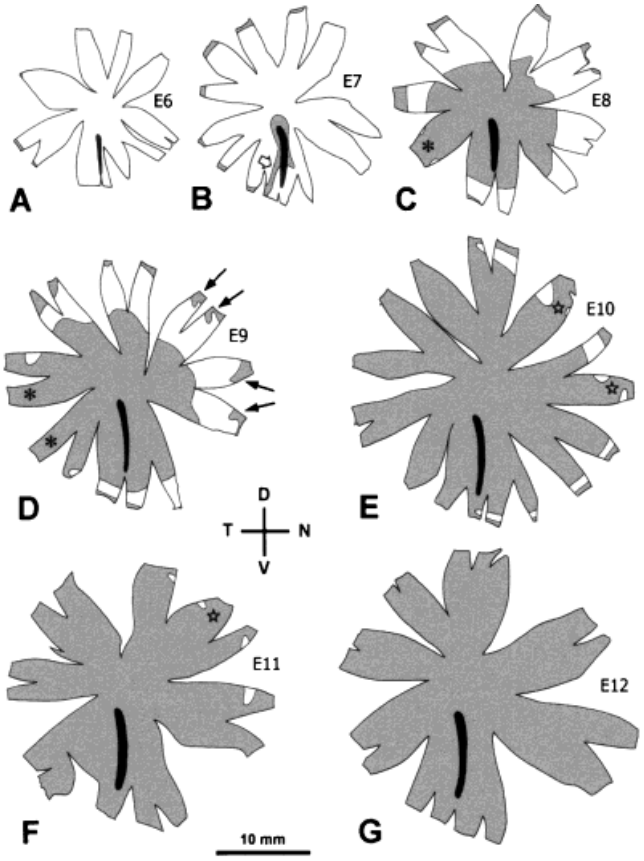

Fig. 5. Distribution maps of microglial precursors migrating tangentially in the vitreal part of the quail retina, obtained from QH1-immunostained whole-mounted retinas of E6 (**A**), E7 (**B**), E8 (**C**), E9 (**D**), E10 (**E**), E11 (**F**), and E12 (**G**) embryos. Gray areas represent areas occupied by microglial precursors migrating tangentially, and white areas indicate regions with no microglial precursors. The narrow band in black represents the P/ONH. Two different areas of migration are identified: the area of central-to-peripheral migration, around the P/ONH, and the area of circumferential migration, around the margin of the retina. In E7 (B), the two areas of microglial cell migration are bridged by a narrow band (arrow) located in the temporoventral quadrant. Both areas of migration merge, first in the temporoventral quadrant of the retina (asterisks in C and D) and then in the entire temporal half (E). In E9 (D), the area of circumferential migration is slightly wider in several parts of the nasodorsal quadrant (arrows). In E10 (E) and E11 (F), these widenings of the area of circumferential migration merge with the area of central-to-peripheral migration through narrow bridges (stars) across the band lacking microglial precursors in the nasal half of the retina. In E12 (G), the entire vitreal surface of the retina is filled with microglial precursors. D, dorsal; V, ventral; T, temporal; N, nasal. Scale bar = 10 mm.

Entry of Dendritic Cells From the Ciliary Body Into the Retinal Margin

In CB/PR wholemounts of E5–E10 embryos, QH1-positive cells were present in the ciliary body anlage. The morphology, density, and distribution of these cells depended on the stage of development and varied in different topographical zones of the ciliary body. At E5, the QH1-positive cells of the ciliary body anlage showed ameboid morphological features (Fig. 7A). These cells were located near the pupil, and were separated from the ora serrata by an area devoid of QH1-positive cells. QH1-positive ameboid cells were observed mainly in the temporal half of the ciliary body and were scarce in the nasal half.

At E6, QH1-positive cells were more abundant in the ciliary body anlage, mainly in the temporal half. Their appearance was more dendritic than at E5, with the cell body and processes tending to be oriented parallel to the radial folds of the ciliary body and perpendicular to the ora serrata (Fig. 7B). In the temporal half (but not in the nasal half), QH1-positive cells were seen throughout the surface of the ciliary body, including areas adjacent to the ora serrata. In these marginal areas of the temporal half, some QH1-positive cells bore processes that traversed the ora serrata to gain access to the retina (Fig. 7B). These observations, together with the presence of the first microglial precursors in the margin of the temporoventral quadrant of the retina (Figs. 5A, 7B), strongly suggested that in temporal areas some QH1-positive cells crossed the ora serrata from the ciliary body toward the retina.

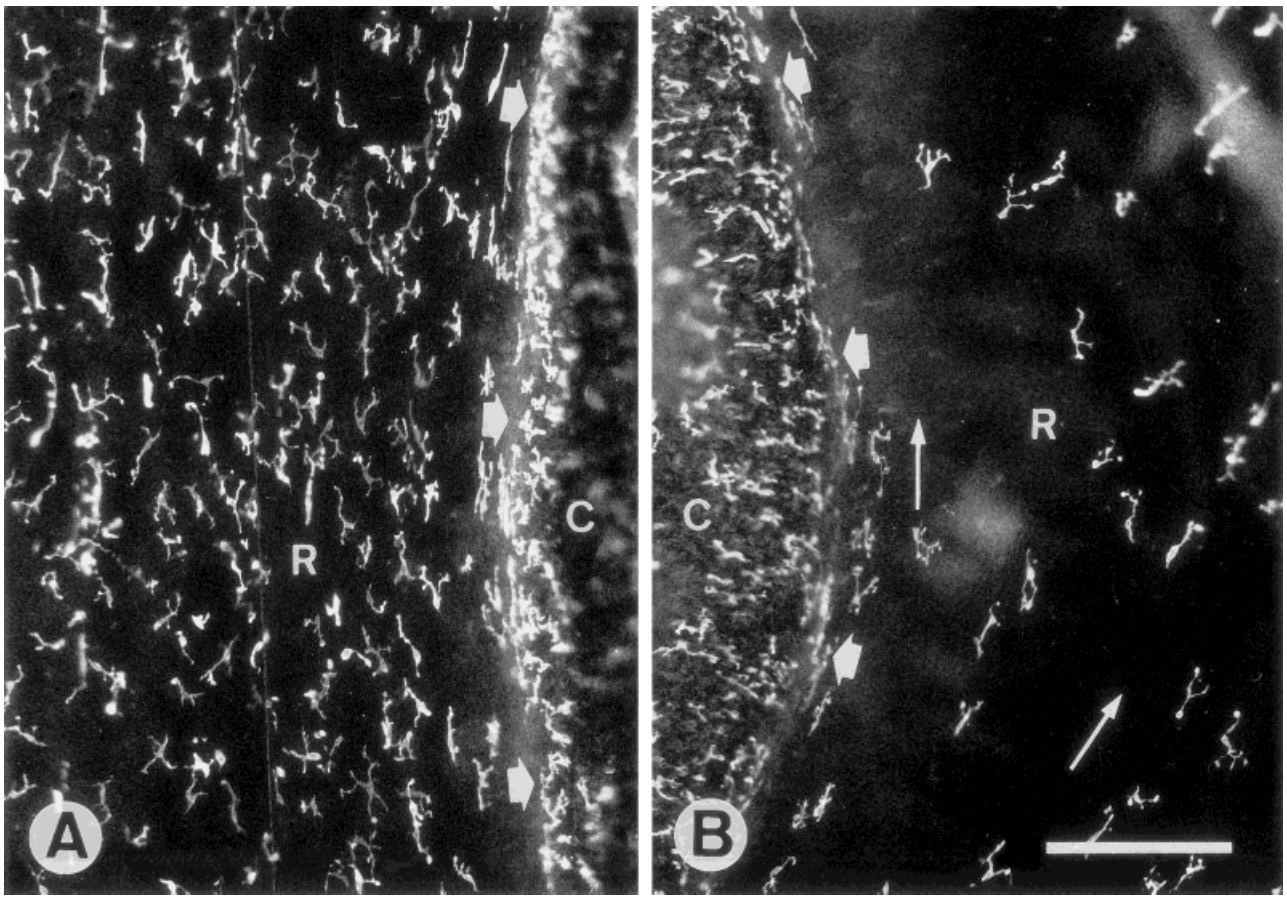

Fig. 6. QH1-immunofluorescence labeled microglial precursors in the area of circumferential migration of the retina on the temporal (**A**) and the nasal (**B**) sides of a CB/PR wholemount from an E10 quail embryo. The thick arrows point out the ora serrata. In both micrographs dorsal is at the bottom. Microglial cell density in the temporal part of the retina (A), where the area of central-to-peripheral migration has merged with the area of circumferental migration, is much higher than in the nasal part (B). In this part (B), cells oriented oblique to the ora serrata (parallel to the oblique thin arrow) are located in a bridge between the areas of central-to-peripheral and circumferential migration. Microglial precursors in the retinal margin are oriented circumferentially (parallel to the vertical thin arrow). R, retina; C, ciliary body. Scale bar = 200 µm.

At E7, QH1-positive cells in the ciliary body were clearly oriented perpendicular to the ora serrata. In temporal areas, some QH1-positive cells were observed midway between the ciliary body and the retinal margin (Fig. 7C). The cell portions that had entered the retina showed a circumferential orientation. In nasal areas of the ciliary body, development was delayed in comparison to temporal areas, and no QH1-positive cells were seen adjacent to the ora serrata.

At E8, QH1-positive cells in the ciliary body anlage were more dendritic in shape and remained oriented perpendicular to the ora serrata (Figs. 2F, 7D,E). QH1-positive cells that seemed to be crossing the ora serrata from the ciliary body toward the retina (Figs. 2F, 7D,E) continued to be observed in the temporal half but also appeared in the nasodorsal quadrant. No QH1-positive cells crossing the ora serrata were seen in the nasoventral quadrant of the ciliary body, although a few of them were already located adjacent to the ora serrata.

At E9, the morphological features and orientation of QH1-positive cells in the ciliary body anlage were similar to those in E8. QH1-positive dendritic cells midway between the ciliary body and the retina were found around the entire perimeter of the ciliary body, including the nasoventral quadrant (Fig. 7F), suggesting that entry of QH1-positive cells from the ciliary body into the retina took place across the entire circumference of the ora serrata.

At E10, the morphological features and orientation of QH1-positive dendritic cells in the ciliary body were similar to those in E9. QH1-positive cells midway between the ciliary body and the retina had almost disappeared in the temporal half, suggesting that no more cells from this half of the ciliary body entered the retina. In contrast, many cells seemed to be entering the nasodorsal quadrant.

The findings described above strongly suggest that, during development, QH1-positive cells from the ciliary body entered the retina, where they migrated circumferentially. Cells first entered temporal areas and spread in a temporal-to-dorsal-to-nasal direction. Once the ventralmost levels were reached, cell entry from the

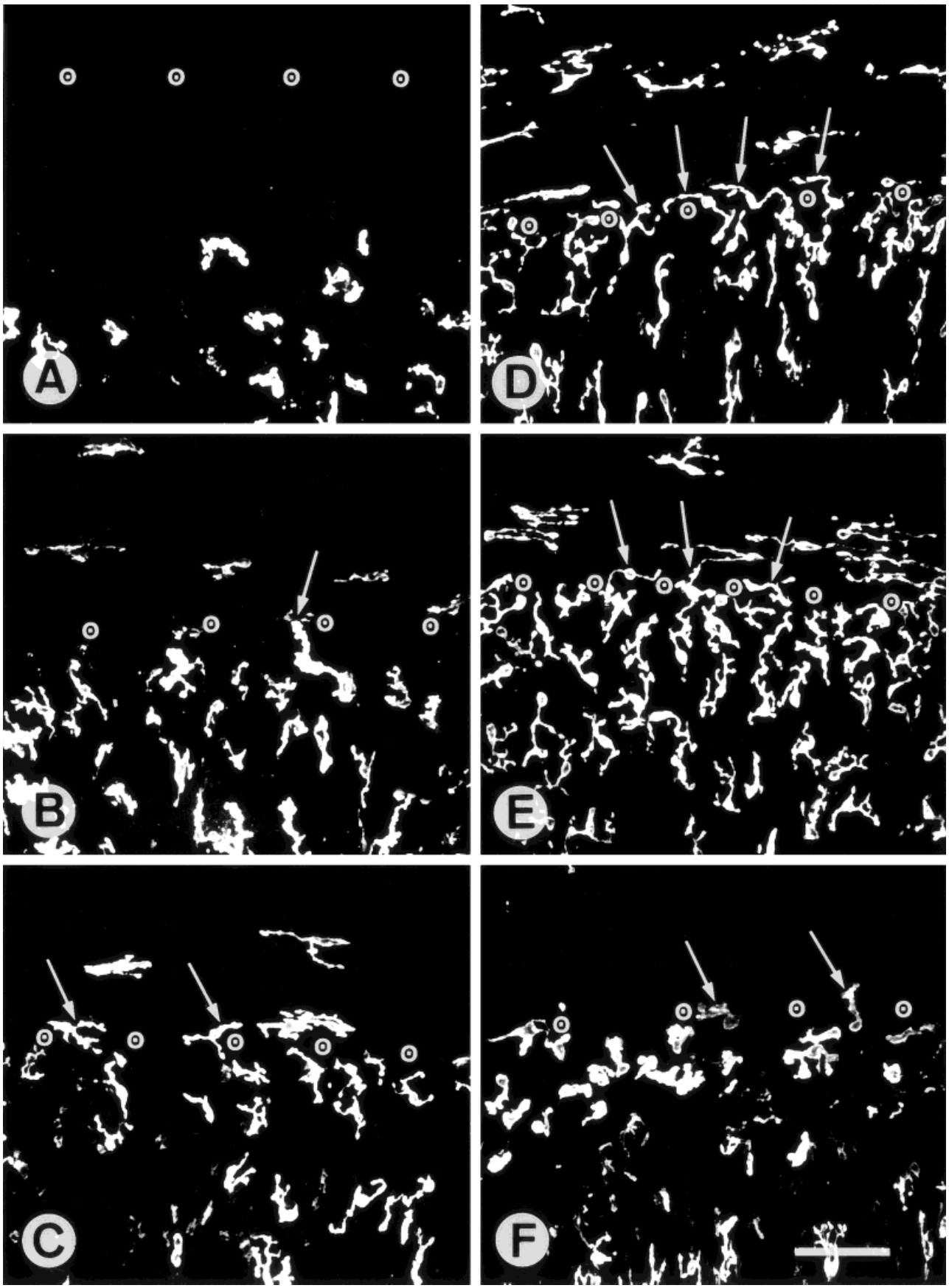

Figure 7.

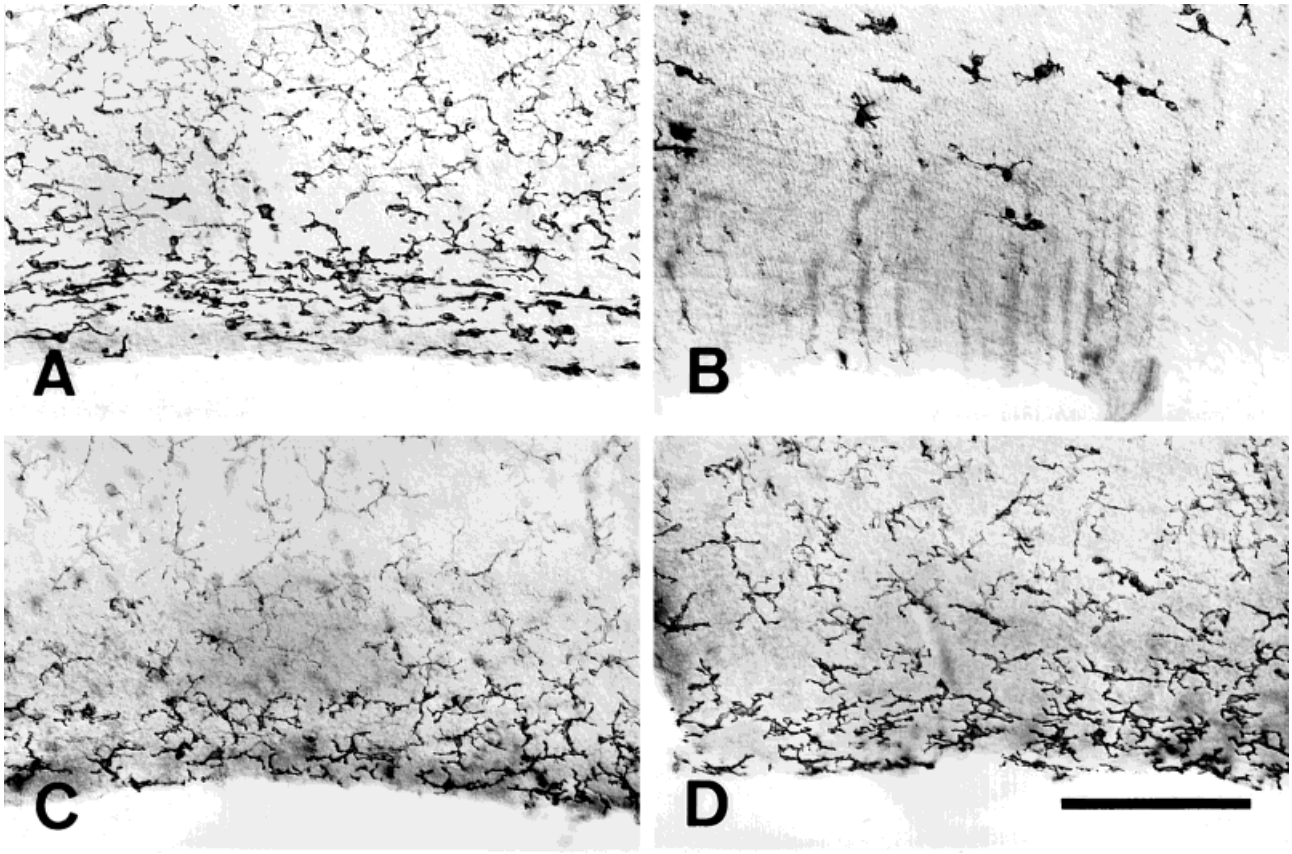

Fig. 8. Comparison of the temporoventral (**A,C**) and the nasoventral quadrants (**B,D**) of QH1-immunolabeled whole-mounted retinas to show the differences in microglial ramification in the retinal margin. A and B are from an E12 quail embryo, and C and D are from a P3 animal. In E12 ramification is incipient in the temporoventral quadrant (A) and has not begun in the nasoventral quadrant (B). In P3, microglial cells have completed their ramification in the temporoventral quadrant (C) but not in the nasoventral one (D), where they still show signs of circumferential orientation. Scale bar = 160 μm.

ciliary body decreased and eventually stopped, following a similar temporal-to-dorsal-to-nasal sequence.

Ramification of Microglial Precursors in the Margin of the Retina

From E12 onward, the QH1-positive cells oriented circumferentially in the retinal margin became ramified. The spatiotemporal pattern of microglial ramification in the retinal margin was similar to that of circumferential migration of microglial precursors: It began in the temporoventral quadrant of the retina and progressed in a temporal-to-dorsal-to-nasal direction, ending in the nasoventral quadrant. Therefore, differences in microglial ramification in the retinal margin were evident when we compared the temporoventral quadrant of the retina to the nasoventral one (Fig. 8).

In the temporoventral quadrant, the process of ramification of circumferentially oriented cells was incipient at E12 (Fig. 8A), more advanced at E15, and almost complete at P3 (Fig. 8C). At this developmental stage microglial cells that had become completely ramified in the retinal margin were no longer oriented circumferentially.

In nasoventral areas of the retinal margin, microglial precursors were ameboid in shape at E12 (Fig. 8B). At E15, they were partially ramified and showed a clearly circumferential orientation similar to that observed in the temporoventral quadrant at E12. At P3, the process of ramification had advanced, but microglial cells still showed a slight circumferential orientation (Fig. 8D), indicating that ramification was not finished. Ramification in the retinal margin of the nasoventral quadrant was completed during the end of the first posthatching week (not shown).

Fig. 7. QH1 immunofluorescence-labeled cells in CB/PR whole-mounts from quail embryos of E5 (**A**), E6 (**B**), E7 (**C**), E8 (**D,E**), and E9 (**F**), as seen with confocal microscopy. Rows of white dots indicate the level of the ora serrata. In all micrographs the ciliary body is at the bottom and the peripheral retina is at the top. A–E are from the temporal side, and F is from the nasoventral quadrant. In E5 (A), QH1-positive ameboid cells within the ciliary body are separated from the ora serrata by an area with no cells. Between E6 and E8 (B–E), QH1-positive cells within the ciliary body are dendritic in shape and occupy areas adjacent to the ora serrata. Some cells show processes (arrows) entering the retinal margin, where they are oriented circumferentially. Other QH1-positive cells are located completely within the retina and show a circumferential orientation. On the ventral side of the ciliary body in E9 (F), some QH1-positive cells bear processes (arrows) that enter the retina. Scale bar = 50 μm.

DISCUSSION

Circumferential Migration of Microglial Precursors and Substrate Orientation in the Retinal Margin

The present study demonstrates that some microglial precursors migrate in the peripheral margin of the embryonic quail retina along a circumferential pathway in a temporal-to-dorsal-to-nasal direction. Thus two types of tangential migration of microglial precursors occur in the quail retina: central-to-peripheral migration from the P/ONH (Navascués et al., 1995) and marginal circumferential migration (this study). Migration of microglial precursors from the ciliary body has been described previously in the margin of the human retina (Diaz-Araya et al., 1995a,b). However, these authors did not find that these precursors followed a circumferential course. Therefore, the circumferential migration of microglial precursors in the margin of the retina is reported here for the first time.

The orientation of ameboid microglial cells migrating tangentially in the margin of the retina coincides with that of the substrate in this part of the retina (Fig. 4). The spatiotemporal pattern of distribution of these microglial precursors also coincides with the pattern of development of circumferential optic fiber fascicles in the marginal region of the retina in chick embryos (Goldberg and Coulombre, 1972). The marginal optic fibers begin to develop at a point of the temporal retina of the chick embryo at E5.5, which is equivalent to E5 in the quail (Zacchei, 1961). In E7 chick embryos (E6 of the quail), these fibers show a circumferential orientation, occupying the temporal half of the retinal margin (Goldberg and Coulombre, 1972). At this stage circumferential fibers are not yet present in the nasal half. Coinciding with the presence of circumferential fibers, microglial precursors first migrate in the marginal zone of the temporoventral quadrant of the E6 quail retina. Between E7 and E12 in the chick embryo (E6–E9 of the quail), the marginal circumferential fibers extend toward the nasal half of the retina (Goldberg and Coulombre, 1972). At the same time microglial cells migrate in the retinal margin in the same direction. Therefore, microglial precursors migrating circumferentially in the retinal margin always move across regions where circumferential fibers are present. Fiber fascicles occupy grooves paved with Müller cell endfeet and flanked by radial processes of Müller cells, so that the circumferential orientation of the optic fibers is identical to that of the grooves on which microglial precursors migrate. The orientation of microglial precursors migrating centrally to peripherally in nonmarginal areas of the retina also coincides with the orientation of both the grooves flanked by Müller cell radial processes (Fig. 4) and the optic fiber fascicles in these areas (Goldberg and Coulombre, 1972; Suburo et al., 1979; Halfter et al., 1985). It therefore appears that mechanical guidance is involved in the mechanism of migration of microglial precursors through all areas of the retina.

Entry Into the Retina of Microglial Precursors From the Ciliary Body

Our study strongly suggests that some microglial precursors enter the quail retina from the ciliary body, in agreement with the findings of Diaz-Araya et al. (1995a,b) in the embryonic human retina. Therefore, microglia of the quail retina appear to originate from both the P/ONH (Navascués et al., 1995) and the ciliary body (this study). QH1-positive cells of the quail ciliary body have morphological features similar to those of the major histocompatibility complex class II-positive dendritic cells in the mammalian ciliary body and iris (Knisely et al., 1991; Flügel et al., 1992; McMenamin et al., 1992, 1994; Steptoe et al., 1996, 1997). This suggests that microglial precursors coming from the ciliary body in the quail retina may be cells of dendritic lineage. Confirmation of this lineage will require experimental demonstration.

The double origin of microglial precursors in the retina is compatible with the notion that microglia of the CNS are a heterogeneous population of cells with different immune phenotypes (Gehrmann and Kreutzberg, 1991; Streit and Graeber, 1993; Mander and Morris, 1995; Wu et al., 1997). This phenotypic heterogeneity has also been described in the human (Penfold et al., 1991, 1993) and rat retina (Dick et al., 1995; Zhang et al., 1997). Two cell populations of different phenotype and origin have been identified in the human retina (Penfold et al., 1993; Diaz-Araya et al., 1995a,b; Provis et al., 1995, 1996). One of them is closely related to blood vessels and seems to be of macrophagic lineage, whereas the other cell population has no topographical relationship with blood vessels and seems to be of dendritic lineage. Microglial cells of macrophagic lineage enter the human retina from the optic disc, whereas those of dendritic lineage enter mainly from the ciliary body (Diaz-Araya et al., 1995a,b). The presence of microglial cells of dendritic lineage has also been reported in the rabbit retina (Cuff et al., 1996). In the quail retina, the entry of microglial cells from the ciliary body (indicating possible dendritic lineage) begins before microglial precursors first enter from the P/ONH (possible macrophagic lineage), a sequence similar to that in mammals (Diaz-Araya et al., 1995a,b). However, unlike the case in mammals, the retina of birds is avascular, so none of the possible subpopulations of retinal microglia can be located near blood vessels.

Once the microglial precursors from the ciliary body have entered the retina, their fate is unknown. Apparently microglial cells from this site migrate first circumferentially around the retinal margin and then mingle with microglial cells entering from the P/ONH. This makes it difficult to follow their subsequent movements, since microglial precursors from the two sites become indistinguishable (both cell populations are labeled with the QH1 antibody).

Asymmetric Spatiotemporal Development of Microglia in the Retina

The spatiotemporal development of the microglia in the retina is asymmetric, showing a temporal-to-nasal gradient. Between E6 and P3, microglial development is most advanced in the temporoventral quadrant, whereas it is least advanced in the nasoventral quadrant. This asymmetry coincides with the temporal-to-nasal differences in the human retina reported by Diaz-Araya et al. (1995a).

In the quail retina, asymmetry in the microglial development is manifested in four ways. First, the central-to-peripheral tangential migration of microglial precursors is more advanced in the temporal than in the nasal half from E7 to E12. Second, marginal circumferential migration of microglial precursors begins in the temporoventral quadrant of the retina and advances in a temporal-to-dorsal-to-nasal direction, ending in the nasoventral quadrant. Therefore, between E6 and E12, marginal circumferential migration is more advanced in the temporal half than in the nasal one. Third, microglial precursors from the ciliary body first enter the temporal half of the retina and then spread in a temporal-to-dorsal-to-nasal direction. The cells therefore enter the nasoventral quadrant last. Fourth, microglial precursors oriented circumferentially along the margin of the retina ramify in the temporal half before the nasal one, and the nasoventral quadrant is again the last area where this process is completed.

Asymmetry also characterizes other developmental processes such as neurogenesis (Prada et al., 1991), programmed cell death (Hughes and McLoon, 1979; Straznicky and Chehade, 1987), and differentiation of neurons (Layer and Kotz, 1983; Halfter et al., 1985; Prada et al., 1991), photoreceptors (Bruhn and Cepko, 1996), and Müller glia (Casado et al., 1996). Therefore, the asymmetric development of microglia in the retina is probably influenced by factors that also affect other developmental processes.

ACKNOWLEDGMENTS

The authors thank Dr. Gervasio Martín-Partido for advice and assistance with confocal microscopy. The monoclonal antibodies QH1 and C4, developed by F. Dieterlen-Lièvre and J.R. Sanes, respectively, were obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa Department of Biological Sciences. Thanks are due to Karen Shashok for help with the English.

REFERENCES

Ashwell K. 1989. Development of microglia in the albino rabbit retina. J Comp Neurol 287:286–301.

Ashwell K, Holländer H, Streit W, Stone J. 1989. The appearance and distribution of microglia in the developing retina of the rat. Vis Neurosci 2:437–448.

Boya J, Calvo J, Carbonell AL. 1987. Appearance of microglial cells in the postnatal rat retina. Arch Histol Jpn 50:223–228.

Boycott BB, Hopkins JM. 1981. Microglia in the retina of monkey and other mammals; its distinction from other types of glia and horizontal cells. Neuroscience 6:679–688.

Bruhn SL, Cepko CL. 1996. Development of the pattern of photoreceptors in the chick retina. J Neurosci 16: 430–1439.

Casado FJ, Pouponnot C, Jeanny JC, Lecoq O, Calothy G, Pierani A. 1996. QR1, a retina-specific gene, encodes an extracellular matrix protein exclusively expressed during neural retina differentiation. Mech Dev 54:237–250.

Cuadros MA, Moujahid A, Martín-Partido G, Navascués J. 1992. Microglia in the mature and developing quail brain as revealed by a monoclonal antibody recognizing hemopoietic cells. Neurosci Lett 148:11–14.

Cuff CA, Berman JW, Brosnan CF. 1996. The ordered array of perivascular macrophages is disrupted by IL-1-induced inflammation in the rabbit retina. Glia 17:307–316.

Diaz-Araya CM, Provis JM, Penfold PL. 1995a. Ontogeny and cellular expression of MHC and leucocyte antigens in human retina. Glia 15:458–470.

Diaz-Araya CM, Provis JM, Penfold PL, Billson FA. 1995b. Development of microglial topography in human retina. J Comp Neurol 363:53–68.

Dick AD, Ford AL, Forrester JV, Sedgwick JD. 1995. Flow cytometric identification of a minority population of MHC class II positive cells in the normal rat retina distinct from CD45lowCD11b/c+CD4low parenchymal microglia. Br J Ophthalmol 79:834–840.

Flügel C, Kinne RW, Streilein JW, Lütjen-Drecoll E. 1992. Distinctive distribution of HLA class II presenting and bone marrow derived cells in the anterior segment of human eyes. Curr Eye Res 11:1173–1183.

Gehrmann J, Kreutzberg GW. 1991. Characterisation of two new monoclonal antibodies directed against rat microglia. J Comp Neurol 313:409–430.

Goldberg S, Coulombre AJ. 1972. Topographical development of the ganglion cell fiber layer in the chick retina. A whole mount study. J Comp Neurol 146:507–518.

Halfter W, Deiss S, Schwarz U. 1985. The formation of the axonal pattern in the embryonic avian retina. J Comp Neurol 232:466–480.

Halfter W, Reckhaus W, Kröger S. 1987. Nondirected axonal growth on basal lamina from avian embryonic neural retina. J Neurosci 7:3712–3722.

Hughes WF, McLoon SC. 1979. Ganglion cell death during normal retinal development in the chick: comparisons with cell death induced by early target field destruction. Exp Neurol 66:587–601.

Hume DA, Perry VH, Gordon S. 1983. Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. J Cell Biol 97:253–257.

Humphrey MF, Moore SR. 1995. Strain differences in the distribution of NDP-ase labelled microglia in the normal rabbit retina. Glia 15:367–376.

Knisely TL, Anderson TM, Sherwood ME, Flotte TJ, Albert DM, Granstein RD. 1991. Morphologic and ultrastructural examination of I-A+ cells in the murine iris. Invest Ophthalmol Vis Sci 32:2423–2431.

Layer PG, Kotz S. 1983. Asymmetrical developmental pattern of uptake of Lucifer yellow into amacrine cells in the embryonic chick retina. Neuroscience 9:931–941.

Libby RT, Xu Y, Selfors L, Brunken WJ, Hunter DD. 1997. Identification of the cellular source of laminin β2 in adult and developing vertebrate retinae. J Comp Neurol 389:655–667.

Mander TH, Morris JF. 1995. Immunophenotypic evidence for distinct populations of microglia in the rat hypothalamo-neurohypophysial system. Cell Tissue Res 280:665–673.

Marín-Teva JL, Almendros A, Calvente R, Cuadros MA, Navascués J. 1998. Tangential migration of ameboid microglia in the developing quail retina: mechanism of migration and migratory behavior. Glia 22:31–52.

Marín-Teva JL, Almendros A, Calvente R, Cuadros MA, Navascués J. 1999. Proliferation of actively migrating ameboid microglia in the developing quail retina. Anat Embryol (in press).

McMenamin PG, Crewe J, Morrison S, Holt PG. 1994. Immunomorphologic studies of macrophages and MHC class II-positive dendritic cells in the iris and ciliary body of the rat, mouse, and human eye. Invest Ophthalmol Vis Sci 35:3234–3250.

McMenamin PG, Holthouse I, Holt PG. 1992. Class II major histocompatibility complex (Ia) antigen-bearing dendritic cells within the iris and ciliary body of the rat eye: distribution, phenotype and relation to retinal microglia. Immunology 77:385–393.

Navascués J, Moujahid A, Quesada A, Cuadros MA. 1994. Microglia in the avian retina: immunocytochemical demonstration in the adult quail. J Comp Neurol 350:171–186.

Navascués J, Moujahid A, Almendros A, Marín-Teva JL, Cuadros MA. 1995. Origin of microglia in the quail retina: central-to-peripheral and vitreal-to-scleral migration of microglial precursors during development. J Comp Neurol 354:209–228.

Navascués J, Cuadros MA, Almendros A. 1996. Development of microglia: evidence from studies in the avian central nervous system. In: Ling EA, Tan CK, Tan CBC, editors. Topical issues in microglia research. Singapore: Singapore Neuroscience Association. p 43–64.

Pardanaud L, Altmann C, Kitos P, Dieterlen-Lièvre F, Buck CA. 1987. Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells. Development 100:339–349.

Penfold PL, Madigan MC, Provis JM. 1991. Antibodies to human leucocyte antigens indicate subpopulations of microglia in human retina. Vis Neurosci 7:383–388.

Penfold PL, Provis JM, Liew SCK. 1993. Human retinal microglia express phenotypic characteristics in common with dendritic antigen-presenting cells. J Neuroimmunol 45:183–192.

Prada C, Puga J, Pérez-Méndez L, López R, Ramírez G. 1991. Spatial and temporal patterns of neurogenesis in the chick retina. Eur J Neurosci 3:559–569.

Provis JM, Penfold PL, Edwards AJ, van Driel D. 1995. Human retinal microglia: expression of immune markers and relationship to the glia limitans. Glia 14:243–256.

Provis JM, Diaz CM, Penfold PL. 1996. Microglia in human retina: a heterogeneous population with distinct ontogenies. Perspect Dev Neurobiol 3:213–222.

Sanyal S, De Ruiter A. 1985. Inosine diphosphatase as a histochemical marker of retinal microvasculature, with special reference to transformation of microglia. Cell Tissue Res 241:291–297.

Schnitzer J. 1989. Enzyme-histochemical demonstration of microglial cells in the adult and postnatal rabbit retina. J Comp Neurol 282:249–263.

Steptoe RJ, Holt PG, McMenamin PG. 1996. Origin and steady-state turnover of major histocompatibility complex class II-positive dendritic cells and resident-tissue macrophages in the iris of the rat eye. J Neuroimmunol 68:67–76.

Steptoe RJ, Holt PG, McMenamin PG. 1997. Major histocompatibility complex (MHC) class II-positive dendritic cells in the rat iris. In situ development from MHC class II-negative precursors. Invest Ophthalmol Vis Sci 38:2639–2648.

Straznicky C, Chehade M. 1987. The formation of the area centralis of the retinal ganglion cell layer in the chick. Development 100:411–420.

Streit WJ, Graeber MB. 1993. Heterogeneity of microglial and perivascular cell populations: insights gained from the facial nucleus paradigm. Glia 7:68–74.

Suburo A, Carri N, Adler R. 1979. The environment of axonal migration in the developing chick retina: a scanning electron microscopic (SEM) study. J Comp Neurol 184:519–536.

Thanos S, Moore S, Hong Y. 1996. Retinal microglia. Progr Ret Eye Res 15:331–361.

Wu CH, Chien HF, Chang CY, Ling EA. 1997. Heterogeneity of antigen expression and lectin labeling on microglial cells in the olfactory bulb of adult rats. Neurosci Res 28:67–75.

Zacchei AM. 1961. Lo sviluppo embrionale della quaglia giapponese (*Coturnix coturnix japonica* T.e.S.). Arch Ital Anat Embryol 66: 36–62.

Zhang J, Wu GS, Ishimoto S, Pararajasegaram G, Rao NA. 1997. Expression of major histocompatibility complex molecules in rodent retina. Immunohistochemical study. Invest Ophthalmol Vis Sci 38:1848–1857.