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Tangential Migration of Ameboid Microglia in the Developing Quail Retina: Mechanism of Migration and Migratory Behavior

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ABSTRACT Long distance migration of microglial precursors within the central nervous system is essential for microglial colonization of the nervous parenchyma. We studied morphological features of ameboid microglial cells migrating tangentially in the developing quail retina to shed light on the mechanism of migration and migratory behavior of microglial precursors. Many microglial precursors remained attached on retinal sheets containing the inner limiting membrane covered by a carpet of Müller cell endfeet. This demonstrates that most ameboid microglial cells migrate tangentially on Müller cell endfeet. Many of these cells showed a central-to-peripheral polarized morphology, with extensive lamellipodia spreading through grooves flanked by Müller cell radial processes, to which they were frequently anchored. Low protuberances from the vitreal face of microglial precursors were firmly attached to the subjacent basal lamina, which was accessible through gaps in the carpet of Müller cell endfeet. These results suggest a mechanism of migration involving polarized extension of lamellipodia at the leading edge of the cell, strong cell-to-substrate attachment, translocation of the cell body forward, and retraction of the rear of the cell. Other ameboid cells were multipolar, with lamellipodial projections radiating in all directions from the cell body, suggesting that microglial precursors explore the surrounding environment to orient their movement. Central-to-peripheral migration of microglial precursors in the retina does not follow a straight path; instead, these cells perform forward, backward, and sideways movements, as suggested by the occurrence of (a) V-shaped bipolar ameboid cells with their vertex pointing toward either the center or the periphery of the retina, and (b) threadlike processes projecting from either the periphery-facing edge or the center-facing edge of ameboid microglial cells. *GLIA 22:31–52, 1998.*

INTRODUCTION

Microglial cells are currently thought to derive from precursor cells related to the monocyte/macrophage lineage (Perry and Gordon, 1991; Thomas, 1992; Theele and Streit, 1993). Microglial development in the central nervous system (CNS) can be divided into three stages: entry of microglial precursor cells into the developing CNS, migration of these cells within the CNS to their final locations, and subsequent differentiation at these locations (Navascués et al., 1996).

With regard to the first step, studies at our laboratory supported the old idea that most microglial precursors enter the CNS from the meninges by traversing the pial surface (Rio-Hortega, 1932; Boya et al., 1979, 1991;

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Caggiano and Brunjes, 1993) at well-confined areas on the developing brain surface (Cuadros et al., 1994; Navascués et al., 1996). After entering the nervous parenchyma, microglial precursors acquire morphological features of ameboid microglia (Perry et al., 1985; Jordan and Thomas, 1988; Perry and Gordon, 1988, 1991; Ling and Wong, 1993; Wu et al., 1994) and migrate for long distances to spread throughout the CNS, eventually reaching their final destination, where they differentiate into ramified microglia. Long migrations of ameboid microglia within the CNS were suggested by Rio-Hortega (1932) and supported by more recent studies (Schnitzer, 1989; Pearson et al., 1993; Cuadros et al., 1994; Navascués et al., 1995; Wolswijk, 1995; Diaz-Araya et al., 1995a,b). Little is known of the pathways, substrates, and mechanisms involved in ameboid microglia migrations, unlike the migration of precursors of other cell types in the CNS including neurons (Rakic, 1990; Edmonson and Hatten, 1987; Lois and Alvarez-Buylla, 1994; Leber and Sanes, 1995; O'Rourke et al., 1995; Jankovski and Sotelo, 1996; Rakic et al., 1996), oligodendrocytes (Small et al., 1987; Leber and Sanes, 1995; Kiernan and ffrench-Constant, 1993; Baron-Van Evercooren et al., 1996), and astrocytes (Watanabe and Raff, 1988; Ling and Stone, 1988; Chan-Ling, 1994; Orentas and Miller, 1996).

Some in vitro studies of cell cultures (Thomas, 1990; Booth and Thomas, 1991; Abd-El-Basset and Fedoroff, 1995; Haapaniemi et al., 1995) and brain slice cultures (Brockhaus et al., 1996) have shed light on some aspects of microglial motility. But in situ studies are necessary to understand mechanisms involved in long migrations of ameboid microglia along the CNS. The developing quail retina is an excellent material in which to study microglia migrations for several reasons. First, all types of microglial cells are immunolabeled with the QH1 antibody (Cuadros et al., 1992). Second, the avian retina is completely avascular, thus avoiding interferences from the covisualization of developing microglia and blood vessels. Third, whole-mounted retinas can be used to study all microglial cells throughout the entire retina as well as their topographical location. And fourth, the chronology of microglial development is well known in the quail retina (Navascués et al., 1995). Microglial precursors enter the developing quail retina via the pecten/optic nerve head area between days 7 and 16 of incubation (E7–E16) and migrate tangentially from central to peripheral areas. Subsequently, microglial precursors migrate radially from vitreal to scleral levels, gaining access to the plexiform layers, where they differentiate into ramified microglia. The pattern of invasion of microglial precursors in the mammalian retina appears to be similar to that in birds: they enter via the optic disc (Diaz-Araya et al., 1995a,b), then migrate tangentially across the retinal surface (Pearson et al., 1993; Diaz-Araya et al., 1995a,b) and radially into the deeper planes (Hume et al., 1983; Diaz-Araya et al., 1995a,b).

In this study we show that ameboid microglia in the developing quail retina use Müller cell endfeet as a substrate to migrate tangentially. Migration of ameboid microglial cells appears to occur through several stages: (1) exploration of the environment by extension of lamellipodia in several directions, (2) polarized distribution of lamellipodia; (3) strong cell-to-substrate attachment, (4) translocation of the soma to the leading part of the cell, and (5) retraction of the rear of the cell. In addition, tangential migration of ameboid microglial cells takes place along a tortuous pathway with forward, backward, and sideways cell movements.

Some of the findings in this paper were presented previously in abstract form (Marín-Teva et al., 1996a).

MATERIALS AND METHODS

Developing retinas of quail (*Coturnix coturnix japonica*) embryos at E7–E16 were used in this study. These developmental stages comprise the period from the beginning to the end of the tangential migration of microglial precursors from the center to the periphery of the retina (Navascués et al., 1995). Many observations were made on E9–E10 retinas, because at these developmental stages a large proportion of the vitreal surface of the retina is supplied with microglial precursors migrating tangentially, whereas radial migration toward the plexiform layers is rarely observed.

Whole-mounted retinas and sheets containing the inner limiting membrane (basal lamina) covered by a carpet of Müller cell endfeet (ILM/MCEF sheets) were studied with immunocytochemical methods as described below. Studies with transmission electron microscopy (TEM) were made on ultrathin sections from either ILM/MCEF sheets or entire retinas, and scanning electron microscopic (SEM) observations were done in ILM/MCEF sheets.

Isolation of Retinas and Preparation of ILM/MCEF Sheets

To isolate developing retinas, the eye was rapidly dissected either in Hank's solution (for ILM/MCEF sheets) or in different fixatives (as indicated below). The cornea, lens, ciliar body, sclera, and vitreous body were removed. These procedures were done quickly to remove the choroid and the pigment epithelium together with the sclera. Retinas were flattened on a slide by making radial incisions around the circumference, and the pecten and optic nerve remains were removed.

Sheets containing the inner limiting membrane and the carpet of Müller cell endfeet were obtained according to the method of Halfter et al. (1987), with light modifications. With this method the vitreous surface of developing retinas was isolated by mechanical disruption of the retina mounted between two adhesive substrates. Developing retinas were isolated in Hanks' solution containing Ca^{2+} and Mg^{2+}. Because ILM/MCEF sheets from retinas isolated in Ca^{2+}-, Mg^{2+}-free

Hanks' solution contained fewer cells than those from retinas isolated in Hanks' solution containing Ca^{2+} and Mg^{2+}, we concluded that the presence of these cations prevented loosening of microglial precursors from Müller cell endfeet. A piece of each isolated retina with an area of approximately 5 × 5 mm was cut out and mounted on a nitrocellulose filter with the vitreous side up.

The flat-mounted retina-filter assembly was fixed in either 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (for immunofluorescence), or 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M cacodylate buffer (for TEM and SEM) for 1 min. It was then placed vitreous side up on a drop of the same fixative on a glass coverslip. A poly-L-lysine-coated (2 mg/ml for 2 h) glass coverslip was placed on the flat-mounted retina-filter assembly, with the vitreous side of the retina facing the polylysine-coated surface. The polylysine-coated glass was gently weighted to press the vitreal retinal surface against the polylysine coating. After 3–4 min, the retina-filter assembly was lifted away from the polylysine-coated glass using a rapid, firm pulling motion. An ILM/MCEF sheet containing microglial precursors migrating on Müller cell endfeet remained attached to the polylysine-coated surface (Fig. 1A), whereas the rest of the retina was removed with the filter.

Electron Microscopy

Quail embryos at E9–E10 were used for TEM and SEM. For TEM, whole retinas were isolated in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), and small pieces of selected zones were immersed in the same fixative for 3 h, 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. Transversal ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM902 microscope.

For TEM we also used ILM/MCEF sheets obtained as described above, but adhered on small pieces of poly-L-lysine-coated (4 mg/ml for 2 h) plastic petri dish. Then the plastic-adhered ILM/MCEF sheets were additionally fixed in 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h, rinsed overnight in 0.1 M cacodylate buffer, postfixed in 2% osmium tetroxide, and stained with 1% uranyl acetate. After dehydration in ethanol, the plastic-adhered ILM/MCEF sheets were embedded in Epon. Ultrathin sections were cut parallel to the plastic surface, thus obtaining ILM/MCEF sheet sections in a plane tangential to the vitreal surface of the retina. The sections were stained with lead citrate and examined.

For SEM, glass-adhered ILM/MCEF sheets were additionally fixed in 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M cacodylate buffer for 30 min, postfixed in 1% osmium tetroxide for 1 h, dehydrated in a graded series of acetones, and dried with the critical-point method. Specimens were then gold sputter-coated and observed with a Zeiss DSM950 scanning electron microscope.

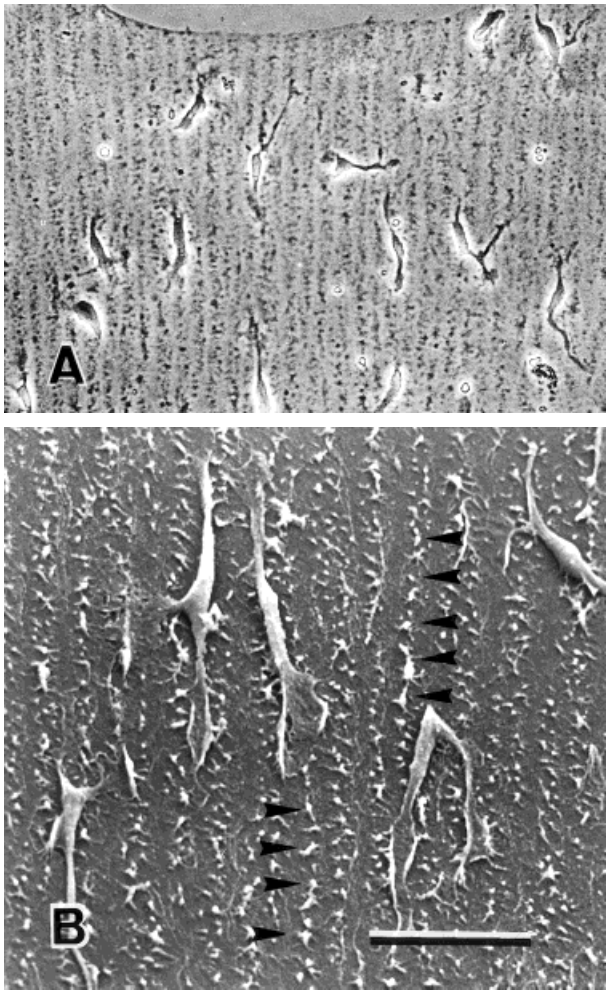

Fig. 1. Ameboid cells on ILM/MCEF sheets as seen with phase-contrast light microscopy (**A**) and SEM (**B**). The cells are located along grooves flanked by parallel rows of broken radial processes of Müller cells (arrowheads). Scale bar: 80 μm in A; 23 μm in B.

Immunocytochemistry

Microglial precursors were demonstrated in the developing quail retina by labeling 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). An anti-tubulin antiserum (Sigma, St. Louis, MO) was also used to label both microglial precursors and Müller cell radial processes in ILM/MCEF sheets.

For QH1 immunocytochemistry on whole-mounted retinas, they were isolated in 4% paraformaldehyde in 0.1 M PBS (pH 7.4), fixed free-floating in the same fixative for 1–3 days, treated with trypsin (Difco, Detroit, MI) in PBS (0.4 mg/ml) at 37°C for 45 min to facilitate removal of pigment epithelium remains (Navascués et al., 1995), and rinsed in PBS. To improve antibody penetration into the retina, the specimens were washed in PBS with 1% Triton X-100 (T-PBS) for

1 h with agitation. Free-floating retinas were then treated with 3% hydrogen peroxide for 5 min to eliminate endogenous peroxidase activity, incubated in normal goat serum (NGS) diluted 1:20 in 1% bovine serum albumin in PBS (BSA-PBS) for 3 h, and incubated in QH1 supernatant diluted 1:2 in BSA-PBS for 40 h with agitation at 4°C. The retinas were then washed in T-PBS, incubated in anti-mouse IgG biotinylated antibody (Sigma) diluted 1:50 in BSA-PBS for 4 h with agitation at room temperature, washed again in T-PBS, and incubated in avidin-biotin-peroxidase complex (Extravidin, Sigma) diluted 1:150 in BSA-PBS for 4 h with agitation. Then the retinas were washed for 30 min in PBS without Triton, and peroxidase activity was revealed during 4–5 min in 0.05% diaminobenzidine and 0.02% hydrogen peroxide in PBS; no counterstaining was used. The retinas were washed in PBS and then in distilled water, and then spread on poly-L-lysine-coated slides with the vitreous side facing up and coverslipped with Aquatex (Merck, Darmstadt, Germany).

For QH1 immunofluorescence on ILM/MCEF sheets, these sheets were fixed in 4% paraformaldehyde in 0.1 M PBS for 15 min. The specimens were rinsed in PBS, immersed in NGS diluted in BSA-PBS for 1 h, incubated in QH1 supernatant diluted 1:2 in BSA-PBS for 1 h at room temperature, and thoroughly washed in PBS. They were then incubated in tetramethylrhodamine isothiocyanate (TRITC)-conjugated anti-mouse IgG (Sigma) diluted 1:60 in BSA-PBS for 1 h, washed in PBS, and coverslipped with glycerol-PBS containing 0.1% *p*-phenylenediamine dihydrochloride. Immunofluorescence observations were made with a Zeiss Axiophot microscope.

For double immunofluorescence with both anti-tubulin and QH1, ILM/MCEF sheets were immersed in 1% paraformaldehyde in 0.1 M PBS with 0.5% Triton X-100 for 2 min to improve the penetration of anti-tubulin, washed in PBS, postfixed in 4% paraformaldehyde in PBS for 10 min, rinsed again in PBS, and immersed in NGS diluted in BSA-PBS. They were then incubated in anti-tubulin antiserum (developed in rabbit) diluted 1:40 in BSA-PBS for 1 h, washed in PBS, and immersed in fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG (Sigma) diluted 1:50 in BSA-PBS for 1 h. The ILM/MCEF sheets were thoroughly rinsed in PBS and processed as described for QH1 immunofluorescence.

Pre-embedding QH1 immunocytochemistry for TEM was also carried out on E9–E10 retinas. The retinas were isolated in 2% glutaraldehyde, 1% paraformaldehyde in 0.1 M PBS and selected pieces of approximately 4 × 7 mm were cut out and immersed in the same fixative for 2 h. Immunocytochemical treatment of these pieces was similar to that described for whole-mounted retinas, but trypsin, Triton X-100 and hydrogen peroxide were omitted. After visualizing peroxidase activity with diaminobenzidine, retinal pieces were postfixed in 1% osmium tetroxide, dehydrated, and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate only and examined in an electron microscope.

RESULTS

Tangential Migration of Ameboid Microglia on Müller Cell Endfeet

In ILM/MCEF sheets, grooves were clearly visible: their floor consisted of Müller cell endfeet and their lateral walls were composed of rows of broken radial processes of Müller cells. Many ameboid cells were seen on these grooves (Figs. 1 and 2A–C), even though the axonal fascicles of the nerve fiber layer were removed during technical preparation. This means that ameboid cells were strongly attached to Müller cell endfeet and radial processes, but not so the fascicles of ganglion cell axons. In some ILM/MCEF sheets, axonal fascicles from the nerve fiber layer were not completely removed and remained on ameboid cells and Müller cell endfeet (Fig. 2D–F).

Immunocytochemical treatment of ILM/MCEF sheets revealed that sheet-adhered ameboid cells were QH1-labeled (Fig. 2). We therefore concluded that ameboid microglial cells migrated on the vitrealmost part of the embryonic retina, to which they strongly adhered. This was additionally supported by TEM observations of ultrathin sections of QH1-immunocytochemically-treated developing retinas (Fig. 3).

Morphology of Tangentially Migrating Ameboid Microglia: Emission of Lamellipodia

Although tangentially migrating ameboid microglia showed variable morphological features, they had some characteristics in common. They showed an oval or fusiform cell body containing the nucleus (Fig. 4A) with numerous coated pits (Fig. 4B) on the cell surface. The cytoplasm of the cell body showed morphological features described as characteristic of ameboid microglia (Ling, 1981; Ling and Wong, 1993), including a well-developed Golgi complex next to the centrosome, from which numerous microtubules radiated (Fig. 4C,D), mitochondria, profiles of rough endoplasmic reticulum, abundant polysomes, and numerous clear or dense vesicles of variable size.

A conspicuous feature of tangentially migrating ameboid microglia was the presence of lamellipodia of variable length (Fig. 2). The cytoplasm of these lamellipodia contained ribosomes and a filamentous network (Fig. 5A,B), probably of actin, but neither microtubules nor other organelles were seen. The lamellipodia of ameboid microglia were usually closely adhered to Müller cell endfeet, as seen in ILM/MCEF sheets (Fig. 2C) and confirmed with TEM of QH1-labeled sections of retina (Fig. 5C), although portions of some lamellipodia were seen to squeeze between ganglion cell axons within the nerve fiber layer.

Lamellipodia emerged either directly from the cell body (Figs. 6A–C and 7A–B) or from cell processes which were short and thick (Figs. 6A,B,D, and 7C,D) or long and thin (Fig. 8A). In some microglial precursors, lamellipodia emerged only from the cell body (Fig. 6C, cell at right); other cells showed both lamellipodia and

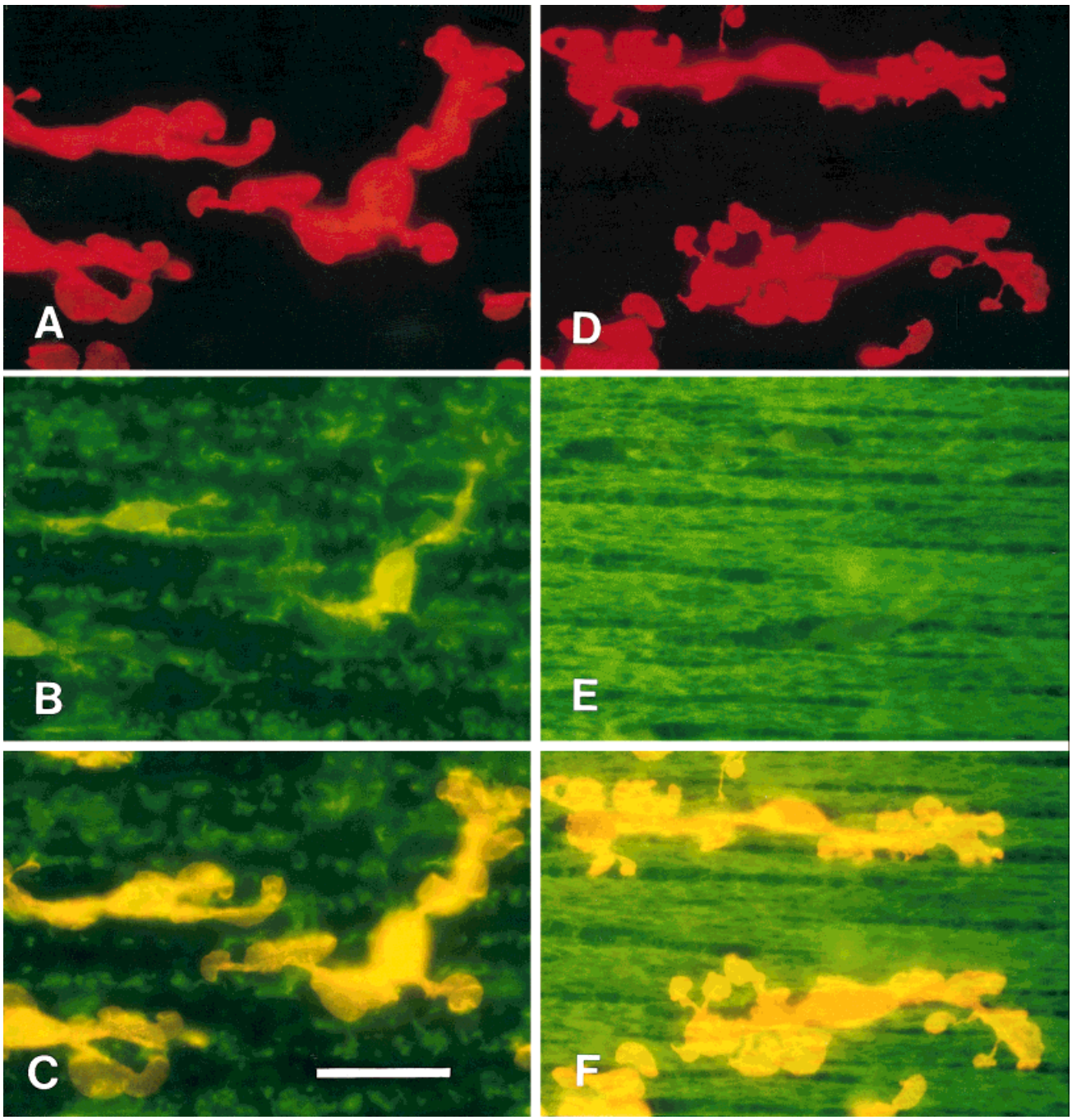

Fig. 2. Double fluorescence immunocytochemistry of ILM/MCEF sheets from E10 quail retinas after TRITC-conjugated labeling for QH1 (A, D) and FITC-conjugated labeling for anti-tubulin (B, E). The QH1 antibody labels ameboid microglial cells (**A, D**) with lamellipodia emerging from both short thick processes and the cell body. Anti-tubulin reveals cell bodies and processes of ameboid microglial cells together with the broken radial processes of Müller cells which flank grooves (**B**), and fascicles of ganglion cell axons, if they remain adhered to the sheet (**E**). Double exposure pictures show the topographical relation of QH1-positive ameboid microglial cells with grooves flanked by radial processes of Müller cells (**C**) and axonal fascicles (**F**). Scale bar: 25 μm.

lamellipodia-bearing processes emerging from the cell body (Figs. 6A, 7A); a third type of microglial cell had a cell body which bore only lamellipodia-bearing processes (Fig. 6C, cell at left, and Fig. 8A). Generally, each cell process ended in a lamellipodium (Figs. 6A,D, and 7D). Other lamellipodia were located midway along the cell processes (Fig. 8B) or emerged laterally from them (Fig. 8C). Frequently, very thin filopodia of variable length emerged from the edge of the lamellipodium (Fig. 8D). These filopodia ended in a sharp-pointed tip (Fig. 8D) or in an additional small fanlike lamellipodium (Fig. 6D,E).

The main axis of many ameboid microglial cells was oriented in a central-to-peripheral direction. Therefore each cell showed topographical polarization, with one cell edge facing the retinal periphery and the opposite edge facing the retinal center. Lamellipodia were present

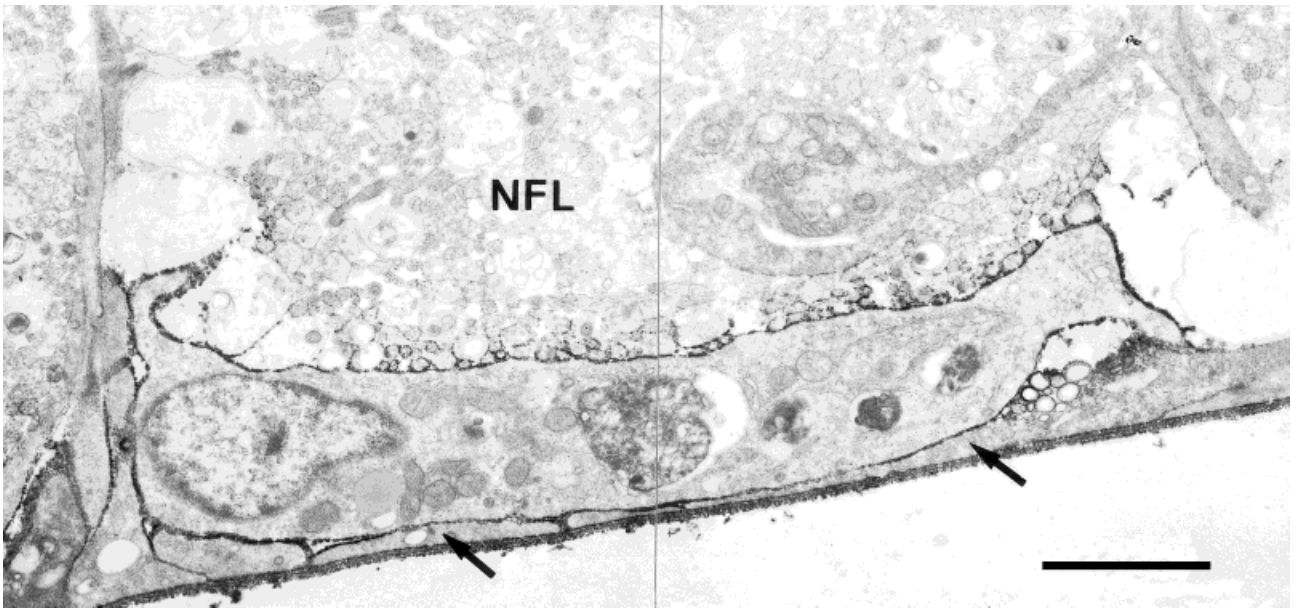

Fig. 3. TEM view of the vitrealmost part of an E10 quail retina labeled with QH1 before embedding. Staining with uranyl acetate only. A QH1-positive microglial cell is seen beneath the nerve fiber layer (NFL), closely adhered to Müller cell endfeet (arrows). Scale bar: 2 μm.

indistinctly at the periphery-facing edge or at the center-facing edge (Figs. 6A and 7) of ameboid microglial cells.

We observed variable relationships between lamellipodia and grooves flanked by Müller cell radial processes. Lamellipodia were seen extending longitudinally through a single groove (Fig. 7D) or laterally across several adjacent grooves in which lamellipodial lobes spread (Figs. 2C and 8E,F).

Together the results described above suggest that ameboid microglial cells migrate on the Müller cell endfeet by emitting lamellipodia that adhere to them. Interestingly, some lamellipodia from neighboring cells were seen in contact with one another, but never overlapping, suggesting that the spread of lamellipodia was stopped by contact inhibition.

Attachment to the Substrate of Migrating Ameboid Microglia

The lateral edges of many lamellipodia were frequently associated to vitrealmost portions of Müller cell radial processes which continued with the endfeet (Figs. 6E and 7B,D). Morphological interactions and surface contacts were seen between short projections from lamellipodia and the vitrealmost portions of Müller cell radial processes (Fig. 9A,B), suggesting that lamellipodia were strongly anchored to these radial processes. Close contacts were also detectable between Müller cell radial processes and the lateral parts of the cell body of ameboid microglial cells (Fig. 9C,D). Interestingly, one or more small cisternae of smooth endoplasmic reticulum always appeared in the cytoplasm of the vitrealmost portions of Müller cell radial processes near the close contact with lamellipodia (Fig. 9B) or cell bodies (Fig. 9D) of ameboid microglia.

The lateral edges of the Müller cell endfeet made contact with one another to form a continuous sheet (Fig. 10A) to which the cell membrane of ameboid microglial cells closely adhered (Fig. 3). At some points the membrane of cell processes (Fig. 10B,C) and the cell body (Fig. 10D,E) of microglial cells were closely attached to the subjacent basal lamina through gaps between adjacent Müller cell endfeet.

The above findings showed that ameboid microglial cells migrating tangentially on the vitrealmost part of the developing retina were transiently anchored to the substrate via two types of attachments: (a) those of the lateral surface of both lamellipodia and cell bodies to Müller cell radial processes, and (b) those of the vitreal face of both cell processes and cell bodies to the basal lamina. These attachments explain why ameboid microglial cells remained on the ILM/MCEF sheets even after the rest of the retina was mechanically removed.

Threadlike Processes Projecting From Some Migrating Ameboid Microglial Cells

Some tangentially migrating ameboid microglial cells bore a very thin threadlike process of variable length (Fig. 11A). This process did not project from lamellipodia but was connected either with the cell body or with a thick cell process. Generally, the threadlike processes ended in a sharp-pointed tip (Fig. 11A), but occasionally they ended in a small lamellipodium or in a macelike structure (Fig. 11B). The threadlike processes were approximately 0.5 μm thick and extended between 10 and 100 μm beyond the surface of the cell body, although extremely long threadlike processes (up to 500 μm) were occasionally seen (Fig. 11C).

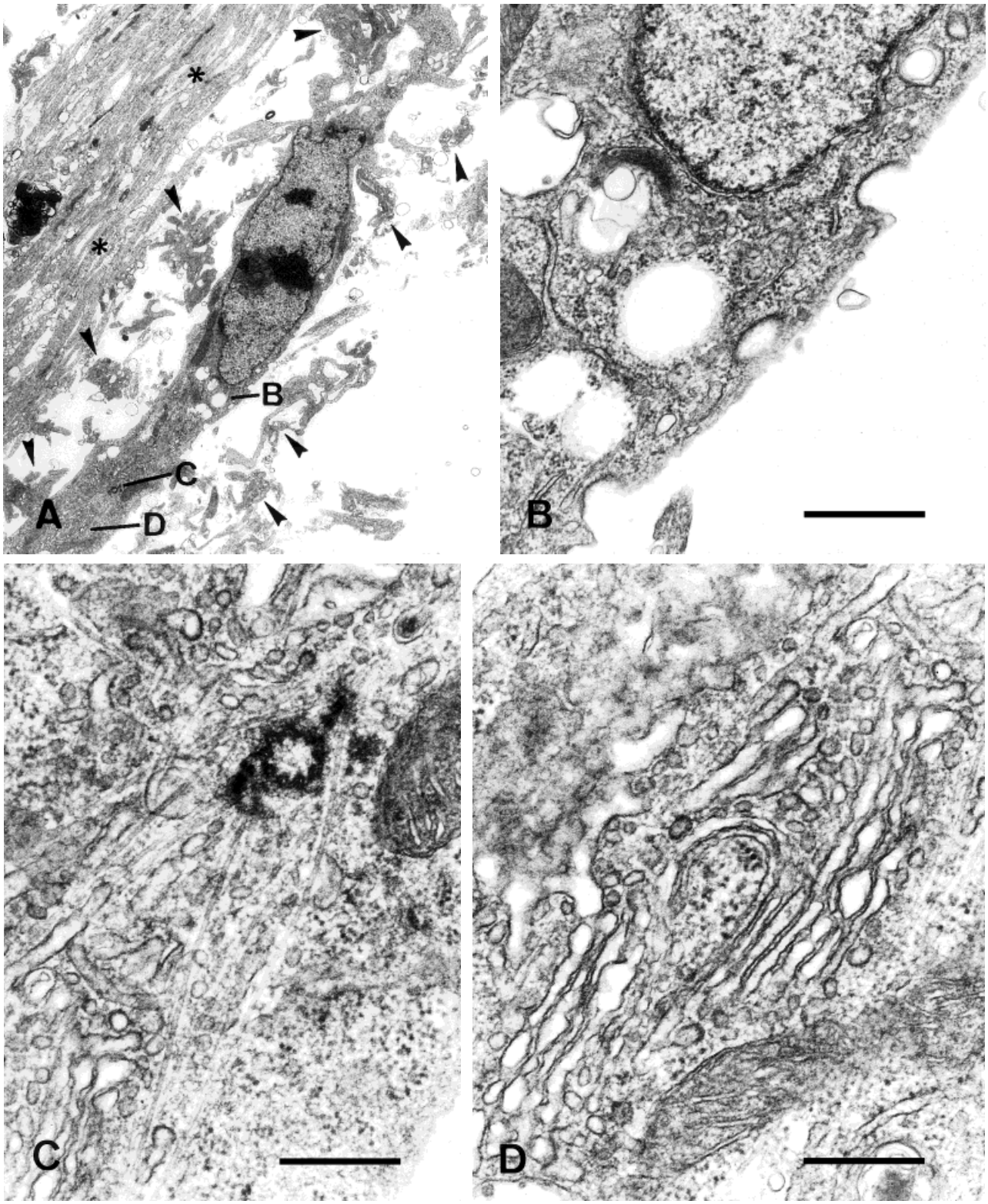

Fig. 4. Morphological features of ameboid microglia migrating tangentially in an E9 retina as seen with TEM in a tangential section of an ILM/MCEF sheet. **A:** A microglial cell with its main axis parallel to the course of fascicles of ganglion cell axons (asterisks) is seen on a groove flanked by radial processes of Müller cells (arrowheads). Areas magnified in B, C, and D are indicated. **B:** Coated pits on the surface of the cell body. **C, D:** The centrosome from which microtubules radiate (C) is located in the vicinity of a well-developed Golgi complex (D). Scale bar: 4.2 μm in A; 0.5 μm in B; 0.3 μm in C, and D.

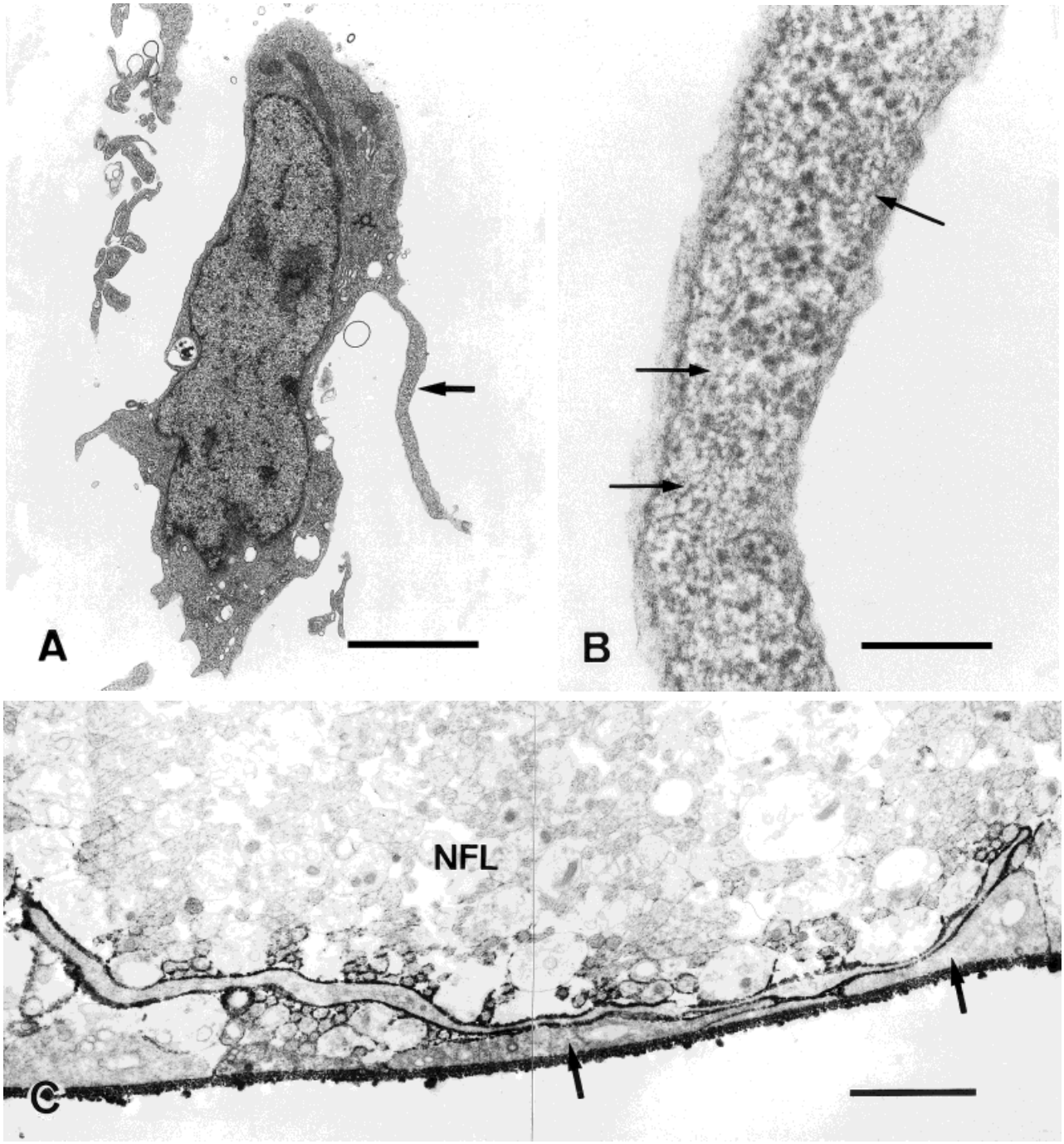

Fig. 5. TEM views of lamellipodia from ameboid microglia migrating tangentially in developing quail retinas. **A:** Ameboid microglial cell in a tangential section of an ILM/MCEF sheet from an E9 retina. A transversally sectioned lamellipodium (arrow) projects from the cell body. **B:** Magnification of the lamellipodium in A. Its cytoplasm contains ribosomes and a filamentous network (arrows). **C:** Section of an E10 retina labeled with QH1 before embedding, showing a QH1-positive lamellipodium of an ameboid microglial cell. Staining with uranyl acetate only. A portion of the lamellipodium (on the right) is closely adhered to Müller cell endfeet (arrows) and the lamellipodial portion on the left is separated from endfeet by ganglion cell axons. Immunolabeling surrounds some axons in close contact with the lamellipodium. NFL, nerve fiber layer. Scale bar: 2.1 µm in A; 0.2 µm in B; 1.8 µm in C.

The threadlike processes projected from either the periphery-facing edge or the center-facing edge of ameboid microglial cells (Fig. 11A). They were rarely seen to emerge laterally from the cell body. Sometimes their trajectory was straight (Fig. 11B), apparently following a single groove between adjacent rows of Müller cell radial processes, but some threadlike processes were curved (Fig. 11D), crossing over several adjacent grooves. Less frequently, the threadlike process turned through 180°, thus changing direction once or twice (Fig. 11E).

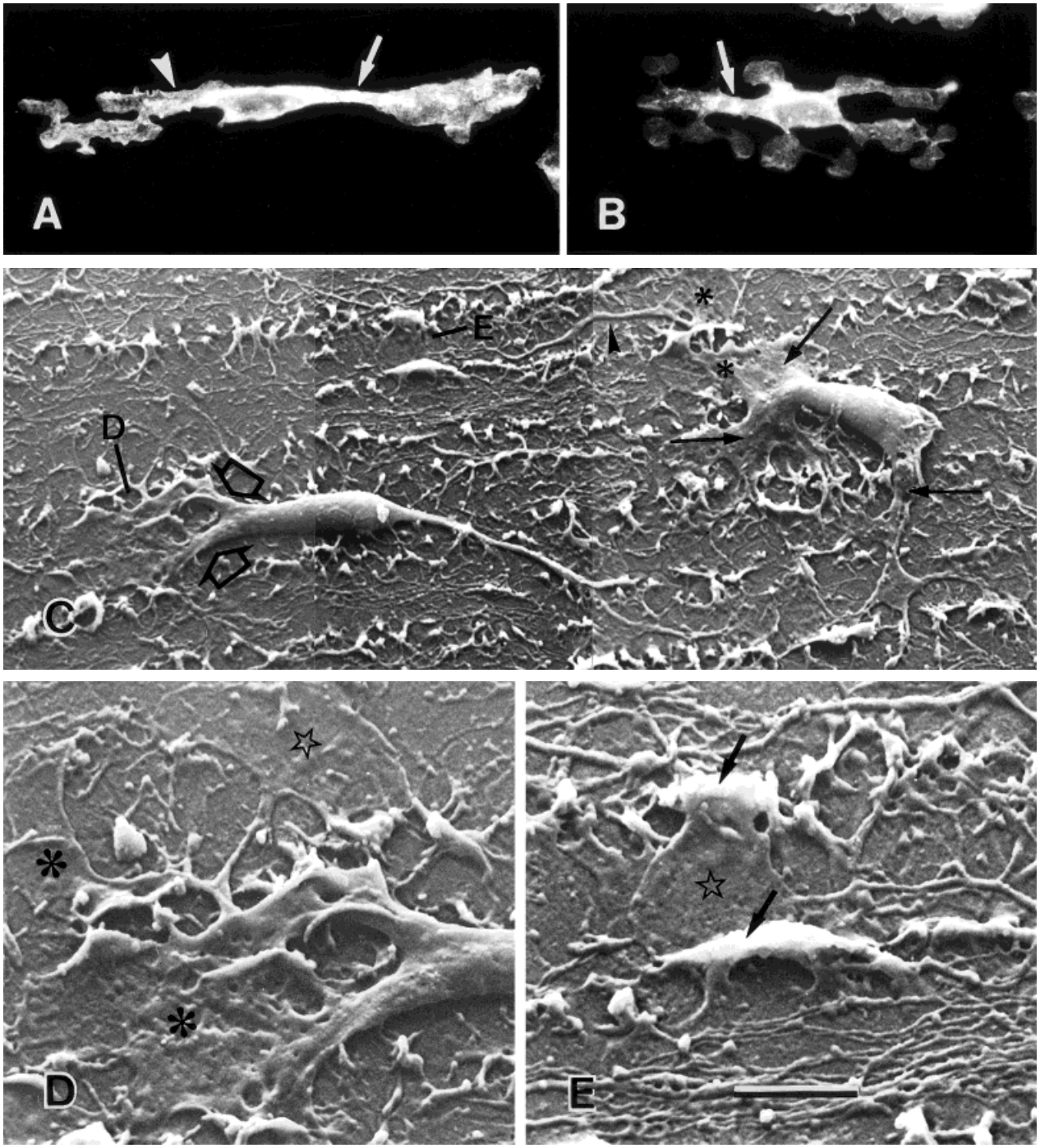

Fig. 6. Morphological features of ameboid microglial cells migrating tangentially in E9 quail retinas, as seen with QH1 immunofluorescence (A, B) and SEM (C, D, E) on ILM/MCEF sheets. In all micrographs the periphery of the retina is toward the left and the center is toward the right. **A:** QH1-positive bipolar cell. A short process (arrow) ending in a lamellipodium emerges from the center-facing pole of the cell body, and a long lamellipodium arises from the periphery-facing pole (arrowhead). **B:** QH1-positive ameboid cell with a lamellipodia-bearing short cell process (arrow) emerging from the periphery-facing cell pole, and several lamellipodia arising from lateral parts and the center-facing pole of the cell body. **C:** An ameboid microglial cells is seen on the left with two short thick processes (open arrows) emerging from the periphery-facing pole of the cell body and a long thin process stretching from the opposite pole. Another ameboid cell (on the right) has several lamellipodia (thin arrows) emerging from the soma. One lamellipodium has two lobes (asterisks) with a thin process (arrowhead) projecting from one of them and ending in an additional small lamellipodium. Areas magnified in D and E are indicated. **D:** Magnification of C. Lamellipodia (asterisks) emerging from short cell processes. A thin filopodium projects from a lamellipodium and ends in an additional fan-like lamellipodium (star). **E:** Magnification of C. A small lamellipodium (star) at the end of a filopodium adheres to broken radial processes of Müller cells (arrows). Scale bar: 25 µm in A, and B; 11 µm in C; 5 µm in D; 4 µm in E.

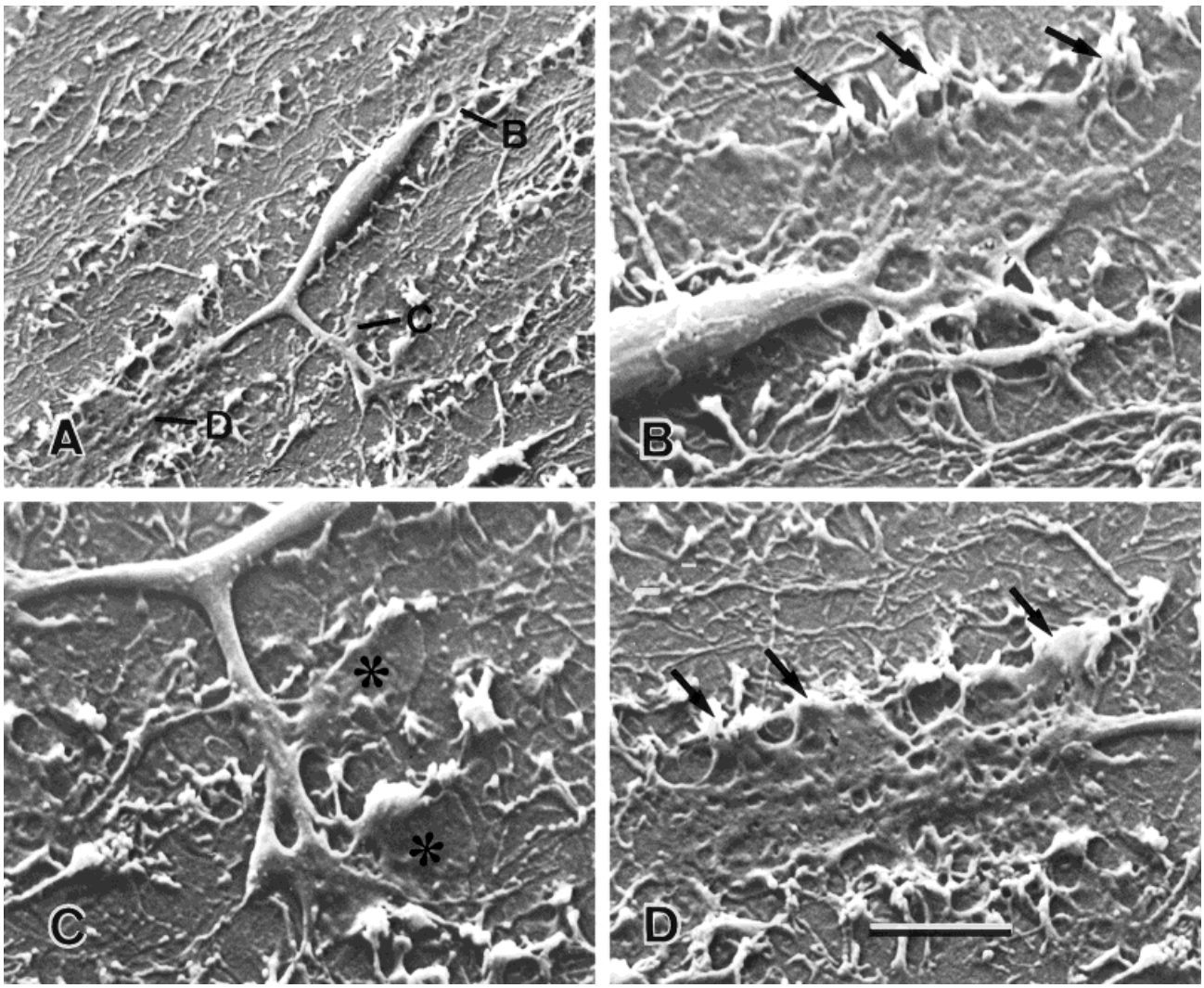

Fig. 7. SEM views of an ameboid microglial cell migrating tangentially on an ILM/MCEF sheet from an E9 quail retina. **A:** General view of the cell. A lamellipodium and a bifurcated thick process emerge from the two poles of the soma. The areas magnified in B, C, and D are indicated. **B:** A lamellipodium arising from a cell pole is adhered to radial processes of Müller cells (arrows). **C:** Small lamellipodia (asterisks) emerging from a short cell process. **D:** Lamellipodium at the end of a cell process adhering laterally to broken radial processes of Müller cells (arrows). Scale bar: 14 μm in A; 5.5 μm in B; 6 μm in C; 7 μm in D.

Occasionally threadlike processes longitudinally connected two adjacent ameboid cells (Fig. 11F) and appeared similar to long cytokinesis bridges.

Long threadlike processes from ameboid cells were frequently fragmented (Fig. 11C,D), and it was not rare to see isolated fragments of threadlike processes on the substrate (Fig. 11G).

The occurrence and length of the threadlike processes were variable as development advanced. In fact, a relatively high proportion of ameboid microglia had these filamentous processes in developmental stages when the first microglial precursors entered the retina (E8–E9). In more advanced stages of invasion of the retina by microglial precursors (E10–E16), smaller proportions of these cells bore threadlike processes. In addition, these processes were generally longer at E8–E9 than at subsequent stages.

Morphological Variability of Tangentially Migrating Ameboid Microglia: Monopolar, Bipolar and Multipolar Ameboid Cells

The morphology of tangentially migrating ameboid microglial cells was variable. A small proportion of them were round or ovoid cells completely lacking lamellipodia or cell processes (Fig. 12A). These cells were dividing cells (Marín-Teva et al., 1996b). The rest of the ameboid microglial cells bore projections (lamellipodia or cell processes) emerging from the cell body and were classified as monopolar, bipolar, or multipolar according to the number and distribution of cell projections.

Monopolar ameboid microglial cells had a single lamellipodium or lamellipodia-bearing process projecting either from the periphery-facing pole (Fig. 12B) or

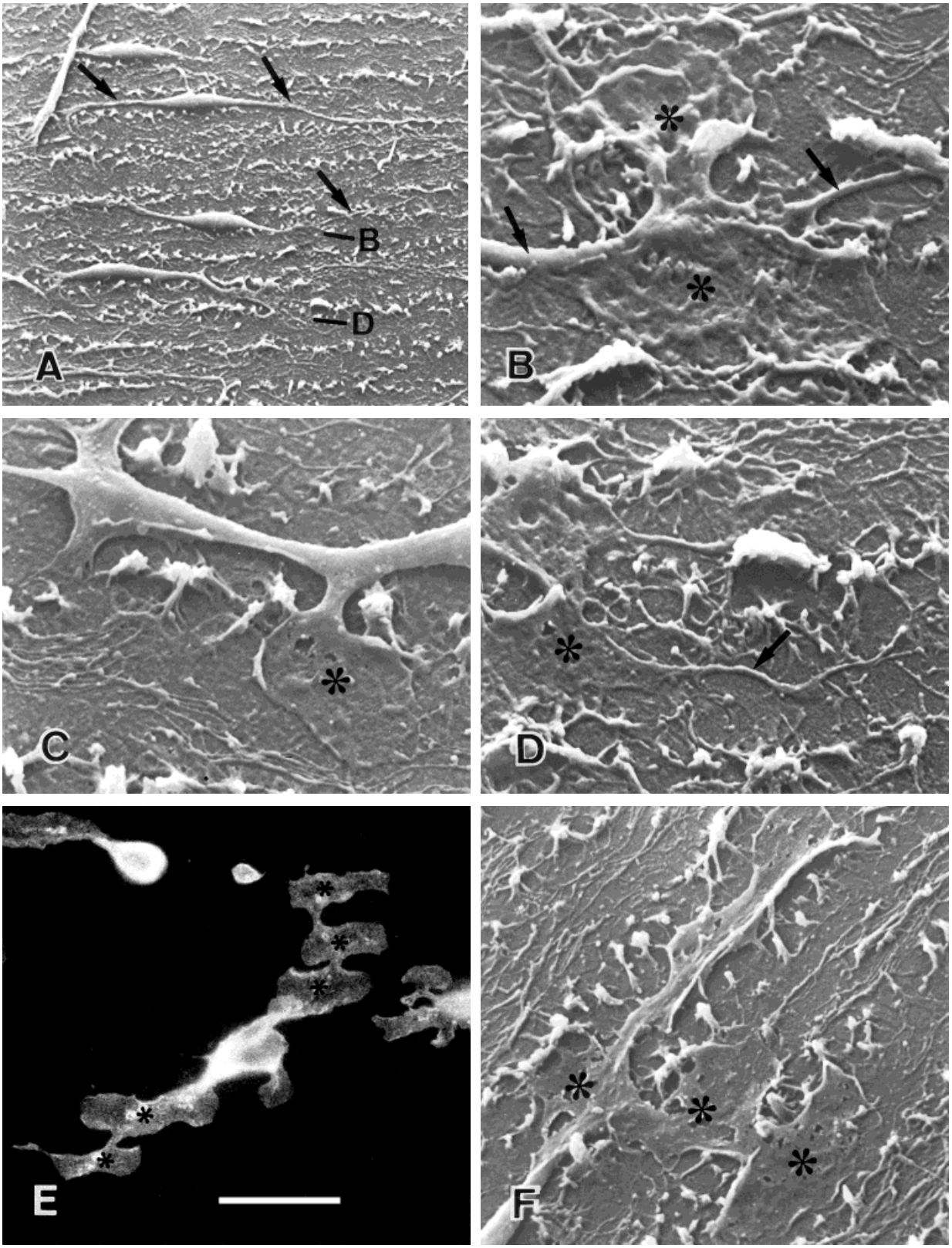

Fig. 8. Ameboid microglial cells migrating tangentially in E9 quail retinas, as seen with SEM (A–D, F) and QH1 immunofluorescence (E) on ILM/MCEF sheets. **A:** Bipolar cells showing long thin processes (arrows) which emerge from both poles. Areas magnified in B and D are indicated. **B:** Lamellipodium with two lobes (asterisks) located midway along a thin cell process (arrows). **C:** Small lamellipodium (asterisk) emerging laterally from a cell process. **D:** A thin filopodium (arrow) arises from a lamellipodium (asterisk). **E:** Ameboid microglial cell showing lamellipodia at both cell poles. Each lamellipodium has several lobes (asterisks) apparently located on adjacent grooves flanked by Müller cell processes. **F:** Lamellipodium showing three lobes (asterisks), each spreading through a different groove. Scale bar: 25 µm in A; 4.5 µm in B, and C; 5.5 µm in D; 20 µm in E; 7.5 µm in F.

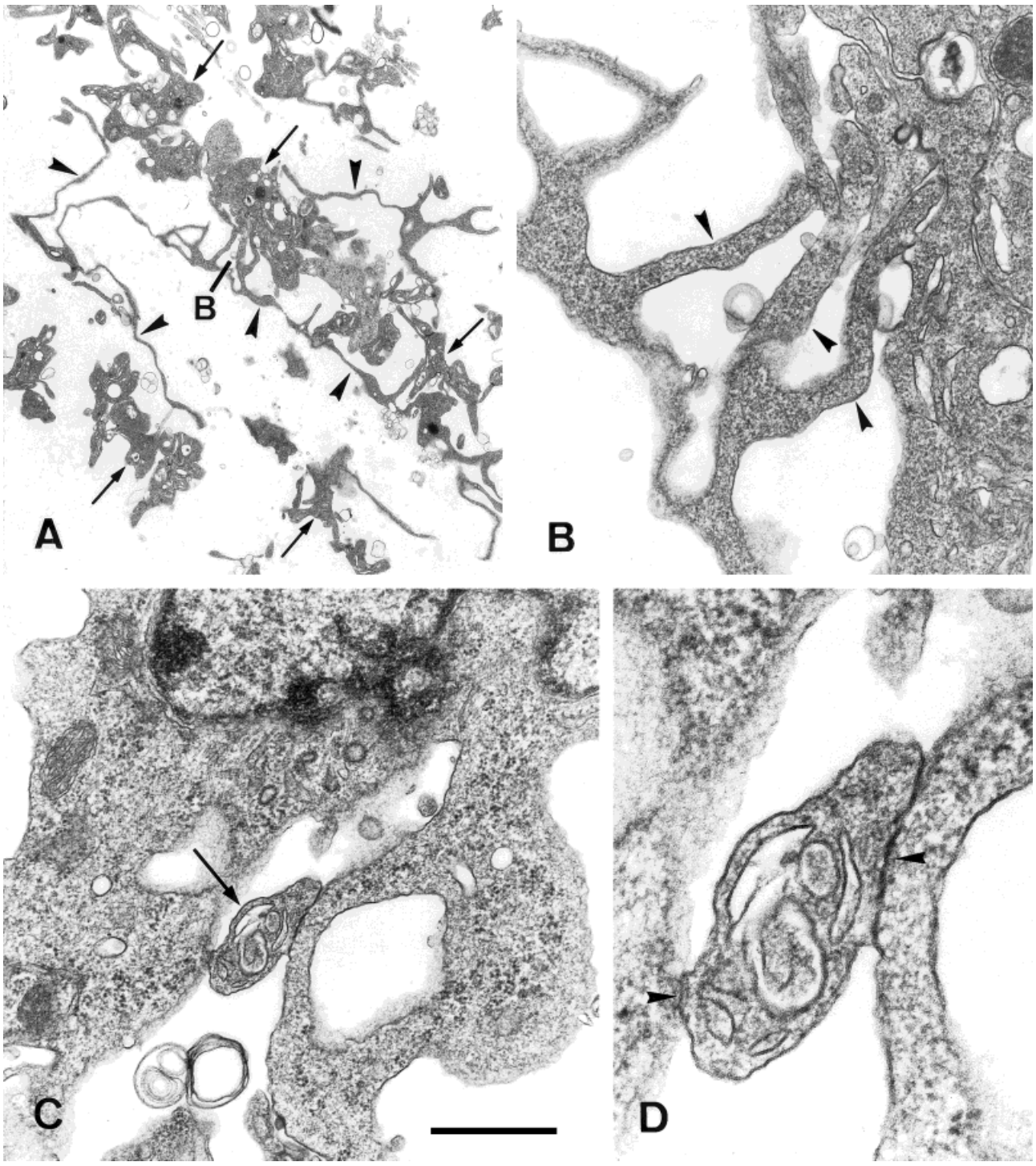

Fig. 9. Attachments of lamellipodia (A, B) and the cell body (C, D) of ameboid microglial cells to the vitrealmost portion of Müller cell radial processes, as seen with TEM in tangential sections from ILM/MCEF sheets of E9 quail retinas. **A:** Transversal sections of Müller cell radial processes (arrows) arranged in rows, and lamellipodial profiles (arrowheads) located on the grooves. The area magnified in B is indicated. **B:** Three lamellipodial projections (arrowheads) adhere closely to the surface of Müller cell radial processes. **C:** Portion of the cell body of an ameboid microglial cell contacting the Müller cell radial process (arrow) which is magnified in D. **D:** Close contacts (arrowheads) between the Müller cell radial process and the microglial cell. Several cisternae of smooth endoplasmic reticulum are present in the radial process. Scale bar: 3 µm in A; 0.5 µm in B, and C; 0.2 µm in D.

from the center-facing pole (Fig. 12C). This relatively large lamellipodium could itself comprise several lobes.

Bipolar ameboid microglial cells were elongated and had two projections extending from opposite poles of the cell body (Figs. 6A and 12D–H). These cells were generally 5–8 µm thick and 50–150 µm long, although cells longer than 150 µm were occasionally seen. Straight and V-shaped bipolar ameboid cells were iden-

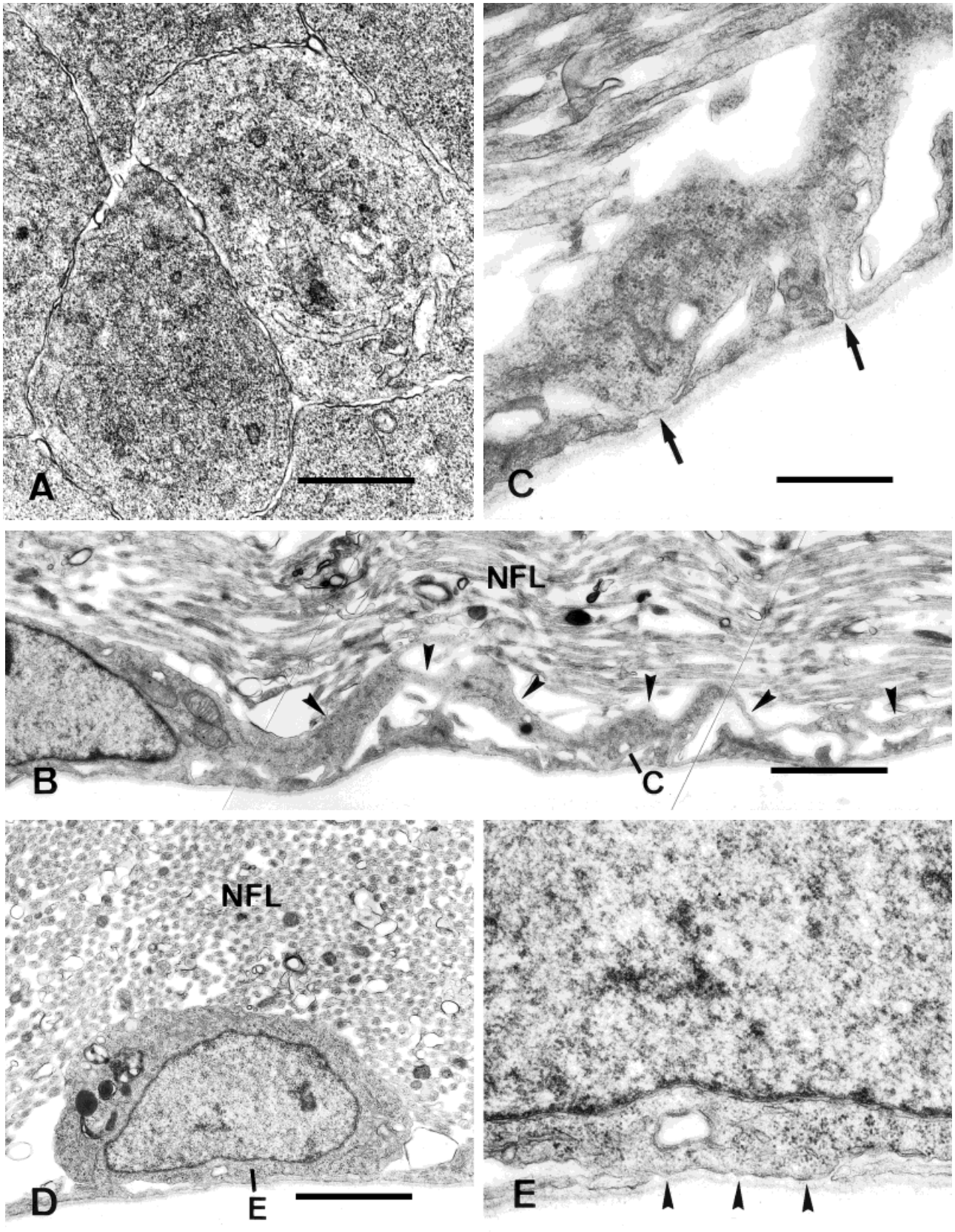

Fig. 10. TEM views of attachments of cell processes (B, C) and the cell body (D, E) of ameboid microglial cells to the basal lamina of the vitreal surface of E9 quail retinas. **A:** Müller cell endfeet forming a continuous sheet, as seen in a tangential section from an ILM/MCEF sheet. **B:** Transversal section of a retina showing a cell process (arrowheads) emerging from the soma of an ameboid microglial cell and coursing along Müller cell endfeet. The area indicated is magnified in **C** to show two low protuberances (arrows) that attach to the basal lamina through gaps between adjacent endfeet. **D:** Microglial cell body closely adhered to Müller cell endfeet in a transversal section of the retina. The area indicated is magnified in **E** to show a gap between adjacent endfeet through which the microglial cell surface attaches to the subjacent basal lamina (arrowheads). NFL, nerve fiber layer. Scale bar: 0.5 µm in A; 2 µm in B, and D; 0.7 in C, and E.

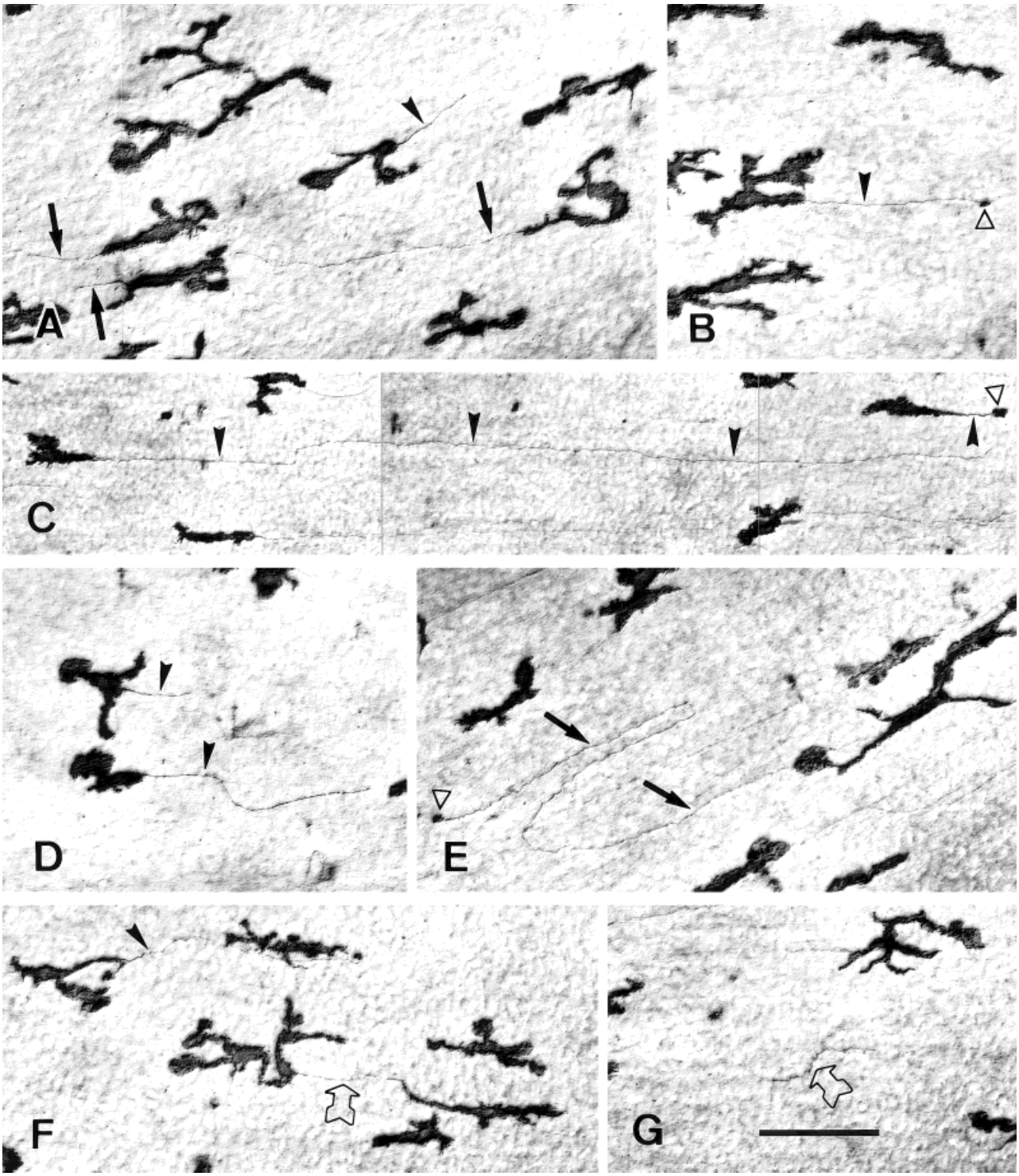

Fig. 11. Thread-like processes from migrating ameboid microglial cells as seen in E9 (**A, D**) and E8 (**B, C, E–G**) whole-mounted retinas after QH1 immunocytochemistry. In all micrographs the periphery of the retina is toward the left and the center is toward the right. Threadlike processes project from the center-facing pole (arrowheads in A–D and F) or the periphery-facing pole (arrows in A and E) of QH1-positive microglial cells. Some threadlike processes end in a macelike structure (triangles in B, C, and E), and others are extremely elongated (C and E). A threadlike process turns through 180° twice (E), and other appears to bridge two microglial cells (open arrow in F). A free thread-like process is seen isolated in the substrate (open arrow in G). Scale bar: 50 μm in all micrographs except C; 80 μm in C.

tified depending on whether the main axis was straight or curved.

Straight bipolar cells were generally oriented in a central-to-peripheral direction, i.e., parallel to the grooves between adjacent rows of Müller cell radial processes (Figs. 6A, 12D,E). Some straight bipolar ameboid cells had similar lamellipodial projections from both poles (Fig. 6A), whereas others bore a larger

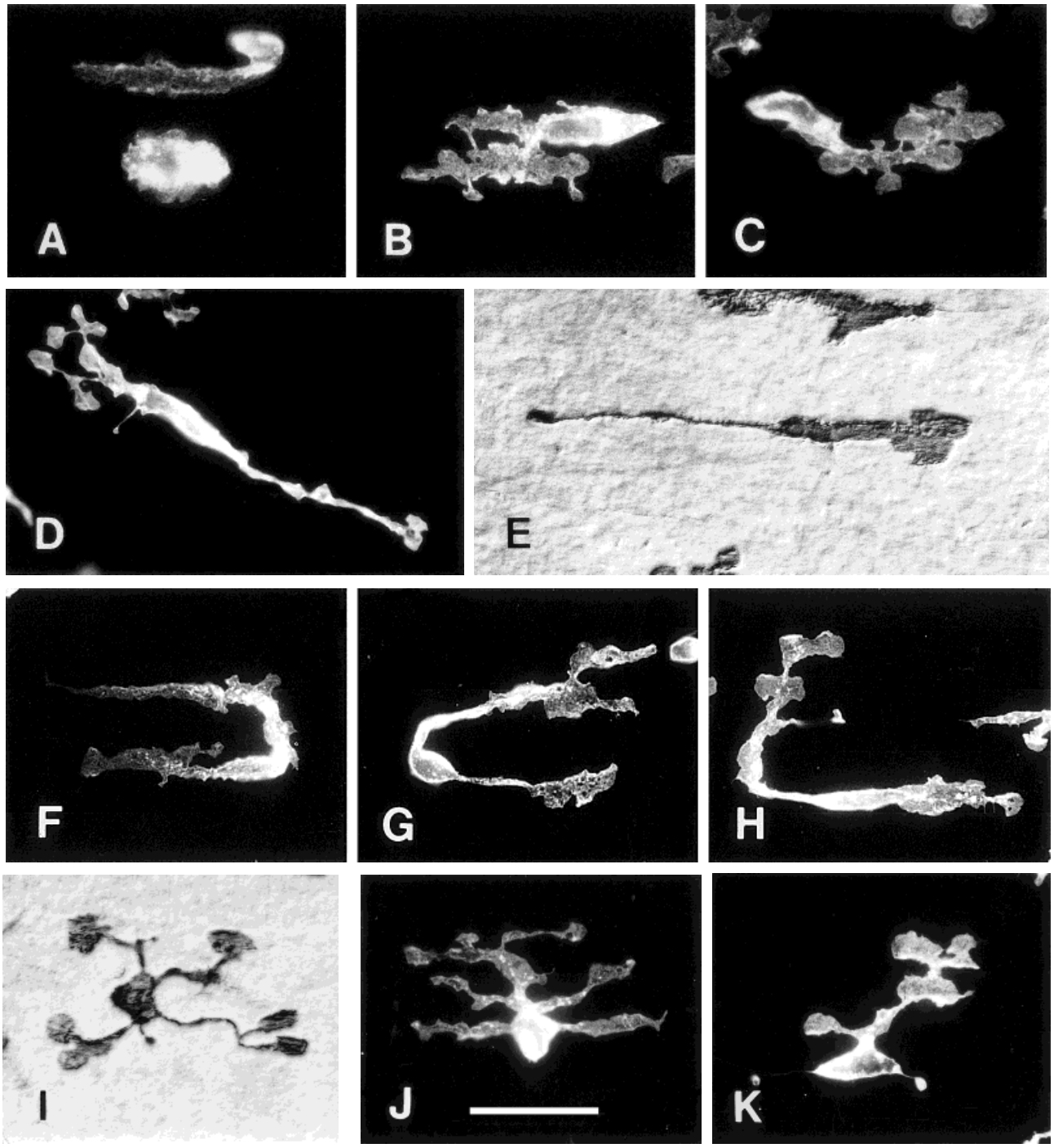

Fig. 12. Variable morphological features of ameboid microglial cells in E9 quail retinas as seen on ILM/MCEF sheets with QH1 immunofluorescence (A–D, F–H, J, and K) and in whole-mounted retinas with QH1 immunolabeling with avidin-biotin-peroxidase for visualization (E, I). In all micrographs the periphery of the retina is toward the left and the center is toward the right. Round (**A**), monopolar (**B, C**), straight bipolar (**D, E**), curved bipolar (**F–H**), multipolar (**I, J**), and intermediate (**K**) cells are seen. In monopolar cells, lamellipodial projections emerge from the periphery-facing (B) or the center-facing (C) pole of the cell body. Straight bipolar cells show lamellipodial projections from the periphery-facing (D) or the center-facing (E) pole of the cell body, and a long process emerging from the opposite pole. Some curved bipolar cells are V-shaped with the vertex pointing toward the center (F) or the periphery (G) of the retina. Other curved bipolar cells (H) are L-shaped with morphological features intermediate between V-shaped and straight bipolar cells. In multipolar cells, lamellipodia-bearing cell processes (I) or lamellipodia (J) emerge from the cell body in several directions. The cell in K appears to be a straight bipolar cell that has retracted its processes from both cell poles and is sending out lamellipodial projections laterally. Scale bar: 30 μm in all micrographs.

lamellipodium at one cell pole and a long thin process at the other pole (Fig. 12D,E). Thin processes were 1–2 μm thick, i.e., much thicker than the threadlike processes described above. They varied in length from 40 to 80 μm and ended in a small lamellipodium (Fig. 12D) or in a macelike swelling (Fig. 12E). These processes emerged

either from the center-facing pole (Fig. 12D) or from the peripheral-facing pole (Fig. 12E) of bipolar cells; therefore opposite polarities were seen in different straight bipolar cells in relation to the position of the long cell process.

The main axis of V-shaped bipolar ameboid microglial cells was divided into two arms, so that the cell body was located at the vertex and cell projections from the two cell poles formed an acute angle (Fig. 12F,G). Interestingly, the vertex of V-shaped bipolar ameboid cells could be oriented toward the center (Fig. 12F) or the periphery (Fig. 12G) of the retina. The other morphological features of the projections in these cells were similar to those described in straight bipolar cells. In some bipolar cells, the cell body and one of the cell projections formed a 90° angle with the other cell projection (Fig. 12H).

Multipolar ameboid microglial cells showed several cell projections radiating in any direction from the cell body. Some cell projections were processes ending in more or less extensive lamellipodia (Fig. 12I), whereas others were lamellipodia that emerged directly from the cell body (Fig. 12J). Intermediate morphologies between multipolar, bipolar, and monopolar ameboid cells were also observed (Fig. 12K), suggesting that these different morphological cell types are interchangeable.

Relationship Between Morphological Features and Chrono-Topographical Location of Ameboid Microglial Cells

The morphological features of ameboid microglial cells generally differed depending on the region of the retina and the developmental stage. These differences became clear when we compared cell morphologies in three regions of the mediotemporal part of the retina at different stages (Fig. 13). These regions were: (a) the zone adjacent to the pecten/optic-nerve-head (ONH zone) from which the microglial precursors enter the retina; (b) the advancing outward edge of the retinal region occupied by migrating ameboid microglia (OE zone); and (c) an intermediate zone equidistant from the two previous ones (I zone).

At E8–E9, many ameboid microglial cells migrating along the ONH and I zones were long bipolar cells with their long axes oriented in a central-to-peripheral direction (Fig. 13A,B), i.e., parallel to the grooves flanked by Müller cell radial processes. At these developmental stages ameboid microglial cells were generally shorter in the OE zone (Fig. 13C) than in the ONH and I zones, and were similarly oriented. At E10, the morphological features and orientation of microglial cells in the ONH and I zones (Fig. 13D,E) were similar to those in these zones at E8–E9. However, many ameboid microglial cells did not parallel the central-to-peripheral direction in the OE zone (Fig. 13F). At E12, most ameboid microglial cells migrating through the ONH zone were elongated and oriented in a central-to-peripheral direction (Fig. 13G). However, many microglial cells in the I zone had no definite orientation (Fig. 13H). In the OE zone, no particular orientation was evident, and many ameboid microglial cells were not elongated, suggesting that they were not migrating.

In summary, these results showed that during all developmental stages studied many ameboid microglial cells migrating along central regions of the retina were elongated and clearly oriented toward the retinal periphery. In contrast, at peripheral regions of the retina from E10 on, many ameboid microglial cells were short and showed no particular orientation. In intermediate regions of the retina up to E12, ameboid microglial cells appeared similar to those in central regions, but from E12 on, these cells were shorter, and like cells in peripheral regions, no longer showed central-to-peripheral orientation.

DISCUSSION

We studied variable morphological features of ameboid microglia migrating tangentially in the developing quail retina in an attempt to gain insights on the mechanisms of migration and migratory behavior of these cells. ILM/MCEF sheets are an excellent material to study migrating microglial precursors in situ, as these preparations offer advantages similar to those of in vitro cell cultures and make it possible to analyze whole nonsectioned cells with a variety of techniques including phase-contrast microscopy, immunocytochemistry, and SEM.

From our results we conclude that: (1) ameboid microglial cells migrate tangentially on Müller cell endfeet; (2) the mechanism of migration of ameboid microglial cells is similar to that described for the locomotion of fibroblasts in culture; (3) ameboid microglial cells appear to alternate phases of active locomotion with phases of "testing" the best path; and (4) the central-to-peripheral migration of the retinal ameboid microglial cells does not follow a straight path, but instead involves forward, backward, and sideways movements. These points are discussed separately below.

Müller Cell Endfeet as the Substrate on Which Ameboid Microglia Migrate Tangentially

The presence of ameboid microglial cells on ILM/MCEF sheets of the developing retina clearly demonstrates that many tangentially migrating ameboid microglial cells in the retina of quail embryos use the Müller cell endfeet as a substrate for movement. We cannot rule out that some microglial cells migrate on ganglion cell axons, at least during a part of their course. In fact, some ameboid microglial cells are seen between axonal fascicles in the nerve fiber layer of the developing quail retina (Navascués et al., 1995), and ameboid microglia also appear to migrate along axons

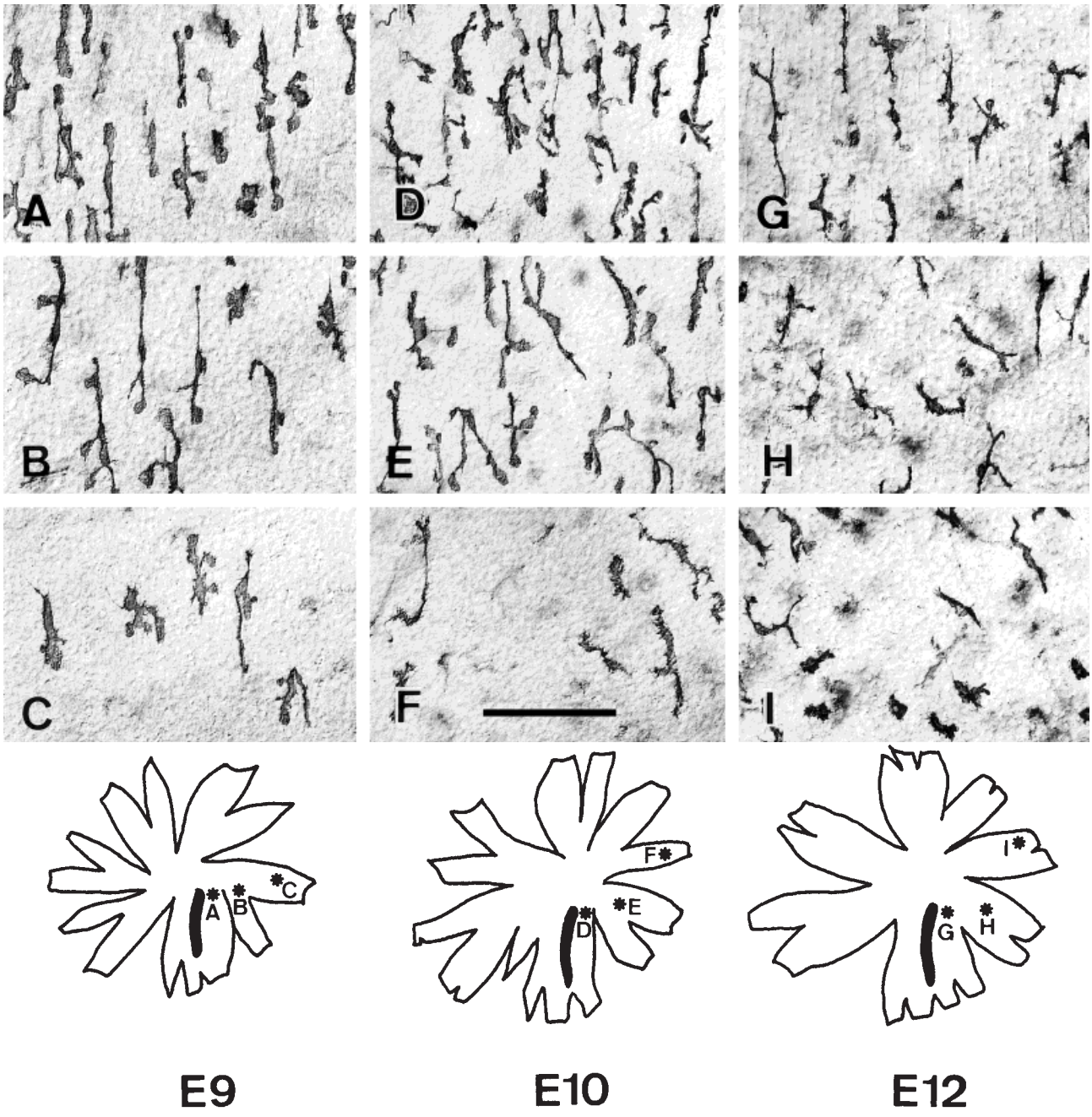

Fig. 13. Morphological features of tangentially migrating ameboid microglia in different areas of QH1-immunolabeled whole-mounted retinas of E9 (A–C), E10 (D–F), and E12 (G–I) quail embryos. The exact locations of different micrographs in each retina are indicated with asterisks on the retinal profiles at the bottom of the figure. The black areas in these profiles represent the optic nerve heads. Micrographs were obtained in each retina in the ONH zone, adjacent to the optic nerve head (**A, D, and G**), in the I zone, midway between the optic nerve head and the retinal periphery (**B, E, and H**), and in the OE zone, at the advancing outward edge of ameboid microglia (**C, F, and I**). QH1-positive ameboid microglial cells are long and central-to-peripherally oriented in all retinal areas at E9 (A, B, and C), in the ONH and I zones at E10 (D, E), and in the ONH zone at E12 (G). Shorter and apparently non-oriented QH1-positive cells are seen in the OE zone at E10 (F) and E12 (I), as well as in the I zone at E12 (H). Scale bar: 90 μm.

within the developing optic nerve (Moujahid et al., 1996) and the white matter of the cerebellum (Navascués et al., 1996; Cuadros et al., 1997).

Endfeet on which microglial precursors migrate appear to belong to Müller cells (not to radial neuronal or glial progenitors) as retinal neurons and Müller cells have withdrawn from the cell cycle at developmental stages studied here. In fact, in the chick retina, nearly all neurons and Müller cells have left the cycle by E10 (Spence and Robson, 1989; Prada et al., 1991), which is equivalent to E8 in quail development.

Müller cell endfeet constitute an oriented substrate, as they form the floor of grooves flanked by rows of Müller cell radial processes ending in the endfeet.

These grooves radiate from the nerve optic head toward the periphery of the retina (Suburo et al., 1979). The coincidence between the orientation of the substrate and the central-to-peripheral directionality of tangentially migrating microglial cells suggests that mechanical guidance is involved in the tangential migration of ameboid microglia through the retina. In this connection, other authors found that fibroblasts or oligodendroglial precursors moving on a surface of parallel grooves in vitro become aligned in the direction of grooves (Clark et al., 1990; Webb et al., 1995). Both the depth and the spacing of the grooves influence the degree of alignment of the cells. Our results are in accordance with this principle, as ameboid microglial cells showed a greater degree of alignment in the direction of grooves flanked by Müller cell radial processes in ONH zones, where such grooves are deep and clearly delimited, than in the retinal periphery, where grooves are more shallow and less clearly delineated.

In addition to their role in the mechanical guidance of ameboid microglial cells, Müller cell endfeet may participate in adhesive guidance of these cells. In fact, the adhesion molecules N-CAM and laminin have been shown to be present on the Müller cell endfeet of the developing chick and quail retina (Halfter et al., 1987; Halfter and Fua, 1987). In addition, cultured Müller cells from adult rabbit retinas have been shown to express laminin (Wakakura and Foulds, 1989).

Müller cell endfeet are firmly in contact with one another, forming a laminar substrate. Ameboid cells migrating on this substrate become flattened and send out lamellipodia, apparently as a result of their attachment to the laminar substrate. In contrast, ameboid microglia in nonlaminar environments within the CNS become rounded and send out pseudopodia, lamellipodia, and occasional filopodia (Rio-Hortega, 1932; Ling, 1976; Boya et al., 1979, 1991; Ling et al., 1980; Murabe and Sano, 1982; Perry et al., 1985; Ashwell, 1990; Cuadros et al., 1994; Brockhaus et al., 1996; Navascués et al., 1996). Therefore, the physical nature of the substrate appears to decisively influence the morphology of migrating ameboid microglial cells, the presence of lamellipodia being a constant feature.

Cell Mechanisms Involved in the Locomotion of Ameboid Microglia During Tangential Migration

To our knowledge, no previous studies have dealt with the mechanism of in situ migration of ameboid microglia within the CNS, although some in vitro approaches have been reported (Booth and Thomas, 1991; Ward et al., 1991; Haapaniemi et al., 1995; Brockhaus et al., 1996). By combining studies of QH1 immunocytochemistry, SEM, and TEM on ILM/MCEF sheets and whole-mounted retinas, we show that the most conspicuous morphological feature of ameboid microglia in the developing quail retina is their flattened morphology, with extensive lamellipodia emerging either from the cell body or from cell processes of variable length. These morphological features are similar to those in cultured microglia under certain conditions (Abd-El-Basset and Fedoroff, 1995; Ilschner and Brandt, 1996). Lamellipodia of ameboid microglia in situ contain ribosomes and a filamentous network, probably of actin, but no microtubules or other cell organelles. This is in accordance with the structural features of lamellipodia in cultured microglia (Abd-El-Basset and Fedoroff, 1995). The morphological features of retinal ameboid microglia migrating in situ are similar to those of cultured fibroblasts moving on adhesive substrates (Abercrombie et al., 1970; Bershadsky and Vasiliev, 1988; Bray, 1992) and neurons migrating in the developing CNS (Book et al., 1991; Liesi, 1992). Therefore the current model of fibroblast locomotion can be used to interpret our results. This model comprises polarized extension of lamellipodia at the leading edge, strong attachment of the cell to the substrate, translocation of the cell body forward, and retraction of the rear of the cell which is frequently seen as a long straight process called the trailing edge (Bershadsky and Vasiliev, 1988; Bray, 1992).

Cell polarization

A prerequisite for directional locomotion appears to be polarization of the moving cell. Abd-El-Basset and Fedoroff (1995) have shown that nonmigratory microglial cells in tissue cultures extend lamellipodia in several directions. When lipopolysaccharide is added to the cultures, some cells become polarized and begin to migrate by forming a large lamellipodium at their leading edge. Nevertheless, Haapaniemi et al. (1995) recently described non-ameboid locomotion of cultured microglia in which no cell polarization appeared to occur. In the developing retina as seen in this study, morphological polarization was evident in monopolar and bipolar ameboid microglial cells but not in round and multipolar ones. Therefore monopolar and bipolar cells are interpreted as cells in the process of active migration, whereas non-polarized cells are thought not to be locomoting (see below).

The changing morphology of different types of polarized ameboid microglial cells reflects stages similar to the different phases in the locomotion of fibroblasts in culture. Bipolar microglial cells bearing a long cell process at one pole and a large lamellipodium at the other pole are similar to fibroblasts in the act of retracting the trailing edge, and monopolar cells are similar to fibroblasts in which retraction is complete.

Cell-to-substrate attachments

In the mechanism of fibroblast locomotion, strong cell-to-substrate attachments must occur. We have shown two types of apparently strong attachment of the microglial precursors to the substrate: attachment of cell processes and bodies to the retinal basal lamina,

and attachment of the lateral surface of lamellipodia and cell bodies to Müller cell radial processes.

Some adjacent Müller cell endfeet are loose allowing microglial cells to attach to the retinal basal lamina through a gap between adjacent endfeet. Several cell types migrating on chick or quail ILM/MCEF sheets in vitro have been also shown to displace endfeet, leaving behind tracks in the endfeet monolayer (Halfter et al., 1988). This appears to demonstrate that cell attachment to the basal lamina is a general mechanism in cell migration on the vitrealmost part of the avian retina. These attachments may be mediated by laminin, which is present in the retinal basal lamina of chick and quail embryos in the second week of incubation (Halfter et al., 1987; Cohen et al., 1987; Morissette and Carbonetto, 1995; Kröger and Mann, 1996). Other molecules, such as a heparan sulfate proteoglycan, have been found in the retinal basal lamina of chick embryos (Halfter, 1993) where they may play a role in microglial cell-basal lamina attachments.

Our SEM and TEM observations clearly show that lamellipodia and cell bodies of ameboid microglial cells attach to the vitrealmost portion of Müller cell radial processes. This suggests that leading lamellipodia spread through the retinal grooves until they attach to Müller cell radial processes. These cell-cell attachments together with cell-basal lamina attachments strongly anchor the migrating microglial precursors to the substrate, allowing subsequent retraction of the trailing edge.

Retraction of the trailing edge

The mechanism of retraction of the trailing edge during locomotion of cultured fibroblasts has been studied in detail (Chen, 1981; Regen and Horwitz, 1992). Two observations in the present study suggest a similar mechanism in polarized ameboid microglial cells. Threadlike processes are seen projecting from a number of migrating microglial precursors; some fragments of these threadlike processes apparently break off and remain scattered across the substrate.

Threadlike processes may be the result of retraction of rear cell processes that are strongly attached to the substrate. Strong attachments would offer resistance to retraction so that the trailing part of the cell might give rise to a thin threadlike process. The occasional presence of small lamellipodia or macelike structures at the end of some threadlike processes appears to support this view. Interestingly, microglial cells in ILM/MCEF sheets cultured in vitro retracted their processes and lamellipodia and frequently bore threadlike processes (results not shown) similar to those observed in situ. Long threadlike processes have been also shown in cultured microglia of newborn rats (Giulian et al., 1995).

An alternative explanation is that the threadlike processes are cytokinesis bridges in microglial precursors which have just divided. The evidence that migrating ameboid microglial cells can divide by mitosis (Marín-Teva et al., 1996b), as well as the presence of threadlike processes connecting two adjacent microglial cells (Fig. 11F), make this interpretation plausible. The daughter cells appear to resume locomotion while they are still joined by a cytokinesis bridge; if they move in opposite directions, the bridge would stretch considerably to give rise to a threadlike process. These two hypotheses are not mutually exclusive: some threadlike processes may be the remains of retracted trailing processes, and others may be cytokinesis bridges.

The presence of isolated fragments of threadlike processes on the substrate can be explained by a mechanism similar to that which occurs in moving cultured fibroblasts. Upon retraction, the fibroblast tail ruptures, leaving a small fragment attached to the substrate (Bard and Hay, 1975; Chen, 1981). Therefore free QH1-labeled threadlike processes in the developing retina may be fragments of the trailing process of migrating microglial precursors.

Orientation of Migrating Ameboid Microglial Cells

As discussed above, monopolar and bipolar ameboid microglial cells are polarized cells which appear to be in the process of active migration. But round and multipolar microglial precursors are also frequent in the developing retina. Round QH1-positive cells are microglial precursors which have stopped to enter in mitosis (Marín-Teva et al., 1996b). Multipolar cells apparently are not in active locomotion: different lamellipodia-bearing processes lie in different grooves flanked by Müller cell radial processes, and some of these lamellipodia must be retracted to allow the cell to move through a groove. These lamellipodia may function as devices for exploring the substrate and recognizing signals that help determine the direction of subsequent movement of microglial precursors. Therefore, multipolar cells seem to be in the act of exploring the surrounding environment to orient their movement.

The orientation phase probably precedes polarization and locomotion of migrating microglial precursors and seems to be essential for central-to-peripheral directional movement of ameboid microglia in the developing retina. This is in accordance with the view of Van Duijn and Van Haastert (1992), who considered locomotion and orientation of directionally moving cells as two different components of cell migration, each controlled independently. Directional movement of locomoting cells can be controlled by chemotaxis or by adhesive gradients on the substrate (Bershadsky and Vasiliev, 1988; Erickson, 1990). Thus lamellipodia may control the orientation of ameboid microglial cells in the developing quail retina by detecting variable concentrations of certain molecules in the environment.

Our observations are compatible with the participation of contact inhibition of locomotion (Bershadsky and

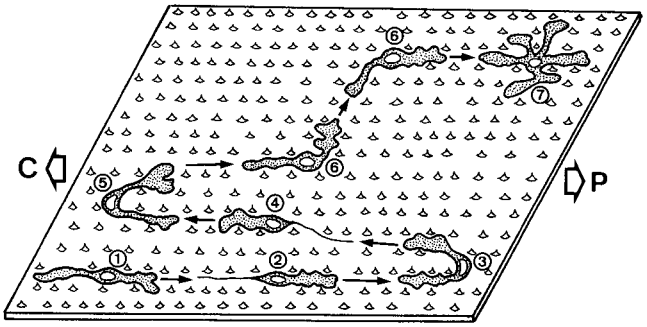

Fig. 14. Schematic drawing of the different steps in a hypothetical course of movement of an ameboid microglial cell on an ILM/MCEF sheet, as inferred from observations in this study. The cell moves forward (steps 1–3), backwards (steps 3–5) and sideways (step 6) along and across grooves flanked by rows of Müller cell processes (drawn as small cones) and oriented from the center (C) toward the periphery (P) of the retina. The moving cell becomes multipolar (step 7) as it prepares for subsequent movements. Step 3, a V-shaped cell with the vertex pointing toward the periphery, shows the cell while it is changing direction through 180°. Step 5, a V-shaped cell with the vertex pointing toward the center, is in the process of changing direction again. The L-shaped cell in step 6 is making a 90° change in direction.

Vasiliev, 1988; Bray, 1992) in the orientation mechanisms of tangentially migrating ameboid microglia in the developing retina. Contacts between the lamellipodia of adjacent cells are occasionally seen, but no overlapping of lamellipodia from adjacent microglial precursors, even when migrating cells are as closely packed as in the ONH zone of the retina. Cell polarization is more evident in this zone than in the OE zone (Fig. 13), where migrating microglial precursors are loosely arranged. Ameboid microglial cells are also highly polarized in zones adjacent to the optic nerve head of the developing human retina (Diaz-Araya et al., 1995a,b). This enhanced cell polarization can be explained by assuming that a high density of migrating cells favors contacts between lateral lamellipodia, which rapidly retract, thus helping to maintain the central-to-peripheral orientation. This explanation is in accordance with the fact that contact inhibition can lead to parallel orientation of cells in dense cultures (Bershadsky and Vasiliev, 1988).

Migratory Behavior of Ameboid Microglial Cells in the Developing Retina

An interesting observation is the presence of V-shaped bipolar ameboid microglial cells in the developing retina, with the vertex pointing either toward the periphery or toward the center of the retina. In both cases, these cells appear to be in the act of turning through 180°. The V-shaped cells with the vertex pointing toward the periphery may be cells that are turning toward the center of the retina (Fig. 14). This is so regardless of which cell arm is considered the leading edge, as both are pointing toward the retinal center. In contrast, V-shaped microglial precursors with the vertex pointing toward the center of the retina can be considered to be cells that are turning toward the periphery (Fig. 14). Therefore the presence of V-shaped microglial precursors with opposite orientations strongly suggests that ameboid microglia can move forward and backward in the developing retina. This is also supported by the occurrence of ameboid microglial cells bearing threadlike processes facing either the center or the periphery of the retina. As discussed above, these thin processes are probably the result of retraction of the rear of microglial precursors. Moreover, the observation of ameboid microglial cells which bear a threadlike process making a 180° turn is additional evidence in support of reversal of the direction of movement of some migrating microglial precursors.

In addition, some microglial precursors appear to move laterally in the retina, crossing over several adjacent grooves (see Figs. 2C and 12H). This lateral movement may be used to change grooves (Fig. 14).

As a whole, the movement of microglial precursors entering the developing retina through the optic nerve head area is central-to-peripheral (Pearson et al., 1993; Navascués et al., 1995; Diaz-Araya et al., 1995a, b). However, individual microglial cells do not take the shortest path, but wander backwards and sideways before they resume their central-to-peripheral course. This behavior appears to be similar to that of cells migrating in a gradient of chemoattractant (Yao et al., 1990; Bray, 1992). The cell mechanisms and molecular signals that participate in the migratory behavior of ameboid microglial cells remain to be elucidated.

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