Microglia in the Optic Nerve Head and the Region of Parapapillary Chorioretinal Atrophy in Glaucoma

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Background: Microglia, the macrophages and immune surveillance cells of the central nervous system, are quiescent normally but become activated in injured neural tissue. We have determined the distribution and potential participation of microglia in glaucomatous optic nerve degeneration.

Methods: Microglia were localized by immunohistochemistry on paraffin sections of age-matched normal and glaucomatous human eyes obtained within 24 hours after death. Monoclonal and polyclonal antibodies that recognize specific epitopes on microglia and other cell types were localized by immunoperoxidase and immunofluorescence.

Results: Stellate cells with thin, ramified processes, positive for HLA-DR and CD45 but negative for glial fibrillary acid protein, were identified as quiescent microglia. These cells were found throughout the normal optic nerve head in the walls of large blood vessels and surrounding capillaries in glial columns and cribriform plates. In glaucomatous eyes with moderate and severe optic nerve head damage, microglia were present as clusters of large amoeboid, activated cells in the compressed lamina cribrosa and as formations of concentric circles surrounding blood vessels. In the parapapillary chorioretinal region of glaucomatous optic nerve heads, large, activated microglia were present as single cells or clusters on the termination of the Bruch's membrane. In addition, along the optic nerve/choriocapillaris-scleral interface, activated microglia appeared to form linear arrays near the choriocapillaris vessels. These cells were parenchymal and not in close association with the vasculature.

Conclusions: In glaucoma, microglia in the optic nerve head become activated and redistributed. Enlarged, activated microglia appear in the parapapillary chorioretinal region, perhaps due to migration from the disorganized prelaminar and laminar tissue. Strategically positioned microglia may also serve a neuroprotective function in relation to a damaged blood-retinal barrier. The activity of microglia in the parapapillary chorioretinal region in glaucoma may be responsible for some of the biomicroscopic and histological changes that are associated with parapapillary chorioretinal atrophy.


In the parapapillary chorioretinal region, the retinal pigment epithelium and Bruch's membrane terminate, and the axons of the retinal ganglion cells bypass the retina and the marginal capillaries of the choroid as they leave the eye to form the optic nerve. In this region, where the optic nerve is not myelinated, the pial membrane begins as an astrocytic covering. However, glial membranes are not barriers to the diffusion of molecules from the blood. In animal studies, this region leaks fluorescein and peroxidase into the optic nerve head and is considered a potential defect in the blood-retinal barrier.

The parapapillary chorioretinal region of the human optic nerve head has been the focus of numerous clinical and biomorphometric studies relating to glaucoma. In patients with glaucomatous optic neuropathy, chorioretinal abnormalities surrounding the optic disc are larger and occur more frequently in the parapapillary region than in normal subjects. Using biomicroscopy in patients with glaucoma, the region of atrophy has been divided into a peripher al zone (“alpha”) with hyperpigmentation and hypopigmentation and a more central zone (“beta”) in which large chorioidal vessels and sclera are visible. Using this analysis, parapapillary chorioretinal atrophy correlated with neuroretinal rim loss and the time and location of intrapapillary glaucomatous changes. In patients with normal-pressure glaucoma due to high myopia or age-related sclerotic changes, a large parapapillary atrophic region is often seen. Recent evaluation of patients with ocular hypertension demonstrated that the presence
MATERIALS AND METHODS

Six normal human eyes from 6 donors, with an age range of 54 to 71 years (mean ± SD, 63 ± 10 years) and 11 eyes from 11 donors with documented primary open-angle glaucoma with moderate or advanced nerve damage, with an age range of 50 to 92 years (mean ± SD, 75 ± 15 years) were obtained from eye banks throughout the United States. Primary open-angle glaucoma was defined by a clinical history of observation and treatment by an ophthalmologist and the presence of optic nerve damage on histological examination. The eyes from donors with glaucoma reportedly had cup-disc ratios of 0.6 to 0.9, demonstrated marked visual field defects, and the donors before their deaths were receiving medications to lower their intraocular pressure. Histological evidence included the presence of a cup, the disorganization of glial columns and cribiform plates, and the appearance of degenerated nerve fibers in cross section.

Normal and glaucomatous eyes were enucleated and fixed in 4% paraformaldehyde within 24 hours after death. The optic nerve heads and a small extension of retina were dissected free of surrounding tissues. Fixed tissue was washed in 0.2% glycine in phosphate-buffered saline (pH 7.4), embedded in paraffin, and oriented for 6-µm sagittal sections.

Slides containing sections were preincubated with 2% nonfat milk or donkey serum for 30 minutes, rinsed, and then incubated with primary antibody overnight. Microglia were identified using a polyclonal antibody to HLA-DR (working dilution 1:100; Accurate Chemical and Scientific Corp, Westbury, NY) and a monoclonal antibody to CD45 (working dilution 1:50; Dako Corp, Carpinteria, Calif). Additional immunofluorescence studies used a monoclonal antibody to glial fibrillary acid protein (GFAP) (working dilution 1:400; Sigma-Aldrich Chemical Co, St Louis, Mo).

Primary antibodies were localized by immunoperoxidase staining with reagents purchased from Vector Laboratories, Burlingame, Calif. The biotinylated secondary antibody was incubated on the sections for 30 minutes, washed with phosphate-buffered saline solution, and reacted with streptavidin-peroxidase conjugate for 30 minutes. Following a wash, sections were incubated with the substrate mixture (1.5 mg of 3,3-diaminobenzidine tetrahydrochloride and 50 µL of 30% hydrogen peroxide in 0.1-mol/L Tris [pH 7.6]). The sections were reacted in the dark until brown staining appeared (about 5–7 minutes), washed in phosphate-buffered saline solution, counterstained with hematoxylin, dehydrated, and coverslipped. Slides were examined in a microscope and images were recorded using a digital camera.

Representative sections of all samples on a given day were stained simultaneously to control variation in the reactions. Negative controls were performed by eliminating primary antibody from the incubation medium or by replacing the primary antibody with nonimmune serum, followed by immunoperoxidase staining. For colocalization protocols, double sequential immunofluorescence procedures (Rhodamine Red and Cy-5; Molecular Probes Inc, Eugene, Ore) were used and the slides were examined by confocal microscopy.

RESULTS

In optic nerves from normal eyes, microglia were distributed throughout the tissue in close proximity to the blood vessels and in the nerve bundles. Immunohistochemical staining for HLA-DR or CD45 gave identical results. Cells positive for HLA-DR or CD45 were evident in the vessel walls of arteries and veins near the vitreal surface (Figure 1, A). In the prelamellar region and in the lamina cribrosa of the optic nerve head, HLA-DR– or CD45-positive cells surrounded capillaries in the glial columns and in the extracellular matrix of the cribiform plates (Figure 1, B). These multiform cells had smaller nuclei than surrounding astrocytes and processes that appeared to contact capillaries. In the lamina cribrosa, there was a regular spacing of the microglia that were parallel to the cribiform plates; several microglia were rarely seen in close proximity in normal eyes (Figure 1, C). In the postlamellar region of the optic nerve, HLA-DR– or CD45-positive cells were in glial columns but appeared more elongated, with long thin processes running parallel to the nerve bundles (Figure 1, D). The density of HLA-DR– or CD45-positive cells was highest in the lamina cribrosa. Elongated cells with long thin processes were sparsely distributed throughout the nerve bundles. Capillaries without HLA-DR– or CD45-positive cells were also apparent. Also present were some HLA-DR– or CD45-positive cells that were not associated with capillaries, but these parenchymal cells were in the minority.

In the parapapillary chorioretinal region of the optic nerve head of normal eyes, microglia were occasionally present (Figure 1, E). In some but not all normal eyes,
a single HLA-DR– or CD45-positive cell was occasion-
ally seen near the last few choriocapillaris vessels and on
the termination of Bruch’s membrane. Also, in some nor-
mal eyes, HLA-DR– or CD45-positive cells sparsely stud-
ded the insertion region of the anterior optic nerve head
and the glial limiting membrane (Figure 1, F).

In the parapapillary chorioretinal region, Bruch’s
membrane, bared of retinal pigment epithelia, separated
retinal and choriocapillaris tissue and extended to the nerve
bundles of the anterior optic nerve head. In almost all tis-
sues from the glaucomatous eyes, intense immunohisto-
chemical staining for HLA-DR and CD45 could easily be
seen as clusters in the parapapillary chorioretinal region
at low magnification. Using the alpha and beta zone no-
menclature derived by Jonas,9 these clusters of cells were
associated with the end of Bruch’s membrane and in the
anterior insertion region of the optic nerve head just be-
hind Bruch’s membrane (Figure 2, A); thus, the microg-
lia accumulated in the beta zone. However, in the optic
nerve head proper, microglia were noticeably sparse in large

Figure 1. Immunohistochemistry for microglia in normal human optic nerve heads. A, Cell positive for CD45 (arrow) is in the wall of the central vein (BV) in the
prelaminar region. B, Cells positive for CD45 are closely associated with capillaries (cap) in the cribiform plates (CP) of the lamina cribrosa and are also found in
the nerve bundles (NB). C, Individual cells positive for CD45 form a regular distribution in parallel to the CP of the lamina cribrosa. D, In the postlaminar region,
the ramified processes of elongated CD45-positive cells (arrow) are parallel to the myelinated nerve fibers. E, In the parapapillary chorioretinal region, bordered by
Bruch’s membrane (BM), the optic nerve head (ONH), and sclera (scl), few if any CD45- or HLA-DR–positive cells are present. A few pigmented cells can be seen
(arrow). F, At the interface between the anterior sclera and optic nerve head, a linear array of HLA-DR–positive cells (thick arrows pointing to brown
immunohistochemical deposits) can be distinguished from pigmented cells (thin arrows) (original magnification $\times 1250$).
areas of the lamina cribrosa of glaucomatous eyes with moderate to severe nerve damage. When present in the optic nerve head, immunohistochemical staining for HLA-DR and CD45 was in close proximity to small blood vessels in compressed areas of the lamina cribrosa and large vessels in the remnant prelaminar areas of glaucomatous eyes.

At high magnification, the distribution of HLA-DR– and CD45-positive cells in the parapapillary choriretinal region was determined. The HLA-DR– and CD45-positive cells had a relatively small nucleus compared with nearby reactive astrocytes, a granular cytoplasm, and a rounded ameboid shape, which is characteristic of activated microglia. Cells that stained positively for HLA-DR and CD45 were in dense clusters on and around the termination of Bruch’s membrane at the optic nerve head (Figure 2, B). These cells were also clustered at the interface of anterior sclera (scl) and the optic nerve head (ONH). Vit indicates vitreous (original magnification ×125).

**Figure 2.** Immunohistochemistry for microglia in the parapapillary choriretinal region of glaucomatous human optic nerve heads. A, Clusters of HLA-DR–positive cells (thick arrows pointing to brown immunohistochemical deposits) in the parapapillary choriretinal region. Cells are clustered on the end of Bruch’s membrane (BM), which extends beyond the termination of the retinal pigmented epithelia (RPE, arrow points to the last cell) and separates the retina and choriocapillaris (chor). Cells are also clustered at the interface of anterior sclera (scl) and the optic nerve head (ONH). Vit indicates vitreous (original magnification × 125). B, Cells positive for CD45 (brown immunohistochemical deposits, arrows) are associated with the end of the Bruch’s membrane. C, Cells positive for CD45 form a linear array near the last choriocapillaris vessel under the end of Bruch’s membrane. D, A CD45-positive cell (thick arrow) embedded in the end of Bruch’s membrane (BM) and distinguishable from pigmented cells (thin arrows) in the region (original magnification × 1250). E, Colocalization of HLA-DR (green) and glial fibrillary acid protein (GFAP) (red) by confocal microscopy demonstrates that microglia (HLA-DR–positive cells) on Bruch’s membrane are distinguishable from reactive astrocytes (GFAP-positive cells) in the optic nerve head (original magnification × 625). F, HLA-DR–positive cells in the glial limiting membrane separating the anterior sclera from the nerve bundles of the optic nerve head (original magnification × 625).
were also in a regularly spaced linear array behind Bruch’s membrane, lining the interface between the end of the choriocapillaris and the limit of the sclera and the optic nerve head tissue (Figure 2, C). Cells were often in parenchymal tissue near the small choriocapillaris vessels in the region (Figure 2, C), but not perivascular, as seen in the optic nerve of normal tissue. Occasionally, individual HLA-DR– and CD45-positive cells were seen embedded in Bruch’s membrane, near its termination, on either the choroidal side or the retinal side (Figure 2, D). In the parapapillary chorioretinal region and on Bruch’s membrane, cells that were positive for HLA-DR and CD45 were not positive for GFAP, as were the reactive astrocytes in the surrounding tissue (Figure 2, E). Cells positive for HLA-DR and CD45 were also present, and distinguishable from GFAP-positive cells, several hundred microns behind the termination of Bruch’s membrane, associated with the glial limiting membrane at the interface between optic nerve bundles and sclera in the insertion region (Figure 2, F).

In the compressed prelaminar and lamina cribrosa regions of the glaucomatous optic nerve head, the regular array of stellar-shaped microglia was no longer evident. In the compressed prelaminar region, HLA-DR– and CD45-positive cells surrounded blood vessels and were also in the parenchyma. When present in the walls of the large, displaced vessels near the vitreous surface, the appearance and distribution of HLA-DR– and CD45-positive cells were similar to that in normal eyes but the density was low. When surrounding small blood vessels, the microglia formed concentric rings at distances that did not appear to touch the vessel walls (Figure 3, A). In some postlaminar optic nerves from normal eyes, large groups of individual HLA-DR– and CD45-positive cells were identifiable as monocytes in the walls of large vessels and distinguishable from microglia in the nerve bundles (Figure 3, B).

In normal-appearing regions of the lamina cribrosa of glaucomatous eyes, HLA-DR– and CD45-positive cells appeared as they did in normal tissue (Figure 3, C). However, large regions of optic nerve heads with moderate to severe nerve damage had very few or no HLA-DR– or CD45-positive cells. Compressed regions of the lamina cribrosa that still retained nerve bundles had elongated cells with thin processes (Figure 3, D). Regions of disorganized tissue had round, vacuolar cells, either as individual cells or in clusters (Figure 3, E). In areas of Schnabel cavernous atrophy, HLA-DR– and CD45-positive cells were sparse; when present they were mostly located near or in remnant extracellular matrix nodules as individual cells (Figure 3, F) and sometimes forming thick, insulating walls surrounding capillaries.

**COMMENT**

Microglia are the resident macrophages and immune-surveillance cells of the central nervous system that are adapted to the specialized microenvironment of neural tissue. In normal tissue, microglia are quiescent and have a stellar shape with a small nucleus and cell body with several ramified processes. Microglia are distributed in a regularly spaced array throughout neural tissue. Such a regular distribution has been recently demonstrated in the human retina where microglia are found, within the perivascular space, surrounded by the glia limitans, and paravascular or encircling the glia limitans. In the retina, microglia were also found in parenchymal tissue, not associated with blood vessels. In the human retina, antibodies to CD45 or HLA-DR were used to identify these microglia in the nerve fiber/ganglion cell layer, the inner nuclear layer, and the inner surface of the outer plexiform layer.

Because microglia are a key factor in the defense of neural tissue, one of their most characteristic features is their rapid activation in response to a wide variety of neural injuries. Activated microglia have been described in several human neurodegenerative diseases, including Alzheimer disease, acquired immunodeficiency syndrome dementia, and Parkinson disease. When activated, microglia change their phenotype markedly and become migratory, antigen-presenting cells that induce the expression of new proteins, cytokines, receptors, and mitogens. Activated microglia have an altered morphology, may undergo mitosis, and can become scavenging, phagocytic cells with cytotoxic and degradative enzymes.

According to the immunohistochemical literature, there is not one antibody, nor differential identification with several antibodies, that can specifically distinguish microglia from macrophages or hematogenous cells like monocytes, leukocytes, or lymphocytes. Microglia are phenotypically altered monocytes that entered the central nervous system during fetal development before the formation of the blood-brain barrier. However, a vast literature on microglia reports on the use of antibodies to specific epitopes to identify these cells in the central nervous system. These cellular markers include HLA-DR (or major histocompatibility class II, also found on monocytes, B cells, activated T cells, and antigen-presenting cells); CD11b (or OX-42 or Mac-1, an integrin aM subunit also found on neutrophils, macrophages, and myeloid cells); and CD45 (or leukocyte common antigen, a glycoprotein on the surface of macrophages, monocytes and lymphocytes). For the purposes of presenting these findings, the cells in the optic nerve head and parapapillary chorioretinal region that were identified with antibodies to HLA-DR and CD45 are referred to as microglia because of their constitutive presence in normal neural tissue and their neural location in glaucomatous tissue. Nevertheless, some of these cells may be hematogenously derived and may have entered the optic nerve under abnormal conditions.

In glaucomatous tissue, activated microglia appear to be strategically positioned in relation to blood vessels. Activated microglia, as clusters of cells in the parapapillary chorioretinal region, appear to form a discontinuous, linear barrier in the peripapillary area near the vessels of the choriocapillaris bordering the neural tissue. In medium-sized vessels in the compressed prelaminar region, activated microglia form concentric rings in the parenchyma around the vasculature. As the glaucomatous optic nerve becomes compressed, disorganized, and remodeled, blood vessels may become leaky and compromise the blood-retinal barrier. Strategically positioned, activated microglia may serve a neuroprotective function with respect to a damaged blood-retinal barrier.
The distribution and activation of microglia in the parapapillary chorioretinal region of glaucomatous eyes may provide, at least in part, a cellular explanation for the progressive changes in parapapillary chorioretinal atrophy that have been seen biomicroscopically, described clinically, and associated, by many authors, with glaucoma.6,10,11,14 Activated microglia are very potent cells that are capable of producing enzymes for tissue degradation and remodeling, phagocytosing damaged neural tissue, synthesizing cytokines and other factors that can activate or inhibit surrounding cells, and presenting antigens to hematogenous cells to provoke immunological responses.22 Further characterization of the functional activity of microglia in glaucomatous tissue is needed. Nevertheless, the cellular activities of microglia in the lamina cribrosa and in the parapapillary chorioretinal region in glaucomatous optic nerve heads are likely to make these areas dynamic regions of change.

Why activated microglia have accumulated on the termination of Bruch’s membrane and the parapapillary chorioretinal region in glaucoma is not clear. Whether the increased density in these areas is due to migration

Figure 3. Immunohistochemistry for microglia in glaucomatous human optic nerve heads. A, Cells positive for CD45 (brown immunohistochemical deposits) form concentric rings around a small blood vessel (BV) in the prelaminar region. B, Monocytes that are in the intima of the central artery and that are CD45 positive (thin arrows) are morphologically distinguishable from CD45-positive microglia (thick arrow pointing to brown immunohistochemical deposit) in the nerve bundles. C, In normal-appearing regions of the lamina cribrosa within eyes with mild glaucoma, the distribution of CD45-positive cells in the cribiform plates (CP) and nerve bundles (NB) is similar to that of normal tissue. D, In compressed areas of cribiform plates, microglia are sparse but are occasionally associated with capillaries (cap). E, In disorganized areas of optic nerve head tissue, round, globular CD45-positive cells are sparsely distributed among remaining astrocytes. F, In areas of Schnabel cavernous atrophy, small CD45-positive cells are attached to the remnant extracellular matrix (ECM) and in close association with remaining astrocytes (arrow) (original magnification ×1250).
of microglia from the optic nerve tissue or local proliferation is also unknown. In this region, there is a defect in the blood-retinal barrier. In normal tissue, the local resident microglia may be strategically placed to protect the area from hemogenous elements. In glaucoma, the ongoing optic nerve degeneration may cause an enlarged area of defect of the blood-retinal barrier in the parapapillary chorioretinal region that attracts and/or stimulates the proliferation and activation of microglia. Whether the intended responses of the microglia are to protect or to destroy the tissue, the presence and activities of these potent cells in the parapapillary chorioretinal region may be inappropriate and, therefore, underlie the progressive degenerative changes seen clinically.

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REFERENCES


