Morphology of the Murine Optic Nerve

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**PURPOSE.** To study the morphology of the murine optic nerve (ON).

**METHODS.** Eyes of C57/Bl6 and BalbC mice were studied by light and electron microscopy. Microvascular castings of the ON region were prepared by transcardial injection of liquid plastic and studied with a scanning electron microscope. Immunohistochemistry was performed using antibodies against glial fibrillary acidic protein (GFAP), annexin 43, carbonic anhydrase II, and collagen types I and III.

**RESULTS.** The transition between nonmyelinated and myelinated portion of the ON started ~0.6 mm behind the globe. A lamina cribrosa was completely absent. Instead, ON axons passed through a scleral hole that was surrounded by a ring of type III and type I collagen fibers. Instead of connective tissue beams within the nerve, layers of elongated astrocytes traversed the ON. All astrocytes stained for GFAP, but not for carbonic anhydrase II. The arterial supply of the nonmyelinated ON derived from branches of the central retinal artery. None of the capillaries derived from choroidal vessels.

**CONCLUSIONS.** The mouse ON head differs from that of other species, because it lacks a lamina cribrosa and a choroidal vascular supply. Studies in glaucomatous mice might help to identify the importance of the lamina cribrosa and the choroidal vascular supply for optic nerve damage in glaucoma. (Invest Ophthalmol Vis Sci. 2002;43:2206–2212)

To study the detailed patho-mechanisms of glaucoma in humans, animal models provide powerful tools. Laser treatment of the trabecular meshwork in monkeys and episcleral vessel occlusion in rats are the most commonly used models. However, both models are restricted to studying changes induced by elevated intraocular pressure. To study single factors in more detail, the mouse is the most restricted laboratory mammal, because it allows cost-effective genetic manipulation.

To help establish the mouse as a powerful model for glaucoma research, the normal structure and anatomy of the optic nerve head must be studied and compared with the situation in humans. For example, Sheldon et al.¹ and John et al.² have documented inherited ocular lesions in aged DBA/2N or 129/SvEv mice that led to glaucoma-like symptoms including optic nerve atrophy with loss of axons and optic nerve head cupping. Electrotinographic changes in this mouse model were also similar to changes in humans with glaucoma.³

Most theories concerning the pathogenesis of glaucoma involve pressure-related changes at the level of the lamina cribrosa (LC). In humans, compression of the lamellar sheets is followed by a backward bowing of the entire LC and disarrangement of collagen fibrils.⁴–⁶ Changes in the composition of the extracellular material⁷–⁹ and in astrocytes of the LC region¹⁰–¹² parallel the optic neuropathy. Toward this end we studied the LC of healthy mice using scanning electron microscopy (SEM), immunohistochemistry, and transmission electron microscopy (TEM).

A second group of pathogenic factors for glaucoma involve vascular factors. In human glaucomatous eyes, a reduced ON capillary bed was described, although the capillary density calculated from serial sections remained unchanged.¹³–¹⁵ Therefore, in addition the vascular supply of the mouse ON head was investigated using SEM of corrosion cast preparations, serial semithin sections, and TEM.

**MATERIALS AND METHODS**

Studies were conducted on 47 adult C57/black 6 and BalbC albino mice, aged 4 to 14 months. Experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the local animal care rules. All animals were deeply anesthetized with ether and intraperitoneal sodium pentobarbital (50 mg/kg body wt). The chest was then opened, and the vessels were transcardially perfused with 20 mL of heparinized saline.

For vascular corrosion casts (30 mice), animals were then transcardially perfused with liquid plastic, containing Araldite CY 225 (45%), hardener HY 2267 (25%), and acetone (30%), derived from a syringe using thumb pressure. After hardening of the plastic for 24 hours, the eyes were enucleated and macerated in concentrated KOH, the solution being changed several times. The eyes were then fixed on a holder, sputtered with gold and studied with a scanning electron microscope (Stereoscan 90; Cambridge Instruments, UK). Because of fragility and partial damage during corrosion cast maceration and preparation, the analysis was limited to casts of 17 animals.

For ultrastructural studies, 5 mice were transcardially perfused with a fixative containing 2.5% glutaraldehyde, 2.5% paraformaldehyde, and 0.01% picric acid in 0.1 M cacodylate buffer, pH 7.2. The eyes were enucleated, immersion fixed for at least 12 hours in the same fixative, and then rinsed in cacodylate buffer. Optic nerve preparations were postfixed in 1% osmium tetroxide, dehydrated, and embedded in Epon-Araldite in the usual manner. From one eye, serial frontal semi- and ultrathin sections through the optic nerve were performed, and similar sections with sagittal orientation were made from the contralateral eye. Semithin sections were either stained with toluidine blue or with additional acid fuchsin to demonstrate elastic fibers; ultrathin sections were stained with uranyl acetate and lead citrate and viewed in a Zeiss electron microscope (EM 902; Zeiss, Oberkochen, Germany).

For immunohistochemistry, 12 animals were transcardially perfused with 4% paraformaldehyde, and then the eyes were enucleated and immersion-fixed in the same fixative for 4 hours. The eyes were then rinsed in phosphate-buffered saline (PBS, pH 7.4) and dissected. The optic nerve was covered in tissue-freezing medium (R. Jung GmbH, Nussloch, Germany), and 14-μm-thick frozen sections were cut and mounted on glass slides.

The sections were preincubated with dry milk solution to reduce nonspecific background staining, followed by overnight incubation of the primary antibody at 4°C. Antibodies used included rabbit anti-GFAP (dilution 1:200; BioGenex Laboratory, San Ramon, CA), rabbit anti-
RESULTS

Connective Tissue

The nonmyelinated portion of the mouse ON became myelinated at a distance of 0.6–0.8 mm behind the globe (Fig. 1).

Serial 1-μm cross sections revealed that there was no connective tissue between the nerve fiber bundles throughout the entire nonmyelinated portion. This was also the case at the level of the sclera, where collagen structures form a lamina cribrosa in other species. Instead, in the mouse eye, a ring of densely packed collagen fibers separated the ON from the sclera. Immunohistochemically, the collagen fibers directly surrounding the ON stained intensely for type III collagen (Fig. 2A) and type I collagen (Fig. 2B). This ring was continuous with the pial connective tissue in the postbulbar portion of the nerve, which showed the same staining pattern (Fig. 2). In the postbulbar nerve, the dura showed intense staining for type III collagen and only a weak staining for type I collagen. At the level of the sclera, the dura sheath was no longer visible but the pial sheath was directly connected to the scleral tissue (Figs. 2, 3A). The pial sheath continued toward the level of Bruch’s membrane (Fig. 3B). In the sclera, there was intense staining for type I and less intense staining for type III collagen (Fig. 2). SEM of the pial sheath surrounding the ON at the level of the sclera showed a smooth ring of connective tissue with no extensions into the area of the ON (Fig. 4A). This smooth ring was incomplete where the central retinal artery and vein entered the ON at the level of the sclera. About one sixth of the ring was occupied by these vessels (Fig. 4A).

At the electron microscopic level the fibers of the pial sheath of the ON in the myelinated, the retrobulbar nonmyelinated portion of the nerve and at the level of the sclera consisted of densely packed bundles of collagen arranged oblique and longitudinally to the long axis of the nerve (Fig. 4B). Elastic fibers were completely absent within the pial sheath. Only at the outer surface the sheath of the ON was connected to the elastic fiber system of the choroid and to the chorioidal stroma by radially oriented collagen fibers. Toward the neuronal tissue of the ON the collagen fibers of the sheath were connected to the basement membrane of the astrocytes surrounding the nerve. At places, 5-μm-long extensions of the collagen sheath followed the basement membrane of the astrocyte processes, giving the internal surface of the ring a somewhat wave-like appearance (Fig. 4B).

In the myelinated part of the ON there were no connective tissue septae separating the nerve fiber bundles from each other.
other. Only some collagen fiber bundles continuous with the pial connective tissue followed the entering vessels into the nerve.

**Glial Tissue**

The bundles of both the nonmyelinated and myelinated portion of the ON were surrounded by glial cells. Immunohistochemically, these cells stained intensely for GFAP (Fig. 5A), indicating that they are astrocytes. GFAP-positive cells also covered the ON papilla and extended toward the adjacent retina. At the level of the sclera, elongated processes of astrocytes ran transversally between the nerve fiber bundles (Fig. 5B). Ultrastructurally, only one type of glial cells could be identified in the nonmyelinated portion of the ON. The cells showed a light nucleus and numerous parallel arranged filaments throughout the cytoplasmic processes (Fig. 5C). Near the nucleus, mitochondriae, ribosomes, and rough endoplasmic reticulum were present. The cells contacted each other with numerous gap junctions (Fig. 5C). Staining with a connexin 43 antibody visualizing gap junctions showed positive staining throughout the entire ON and correlated in its distribution with the staining for GFAP-positive cells.

Only in the myelinated portion of the ON, cells stained for carbonic anhydrase II (Fig. 6A). The staining pattern was different from the GFAP staining in this region. Whereas GFAP-positive cell processes were mainly seen surrounding nerve fiber bundles, carbonic anhydrase II staining was only seen around the single axons (Fig. 6B), assuming that oligodendrocytes stained for this enzyme. Ultrastructurally the oligodendrocytes were characterized by their dense nucleus and a cytoplasm with sparse neurofilaments that was continuous with the myelin sheaths of the axons (Fig. 6C).

**Vascular Supply**

In the mouse eye, the ophthalmic artery passed the orbit toward the sclera region of the globe. There, the central retinal artery branched off the ophthalmic artery and obliquely entered the ON through the scleral ring as described above (Fig. 4A). The central retinal vein ran closer to the ON than the artery (Fig. 3). Opposite to the ostium of the central retinal artery, the intima of the ophthalmic artery formed a V-shaped ridge extending into the lumen of the vessel (Fig. 7A). Serial histologic sections revealed that the internal elastic lamina of the artery was interrupted at the level of the V and that a triangular shaped cellular cushion covered by endothelial cells extended into the lumen (Fig. 8). At the electron microscopic level, these cells had the appearance of smooth muscle cells that were connected to each other or to connective tissue containing numerous elastic fibers in contact with the internal elastic lamina of the vessel. No nerve terminals were found in these muscular cushions.

In a short distance from the central retinal artery, still at the level of the sclera, the ophthalmic artery formed two nasally and temporally oriented major ciliary arteries. In the vicinity of the optic nerve, the temporal ciliary artery formed two

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932915/)
FIGURE 4. (A) Scanning electron micrograph of an enzyme digested preparation of the posterior eye segment (magnification, ×200). Note the circular arrangement of collagen fibers (arrows) at the level of the optic nerve (ON). There are no extensions of fibers into the ON region. The arrow marks the region where the central retinal vessels pass the ring to enter the ON. (B) Transmission electron micrograph of the pial sheath of the ON directly behind the sclera (magnification, ×8700). The small longitudinally arranged collagen fibers of the innermost layer of the pia are connected to the basement membrane (arrows) of the astrocytes surrounding the ON. The sheath therefore has a somewhat wave-like appearance.

FIGURE 5. (A) Immunohistochemical staining of the optic nerve (ON) with antibodies against glial fibrillar acidic protein (GFAP; magnification, ×130). Stained cells are present in the ON fiber layer of the retina, in the nonmyelinated, and in the myelinated part of the ON. Arrowhead marks the central retinal vessels entering the ON. (B) Confocal image of the ON at the level of the sclera (GFAP staining, magnification, ×400). Note the transverse-oriented processes of the astrocytes (arrowheads). The nerve fiber bundles between these processes are unstained. (C) Transmission electron micrograph of the transverse-oriented astrocyte processes in the nonmyelinated portion of the ON (magnification, ×10,000). Note the parallel arranged filaments in the cell processes. The processes are connected to each other by gap junctions (arrowhead).

FIGURE 6. (A) Immunohistochemical staining of the optic nerve (ON) head with antibodies against carbonic anhydrase II (magnification, ×130). In the ON, carbonic anhydrase II staining is only present in the myelinated part. In the retina, Müller cells and some amacrine cells are stained. (B) Confocal image of the ON at the transition zone between the myelinated and nonmyelinated portion (carbonic anhydrase II staining; magnification, ×540). Note the parallel staining along the unstained individual axons in the myelinated portion of the ON (arrows). (C) Transmission electron micrograph of an oligodendrocyte (magnification, ×13,000) showing the nucleus and the sparse cytoplasm continuous with the myelin sheaths.
branches, the first one supplying the choriocapillaris of the inferior, the second one the choriocapillaris of the superior quadrant (Figs. 7A, 7B). There were no branches of the ciliary arteries passing toward the optic nerve, and there was no arterial Zinn Haller ring in the mouse eye. The venous blood of the choroid surrounding the ON drained into posterior ciliary veins (Fig. 7B).

The nonmyelinated portion of the ON was supplied exclusively by branches of the central retinal artery. Serial sections showed no connections between these vessels and the choroidal vasculature (Fig. 3). The central retinal artery ran within the center of the intraocular ON without forming any branches. Only at the level of the innermost retina, six to eight branches were formed. From these retinal vessels, two to three capillaries ran nearly vertically backward to supply the nonmyelinated portion of the ON (Fig. 3A). Here, the branches anastomosed with capillaries deriving from pial vessels supplying the myelinated portion of the ON (Fig. 7C).

EM investigation of the vasculature showed, that vessels deriving from the pial arteries were surrounded by a small connective tissue sheath separating the vessel wall from the glial cells surrounding the nerve fiber bundles. No cellular contact was seen between astrocytes and pericytes or vascular endothelial cells. The vessel wall consisted of endothelial cells surrounded by an incomplete sheath of pericytes located within the basement membrane of the capillaries (Fig. 9A). At the transition zone between the myelinated and nonmyelinated portions of the nerve, the connective tissue disappeared, and the capillaries came in direct contact with the glial cells. The basement membrane of the endothelial cells/pericytes and of the astrocytes merged and could no longer be distinguished.

**Figure 7.** Scanning electron micrographs of corrosion cast preparations of the optic nerve head vasculature. (A) At the branching of the central retinal artery, a V-shaped impression is seen in the ophthalmic artery (arrow). The ophthalmic artery then divides into the temporal (t) and nasal (n) major ciliary arteries supplying the choroid (magnification, ×225). (B) Higher magnification of the vessels surrounding the optic nerve (ON) at the level of the choroid and sclera (magnification, ×570). t, temporal; n, nasal major ciliary artery; s, superior ciliary artery; p, posterior ciliary veins. (C) The capillaries in the myelinated portion of the ON (arrows) are in contact with the pial vessels running parallel to the long axis of the nerve (arrowheads). The central retinal vein (V) is located closer to the ON than the artery (A). Magnification, ×900.

**Figure 8.** Light micrograph of the ophthalmic artery at the level of the cushions (magnification, ×320). Note that the internal elastic membrane at the region of the intima cushions is interrupted. The cushions consist of smooth muscle cells.
from each other. The capillary wall itself had the same structural elements as in the postlaminar portion (Fig. 9B).

**DISCUSSION**

Our study clearly shows that the mouse ON shares similarities with the human ON, but also shows profound differences. Similar to the human ON, the intraocular portion of the nerve in the mouse is nonmyelinated, and the axon bundles are surrounded only by astrocytes. Oligodendrocytes occur first in the retrobulbar portion, where myelinization of the nerve fibers start. In contrast to primate eyes, the transition zone between the nonmyelinated and myelinated portions is not located at the level of the sclera, but further posterior, and a LC is completely absent. Instead, layers of astrocyte processes transverse the nonmyelinated nerve at the level of the sclera. The basement membranes of the astrocytes at the rim of the nerve are directly connected to the pial sheath. Because the different subtypes of astrocytes that have been described in the human and primate ON are only present at the level of the LC, the lack of the LC might also explain the uniformity of astrocytes in mice ON.

The absence of an LC in mouse eyes has previously been described by Tansley and more recently by Fujita et al. In a comparative study using different classes of vertebrates, Fujita et al. addressed the question as to whether a lack of LC is correlated with the localization of a transition zone between the myelinated and nonmyelinated portion of the nerve. They did not find a correlation. The problem, what controls a LC formation and differentiation, is not solved. Fujita et al. hypothesized that the presence of a LC depends on the diameter of the nerve. A large nerve sustains more mechanical stress in response to IOP, and LC development could be a response to this stress. However, this cannot be the only explanation, because the rat and rabbit (the latter having an ON much larger than the rat) have similar, sparse connective tissue bundles in the anterior ON.

The role of the LC in ON damage in glaucoma is also not clearly understood. Experimentally induced elevation of IOP in monkey eyes leads to blockage of axonal transport and finally to nerve fiber degeneration. It is not clear how interactions between connective tissue elements and nerve fibers lead to nerve fiber damage in primary open angle glaucoma. Using tracer studies in buphthalmic rabbit eyes, Bunt-Milam et al. found blockage of axonal transport only in those axons directly adjacent to connective tissue beams. A comparison between ON changes in glaucomatous monkey and human eyes with that seen in mouse eyes will provide a useful tool to investigate whether the ON degeneration is due to increased IOP alone or to the IOP-induced LC changes.

Another important difference between the murine and primate ON head is the vascular supply (Fig. 10). In human eyes, most of the area of the LC and the nerve head is supplied by the arterial circle of Zinn and Haller formed by the choroidal arteries. Although in rat corrosion cast preparations the presence of an arterial circle around the optic nerve was described controversially, in mouse eyes no additional arterial circle was found around the ON.

A peculiarity, frequently found in rodent arteries before ramification, is the presence of intra-arterial cushions. They are described in numerous rat organs, including the ophthalmic artery. In humans these arterial cushions were also described in the nasal mucosa, uterus, and penis, but not...
in the ophthalmic artery. In the mouse ophthalmic artery, a v-shaped cushion was regularly present just before the branching of the central retinal artery. The functional significance of these cushions is not clear. They might modify blood flow in the entrance region of branching vessels.

Because the mouse anterior ON is exclusively supplied by vessels from the retina, but not from the choroid, a direct effect of intraocular pressure on ON circulation appears unlikely. Studies in ocular hypertensive mouse models might help to differentiate between direct effects of IOP on ON function and those caused by additional ischemia.

References

8. Quigley HA, Brown A, Dorman-Prase ME. Alterations in elastin of the central retinal artery. The functional significance of these cushions is not clear. They might modify blood flow in the entrance region of branching vessels.

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References