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Microglial Responses in the Quail Retina Following Intraorbital Transection of the Optic Nerve

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ABSTRACT

Quail retinae were stained immunocytochemically with monoclonal antibody QH1, a specific marker for quail microglia, at various times up to 6 mo after unilateral intraorbital optic nerve transection, which is known to induce degeneration of ganglion cell axons and perikarya in the retina. A few days after transection, activated microglia displaying an amoeboid shape and strong QH1-immunoreactivity began to appear in the inner part of the retina including the inner plexiform layer. The number of these amoeboid cells transiently increased, particularly in the ganglion cell and in the optic nerve fiber layers, where retrograde neuronal degeneration actively occurs, and returned to the normal value 6 mo post-transection. In addition, we observed that numerous QH1-labeled macrophages intimately adhered to the surfaces of the pecten and the optic disc, and subsequently radially oriented in the retina. Furthermore, some of these vitreal macrophages and retinal microglia were labeled with PCNA, indicated migration and proliferation around the optic disc. The results of this study, for the first time, describe the temporal and spatial aspects of microglial activation in the avascular retina, and suggest that the proliferation of microglia and the migration of macrophages, attributable to a massive increase of activated microglia at the lesion site during microglial activation, is a property shared by vascular and avascular central nervous system (CNS) tissue.

Key words: Optic nerve transection, microglia, macrophages, proliferation, migration

INTRODUCTION

Transection of the optic nerve of adult mammals leads not only to Wallerian degeneration of the axons in the nerve, but also to degeneration of most ganglion cell perikarya residing in the retina (Springer and Willson, 1989; Stoll et al., 1989). Microglial cells, known to be present in all mammalian retinae examined so far (Vrabec, 1970; Boycott and Hopkins, 1981; Hume et al., 1983; Sanyal and De Ruiter, 1985; Schnitzer, 1989), actively phagocytose degenerating ganglion cells following lesions of the retina in rats and rabbits (Miller and Oberdorfer, 1981; Barron et al., 1986; Schnitzer and Scherer, 1990). On the other hand, the hematogenous origin of brain and retinal microglia has been assumed to depend on the migration of monocytes from the leptomeninges into neighboring CNS tissue late in

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embryonic development, and their subsequent transformation into microglia (del Rio-Hortega, 1932; Perry and Gordon, 1988). However, the origin and the migratory path of retinal microglia in the repair process at injury sites is not well defined.

Avian retinae are remarkably different from their mammalian counterparts; astrocytes are lacking in the avian retinae, and Müller cells constitute the only astrocytic-like cell population (Reichenbach et al., 1993), whereas the avian retina contains a large number of oligodendrocytes (Cho et al., 1997, 1999). Avian retinae are completely avascular, and include a structure called the pecten, a richly vascularized organ, which projects into the vitreous body from the optic disc (Romanoff, 1960; Meyer, 1977). The avascular nature of this retina makes it possible to test the hypothetical relationship between microglial activation and tissue vascularization, since the presence of microglial cells has been reported in the adult quail retina by using QH1 antibody, a marker for microglia and blood vessels in this species (Navascues et al., 1994). In addition, the absence of blood vessels in the retina greatly facilitates studies of microglia.

However, to our knowledge, no studies have been published on microglial activation in the avian retina. In the present study, we examined microglial response in the adult quail retina, with regard to the spatial and temporal aspects of activation, and proliferation, and regarding the possible migratory path of microglia using an optic nerve transection model, an injury that does not breach the blood-retinal barrier. We compared the findings obtained with those of a mammalian counterpart.

MATERIALS AND METHODS

Animal and tissue preparation

All animal experiments were approved by the local Institutional Animal Care and Use Committee. Forty adult Japanese quail were used in this study. Under deep anesthesia (20 mg/kg Ketamine; 80 mg/kg Xylazine), the left optic nerve was approached intraorbitally, and completely transected by cutting the nerve immediately after the eyeball. The transection was performed unilaterally, the right retina served as a control. Quails treated in the above manner were allowed to survive for postoperative periods of 4, 7, 10, 14, 21, 28 and 180 d. The retinae of non-operated quails served as normal controls. Following enucleation, the cornea, lens, and vitreous body were removed and eyecups were immersion fixed for 3 hr at 4°C in 4% paraformaldehyde. They were cryoprotected by serial sucrose treatment. The blocks containing the eyecups were rapidly frozen with liquid nitrogen and cut into 10μm thick transverse sections on a freezing microtome. Sections were then thaw mounted on gelatin-coated microscopic slides and stored at -20°C until required for immunohistochemistry.

Immunohistochemistry

Sections were sequentially treated with 0.3% H_2O_2 in methanol for 30 min, incubated in 1% normal chicken serum in PBS for 1hr, and then incubated in diluted mouse anti-QH1 antibody solution (1 : 300, Developmental Studies Hybridoma Bank, University of Iowa) overnight at 4°C. They were then exposed to biotinylated horse anti-mouse IgG (Vector, USA) and streptavidin peroxidase complex (Vector, USA), and finally treated with 3,3'-diaminobenzidine (Sigma, USA) solution containing 0.003% H_2O_2 to visualize the antigen-antibody reaction. The sections stained with anti-QH1 controls for immunohistochemical staining included the omission of primary or secondary antibodies. To reveal the colocalization of proliferating microglia, we performed double-labeling immunofluorescence staining. The two primary antibodies were applied to sections simultaneously, and this was followed by the simultaneous application of the two secondary antibodies. The dilutions used for primary antibodies were 1 : 100 for anti-PCNA (Sigma, USA), which is a specific marker for proliferating cell nuclear antigen and 1 : 3000 for Ricinus communis agglutinin 1 (RCA-1; Vector), which is the specific marker for microglial cells (Mannoji et al., 1986). Specific labeling by the secondary antibodies was performed using CyTM3 labeled goat anti-mouse IgG 1 : 100 (Amersham, USA) and CyTM2 conjugated streptavidin IgG 1 : 500 (Amersham, USA). Each step was followed by washing 3 times for 10 min with PBS. Sections were coverslipped in crystal mounts (Biomeda, USA), and observed under a Carl Zeiss fluorescent microscope. Some retinal whole mounts were prepared by using the free-floating

method described previously (Cho et al., 1997).

Quantitative analysis

Sagittal sections though the optic disc were evaluated to obtain microglial densities in the three main layers of the retina; the nerve fiber layer (NFL) including the ganglion cell layer (GCL), and the inner plexiform (IPL) and outer plexiform (OPL) layers. The central part of sections obtained from lesioned and control sample at equal distances from the optic disc were chosen because this portion of the retina contains a rich population of microglia in the three layers described above (Navascues et al., 1994). The cell counts were made in the retina within 1 mm from the optic disc at a final magnification of ×100. At this magnification, the number and position of all QH1-labeled cell bodies residing in all retinal layers could be determined. No attempt was made to distinguish between ramified and amoeboid cells. Five representative retinae from different animals were measured and mean cell densities, expressed as number per millimeter, were calculated. The student's *t*-test was used for the statistical analysis.

RESULTS

Morphology and distribution of microglial cells

In the normal retina, as described previously (Navascues et al., 1994), QH1-immunoreactivities are localized specifically in ramified (resting) microglial cells, which have small bodies with scanty cytoplasm and long slender processes extending radially over the retinal layers. These bodies were found mainly in the IPL and NFL, although some were found in the OPL (Fig. 1A). These findings on the topography and shape of microglial cells in the quail retina are generally in accordance with previous findings in the adult rabbit retina (Schnitzer and Scherer, 1990). Following optic nerve transection (ONT), staining intensity, shape, number and the distribution of retinal microglial cells were rapidly changed. About 4 days after ONT, strong QH1-immunoreactivities were found in the amoeboid cells (Fig. 1B). These were more numerous in the inner retinal layers, such as the IPL, NFL and GCL, than in the outer retinal layers. Similar findings were noted 10 d after transection: strong $QH1^+$ amoeboid cells were found in the OPL in addition to the inner retinal layers (Fig. 1C). At 2 and 3 weeks after transection, larger amoeboid cells with short and stout processes appeared in the NFL and GCL where retrograde degeneration of ganglion cells was actively observed (Fig. 1D, E). Six mo after ONT, retinal microglia resumed a ramified shape, and activated microglial cells were no longer apparent (Fig. 1F). Whole mount preparations demonstrated the morphologies of resting and activated microglial cells. In the IPL of the normal retina, $QH1^+$ cells displayed a typical morphology of ramified microglia, and were scattered in a mosaic-like fashion throughout this layer (Fig. 2A). However, microglial cells in the NFL were poorly ramified,

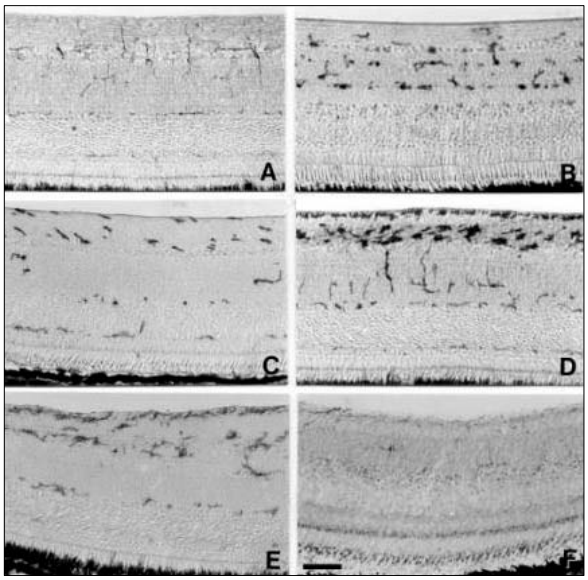

Fig. 1. Representative retianl sections stained immunohistochemically with QH1 antibody showing alternations in the morphology and distribution of microglia after optic nerve transection (ONT). In the normal retina (A), QH1 labeled ramified cells whose cell bodies were localized mainly in the IPL. Following transection, QH1-immunoreactivity and the cell number of microglial cells were transiently increased (B-E). Amoeboid cells showing strong QH1-immunoreactivity appeared primarily in the inner portion of the retina 4 and 10 d after ONT (B, C). Two weeks after ONT, these amoeboid microglia exhibited enhanced immunoreactivity and larger cell bodies with short and stout processes (D). Thereafter these cells gradually diappeared and amoeboid cells were no longer observed 6 mo after ONT (F). Photomicrographs were taken from the retinal area 1 mm anterior from the optic disc in each experimental group. Normal control, A; Postoperational day 4 (B), 10 (C), 14 (D), 21 (E) and 180 (F). Scale bar=50μm.

with bipolar processes that were mainly oriented parallel to the optic axons (Fig. 2C). They were more numerous close to the optic disc than in the peripheral areas. Following ONT, microglia in both the IPL and NFL were more intensely QH1-labeled, and exhibited larger cell bodies with shorter, thicker and more branched processes, although their characteristic pattern of distribution in both layers was largely preserved during the ganglion cell degeneration (Fig. 2B, D). We counted the number of microglial cells in the retinal layer within 1 mm of length from the optic disc in sagittal section, results are shown in Table 1. These results demonstrate that overall density of microglia began to increase significantly in a few days after ONT ($P<0.001$). This increase peaked (2.8 fold) 2 weeks after lesion, thereafter the number of $QH1^+$ microglial cells decreased gradually and returned to the normal value 6 mo after ONT. When we compared the three main layers of the lesioned retina, namely the NFL including GCL, IPL, and OPL, with the respect to cell density, the cell counts in the NFL always led the total increase of cell density in the lesioned retina, while those in other two layers remained roughly constant. This means that increases in the microglial cell population were confined to the retinal layers where the retrograde degeneration of ganglion cells actively occurred.

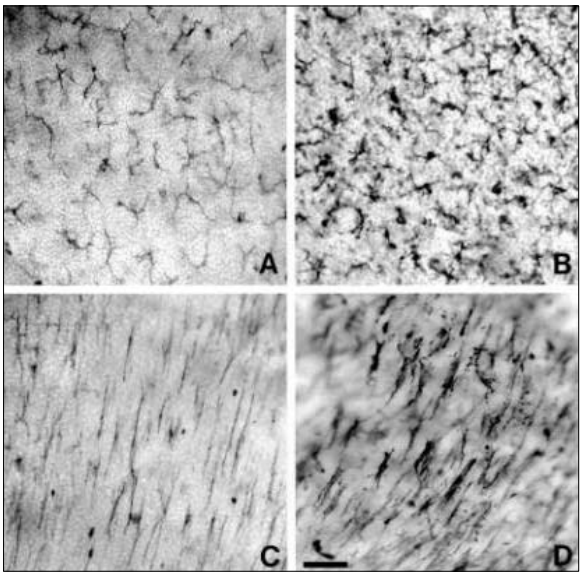

Fig. 2. $QH1^+$ microglial cells in the whole-mounted retina of controls (A, C) and 14 d after ONT (B, D). In the normal retina, $QH1^+$ cells in the IPL displayed typical morphology of ramified (resting) microglia (A), but microglial cells in the NFL were poorly ramified, with processes mainly oriented parallel to the the optic axons (C). Following ONT, microglia in both layers were more intensely QH1-labeled, and exhibited larger cell bodies with shorter and thicker processes, although their characteristic pattern of distribution in both layers was largely preserved during ganglion cell degeneration (B, D). Photomicrographs were taken from the retinal area 1 mm anterior to the optic disc in each experimental group. Scale=50μm.

Proliferation and migration of microglial cells

The increased number of microglial cells present in the NFL and GCL could have been caused by the proliferation of cells situated in the retina or, alternatively, to invasion by macrophages. To test these possibilities, we first examined the apperance of macrophages from the pecten and the adjacent retinal portion, since it has been suggested that the pecten is the main source of macrophages that migrate into the retina during the embryonic period (Navascues et al., 1995; Marin-Teva et al., 1998). In the normal retina, $QH1^+$ cells could not be detected on the surface of the pecten, although the pecten and its attachment to the optic disc appeared to be darkly stained due to the inherent blood

Table 1. Numbers of QH1-labeled microglia in the different layers following optic nerve transection

Layer	Postoperative day (D)					
	Control	4 D	10 D	14 D	21 D	180 D
NFL	11.0±1.7	17.7±0.6*	42.7±1.5*	84.3±2.1*	39.7±1.5*	12.0±2.0
IPL	19.7±1.5	47.0±2.6*	17.0±2.6	13.3±1.5	15.3±1.5	14.7±1.5
OPL	5.7±1.2	5.3±0.6	9.3±0.6	9.3±1.5	7.3±1.2	5.7±1.2
Total	37.0±3.6	70.0±2.0*	69.0±3.6*	107.0±5.0*	62.3±1.2*	32.3±3.8

Figures are means±S.D. The numbers of cells per section were determined in three animals of each group. *Statistically significant differences between control and lesioned retinae at the same layer ($P<0.001$).

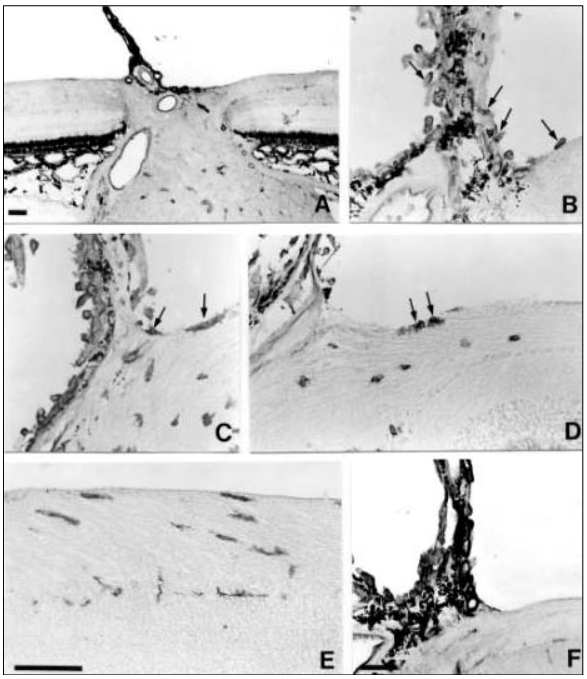

Fig. 3. Sections of the pecten and of the adjacent retina stained immunohistochemically with QH1 antibody, showing a possible $QH1^+$ macrophage migratory path from the pecten to the retina following ONT. No $QH1^+$ cells were detected on the vitreal surface of the pecten or retina in the controls (A), although the inside of the pecten and its attachment to the optic disc appeared to be dark due to the inherent blood vessels and pigment cells residing in these organs. A few days after ONT, however, many $QH1^+$ cells (arrows) were observed along the vitreal surfaces of the pecten (B), and retina (C, D). These QH^+ vitreal macrophages were continually found up to 2 weeks postlesion, but then disappeared, and were absent at 4 weeks postlesion (F). Note that $QH1^+$ cells were elongated or amoeboid in shape and intimately adhered to the vitreal surface of the pecten and to its attachment with the retina (B, C, D). In the retina, $QH1^+$ cells often appeared underneath the internal limiting membrane or situated along the neve fibes of the retina (E). Normal control, A; Postoperational day 4 (B, C), 7 (D), 10 (E), and 28 (F). Scale=100μm in A; 25μm in E (applies to B-E); 50μm in F.

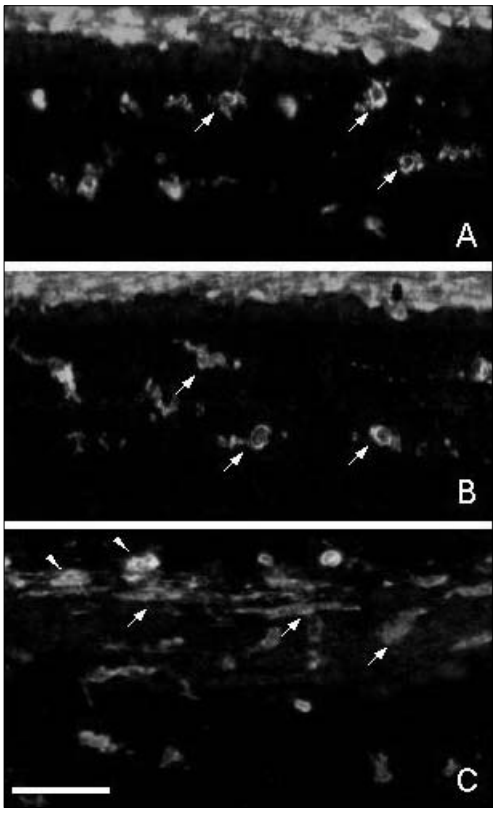

Fig. 4. Double-labeled retina with RCA-1 and PCNA showing proliferating microglial cells after ONT. Four days later PCNA was found in the nuclei of some RCA-1-labeled microglia cells (arrows) in the IPL throughout the central (A) and peripheral (B) area. At 7 d after ONT, PCNA immunoreactivities were mainly found in the microglial cells (arrows) located in the NFL (C). Note $PCNA^+$ microglial cells (arrow heads) positioned on the inside or vitreal side of the internal limiting membrane and in close contact with the membrane (C). Scale bar=25μm.

vessels and pigment cells reside in these organs (Fig. 3A). A few days after ONT, however, many $QH1^+$ cells were observed along the vitreal surface of the pecten and adjacent retina (Fig. 3B, C, D). These QH^+ macrophages were continually found up to 2 weeks postlesion, but then disappeared (Fig. 3F). $QH1^+$ macrophages were elongated or amoeboid in shape, without any cellular processes, and intimately adhered to the vitreal surface of the pecten or midway between the pecten and the retina (Fig. 3B, C, D). In the retinal portion, $QH1^+$ macrophages were often observed underneath the internal limiting membrane or situated along the nerve fibers of the retina (Fig. 3E). These findings suggest that macrophages are derived from the pecten and migrate into the injured portion of the reina. We also investigated whether microglial cells proliferate in the injured retina by double-labeling using PCNA antibody and lectin RCA-1. Immunofluorecent microscopy revealed that PCNA was colocalized in many microglial cell bodies in the IPL throughout the central (Fig. 4A) and the peripheral (Fig. 4B) retina 4 d post-lesion. At 7 d after ONT, PCNA immunoreactivities were mainly found in cells located in the NFL or those cells positioned in close contact with the inner limiting membrane (Fig. 4C). On the other hand, PCNA-labeled microglial

cells were not observed in the contralateral control retina from the same animal.

DISCUSSION

The present study demonstrates for the first time the response of microglial cells in the avian retina after retrograde degeneration of the ganglion cell axons and perikarya. In particular, it was found that strong QH1-immunoreactive amoeboid cells appeared a few days after optic nerve transection and their numbers began to increase. This increase continued for up to 2 weeks post-lesion, and gradually returned to the normal value 6 mo after ONT. Moreover, this increase of microglial cells occurred mainly in the NFL and GCL, while those in the IPL and OPL remained constant. Microglial increases in these layers have been described in the rabbit (Schntizer and Scherer, 1990) and the rat (Thanos, 1991a, b; Thanos et al., 1993). Thus, it appears that increased microglial cell number is largely confined to the retinal layers, in which ganglion cell degeneration is actively occurring. This increased number of microglial cells in the retina could be due either to the proliferation of cells located in the retina or, alternatively, to the migration of microglia to the injured site. We observed a considerable number of PCNA-labeled microglia after ONT in the lesioned retina, suggesting that some microglial cells in the retina are capable of proliferation. This is in accordance with observations of the degenerating optic nerve in the rat (Skoff, 1975) and rabbit (Schntizer and Scherer, 1990). Interestingly, we found that majority of $PCNA^+$ microglial cells, which appeared at earlier time points, were in the IPL, although a few $PCNA^+$ microglial cells appeared at later time points in the NFL. Furthermore, when PCNA-labeled microglia appeared in the IPL, this was accompanied by a concomitant increase of microglia density in this layer. This suggests that some microglia proliferating in the IPL may migrate to the NFL. This notion is also supported by previous studies, which reported that microglia proliferate far away from the lesion site (Riva-Depaty et al., 1994; Ziaja and Janeczko, 1999) and migrate through the rat retina to the layers of neuronal degeneration (Thanos and Richter, 1993). On the other hand, it has long been believed that macrophages invade the degenerating CNS from the blood stream. However, direct evidence is lacking because of the non-availability of cell markers capable of distinguishing macrophages from microglia. In this study, however, we were able to demonstrate the existence of long distance macrophage migration due to the unique structure of the avian retina. Avian retinae are completely avascular and include a structure called the pecten, which is a richly vascularized organ that projects into the vitreous body from the optic disc (Romanoff, 1960; Meyer, 1977). This organ provides nutrients and oxygen to the retina through the vitreous body. During the embryonic period, the pecten is the source of amoeboid macrophages, microglial precursors. They migrate from the pecten to the retina along the vitreal side 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 (Cuadros et al., 1993; Marin-Teva et al., 1998). A similar mode of migration was found in this study. A few days after ONT, we observed numerous vitreal macrophages intimately adherent to the surfaces of the pecten and retina. Subsequently, they were found located underneath the internal limiting membrane and along the nerve fibers of the retina. Further tracing of the migratory paths of these macrophages was not possible, because when macrophages migrate into the retinal layers they are no longer distinguishable from the intrinsic microglial cells. This sequence of events, however, suggests that $QH1^+$ macrophages derived from the pecten migrate into the injured site of the retina where they contribute to microglial cell numbers in the NFL. On the other hand, we did not observe PCNA-labelling in the vitreal macrophages of the pecten, although a few PCNA-labeled cells were found in the NFL, though it appears that at least some of PCNA-labeled cells in the NFL are migrated macrophages because they were located on the inside or vitreal side of the internal limiting membrane.

In conclusion, this study demonstrates for the first time microglial activation, migration and proliferation in the avascular retinal tissue. Further studies underlying the fate of the migrating macrophages in relation to intrinsic microglial cells should improve our understanding of microglial function in injured

CNS tissue.

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REFERENCES

Barron KD, Dentinger MP, Krohel G, Easton SK and Mankes R (1986) Qualitative and quantitative ultrastructural observations on retinal ganglion cell layer of rat after intraorbital optic nerve crush. *J Neurocytol* 15:345-362.

Boycott BB and Hopkins JM (1981) Microglia in the retina of monkey and other mammals: its distinction from other types of glia and horizontal cells. *Neuroscience* 6:679-688.

Cho SS, Lucas JJ and Hyndman AG (1999) Transferrin binding protein is expressed by oligodendrocytes in the avian retina. *Brain Res* 816:229-233.

Cho SS, Lucas JJ, Roh EJ, Yoo YB, Lee KH, Park KH, Hwang DH and Baik SH (1997) Distribution of transferrin binding protein immunoreactivity in the chicken central and peripheral nervous systems. *J Comp Neurol* 382:260-271.

Cuadros MA, Martin C, Coltey P, Almendros A and Navascues J (1993) First appearance, distribution, and origin of macrophages in the early development of the avian central nervous system. *J Comp Neurol* 330:113-129.

del Rio-Hortega P (1932) Microglia. In: Cytology and Cellular Pathology of the Nervous System. *Penfield W ed* pp 482-534.

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

Mannoji H, Yeger H and Becker LE (1986) A specific histochemical marker (lectin Ricinus communis agglutinin-1) for normal human microglia, and application to routine histopathology. *Acta Neuropathol* (*Berl*) 71:341-343.

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

Meyer DB (1977) The avian eye and its adaptations. In: The Visual System in Vertebrates. Crescitelli F ed. Springer-Verlag, *Berlin* pp 549-611.

Miller NM and Oberdorfer M (1981) Neuronal and neuroglial responses following lesions in the neonatal rats. J Comp Neurol 202:493-504.

Navascues J, Moujahid A, Quesada A and Cuadros MA (1994) Microglia in the avian retina: immunocytochemical demonstration in the adult quail. *J Comp Neurol* 350:171-186.

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

Perry VH and Gordon S (1988) Macrophages and microglia in the nervous system. *Trends Neurosci* 11:273-277.

Reichenbach A, Stolzenburg JU, Eberjardt W, Chao TI, Dettmer D and Hertz L (1993) What do retinal Mller (glial) cells do for their neuronal "small siblings"? *J Chem Neuroanat* 6:201-213.

Riva-Depaty I, Fardeau C, Mariani J, Bouchaud C and Delhaye-Bouchaud N (1994) Contribution of peripheral macrophages and microglia to the cellular reaction after mechanical or neurotoxin-induced lesions of the rat brain. *Exp Neurol* 128: 77-87.

Romanoff AL (1960) The avian embryo. Structural and functional development. Macmillan, New York.

Sanyal S and De Ruiter A (1985) Inosine diphosphatase as a histochemical marker of retinal microvasculature, with special reference to transformation of microglia. *Cell Tiss Res* 241: 291-297.

Schnitzer J and Scherer J (1990) Microglial cell responses in the rabbit retina following transection of the optic nerve. *J Comp Neurol* 302:779-791.

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

Skoff RP (1975) The fine structure of pulse labeled (3-H-thymidine cells) in degenerating rat optic nerve. *J Comp Neurol* 161:595-611.

Springer AD and Wilson BR (1989) Light microscopic study of degenerating cobalt-filled optic axons in goldfish: role of microglia and radial glia in debris removal. *J Comp Neurol* 282:119-132.

Stoll G, Trapp BD and Griffin JW (1989). Macrophage function during Wallerian degeneration of rat optic nerve: Clearance of degenerating myelin and Ia expression. *J Comp Neurol* 9: 2327-2335.

Thanos S (1991a) Specific transcellular carbocyanine-labelling of rat retinal microglia during injury-induced neuronal degeneration. *Neurosci Lett* 127:108-112.

Thanos S (1991b) The relationship of microglial cells to dying neurons during natural neuronal cell death and axotomy-induced degeneration of the rat retina. *Eur J Neurosci* 3: 1189-1207.

Thanos S, Mey J and Wild M (1993) Treatment of the adult retina with microglia-suppressing factors retards axotomy-induced neuronal degradation and enhances axonal regeneration in vivo and in vitro. *J Neurosci* 13:455-466.

Thanos S and Richter W (1993) The migratory potential of vitally labelled microglial cells within the retina of rats with hereditary photoreceptor dystrophy. *Int J Dev Neurosci* 11:671-680.

Vrabec F (1970) Microglia in the monkey and rabbit retina. *J Neuropathol Exp Neurol* 29:217-224.

Ziaja M and Janeczko K (1999) Spatiotemporal patterns of microglial proliferation in rat brain injured at the postmitotic stage of postnatal development. *J Neurosci Res* 58:379-386.