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Origin of Microglia in the Quail Retina: Central-to-Peripheral and Vitreal-to-Scleral Migration of Microglial Precursors During Development

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ABSTRACT

The origin, migration, and differentiation of microglial precursors in the avascular quail retina during embryonic and posthatching development were examined in this study. Microglial precursors and developing microglia were immunocytochemically labeled with QH1 antibody in retinal whole mounts and sections. The retina was free of $QH1^+$ macrophages at embryonic day 5 (E5). Ameboid $QH1^+$ macrophages from the pecten entered the retina from E7 on. These macrophages spread from central to peripheral areas in the retina by migrating on the endfeet of the Müller cells and reached the periphery of the retina at E12. While earlier macrophages were migrating along the inner limiting membrane, other macrophages continued to enter the retina from the pecten until hatching (E16). From E9 on, macrophages were seen to colonize progressively more scleral retinal layers as development advanced. Macrophages first appeared in the ganglion cell layer at E9, in the inner plexiform layer at E12, and in the outer plexiform layer at E14. Therefore, it seems that macrophages first migrated tangentially along the inner retinal surface and then migrated from vitreal to scleral levels to gain access to the plexiform layers, where they differentiated into ramified microglia. Macrophages appeared to differentiate shortly after arrival in the plexiform layers, as poorly ramified $QH1^+$ cells were seen as early as E12 in the inner plexiform layer and at E14 in the outer plexiform layer. Radial migration of macrophages toward the outer plexiform layer continued until posthatching day 3, after which retinal microglia showed an adult distribution pattern. We also observed numerous vitreal macrophages intimately adhered to the surface of the pecten during embryonic development, when macrophages migrated into the retina. These vitreal macrophages were not seen from hatching onwards, when no further macrophages entered the retina.

Indexing terms: macrophages, cell migration, differentiation, pecten, immunocytochemistry

Since publication of the pioneering studies by Rio-Hortega (1932), the origin of microglia has been a controversial issue (reviewed by Theele and Streit, 1993; Ling and Wong, 1993). At present, it is well established that resting microglia come from ameboid microglia, which, in turn, derive from macrophages invading the nervous system during development (see reviews by Jordan and Thomas, 1988; Perry and Gordon, 1988; Streit et al., 1988; Perry and Lawson, 1992; Thomas, 1992; Ling and Wong, 1993). Although the hemopoietic origin of ameboid microglia is now largely accepted (Ling, 1981; Thomas, 1992; Cuadros et al., 1992; Ling and Wong, 1993; Theele and Streit, 1993), there are two contradictory views on the source of hemopoietic microglial precursors infiltrating the nervous system. Some authors maintain that microglia derive from circulating monocytes that enter the brain parenchyma by traversing the endothelium of the blood vessels (Ling et al., 1980; Valentino and Jones, 1981; Perry et al., 1985; Chugani et al., 1991; Milligan et al., 1991a; Perry and Lawson, 1992; Ling and Wong, 1993). Others suggest that ameboid microglia come from pial macrophages that traverse the basement membrane of the nervous system (Rio-Hortega, 1932; Boya et al., 1979, 1991). These two hypotheses are not incompat-

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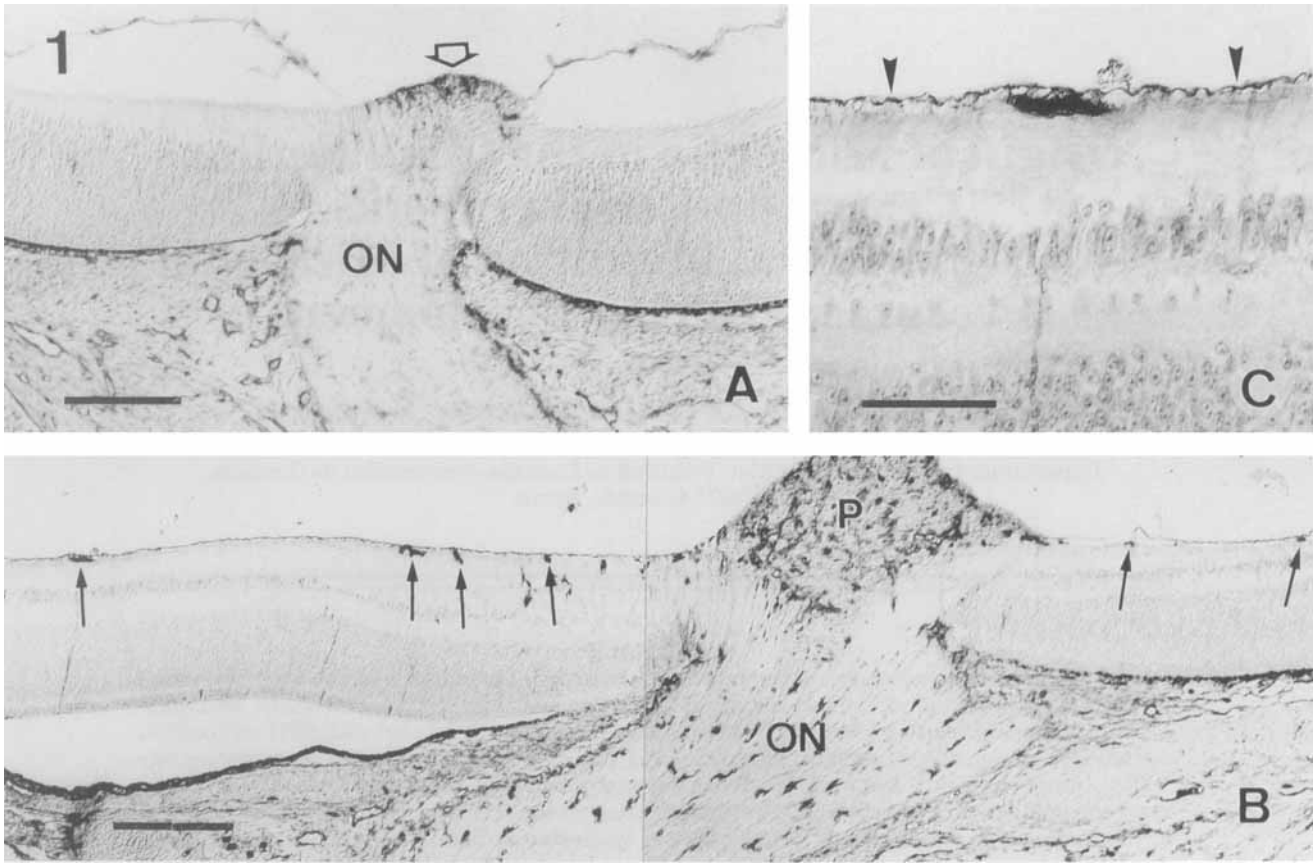


Fig. 1. QH1-immunostained sections of quail embryo retinas at the level of the optic nerve head (ON). **A:** E6 retina. The nerve fiber layer is well developed, but plexiform layers are not yet identifiable. The pecten is not developed, but the zone opposite the optic nerve head (arrow), from which the pecten will develop, bulges into the vitreous body and contains QH1$^+$ blood vessels. No QH1$^+$ cells are detected in the retina. **B:** E8 retina. Plexiform layers are evident. The developing pecten (P) protrudes into the vitreous body and contains abundant QH1$^+$ blood vessels. QH1$^+$ cells (arrows) are located in the nerve fiber layer, closely adhering to the internal limiting membrane. **C:** Higher magnification of the QH1$^+$ cell at left in B. The cell is elongated and closely adheres to the internal limiting membrane (arrowheads). Scale bars = 150 μm in A,B, 40 μm in C.

ible; both routes of entry may be possible (Jordan and Thomas, 1988; Theele and Streit, 1993; Cuadros et al., 1994).

The mammalian retina has been used as a suitable model to study mature and developing microglia (Vrabec, 1970; Boycott and Hopkins, 1981; Hume et al., 1983; Terubayashi et al., 1984; Sanyal and De Ruiter, 1985; Boya et al., 1987; Schnitzer, 1989; Ashwell, 1989; Ashwell et al., 1989). In mammals, close relationships have been reported between blood vessels and both macrophages and developing microglia (Hume et al., 1983; Sanyal and De Ruiter, 1985; Linden et al., 1986; Boya et al., 1987; Ashwell et al., 1989; Penfold et al., 1990), and a vascular origin (from circulating monocytes) also has been suggested for the retinal microglia (Hume et al., 1983; Boya et al., 1987). However, experiments using labeling with colloidal carbon (Linden et al., 1986), similar to the study by Ling et al. (1980) to demonstrate the monocytic origin of microglia in the corpus callosum, failed to find labeled microglia in the rat retina at postnatal day 8, when microglia are already present in deep retinal layers (Boya et al., 1987; Ashwell et al., 1989). The rabbit retina, which is partially avascular, contains microglia in both vascular and avascular parts (Ashwell, 1989; Schnitzer, 1989). In addition, microglial cells are detectable in the rabbit retina well before the first appearance of intraretinal blood vessels (Schnitzer, 1989). Thus, these studies do not support the origin of retinal microglia from circulating monocytes.

In the adult quail retina, ramified microglia show morphological features and a pattern of distribution similar to those in the adult mammalian retina (Navascués et al., 1994). The avian retina is completely avascular and includes a structure called the pecten, a richly vascularized organ projecting into the vitreous body from the optic disc (Romanoff, 1960; Meyer, 1977). The present study was designed to investigate the intriguing issue of the origin of microglia in avascular retinas. Microglial cells and microglial precursors were identified with the monoclonal antibody QH1, which labels all forms of developing and mature microglia in embryonic and posthatching stages of quail development (Cuadros et al., 1992, 1993). This antibody was previously used to study ramified microglia in the retina of the adult quail (Navascués et al., 1994).

Our results showed that ameboid macrophages from the pecten migrated along the inner limiting membrane (ILM), spreading from central to peripheral areas in the retina. Subsequently, macrophages migrated from vitreal to scleral layers to reach the inner and outer plexiform layers, where they differentiate into ramified microglia.

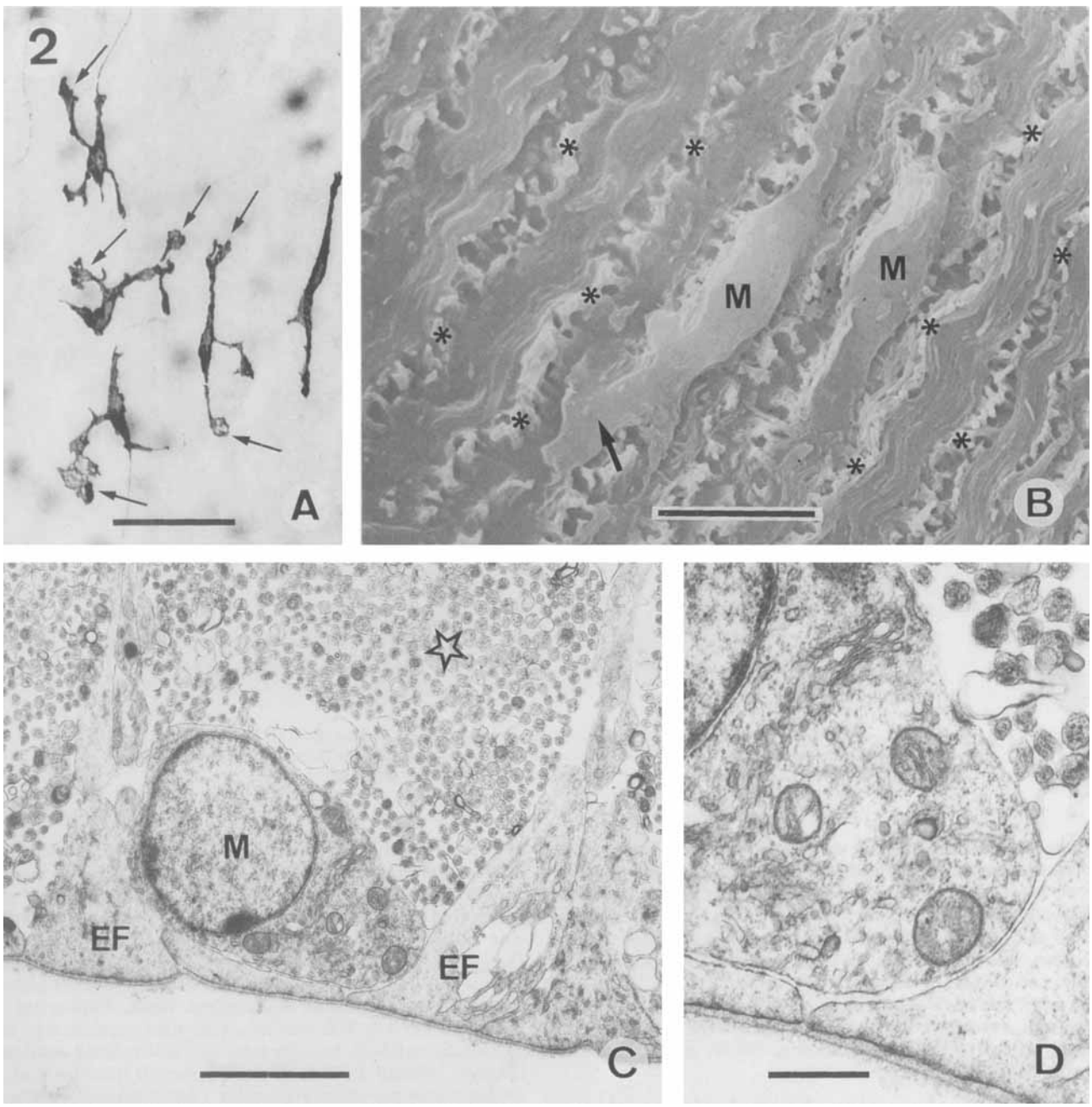


Fig. 2. Cells with migratory characteristics in the nerve fiber layer of E9 quail embryo retinas. **A:** Labeled cells in a QH1-immunostained retinal whole mount, focused on the nerve fiber layer. The QH1$^+$ cells are elongated, ameboid in shape, and oriented parallel to the ganglion cell axons (not stained), which course from bottom to top. Lamellipodia (arrows) are seen at the end of cell processes. **B:** Scanning electron microscopic (SEM) view of two elongated cells (M) in the nerve fiber layer closely adhering to the Müller cell endfeet. A lamellar pseudopod (arrow) leads the cell on the left. Asterisks mark rows of radial processes of Müller cells that have been broken by detachment with adhesive tape (see Materials and Methods). Nerve fiber bundles are seen between rows of cell processes. **C:** Transmission electron microscopic (TEM) view of the nerve fiber layer showing a transversely sectioned cell (M) closely adhering to the endfeet of Müller cells (EF). Its nucleus shows a thin band of heterochromatin just beneath the nuclear envelope. Ganglion cell axons (star) are seen in cross section. **D:** Higher magnification of the cell in C showing the intimate adhesion between its cell membrane and Müller cell endfeet. Golgi complex vesicles and numerous small vacuoles are noted in the cytoplasm. Scale bars = 50 μm in A, 10 μm in B, 2 μm in C, 0.3 μm in D.

MATERIALS AND METHODS

Materials

Embryonic and posthatching quails (*Coturnix coturnix japonica*) were obtained at the following days of incubation (E) and posthatching days (P): E5, E6, E7, E8, E9, E10, E12, E14, E16, P1, P3, P7, and P45 (adulthood). The adult animals were those used elsewhere to study microglia in the adult quail retina (Navascués et al., 1994). At each developmental stage, one set of animals was used to prepare retinal

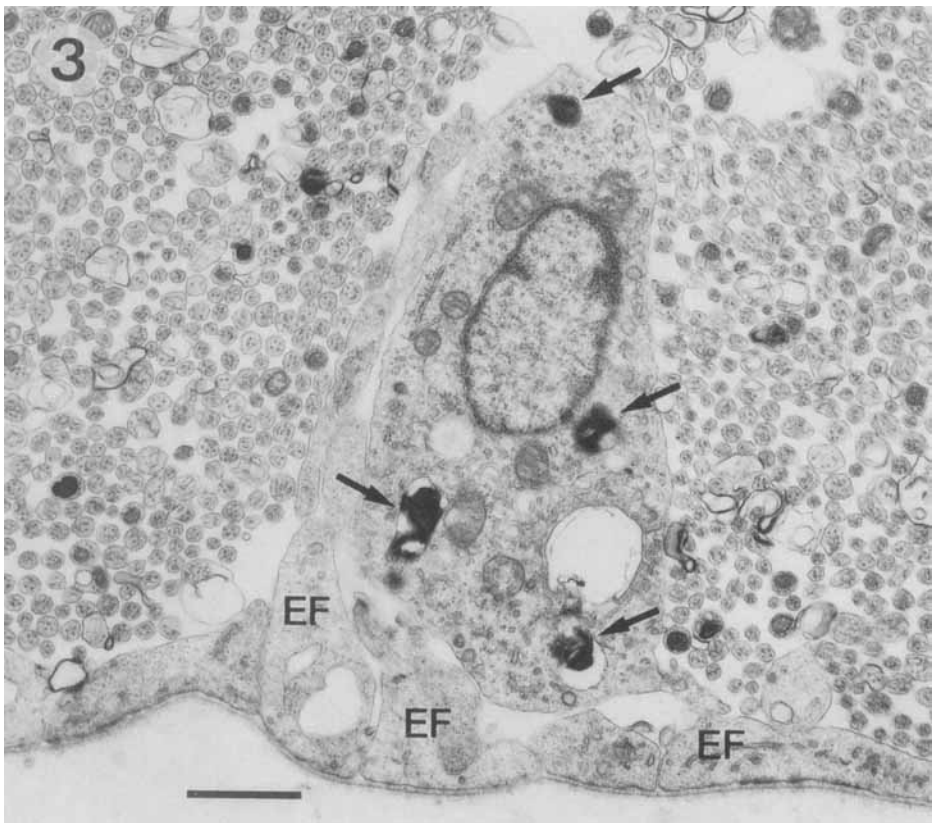


Fig. 3. TEM view of a macrophage intimately adhering on Müller cell endfeet (EF). This cell is identified as a macrophage because of the band of heterochromatin beneath the nuclear envelope and the numerous dense vesicles (arrows) similar to secondary lysosomes in the cytoplasm. Scale bar = 1 μm.

whole mounts, and others were used to obtain histological sections of retinas. Both retinal whole mounts and sections were treated immunocytochemically with the monoclonal antibody QH1, which recognizes an antigen present in all quail cells of hemangioblastic lineage except mature erythrocytes (Pardanaud et al., 1987), including resting microglia in the central nervous system (Cuadros et al., 1992) and the adult retina (Navascués et al., 1994). In addition to this material, retinas from E9 and E10 embryos were used for transmission (TEM) and scanning (SEM) electron microscopic studies.

Immunocytochemistry of histological sections

Hatched quails and quail embryos were decapitated, and the head was immersed in Bouin's solution, in which the eyes were rapidly dissected. Hatched quails were anesthetized with ether before decapitation. The method of killing the quails used in this study was in accordance with specifications on euthanasia methods from the unit of Laboratory Animals of the University of Granada. The eyes were fixed in Bouin's solution for 48 hours and embedded in paraffin. Serial sections 10 μm in thickness were obtained and mounted on gelatinized slides. For each eye, slides containing different levels of the retina were selected for QH1 immunocytochemical staining.

Endogenous peroxidase activity in erythrocytes was eliminated by treating the sections for 30 minutes with 0.3% hydrogen peroxide in phosphate-buffered saline (PBS). Nonspecific antibody binding was blocked by incubation for 30 minutes in normal goat serum diluted 1:20 in 1% bovine serum albumin in PBS (BSA-PBS). Incubation in the primary antibody lasted for 12 hours. The primary antibody was either QH1 ascites fluid (kindly provided by Dr. Dieterlen-Lièvre), diluted 1:100, 1:300, or 1:800 as appropriate for different batches of antibody, or QH1 supernatant (Developmental Studies Hybridoma Bank, University of Iowa) diluted 1:2. The sections were then incubated in the secondary antibody [mouse anti-IgG biotinylated antibody (Sigma), diluted 1:50 in BSA-PBS] for 40 minutes and in avidin-biotin-peroxidase complex (ExtrAvidin-Peroxidase; Sigma) diluted 1:400 in BSA-PBS for 40 minutes. Peroxidase activity was revealed in a solution containing 0.05%

Fig. 4. Labeled macrophages in a QH1-immunostained retinal whole mount of an E8 quail embryo. **A:** Retinal region just dorsal to the insertion of the pecten. In this whole mount, the pecten has been removed so that the triangular zone (P) at the bottom of the figure corresponds to the tissues of the base of the pecten. Numerous elongated, ameboid QH1$^+$ cells appear to be migrating along radial trajectories joined at the insertion of the pecten, i.e., the paths followed by ganglion cell axons. QH1$^+$ spots (arrowheads) are photoreceptors and scleral portions of Müller cells, a proportion of which are labeled with the QH1 antibody. **B,C:** Higher magnification of QH1$^+$ ameboid cells located in the nerve fiber layer. These cells show pseudopodial processes, many of which end in lamellipodia (arrows). Scale bars = 150 μm in A, 50 μm in B,C.

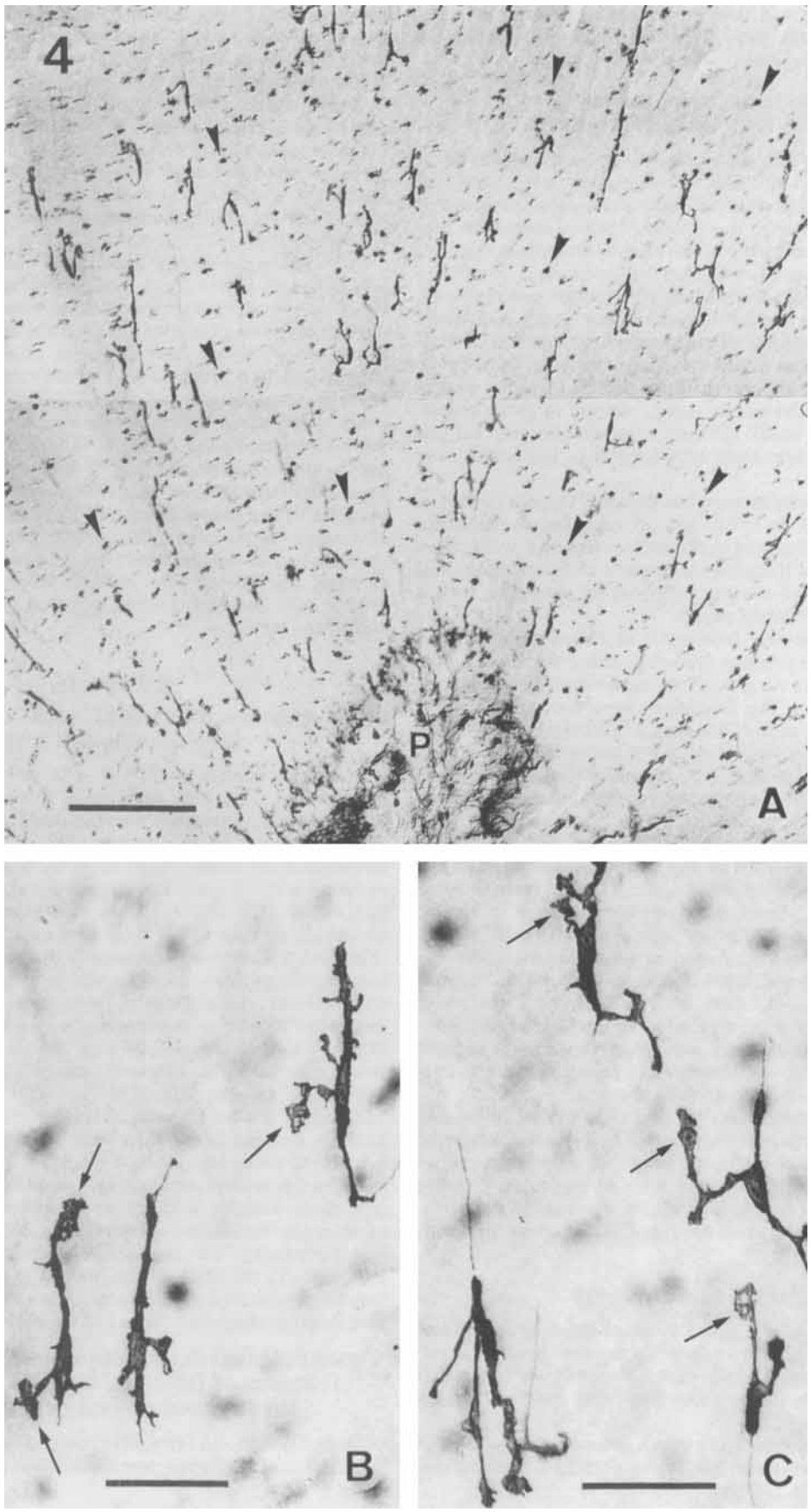


Figure 4

diaminobenzidine and 0.02% hydrogen peroxide in PBS. Finally, the sections were counterstained with hematoxylin, dehydrated, and mounted in DePex (Gurr).

Immunocytochemistry of whole-mounted retinas

After rapid eye dissection in 4% paraformaldehyde in PBS, the cornea, lens, and vitreous body were removed; then, the sclera and choroid (when developed) were also removed. Occasionally, the choroid carried away the pigment epithelium, allowing us to obtain pigment-free neural retina; more frequently the pigment epithelium remained joined to the neural retina when the choroid was removed. Retinas were flattened on a piece of filter paper moistened with fixative, by making radial incisions around the circumference. Then the pecten was usually removed in order to facilitate subsequent observations in retinal regions next to the pecten. Nevertheless, it was preserved in some retinal whole mounts to study QH1-immunostained cells on the surface of the pecten. Retinas were left in the fixative for 3–5 hours.

After fixation, retinas were treated with trypsin (Difco) in PBS (0.4 mg/ml) at 37°C for 30 minutes to facilitate removal of the pigment epithelium. Retinas were then rinsed in PBS and flattened on a piece of filter paper, and the pigment epithelium was removed by sweeping with a small piece of moist filter paper.

Immunocytochemical treatment of free-floating retinas was similar to the process described above for histological sections, with some variations to improve antibody penetration into the retina. The specimens were washed overnight in PBS, then washed for 30 minutes in PBS with 1% Triton X-100 (T-PBS) and incubated for 2 hours in normal goat serum diluted 1:20 in BSA-PBS. Because the quail retina is avascular, it was not necessary to eliminate endogenous peroxidase activity of erythrocytes. Retinas were then incubated for 40 hours at 4°C in QH1 ascites fluid diluted 1:100–1:800 in BSA-PBS or QH1 supernatant diluted 1:2. After three 10 minute washes in T-PBS, retinas were incubated for 3–4 hours at room temperature in mouse anti-IgG biotinylated antibody diluted 1:50 in BSA-PBS, washed in T-PBS, and incubated for 4 hours in avidin-biotin-peroxidase complex diluted 1:400 in BSA-PBS. Retinas were washed for 1–3 hours in PBS without Triton, and peroxidase activity was revealed as described for histological sections. Development was observed under a light microscope and was complete in 2–5 minutes. No counterstaining was used in retinal whole mounts.

Retinas were washed in PBS and then in distilled water before they were mounted on gelatinized slides. They were adhered to the slides by drying in air at room temperature for 2–3 days. Adhered retinas were hydrated in distilled water, dehydrated in a graded series of ethanol concentrations, cleared in xylol, and mounted under coverslips with DePeX (Gurr).

Electron microscopy

Quail embryos at E9 and E10 were used for SEM and TEM, respectively. For each embryo, the head was immersed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), in which the retina from the right eye was rapidly dissected.

For TEM, small pieces of retina, measuring 1–2 mm in width and 3–4 mm in length, were obtained from retinal zones dorsal or lateral to the pecten, from zones of transition between the pecten and the retina, and from the pecten itself. These small pieces were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer for 5 hours. They were then rinsed in buffer alone, postfixed in 1% osmium tetroxide, dehydrated in a graded series of acetone concentrations and propylene oxide, and embedded in Spurr's resin. Semithin sections were cut and stained with 1% toluidine blue. Ultrathin sections of selected zones were stained with uranyl acetate and lead citrate and examined with a Zeiss EM902 electron microscope.

For SEM, retinas were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer for 1 week. Prolonged fixation facilitated subsequent dissection, avoided tissue disruption, and did not affect its preservation for SEM observations. Small pieces of each retina were dissected, rinsed in buffer alone, dehydrated in a graded series of acetones, and dried with the critical-point method. Dried pieces were affixed with conductive silver paint to aluminum stubs, with their vitreal surface facing upwards. The ILM was removed by gently applying a small piece of double adhesive tape. The tape, with the endfeet of Müller cells and some axonal fascicles of the nerve fiber layer (NFL) adhering to it, was also affixed to aluminum stubs. Specimens were then gold sputter-coated and observed with a Zeiss DSM950 scanning electron microscope. We studied pieces with endfeet of Müller cells and their counterparts in pieces of the facing side of the retina.

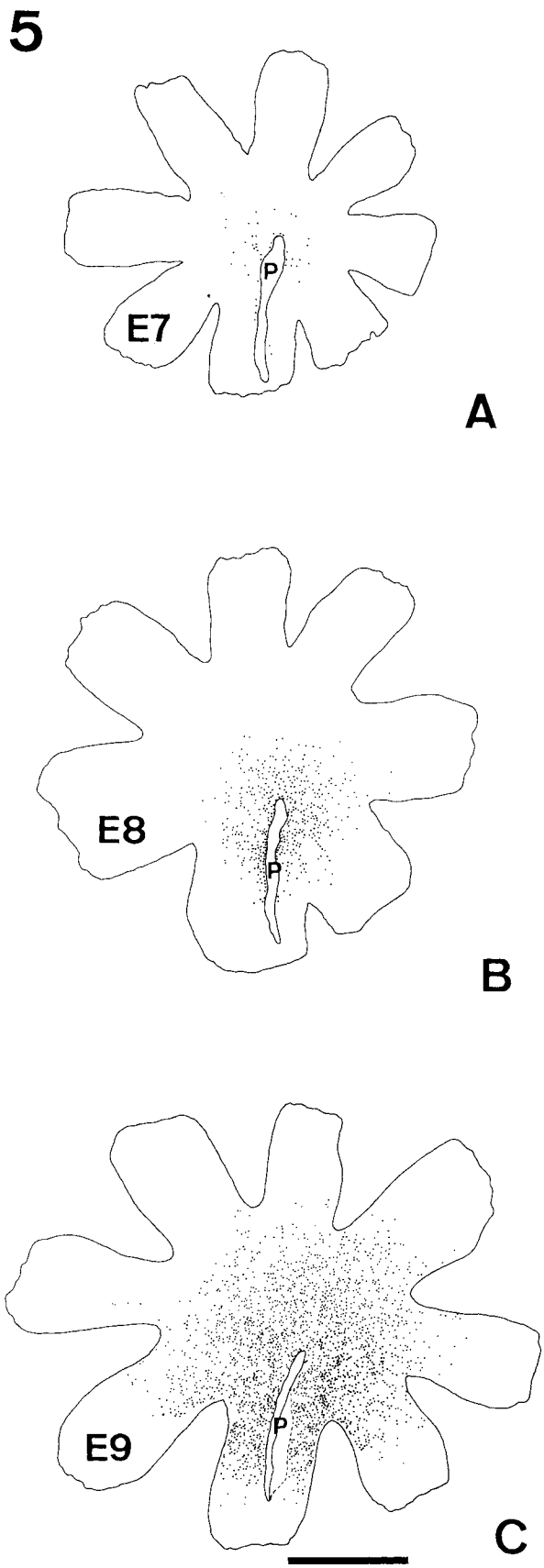
RESULTS

First appearance of QH1$^+$ macrophages in the developing quail retina

At stages E5–E6, no QH1$^+$ cells were observed in the quail retina (Fig. 1A). At E7–E8, a few QH1$^+$ cells with migratory characteristics were detected in the region of the retina adjacent to the pecten (Fig. 1B), which at these developmental stages began to develop, projecting into the vitreous body. These cells were intimately adhered to the ILM (Fig. 1C). Observations in whole-mounted retinas showed that these QH1$^+$ cells were ameboid and elongated (Fig. 2A), with several pseudopods that frequently ended in laminar expansions, as is typical of migrating cells. Cells with similar morphological features closely adhering to endfeet of Müller cells were seen with SEM and TEM (Fig. 2B,C). These ameboid cells had lamellar pseudopods and were oriented parallel to both axonal bundles and rows of Müller cell endfeet (Fig. 2B). Their cell membrane closely adhered to the membrane of Müller cells (Figs. 2C,D, 3), and the nucleus had a thin band of heterochromatin just beneath the nuclear envelope (Figs. 2C, 3). Their cytoplasm contained a prominent Golgi complex (Fig. 2D) and numerous clear vesicles and dense granules (Fig. 3), which appeared to be secondary lysosomes. Thus, morphological and structural features of these cells as well as their labeling with the QH1 antibody allowed us to conclude that they were macrophages migrating on the Müller cell endfeet through the inner surface of the retina.

Topographical distribution and morphological features of QH1$^+$ microglial precursors throughout retinal development

Days E7–E10. A few QH1$^+$ macrophages in the retina of E7–E8 quail embryos were detected in the immediate



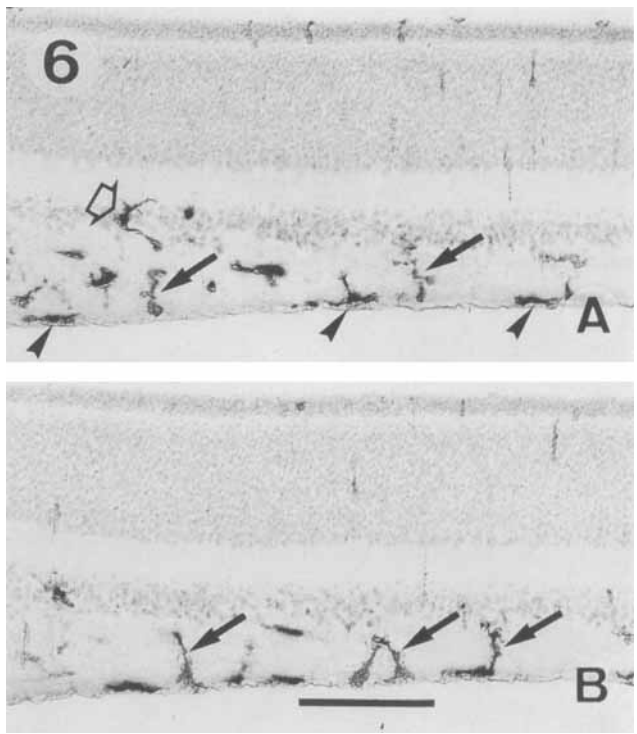


Fig. 6. QH1-immunostained histological sections of E9 quail embryo retina at a region adjacent to the insertion of the pecten. **A:** Some elongated QH1$^+$ macrophages (arrowheads) can be seen intimately adhering to the internal limiting membrane. Other macrophages are oriented perpendicular to the internal limiting membrane and are located in the nerve fiber layer (solid arrows) or in the ganglion cell layer (open arrow). **B:** Some QH1$^+$ macrophages closely adhering to the internal limiting membrane have vertical pseudopodial processes (arrows), which go deep into the nerve fiber layer. Scale bar = 100 μm.

vicinity of the base of the pecten (Figs. 4, 5). In E7 retinas, very few macrophages were observed (Fig. 5A). They increased in number in subsequent stages, and the outward edge of the retinal region occupied by QH1$^+$ macrophages progressively moved away from the zone of insertion of the pecten in the retina (Fig. 5B,C). Thus, the macrophage-free surface in the retinal periphery progressively decreased as development advanced.

From their first appearance in the retina until E10, most macrophages had the elongated, ameboid shape described above, were located in the NFL, and were aligned parallel to the course of ganglion cell axons (Fig. 4). These macrophages appeared to be in the process of migration along the ILM. Some macrophages sent out a slender process leading

Fig. 5. Camera lucida drawings from QH1-immunostained retinal whole mounts showing the distribution of QH1$^+$ macrophages at stages of early migration in the quail embryo retina. Each dot represents a single macrophage. The pecten has been removed in whole-mounted retinas and the elongated zone (P) represents the contour of the insertion of the pecten into the retina. **A:** E7 retina. **B:** E8 retina. **C:** E9 retina. The region delimited by a discontinuous line represents a portion of the pecten that was not removed during preparation of the retinal whole mount. This portion of pecten, which is pigmented, hides the macrophages located in the retinal portion beneath it. Scale bar = 3 μm.

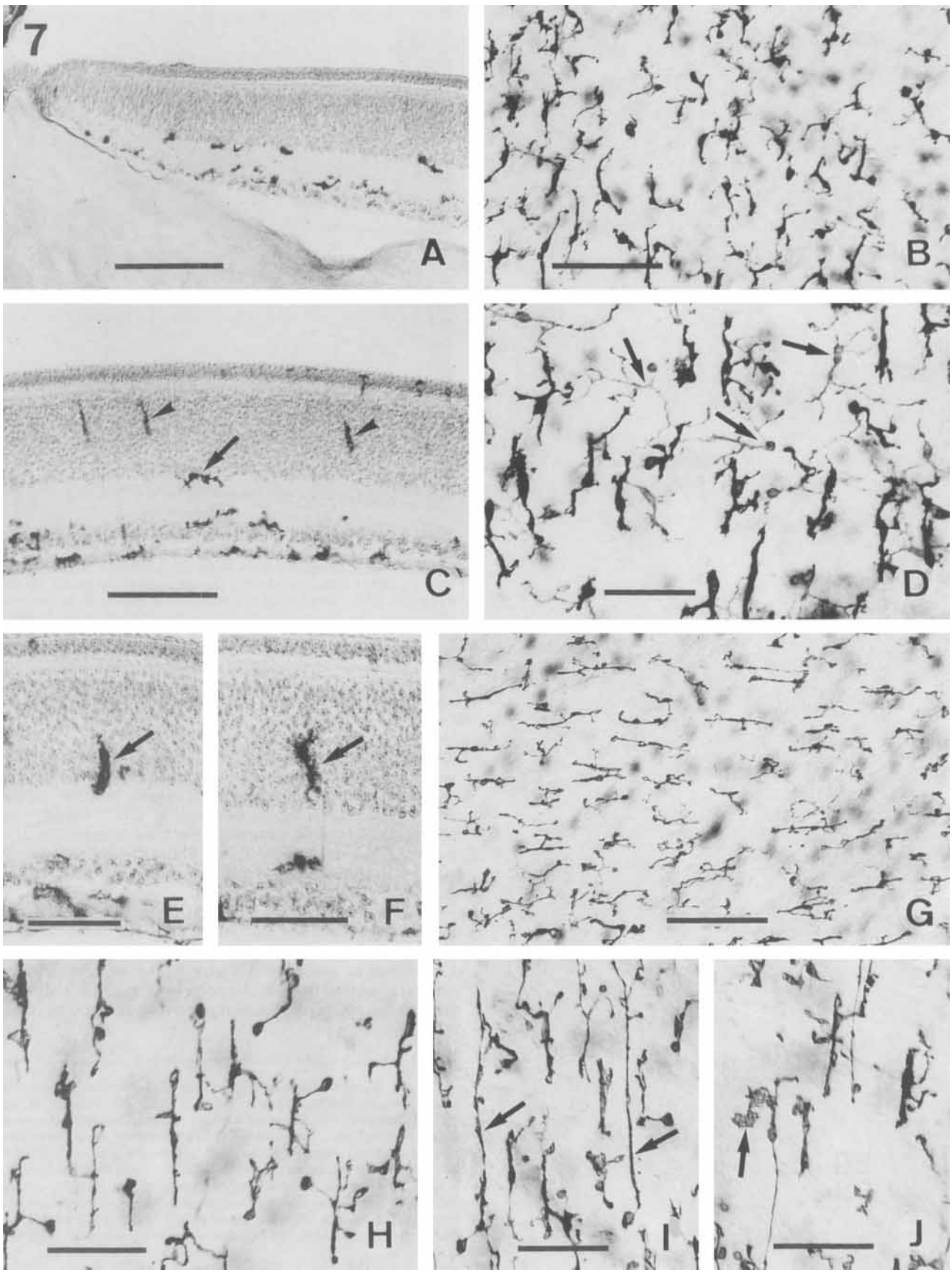


Figure 7

some pseudopods (Fig. 4C). From these observations we concluded that macrophages migrated tangentially in the retina from the insertion of the pecten toward peripheral regions, i.e., centrally to peripherally.

In E9–E10 retinas, many QH1$^+$ macrophages were oriented parallel to the ILM and were intimately adhering to it (Fig. 6A,B). In addition to these tangentially migrating macrophages, ameboid macrophages oriented perpendicular to the ILM were also seen in regions of the retina adjacent to the insertion of the pecten. They appeared to squeeze through the optic fiber bundles or the ganglion cell bodies (Fig. 6A). No QH1$^+$ macrophages were seen in retinal levels scleral to the ganglion cell layer (GCL). Moreover, some ILM-adhered macrophages sent perpendicular pseudopodial processes toward the depth of the NFL (Fig. 6B). These observations suggested that some macrophages migrating along the Müller cell endfeet became radially oriented to migrate toward more scleral levels of the retina, i.e., vitreally to sclerally.

The QH1 antibody labeled a small proportion of photoreceptors and Müller cells (Fig. 4) from these early developmental stages to adulthood (Navascués et al., 1994). Nevertheless, this labeling did not interfere with the study of QH1$^+$ macrophages, since the labeled photoreceptors and Müller cells had precise morphological features and locations in the retinal layers that made them easily distinguishable from macrophages.

Day E12. In the E12 quail embryo retina, QH1$^+$ macrophages were detected across the entire surface, including peripheralmost regions (Fig. 7A,B). Therefore, the first centrally to peripherally migrating macrophages had already reached the periphery of the retina by this developmental stage. Observations in retinal whole mounts showed that macrophages in peripheral regions of the retina were elongated and ameboid in shape (Fig. 7B).

In central retinal regions, in addition to elongated ameboid QH1$^+$ macrophages in the NFL, ramified QH1$^+$ cells were seen in the inner plexiform layer (IPL; Fig. 7C,D). These ramified cells were located exclusively in central regions. In histological sections, ameboid QH1$^+$ macrophages oriented perpendicular to the retinal surface were identifiable in the inner nuclear layer (INL; Fig. 7C,E,F). Together, these observations showed that, at E12, radially migrating macrophages arrived at more scleral levels of the retina than those occupied at E9–E10 and began to ramify in the IPL.

In addition to the advancing vitreal-to-scleral migration and the beginning of ramification, intense central-to-peripheral migration of macrophages appeared to continue along the ILM of the retina. In fact, numerous elongated ameboid QH1$^+$ macrophages were present on the vitreal surface throughout the entire retina (Fig. 7B,D,G). In central regions, these macrophages were oriented mainly parallel to the course of the ganglion cell axons (Fig. 7D). This orientation was particularly evident near the insertion of the pecten (Fig. 7G–J), where macrophages were very thin and elongated, some of them reaching up to 300 μm in length (Fig. 7I). Occasionally, these extremely long macrophages also showed lamellipodia (Fig. 7J).

Day E14. At this developmental stage, ameboid QH1$^+$ cells bearing pseudopodial processes oriented perpendicular to the retinal surface continued to be observed (Fig. 8A,B). Such cells were seen through the NFL (Fig. 8A), the IPL, and the INL (Fig. 8B). These observations suggested that intense vitreal-to-scleral migration of QH1$^+$ macrophages continued in the E14 retina.

This was the first stage in which a few slightly ramified QH1$^+$ cells were seen in the outer plexiform layer (OPL; Fig. 8C,D). These cells were seen exclusively in retinal regions adjacent to the pecten insertion.

At E14, there were more ramified QH1$^+$ cells in the IPL (Fig. 8E) than at E12. Moreover, many of these cells were more ramified than at E12. Ramified cells in the IPL were detected not only in central regions of the retina but also in intermediate and peripheral regions.

At E14, elongated ameboid QH1$^+$ macrophages in the NFL, oriented parallel to the ganglion cell axons, were still abundant in central, intermediate (Fig. 8E), and peripheral regions, although they were less numerous than at E12. This indicated that tangential migration of macrophages through the vitreal surface of the retina continued, although at a slower pace.

Days E16 and P1. At E16, few ameboid QH1$^+$ macrophages remained in the NFL (Fig. 9A,B), scattered throughout the retina. In contrast with E12, no thin, elongated macrophages were observed in the retinal zone adjacent to the insertion of the pecten. These observations appeared to indicate that tangential migration of macrophages through the vitreal surface of the retina had all but ended.

We deduced that vitreal-to-scleral radial migration of macrophages continued at E16, because, in transverse sections of E16 retinas, ameboid QH1$^+$ macrophages oriented perpendicular to the retinal surface were still seen throughout the NFL, IPL, and INL (Fig. 9A). Cell density of ramified QH1$^+$ cells in the IPL was greater than at E14, and ramification of the cell processes was also more complex (cf. Fig. 9B and Fig. 8E).

The zone occupied by ramified QH1$^+$ cells in the OPL (Fig. 9C) was larger than at E14. In fact, ramified cells were scattered throughout the entire retina, including peripheral zones (Fig. 9D), although they were few in number. Cells in the OPL were more ramified than at E14 (cf. Fig.

Fig. 7. QH1$^+$ ameboid macrophages and poorly ramified cells in immunostained E12 quail embryo retinas. **A:** Peripheralmost retina in transverse section, showing QH1$^+$ macrophages adhering to the inner limiting membrane, and in the inner plexiform layer. **B:** Peripheral region in a whole-mounted retina focused on the nerve fiber layer. Numerous ameboid QH1$^+$ macrophages are seen. **C:** Transverse section of the central region of a retina showing ameboid QH1$^+$ macrophages on the vitreal surface, in the ganglion cell layer, and in the inner plexiform layer. A QH1$^+$ cell (arrow) bearing two processes is seen at the border between the inner plexiform layer and the inner nuclear layer. Ameboid QH1$^+$ cells oriented perpendicular to the retinal layers (arrowheads) are seen in the inner nuclear layer. **D:** Central region in a whole-mounted retina focused on the nerve fiber layer and the inner plexiform layer. Numerous strongly QH1-labeled ameboid macrophages are oriented parallel to the ganglion cell axons (not stained), which course from the bottom to the top in the figure. In addition, poorly ramified QH1$^+$ cells (arrows) are seen at a depth of focus corresponding to the inner plexiform layer. These latter show weaker immunostaining than cells in the nerve fiber layer, probably due to incomplete antibody penetration. **E,F:** Ameboid QH1$^+$ macrophages (arrows) oriented perpendicular to the retinal layers, located at the border between the inner plexiform layer and the inner nuclear layer (E) or within the inner nuclear layer (F). **G–J:** Pecten-adjacent region in a whole-mounted retina focused on the nerve fiber layer. Elongated, ameboid QH1$^+$ macrophages oriented parallel to the ganglion cell axons (not stained), which course from left to right in G and from bottom to top in H–J. Some macrophages are very long and thin (arrows in I), and others show additional lamellipodia (arrow in J). Scale bar = 100 μm in A–C,G, 50 μm in D–F,H–J.

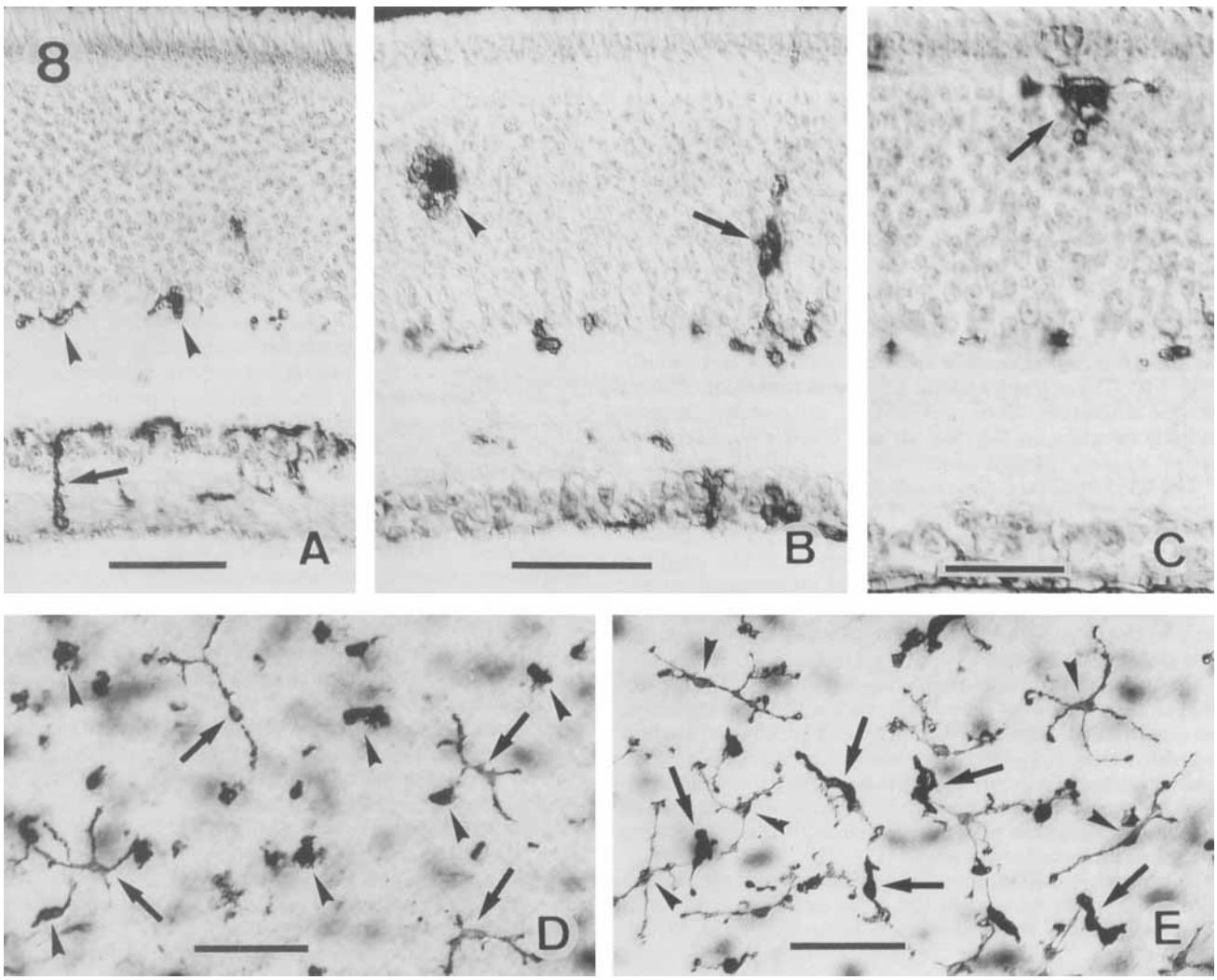


Fig. 8. Ameboid QH1$^+$ macrophages and poorly ramified QH1$^+$ cells in immunostained E14 quail embryo retinas. **A:** Transverse section of the retina showing an ameboid QH1$^+$ macrophage (arrow) with a pseudopodial process perpendicular to the retinal surface and traversing the nerve fiber layer. Two QH1$^+$ cells (arrowheads) are seen at the border between the inner plexiform layer and the inner nuclear layer. **B:** Ameboid QH1$^+$ macrophages in the inner nuclear layer. One of them (arrowhead) is round, whereas the processes emanating from the other (arrow) are oriented perpendicular to the retinal surface. **C:** QH1$^+$ cell (arrow) in the outer plexiform layer, bearing short cell processes. **D:** Zone adjacent to the insertion of the pecten in a whole-mounted retina, focused on the outer plexiform layer to show QH1$^+$ cells (arrows) with scarce, short processes. Arrowheads point out some QH1-labeled photoreceptors. **E:** Intermediate zone between central and peripheral regions in a whole-mounted retina, focused on the nerve fiber layer and the inner plexiform layer. Strongly QH1-labeled ameboid macrophages (arrows) are seen in the nerve fiber layer. In addition, poorly ramified cells (arrowheads) with weak QH1 labeling are located in the inner plexiform layer. Scale bars = 50 μm in A,D,E, 40 μm in B, 30 μm in C.

9C and Fig. 8D), although they were less highly ramified than at later stages. The findings at P1 were similar to those at E16 (Fig. 9E).

Day P3. At this developmental stage, the quail retina showed histological features similar to those in adulthood. Virtually no QH1$^+$ macrophages were found on the ILM, indicating that no macrophages migrated tangentially through the vitreal surface.

In ramified QH1$^+$ cells in both the IPL (Fig. 10A,B) and the OPL (Fig. 10A,C), processes were more profusely ramified than at E16. These cells were similar in shape to QH1$^+$ microglial cells seen in the same layers of the adult retina (Navascués et al., 1994), suggesting that they had reached their differentiated morphological state. The density of ramified QH1$^+$ cells in the OPL through central and peripheral retinal regions was higher than at E16 and was similar to that in the adult retina.

Vitreal-to-scleral migration of macrophages appeared to be ending, in that only few QH1$^+$ macrophages were seen in the INL, and they were no longer seen at subsequent stages. The presence of macrophages in the INL was the only noticeable difference between P3 and adult retinas in the pattern of distribution of QH1$^+$ cells.

Day P7 and adulthood. In E7 retinas, the pattern of distribution of QH1$^+$ cells was identical to that of microglial cells in the adult retina (Navascués et al., 1994). Ramified QH1$^+$ cells were located in the IPL and the OPL (Fig. 10D). In the IPL, they bore processes ramifying through the

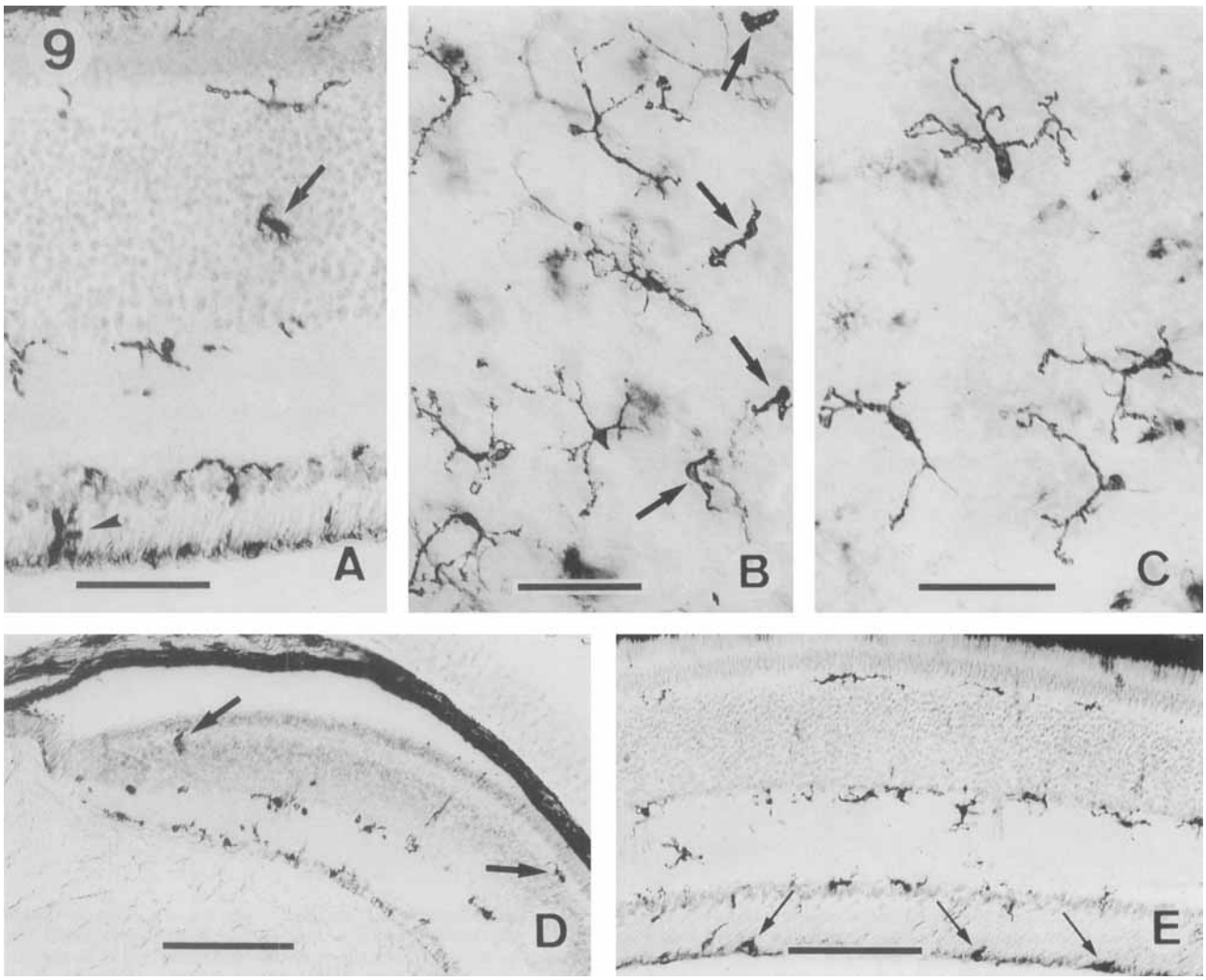


Fig. 9. Ameboid QH1$^+$ macrophages and poorly ramified QH1$^+$ cells in immunostained retinas of E16 (A–D) and P1 (E) quail embryos. **A:** Transverse section of an E16 retina showing ameboid QH1$^+$ macrophages in the nerve fiber layer (arrowhead) and the inner nuclear layer (arrow). Ramified QH1$^+$ cells are seen in the inner and outer plexiform layers. **B:** Whole-mounted E16 retina focused on the inner plexiform layer and the nerve fiber layer. Ramified QH1$^+$ cells show secondary branches and are uniformly distributed in the inner plexiform layer. Some ameboid macrophages (arrows) are seen in the nerve fiber layer. **C:** Whole-mounted E16 retina focused on the outer plexiform layer to show slightly ramified QH1$^+$ cells. **D:** Histological section from the periphery of the retina. In addition to QH1$^+$ cells in the nerve fiber layer and the inner plexiform layers, two QH1$^+$ cells (arrows) are seen in the outer plexiform layer. **E:** Transverse section of a P1 retina showing ramified QH1$^+$ cells in both plexiform layers. Some ameboid macrophages (arrows) are seen on the internal limiting membrane. Scale bars = 50 μm in A–C, 100 μm in D,E.

entire thickness of this layer. In the OPL, processes of QH1$^+$ cells ramified only in a horizontal plane.

Vitreal QH1$^+$ macrophages adhered to the pecten

Round QH1$^+$ macrophages in the vitreous body adhered to the basal lamina of the pecten throughout embryonic development and were investigated as a possible source of retinal microglial precursors. At E5, round QH1$^+$ cells were intimately adhered to the vitreal side of the surface of the chorioid fissure (Fig. 11A), which was where the pecten developed at subsequent stages. From E7 onwards, when the pecten projected into the vitreous body, numerous round QH1$^+$ cells were seen intimately adhered to the basal lamina of the pecten (Fig. 11B,C,D). These cells had a ruffled surface and lamellipodial processes (Fig. 11C,D), and their morphological features were similar to those of macrophages in the ventricle of the central nervous system of the chick embryo (Cuadros et al., 1993). They were actively proliferating, as deduced from frequent observations of mitotic macrophages adhered to the pecten in ultrathin sections (not shown).

The number of QH1$^+$ cells adhered to the pecten increased until E10, when they were most numerous (Fig. 12A), then decreased steadily during E12 and E14. At E16, the few QH1$^+$ macrophages that appeared on the pecten (Fig. 12B) were not round in shape but extremely flattened. No pecten-adhered QH1$^+$ macrophages were seen at post-hatching developmental stages or adulthood.

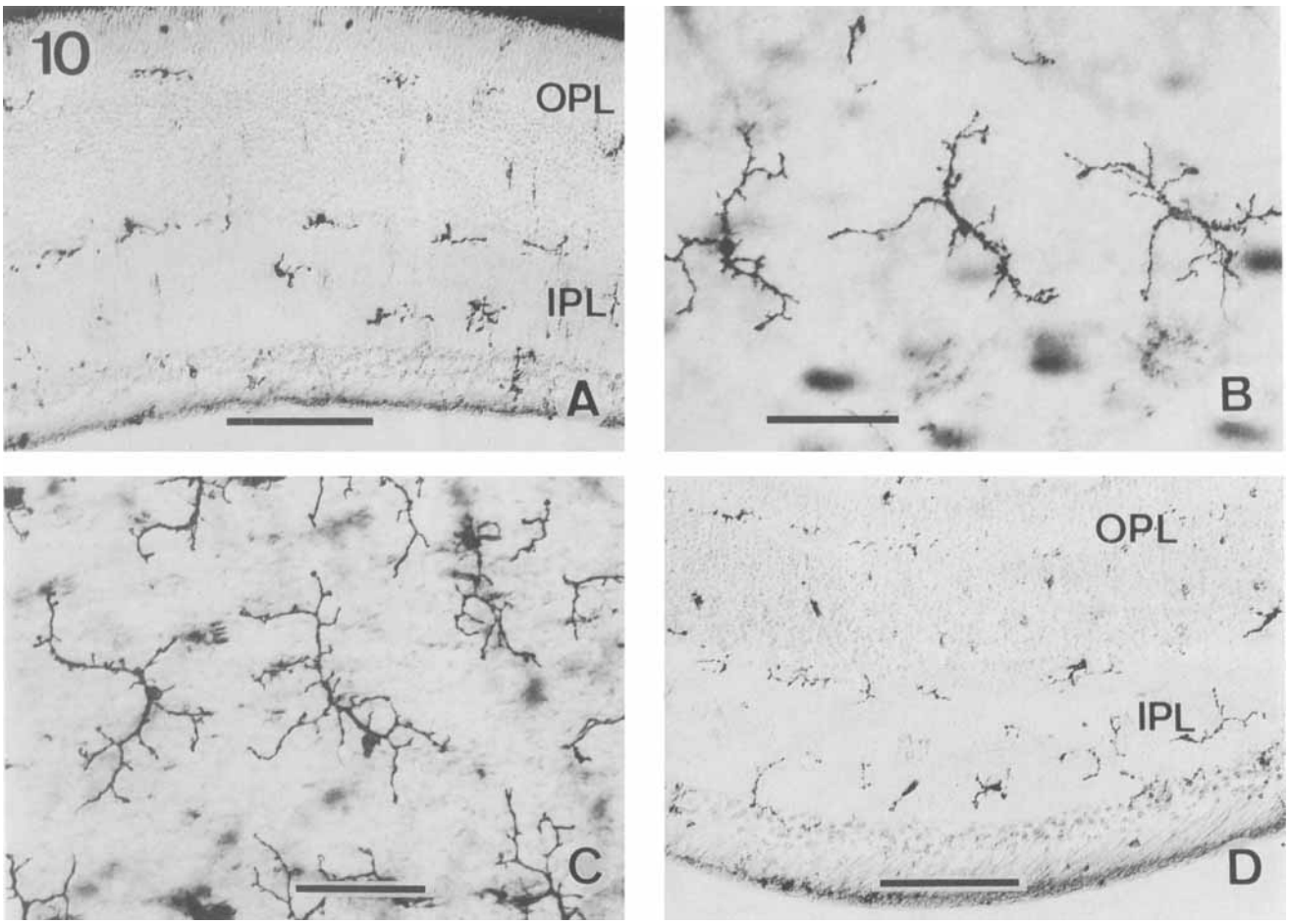


Fig. 10. Ramified QH1$^+$ cells in immunostained retinas of P3 (A–C) and P7 (D) quail embryos. **A:** Transverse section of a P3 retina showing ramified QH1$^+$ cells in the inner (IPL) and outer (OPL) plexiform layers. **B:** Whole-mounted P3 retina focused on the inner plexiform layer to show adult-like ramified QH1$^+$ cells. **C:** Whole-mounted P3 retina focused on the outer plexiform layer, showing adult-like ramified QH1$^+$ cells. **D:** Histological section of a P7 retina. Ramified QH1$^+$ cells show a pattern of distribution similar to that in adults in the inner (IPL) and outer (OPL) plexiform layers. Scale bars = 100 μm in A,D, 50 μm in B,C.

QH1$^+$ macrophages within the pecten and migration toward the retina

In developmental stages when the pecten projects into the vitreous body, i.e., from E7 onwards, in addition to round QH1$^+$ macrophages adhering to the basal lamina, ameboid QH1$^+$ cells were seen within the pigmented lamina of the pecten, at both its apical (Fig. 13A) and basal (Fig. 13B) portions. With TEM, cells with morphological features of macrophages were also seen within the pecten (Fig. 13C). These cells contained a nucleus with a thin band of heterochromatin just beneath the nuclear envelope and cytoplasm with clear vesicles and dense granules that appeared to be primary and secondary lysosomes.

Numerous elongated QH1$^+$ macrophages, which appeared to be actively migrating, were seen in zones of transition between the vascularized tissue of the pecten and the NFL of the retina (Figs. 13B, 14A,B). With TEM, macrophages similar to those in the pecten were detected in transition zones (Fig. 14C). Their cytoplasm contained clear vesicles and dense granules, some of which contained concentric systems of thin dense lamellae (Fig. 14C), which appeared to be secondary lysosomes. These migrating macrophages located midway between the pecten and the retina were first detected at stages E7–E8 in the dorsal region of the zone of insertion of the pecten in the retina. From E8–E9 onwards, they were seen in both the dorsal region (Fig. 13B) and on both sides along the zone of insertion of the pecten (Fig. 14A,B). In E12 whole-mounted retinas, large numbers of QH1$^+$ macrophages located midway between the pecten and the retina were observed both in the dorsal region (Fig. 15A,B) and on both sides of the insertion of the pecten (Fig. 15C,D). Slightly fewer macrophages were seen at these sites at E14. At E16, only a few QH1$^+$ macrophages were detected midway between the pecten and the retina. Finally, no macrophages were seen in these locations at posthatching stages. Taken together, these observations strongly suggested that macrophages migrating tangentially into the retina came from tissues of the pecten.

DISCUSSION

We have used labeling of the microglia by monoclonal antibody QH1 to study the development of these cells in the

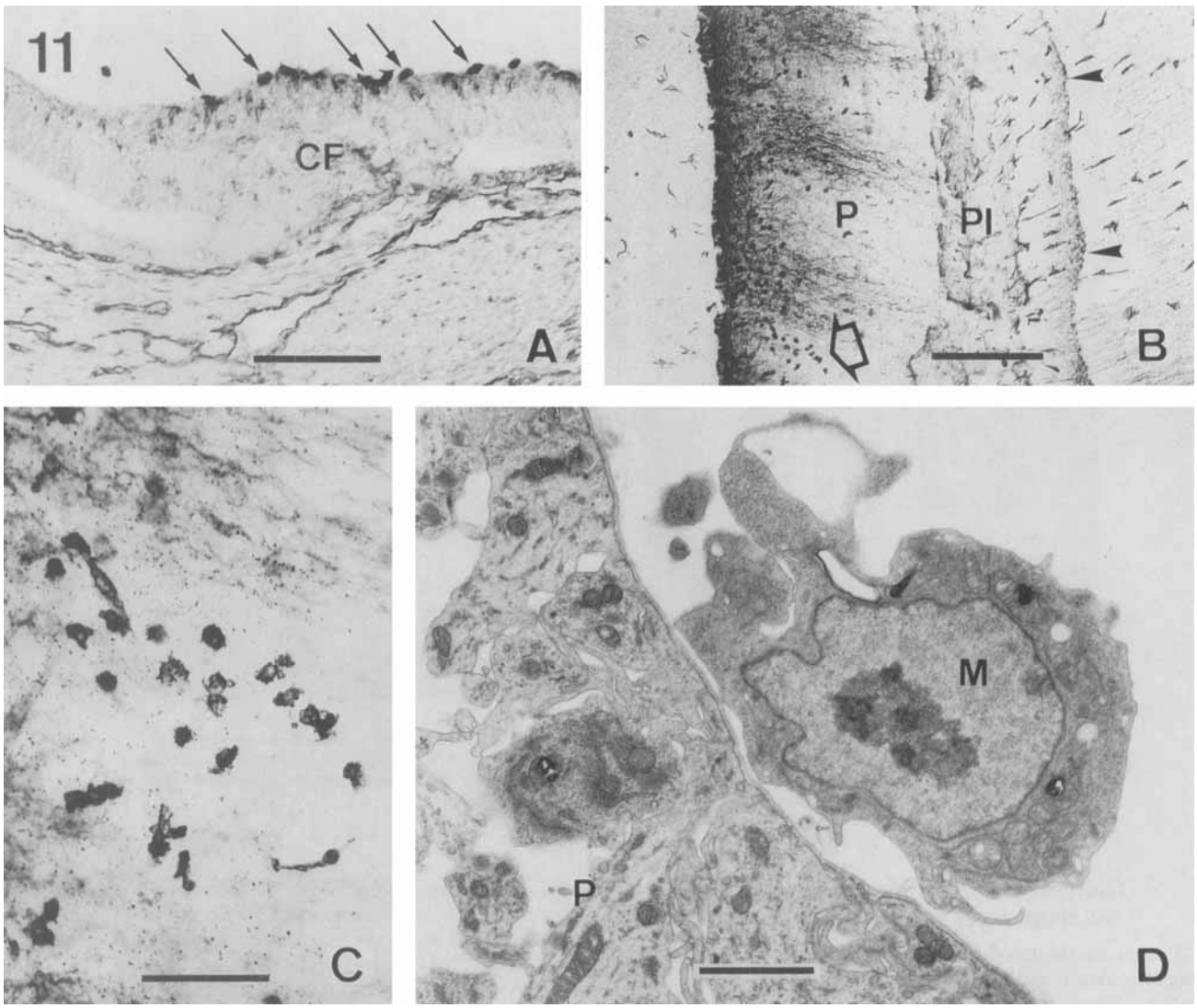


Fig. 11. Vitreal macrophages closely adhering to the pecten surface. **A:** Immunostained histological section of an E5 quail retina showing round QH1$^+$ macrophages (arrows) intimately adhering on the vitreal surface of the choroid fissure (CF). **B:** QH1-immunostained retinal whole mount of an E9 quail embryo, in which the pecten (P) is flattened as a consequence of flattening of the retinal whole mount. Thus, one of its faces contacts the vitreal surface of the retina and cannot be seen, whereas the other is observed from its strongly pigmented apex to its insertion in the retina (PI). The pigmented line to the right of the insertion of the pecten (arrowheads) corresponds to remains of pigment epithelium, which were not removed during retinal dissection. The open arrow points out a zone seen at higher magnification in C. **C:** Round QH1$^+$ macrophages on the pecten surface. Small dots are pigment granules of the pecten. **D:** TEM view of a vitreal macrophage (M) closely adhering to the surface of the pecten (P) in an E9 quail embryo. The surface of the macrophage contacts the basal lamina of the pecten at several points. Scale bars = 100 μm in A, 200 μm in B, 50 μm in C, 2 μm in D.

quail retina. This material is especially suitable for study, in that the avian retina is avascular (Meyer, 1977) and blood vessels do not interfere with observations of microglia. In addition, the possible relationship between microglia and inherent retinal vessels can be tested. Retinal whole mounts can also be made, providing complete pictures of the morphology and distribution of microglial cells at each developmental stage. Finally, the layered organization of the retina facilitates comparisons of microglial cell distribution at different depths within the retina during development.

From our results, we propose that microglial development in the quail retina occurs through several steps: 1) Macrophages enter retinal neural tissue from the pecten, 2) these macrophages migrate tangentially along the ILM to spread centrally to peripherally, 3) macrophages then move radially to both plexiform layers, and 4) macrophages differentiate to become ramified microglia. This sequence of events is summarized in Figure 16. In this model of microglial invasion, macrophages that colonize the peripheral retina migrate tangentially for long distances, whereas those occupying final locations in the central retina migrate short distances. Macrophages that remain in retinal regions next to the pecten and optic nerve head need only move radially to reach their final locations in the plexiform layers.

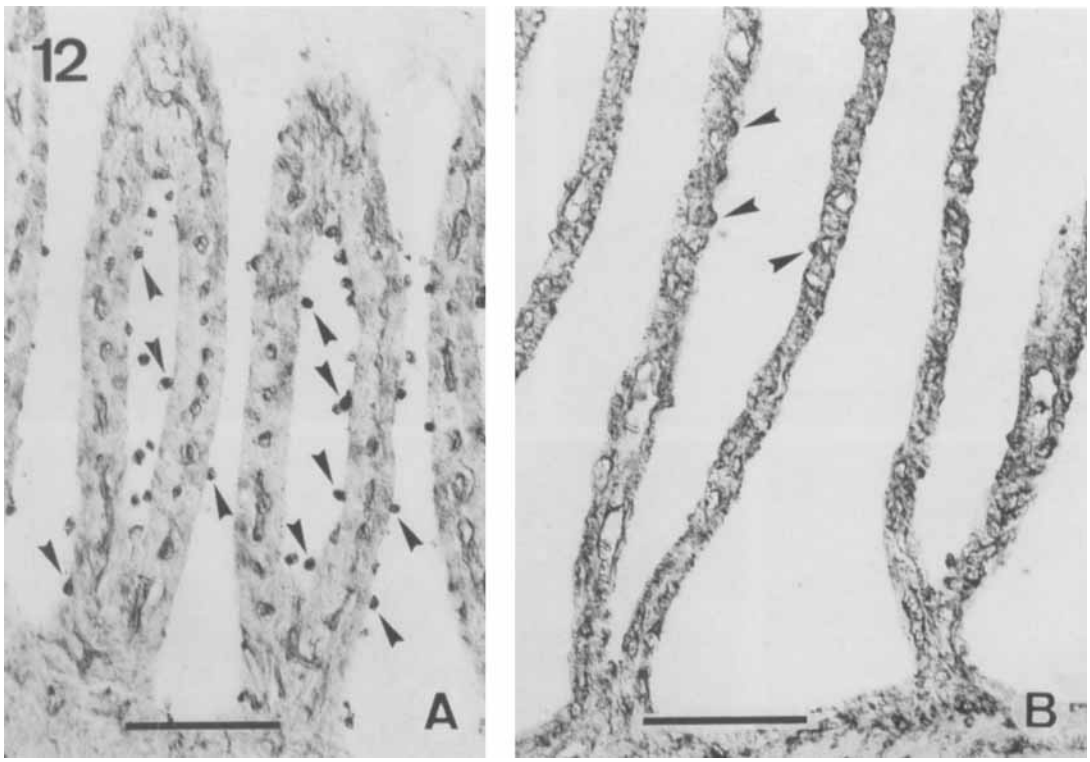


Fig. 12. Immunostained sections of the pecten parallel to its longitudinal plane. **A:** Pecten lamellae from an E10 quail embryo. Numerous round QH1$^+$ macrophages (arrowheads) are adhered to the surface of the pecten. **B:** Pecten lamellae from an E16 quail embryo, showing few QH1$^+$ macrophages adhering to the pecten (arrowheads). Scale bars = 50 μm.

Central-to-peripheral migration of macrophages into the quail retina

It can be deduced from our results that macrophages migrate from central to peripheral areas of the retina during developmental stages E7–E16. This central-to-peripheral migration is the most plausible interpretation of our observations at the early stages of migration (E7–E12), when the surface area of retina occupied by ameboid macrophages increases from the insertion of the pecten toward the retinal periphery as development advances (Fig. 5). At developmental stages when the entire retina is covered with macrophages (from E12 on), central-to-peripheral migration of macrophages is more difficult to deduce from our static observations. Nevertheless, observations of numerous elongated ameboid macrophages, oriented parallel to the course of the ganglion cell axons, and especially long and numerous in the zone of transition between the pecten and the retina, strongly suggest that macrophages continue to arrive in the retina until E16. The central-to-peripheral pattern of macrophage migration is similar to that shown to occur in other events in the vertebrate retina, such as neurogenesis (Kahn, 1974; LaVail et al., 1991; Prada et al., 1991), synaptogenesis (Maslim and Stone, 1986), cell death (Rager and Rager, 1978; Robinson, 1988), vascularization (Chan-Ling et al., 1990), and migration of astrocytes (Ling and Stone, 1988; Ling et al., 1989; Chan-Ling and Stone, 1991; Huxlin et al., 1992). Although several studies have dealt with microglial development in the mammalian retina (Hume et al., 1983; Sanyal and De Ruiter, 1985; Boya et al., 1987; Ashwell, 1989; Ashwell et al., 1989; Schnitzer, 1989), central-to-peripheral migration of microglial precursors has not been reported until recently (Pearson et al., 1993).

What is the stimulus for macrophage immigration into the retina? Some studies have provided evidence that cell death acts as a stimulus for recruitment of macrophages in different regions of the nervous system (Perry et al., 1985; Milligan et al., 1991a,b), including the retina (Hume et al., 1983; Thanos and Richter, 1993; Pearson et al., 1993). However, this hypothesis does not explain the absence of macrophages in other brain regions where cell death is known to happen (Milligan et al., 1991a). In addition, microglial precursors appear to enter the mammalian retina well before the period of neuronal death (Ashwell, 1989; Ashwell et al., 1989). In the quail retina, dying ganglion cells are not seen at E6 but are detected at E8 (Yew, 1979). In the chick embryo, the first dying ganglion cells are detected in the central region at E9 (Rager and Rager, 1978), which is equivalent to E7 in the quail embryo (Zacchei, 1961), when macrophages begin to enter the retina. Subsequently, a wave of cell death spreads from the central part of the retina toward its periphery, so that dying cells are distributed throughout the whole retina at E12 (Rager and Rager, 1978), which corresponds to E9 in the quail. Our study in the quail retina reveals that, as with cell death, macrophages also spread centrally to peripherally but do not reach the retinal periphery until E12, well after cell death is first detected at this location. Therefore, in the

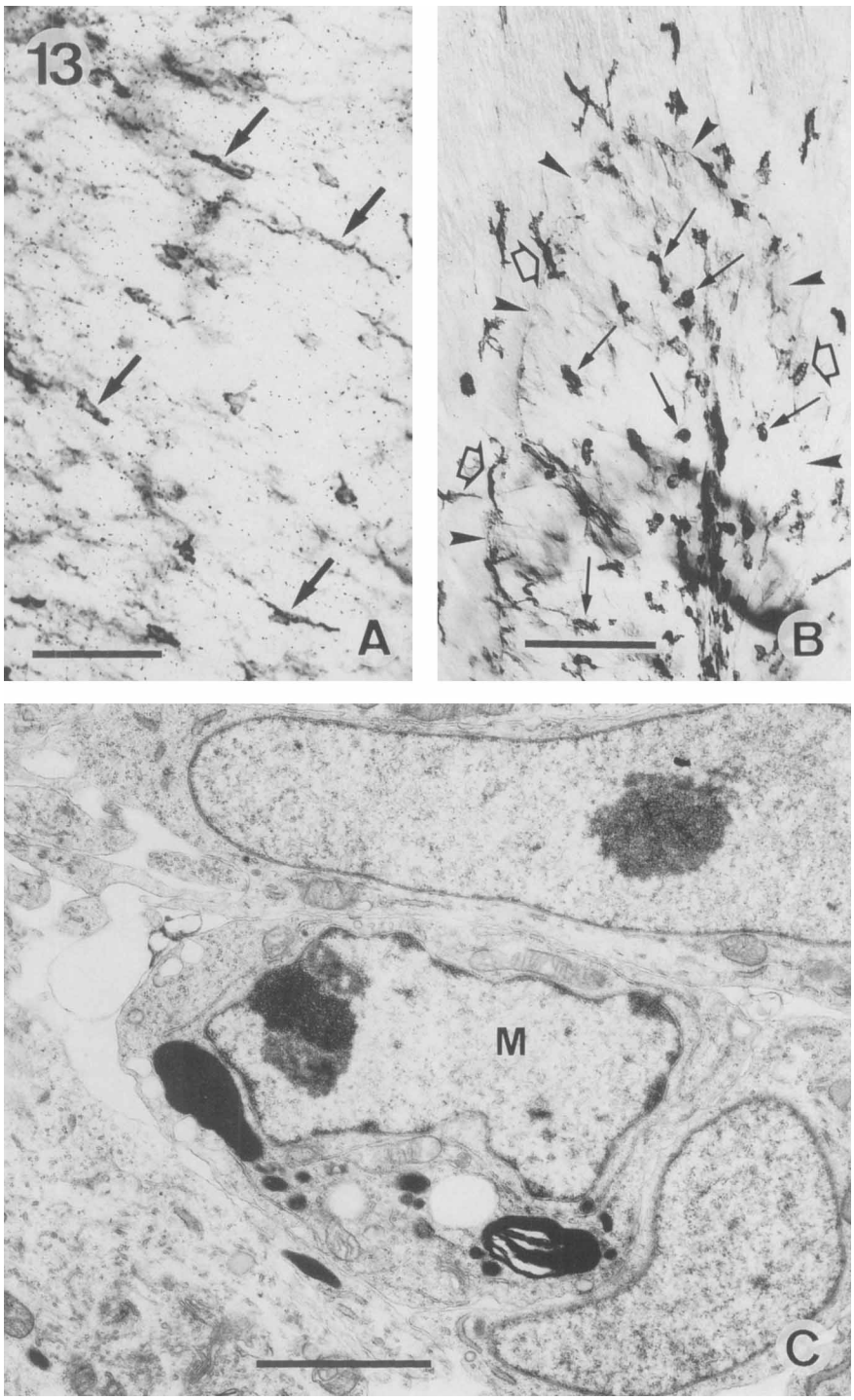


Fig. 13. Macrophages in pecten tissues in E9 (A,B) and E10 (C) quail embryos. **A:** Medial-apical zone of an immunostained whole mount of pecten similar to that in Figure 11B. Elongated QH1$^+$ cells (arrows) are seen in the pecten. **B:** Zone of insertion of the pecten into the retina from an immunostained retinal whole mount, from which the pecten was removed during the dissection. The base of the pecten is outlined with arrowheads. Numerous ameboid QH1$^+$ macrophages (solid arrows) are seen in the base of the pecten; others are located in the retina. Some QH1$^+$ macrophages (open arrows) are midway between the pecten and the retina. **C:** TEM view of the tissue in the base of the pecten of an E10 quail embryo. A macrophage (M) is identifiable by heterochromatin patches beneath the nuclear envelope and lysosome-like dense vesicles in the cytoplasm. Scale bars = 50 μm in A,B, 2 μm in C.

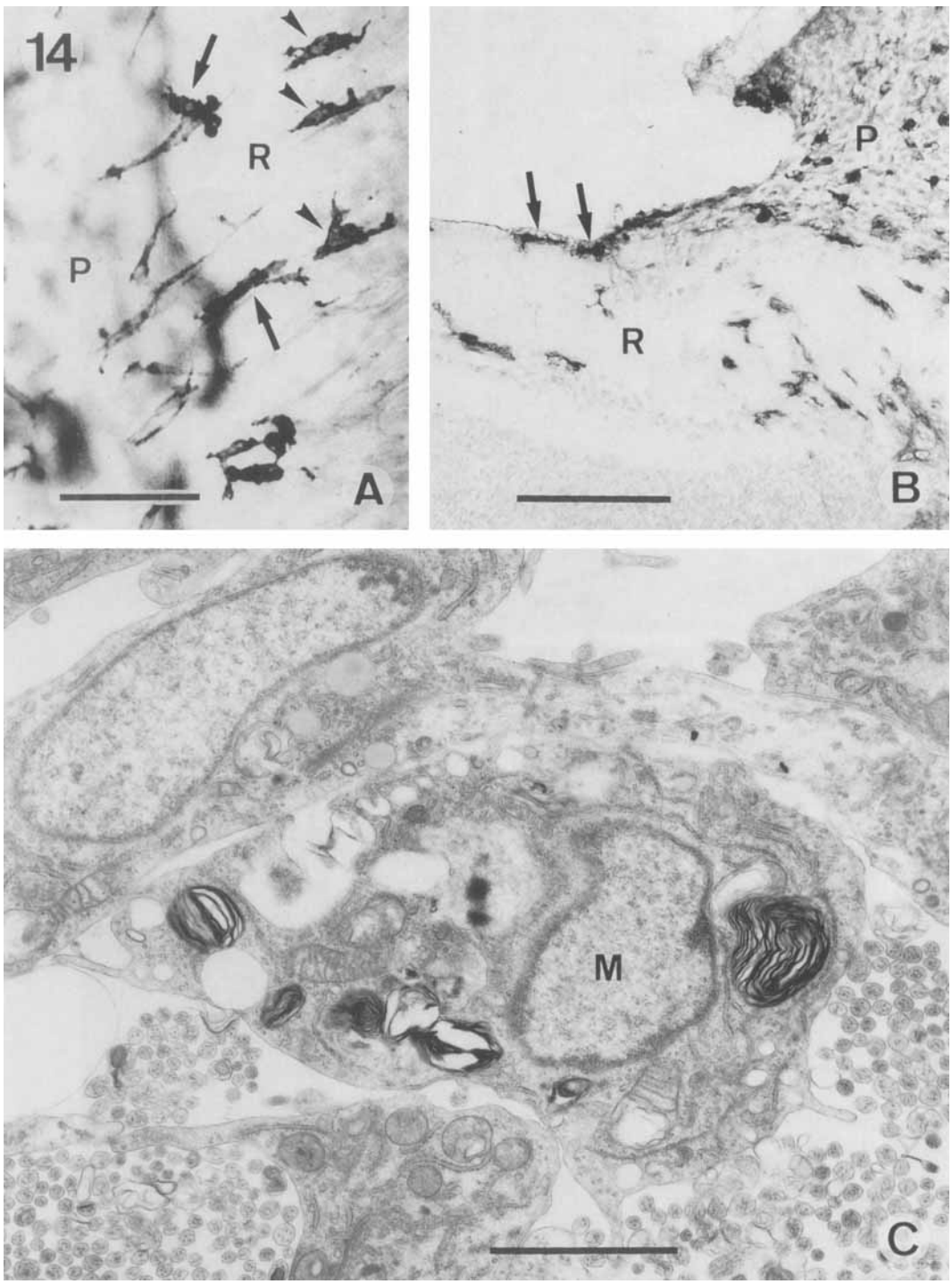


Fig. 14. Macrophages midway between the pecten and the retina in E9 (A,B) and E10 (C) quail embryos. **A:** Retinal region (R) lateral to the base of the pecten (P) in an immunostained whole-mounted retina from which the pecten has been removed. Some ameboid QH1$^+$ macrophages (arrows) are seen midway between the pecten and the retina; others (arrowheads) are located in the retina. **B:** Transition zone between the pecten (P) and the retina (R) in an immunostained histological section. Some ameboid QH1$^+$ macrophages (arrows) in close contact with the internal limiting membrane are seen midway between the pecten and the retina. **C:** TEM view of a macrophage (M) in the zone of transition between the pecten and the retina. Its cytoplasm contains dense vesicles. The cell on the left of the macrophage is from the pecten tissue. Ganglion cell axons are seen in cross section at the bottom. Scale bars = 50 μm in A, 100 μm in B, 2 μm in C.

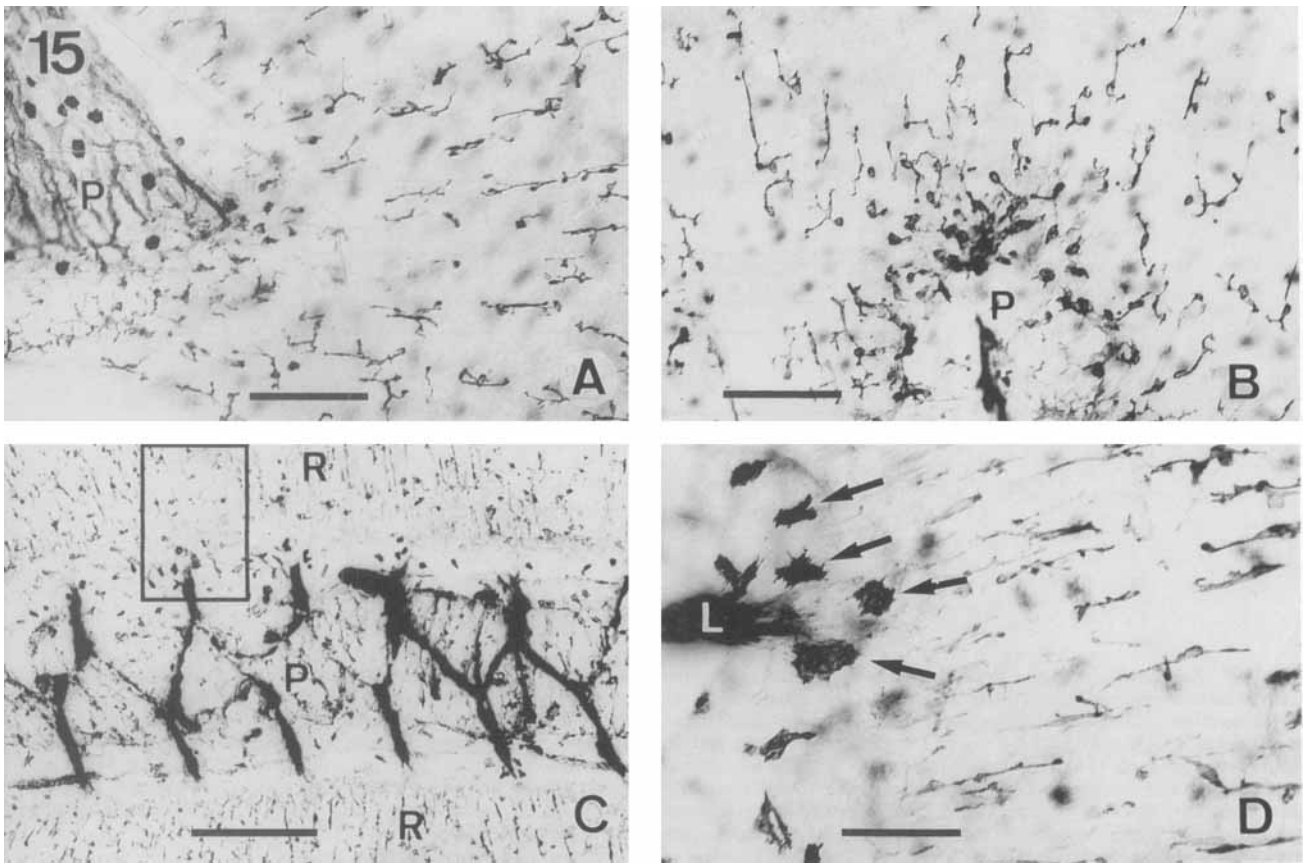


Fig. 15. Retinal regions adjacent to the pecten in immunostained E12 whole-mounted retinas, showing elongated QH1$^+$ macrophages, which appear to be in the process of active migration. **A:** Elongated macrophages in the retinal region adjacent to the dorsal end of the pecten (P), preserved in the retinal whole mount. Round macrophages can be seen adhered to the pecten. **B:** Elongated macrophages in the same region as in A from a retinal whole mount from which the pecten has been removed. The dorsal end of the zone of insertion of the pecten into the retina (P) is seen. **C:** Retinal zones (R) located lateral to the pecten (P), most of which has been removed, although the basal part of its lamellae (dense zigzag lines) has been preserved. The boxed area is enlarged in D. **D:** Elongated macrophages in the retinal region lateral to the pecten. Macrophages (arrows) showing shapes intermediate between rounded and elongated are seen in the base of the pecten lamella (L). Scale bars = 100 μm in A,B, 200 μm in C, 50 μm in D.

avian retina, the beginning of macrophage immigration coincides with the first appearance of ganglion cell death, although ganglion cell death appears to spread toward the periphery more rapidly than macrophages. In addition, the first macrophages to invade the retina do not immediately reach the location where cell death takes place, i.e., the ganglion cell layer, but rather move along the ILM. Therefore, it remains to be elucidated whether the relationship between cell death and migration of macrophages is only coincidental or whether there is a direct link between the two phenomena.

Electron microscopic observations reveal that macrophages on the ILM are in close contact with endfeet of Müller cells. The surface of these cells may have a role in guiding the tangential migration of macrophages. This would not be surprising, in that Müller cells also participate in other adhesive events such as migration of ganglion cell axons (Silver and Rutishauser, 1984; Drazba and Lemmon, 1990).

Vitreal-to-scleral migration of macrophages in the retina

Shortly after the beginning of tangential migration of macrophages into the retina, a proportion of macrophages is seen in more and more scleral retinal layers as development advances. From these observations, we deduce that macrophages migrating through the vitreal part of the retina changed the direction of their migration and began to move radially toward more scleral parts. Through this radial migration, macrophages first reached the IPL at E12 and the OPL at E14. No migration beyond the OPL was evident. Furthermore, the first macrophages to be detected in both plexiform layers were seen next to the pecten; they subsequently appeared in progressively more peripheral parts of the retina.

An argument against the above interpretation is that there may be three levels (NFL, IPL, and OPL) of tangential migration from the pecten insertion, rather than radial migration of macrophages previously migrating on the vitreal part of the retina. Three findings contradict this possibility. First, radially oriented macrophages are frequently seen in the NFL, IPL, and INL up to P3. Second, macrophages in the INL are detected only at developmental stages just previous to and coincident with the appearance of macrophages in the OPL (E12–P3) and are no longer identifiable after microglia differentiate in this layer. This observation is only explainable if macrophages radially

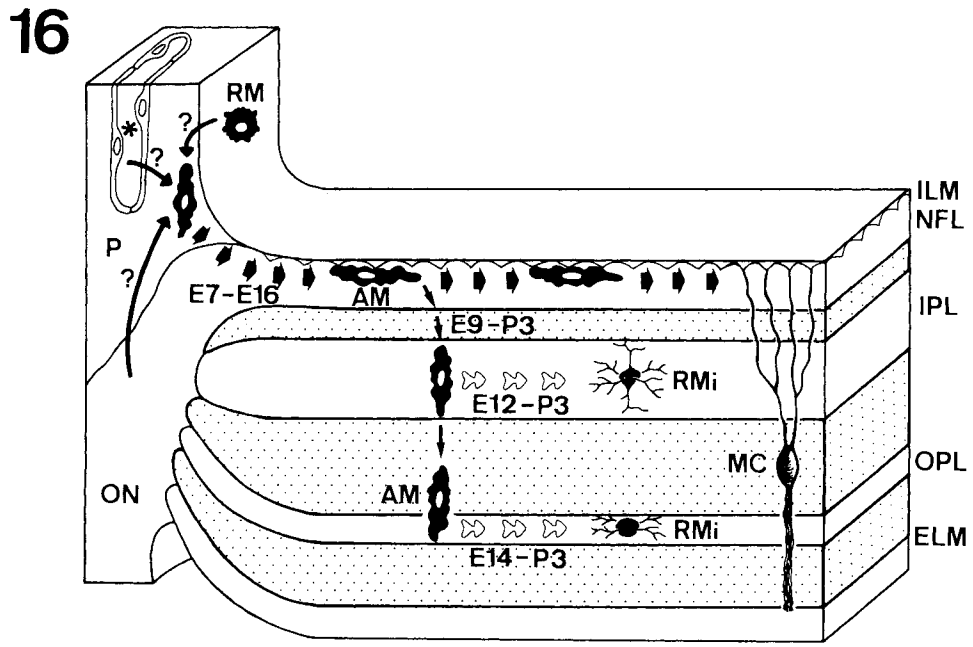


Fig. 16. Schematic drawing summarizing the major findings in this study. Ameboid macrophages (AM) in the base of the pecten (P) enter the retina during E7–E16 and tangentially migrate (thick solid arrows) on endfeet of Müller cells (MC), spreading throughout the entire retina. From E9 to P3, ameboid macrophages on the inner limiting membrane (ILM) change the direction of their movement and migrate radially (thin arrows) to colonize the inner (IPL) and outer (OPL) plexiform layers. In these layers, ameboid macrophages differentiate (thick open arrows) into ramified microglia (RMi). Morphological differentiation of microglia begins at E12 in the inner plexiform layer and at E14 in the outer plexiform layer and is complete in both layers at approximately P3. Three possible sources (curved arrows with question marks) of ameboid macrophages within the pecten are compatible with our observations: vitreal round macrophages (RM) adhering to the pecten, monocytes in the blood vessels (asterisk) of the pecten, and macrophages in the optic nerve (ON). ELM, external limiting membrane; NFL, nerve fiber layer.

traverse the INL before they reach the OPL. Third, QH1$^+$ cells in the OPL are ramified from their first appearance in this layer. This morphological feature appears to be compatible with the end of the radial migration but not with tangential migration through the OPL.

Radial migration of macrophages has been previously described in the developing mouse retina (Hume et al., 1983). Some retinal neurons, such as amacrine cells, also appear to migrate radially, although in the opposite direction, i.e., from scleral to vitreal parts (Hinds and Hinds, 1983; Prada et al., 1987). In the developing mammalian and avian brains, radial glia have been shown to guide neurons migrating radially (Rakic, 1971; Hatten, 1990; Gray and Sanes, 1991). Preliminary results in our laboratory (not shown) suggest that macrophages migrate radially on processes of Müller cells, the retinal equivalent of radial glia.

Migration of microglial cells from the GCL and IPL toward the photoreceptor layer was demonstrated in retinas of rats with inherited retinal dystrophy (Thanos, 1992; Thanos and Richter, 1993). In these animals, sick photoreceptors appear to exert chemotactic attraction on microglial cells, probably via inflammatory substances (Thanos, 1992). Vitreal-to-scleral movement of macrophages in the developing quail retina may also be mediated by factors secreted by dying neurons in retinal layers sclerad to the NFL. In fact, we saw large amounts of cell debris in the INL at developmental stages when radial migration of macrophages takes place (not shown).

Possible origin of macrophages entering the quail retina

In a previous study in our laboratory, we found that macrophages appeared in the posterior pole of the neural retina of chick embryos at E3.5 (Cuadros et al., 1991). Some of these cells were located deep in the retinal neuroepithelium, in association with dying neuroepithelial cells (Cuadros and Ríos, 1988; Martín-Partido et al., 1988; Cuadros et al., 1991). These macrophages were no longer observable at E5 in quail embryos, as is shown in this study. Therefore, they seem to be transitory cells that disappear once they have removed neuroepithelial cell debris.

Macrophages that differentiate into ramified microglia enter the quail retina from E7–E8 on and appear to come from the pecten. Therefore, the pecten is the nearest source of retinal microglial precursors, but what is the early origin of these macrophages? Three possibilities are compatible with our results (Fig. 16). First, retinal macrophages might come from vitreal macrophages adhered to the pecten surface, which might cross the basal lamina to reach the pecten. Second, they might derive from blood vessels in the pecten. This possibility is compatible with the hypothetical monocytic origin of microglia. Third, macrophages may reach the pecten from the optic nerve head.

None of these possibilities can be ruled out on the basis of our results. However, we show that numerous vitreal macrophages intimately adhere to the pecten surface in developmental stages when macrophages migrate into the

retina. These macrophages are no longer seen in developmental stages when migration into the retina has ceased. This chronological parallel between the appearance of macrophages adhering to the pecten and the migration of macrophages into the retina is compatible with the origin of retinal microglia from vitreal macrophages. This is in accordance with the suggestion by Ashwell (1989) that vitreal cells coming from the hyaloid circulation enter the rabbit retina through the vitreal surface and become retinal microglia. In that the quail retina is avascular, the present study demonstrates that macrophage/microglia precursors must migrate long distances to reach their final destination, which is not accessible via blood vessels. Studies of the developing rabbit retina, which is partially avascular, also support the idea that the appearance and distribution of microglia are not dependent on the presence of inherent retinal vessels (Ashwell, 1989; Schnitzer, 1989).

Microglial ontogeny in the retina: Comparison with other parts of the nervous system

The coincidence between the arrival of the first radially migrating macrophages in the plexiform layers and the first appearance in these layers of poorly ramified $QH1^+$ cells (at E12 in the IPL and at E14 in the OPL) strongly suggests that ameboid macrophages migrating into the retina are precursors of the poorly ramified cells in the plexiform layers. As development advances, these cells become more completely ramified, acquiring the features of mature microglia. This sequence of events supports the hypothesis that ameboid macrophages are the precursors of mature microglia through intermediate forms, as occurs in the mammalian brain (Perry and Gordon, 1988; Ling and Wong, 1993) and retina (Hume et al., 1983; Sanyal and De Ruiter, 1985; Ashwell, 1989; Ashwell et al., 1989) as well as in the quail brain (Cuadros et al., 1992, 1994).

The strategy used by microglial precursors to colonize the retina before they differentiate appears to be similar to that observed in the quail optic tectum (Cuadros et al., 1994). In the retina, microglial precursors derive from the pecten, migrate along the ILM to spread across the entire surface of the retina, and then migrate radially to colonize different depths of the retinal wall, where they differentiate into ramified microglia (see Fig. 16). Similarly, in the tectum, microglial precursors mostly enter the nervous parenchyma at a ventromedial caudal zone of the pial surface, spread tangentially through the entire tectum along an axonal tract (the stratum album centrale), and then migrate radially toward more superficial layers, where they differentiate into ramified microglia.

Observations in different parts of the mammalian brain are also compatible with this strategy of colonization by microglial precursors. Thus, the transient accumulation of ameboid macrophages in the corpus callosum (Ling, 1981; Valentino and Jones, 1981; Perry et al., 1985; Kaur et al., 1990; Milligan et al., 1991a) as well as in the white matter of the neocortex (Murabe and Sano, 1982; Innocenti et al., 1983a,b; Lent et al., 1985; Perry et al., 1985) and cerebellum (Ashwell, 1990; Boya et al., 1991; Milligan et al., 1991a) suggests that axonal tracts are used as a pathway for the migration of macrophages into different parts of the central nervous system, where they will differentiate.

The development of microglia in the quail retina and optic tectum seems to occur through four events, which may be common throughout the nervous system. First, microglial precursors enter the nervous parenchyma at distinct anatomical regions and migrate long distances to reach their final locations. Second, there is tangential migration of microglial precursors, which spread in each part of the nervous system. Third, microglial precursors migrate radially to different depths in the neural wall. Fourth, once these different depths have been reached, microglial precursors differentiate to become ramified microglia. Studies are currently in progress to test whether these events are common to microglial development in other parts of the nervous system.

ACKNOWLEDGMENTS

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