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Cytoskeleton and Tissue Origin in the Anterior Cynomolgus Monkey Eye

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Abstract: We studied cytoskeletal proteins and other markers for embryologic origin in the outflow pathways of the aqueous humor, cornea, sclera, and ciliary muscle of the cynomolgus monkey. The corneal endothelium and trabecular cells stained with markers for vimentin, smooth muscle cell α-actin, F-actin, spectrin, vinculin, and talin. The endothelium of Schlemm's canal stained with markers for vimentin, spectrin, and F-actin. These results suggest that trabecular cells are a kind of myofibroblast and support the belief that the endothelial cells of Schlemm's canal are vascular in origin. Fibrillary staining with antibodies to vimentin, spectrin, neurofilament protein, and glial acid fibrillary protein was observed along and between the ciliary muscle cells. Cells in the deep sclera adjacent to the supraciliary space stained with antibodies to smooth muscle α-actin, α-vinculin, talin, and desmin. These cells may anchor ciliary muscle cells into the sclera or may be developmental remnants of ciliary muscle cells. Leu 19 immunoreactivity was found in the corneal endothelium, in all trabecular cells, in ciliary muscle cells, and in keratocytes and fibroblasts in the superficial part of the cornea and sclera. All of these cells are therefore likely to express neural cell adhesion molecules indicating neuroectodermal origin. **Jpn J Ophthalmol 1997;41:138–149** © 1997 Japanese Ophthalmological Society

Key Words: Ciliary muscle, cornea, cytoskeletal proteins, tissue origin, trabecular meshwork.

Introduction

Several filament-forming proteins form the cytoskeleton of most cells, which is of utmost importance for cell shape and function.[1,2] Two of these, actin and tubulin, are better understood than those of the intermediate filaments, which form intermediate size filaments, about 10 nm in diameter, between those of actin and tubulin.

Actin is present in vascular smooth muscle cells in its alpha isoform and in most cells in its monomeric G and polymeric filamentous F-form. Intercellular cytoplasmic intermediate filaments vary depending on cell origin, and possibly also on function. Five main types have been described: tonofilaments in the epithelia, neurofilaments in neuronal cells, glial filaments in astroglia and related cells, desmin filaments in muscle cells and vimentin in mesenchyme-derived cells. Vimentin filaments or subunits may also be present in cells other than their cell-type class of intermediate filament.[3] Tubulin is present in almost all cells.

Aqueous humor leaving the anterior chamber must first pass through the spaces of the trabecular meshwork and then be drained via Schlemm's canal and collector channels or via uveoscleral routes into the episcleral tissues.[4] There are a number of studies of the outflow routes for aqueous humor and the adjacent tissues showing the presence of actin, intermediate filaments, and tubulin[5–14]; agents affecting the cytoskeleton have been reported to reduce the flow resistance in these routes as a result of effects on their ultrastructure.[15–18] However, there has been no in situ study of the cytoskeleton in the outflow routes, cornea, and ciliary body of monkey eyes.

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The trabecular cells have many functions such as phagocytic activity, regulation of aqueous humor flow by changing their shape, and maintenance of the trabecular sheets. The inner wall cells of Schlemm's canal form invaginations (giant vacuoles) which develop transcellular pores that drain the aqueous humor into Schlemm's canal. Therefore, for functional reasons, different cytoskeletal filaments might be involved in the trabecular cells than in the inner wall of Schlemm's canal.

Schlemm's canal originates from the vascular system in the sclera[19] but it is believed that the anlage of the trabecular cells arises from the neural crest, although this is not yet clarified.[20] Thus, also for developmental reasons, there might be differences in cytoskeletal proteins.

Actin filaments and intermediate-sized filaments were observed by electron microscopy in the trabecular cells and inner wall cells of Schlemm's canal. The α-isoform of actin has been found immunohistochemically in the ciliary muscle and in special cells in the trabecular meshwork, and also in cells adjacent to the outer wall of Schlemm's canal and collector channels.[6] The filamentous form of actin, however, was present in all cells of the outflow routes.[6] Other immunohistochemical studies have demonstrated that vimentin[8] exists in cultured human trabecular cells as well as in trabecular cells in situ.[14] Desmin was reported in human trabecular cells by Iwamoto and Tamura[10] but was not observed in other studies.[12,14] Tubulin has been described in trabecular cells.[12] There are regional differences in both function and ultrastructure in the trabecular meshwork[21]; there may also be differences in origin. The trabecular cells in the anterior part of the trabecular meshwork were intensely stained with an antibody to neuron specific enolase,[22] whereas those in the posterior part were irregularly stained.[20] These results suggested to Tripathi and Tripathi[21] that the trabecular cells in the posterior region of the meshwork in the human eye had lost the ability to express neuron specific enolase, or that they were not derived from neural crest cells.

Avian corneal endothelium has been regarded as a derivative of the primary mesenchyme, which also gives rise to the vascular endothelium.[23] However, by transplanting quail neural crest into homologous regions of host chick embryos it was shown that the corneal endothelium, stromal cells, and ciliary muscle originated from the neural crest.[24] These results were supported by experiments with positive reactions to antibodies to neuron specific enolase in the human corneal endothelium and keratocytes.[20,25] According to these studies, reactivity to antibodies to neuron specific enolase in the keratocytes differed in the various regions suggesting that some of them lost the ability to express neuron specific enolase or that they were of mixed origins.[25]

CD56 and CD57 are newly defined clusters of leukocyte differentiation[26] antigens corresponding to the NK3 cell-associated molecules NKH-1 and HNK-1, respectively. CD56 and CD57 are related to the neural cell adhesion molecule N-CAM[27,28] and antibodies including Leu19 and Leu7, respectively. Recently, N-CAM, Leu7 and Leu19 antigens were detected in cells of neuroectodermal origin,[26,29,30] therefore, these markers for detecting neuroectodermal origin were used to investigate the origin of the anterior segment of the monkey eye.

Materials and Methods

Four adolescent cynomolgus monkeys (Macaca fascicularis) were used in this study. Animals used in this study were treated in accordance with the ARVO resolution on the care and handling of animals used in vision and ophthalmic research. The monkeys were killed by an overdose of pentobarbital sodium, 120 mg/kg body weight. From 2, the anterior segments of both eyes were removed, cut into quadrants and frozen in chilled isopentane. In 2 monkeys, the anterior chambers were perfused with periodate-lysine-paraformaldehyde (PLP) in one and 10% buffered formalin in the other, for 30 minutes before death. After removal, the anterior parts were separated and immersed in the same fixative for 3.5 hours. During fixation, the anterior part was dissected into small pieces. Specimens were washed in 0.1 mol/L Sörensen's phosphate buffer overnight; two specimens were then processed for paraffin embedding and the rest were frozen in isopentane that had been chilled with liquid nitrogen. Five micrometer serial meridional sections were cut by a Leitz or Leinert Cryostat at 20–25°C and fixed in acetone at 20°C for 5 minutes before being air dried. Meridional sections were made from all monkey specimens and tangential sections were made from the specimens that were fixed with formalin, in order to observe a wide range of the trabecular meshwork and Schlemm's canal. The sections were collected on objective glasses and then air dried. Specimens embedded in paraffin were cut in serial sections about 3 μm thick. Trypsin digestion preceded staining. The primary antibodies used are listed in Table 1.

The standard indirect immunofluorescent techniques or the peroxidase antiperoxidase (PAP)

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method was used. Secondary fluorescent antibodies and/or PAP reagents were obtained from Dakopatts, Copenhagen, DK. Rhodamine-conjugated phalloidin was used to detect F-actin. The sections were examined in a Leitz Orthoplane microscope or an Olympus Vanox microscope equipped with epifluorescence.

Results

Immunoreactivities are summarized in Table 2.

Vimentin

Endothelial cells of Schlemm's canal and trabecular cells stained with antivimentin (Figures 1a–c). In the cryosections, different antivimentins gave the same results. However, in paraffin sections stained with vimentin 3B4 antibodies, all trabecular cells and the endothelium of Schlemm's canal showed strong staining while, in those stained with vimentin 9, the trabecular cells showed no or only faint staining (Figure 1c). In the inner wall cells of Schlemm's canal, strong staining was observed close to the nuclei and at the invaginations (Figure 1c). Corneal endothelium and keratocytes (the stromal fibroblast in the cornea) were strongly stained with all antivimentin antibodies (Figures 1a,b). Because keratocytes are flat and run parallel to the corneal curvature, tangential sectioning was very useful for showing their cytoskeleton. Staining with antivimentin antibodies was observed in the foot plates of the cytoplasm of keratocytes where they attached to the neighboring keratocytes (Figure 1d). Fibroblasts in the sclera were also strongly stained with antivimentin; however, there was no close relationship between neighboring fibroblasts in the sclera in tangential sections.

The blood vessels in the sclera and the nerve fibers in the ciliary muscle were stained. Ciliary muscle cells did not stain but staining was observed between the cells (Figure 1b).

Desmin

The antibody to desmin reacted with the ciliary muscle cells (Figures 2a–c) and the muscular wall of blood vessels (Figures 2a,d). Trabecular cells and the

Table 1. Panel of Antibodies and Ligands Used in the Present Study

Antibodies Against Intermediate Filament Proteins	Source
Vimentin	
Vim 9	Monosan, Sanbio, The Netherlands
Vim 3B4	Boehringer Mannheim, Germany
Polyclonal	IsmoVirtanen, Helsinki, Finland
Desmin	
D33	Dakopatts, Copenhagen, Denmark
37EH	Locus, Helsinki, Finland
Keratin	
PKK1 (cytokeratins 8, 18, 19)	Locus, Helsinki, Finland
PKK2 (cytokeratins 7, 17, 19)	Locus, Helsinki, Finland
PKK2 (cytokeratin 18)	Locus, Helsinki, Finland
Neurofilament	
NF clone 2F11	Dakopatts, Copenhagen, Denmark
Glial fibrillary acid protein	
GFAP	Dakopatts, Copenhagen, Denmark
Cytoskeleta 1 associated proteins	
Spectrin	Locus, Helsinki, Finland
α-spectrin clone 1A1	Locus, Helsinki, Finland
Vinculin clone FB11	Locus, Helsinki, Finland
Talin clone 8d4	Sigma Immunochemicals, St. Louis, MO, USA
Microfilament	
α-smooth muscle actin	Sigma Immunochemicals, St. Louis, MO, USA
F-actin	Molecular Probes, Eugene, OR, USA
Rhodamine-phalloidin	Molecular Probes, Eugene, OR, USA
Microtubules	
β-tubulin code N1 357	Amersham, England
Others	
Leu 19(CD56)	Becton Dickinson, Mountain View, CA, USA
Leu 7 (CD57)	Becton Dickinson, Mountain View, CA, USA
Neuron-specific enolase (NSE) code no A589	Dakopatts, Copenhagen, Denmark

endothelium of Schlemm's canal were negative, but there were occasional spotty, positive reactions in the outer wall of Schlemm's canal and in the trabecular meshwork (Figure 2c) that were restricted to the area of the ciliary muscle and varied in number in the sections examined. There were thick bands of these positive cells in some sections but almost no reaction was found in other sections. The arteries in the sclera were more strongly stained than the veins (Figure 2d).

Keratin

The corneal epithelium stained with all types of antibodies to keratin. With PKK2, 3, or 4 deep layers of corneal epithelium stained more strongly than the superficial layers. With PKK3, there was no difference in the staining pattern of the different layers. The staining pattern of PKK1 differed from the others: there was positive staining in the corneal endothelium, ciliary muscle, and nonpigmented epithelium (not shown).

Neurofilament (NF) and Glial Fibrillary Acidic Protein (GFAP)

Nerve fibers in the ciliary body were stained with antibody to NF and GFAP. Fibrillary staining was observed along the ciliary muscle bundles. The cornea, trabecular meshwork, and Schlemm's canal were negative to the NF and GFRAP antibodies.

Actin

The smooth muscle α-actin antibody stained the corneal endothelium, ciliary muscle and blood vessels (Figure 3a). Trabecular cells were faintly positive (Figure 3b), but the endothelium of Schlemm's canal did not stain. In the deep sclera adjacent, a number of slender cells adjacent to supraciliary spaces were stained in some sections (Figures 3a–c); positive reactions were restricted to the area close to the ciliary muscle. Arteries and veins in the sclera were easily differentiated by staining intensity (Figure 3d). Rhodamine phalloidin stained filamentous F-actin in the endothelium of Schlemm's canal, the

Table 2. Immunoreactivities

	Schlemm's Canal		Trabeculum	Cornea			Sclera	Ciliary Body		Nerves	
	Inner Wall	Outer Wall	Trabecular Cell	Epithelium	Keratocytes	Endothelium	Fibroblast	Ciliary Muscle	Blood Vessels	Axon	Schwann
Vimentin											
vim 9	+	+	+	−	+	+	+	−[a]	+	−	+
vim 3B4	+	+	+	−	+	+	+	−[a]	+	+	−
Desmin											
D33	−	−(+)	−(+)	−	−	−	(−,+)[b]	+	+−	−	−
37EH	−	−(+)	−(+)	−	−	−	(−,+)[b]	+	+	−	−
Karatin											
PKK1	−	−	−	+	−	±	−	+	−	−	−
PKK2,3	−	−	−	+	−	−	−	−	−	−	−
Neurofilament	−	−	−	−	−	−	−	−[a]	−	+	−
GFAP	−	−	−	−	−	−	−	−	−	−	+
Spectrin	+	+	+	+	+	+	+	−[a]	+	−	+
Vinculin	±	±	±	±	−		(−,+)[b]	+	+	−	−
Talin	+	+	+	±	−	+	(−,+)[b]	+	+	−	
Actin											
α-smooth muscle actin	−	−	±	−	−	+	(−,+)[b]	+	+	−	−
F-actin	+	+	+	+	±	+		+	+		
Tubulin											
β-tubulin	+	+	+	+	+	+	+	+	+	+	+
Leu 19 (CD 56)	−	−	+	−	(−,+)[c]	+	(−,+)[c]	+	−	−	+
NSE	−	−	±	−	±	+	−	+	−	+	−
Leu 7 (CD57)	−	−	−	−	−	−	−	−	−	−	+

In this list, the results of paraffin sections (see RESULTS) are discarded. ±: faintly positive; (−,+): some were negative and the other were positive; −(+): occasional dotty positive reaction.

[a] Fibrillary positive staining along the ciliary muscle.

[b] Positive reactions were restricted in the deep sclera adjacent to the supraciliary spaces.

[c] Keratocytes and fibroblasts were positive only in one-third or half of the superficial part of cornea and sclera.

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trabecular cells (Figure 3e), the corneal epithelium, keratocytes, corneal endothelium, fibroblasts in the sclera, ciliary muscle, and the blood vessels. In tangential sections, the area around Schlemm's canal, the trabecular meshwork, and the ciliary muscle were stained (Figure 3f).

Spectrin

The endothelium of Schlemm's canal and the trabecular cells in the anterior part of the meshwork stained with antispectrin (Figure 4). The corneal endothelium, corneal epithelium, keratocytes, and fibroblasts in the sclera and blood vessels were strongly stained with antispectrin. Positive reactions in the corneal epithelium were observed at the periphery of the cytoplasm. The superficial layer of the corneal epithelium was more intensely stained than the deeper layers. There was positive fibrillary staining around the ciliary muscle cells, resembling the staining pattern of antivimentin.

Vinculin and Talin

Antibodies to vinculin (α-vinculin) stained ciliary muscle and blood vessels. Trabecular cells (Figure 5) and the endothelium of Schlemm's canal showed

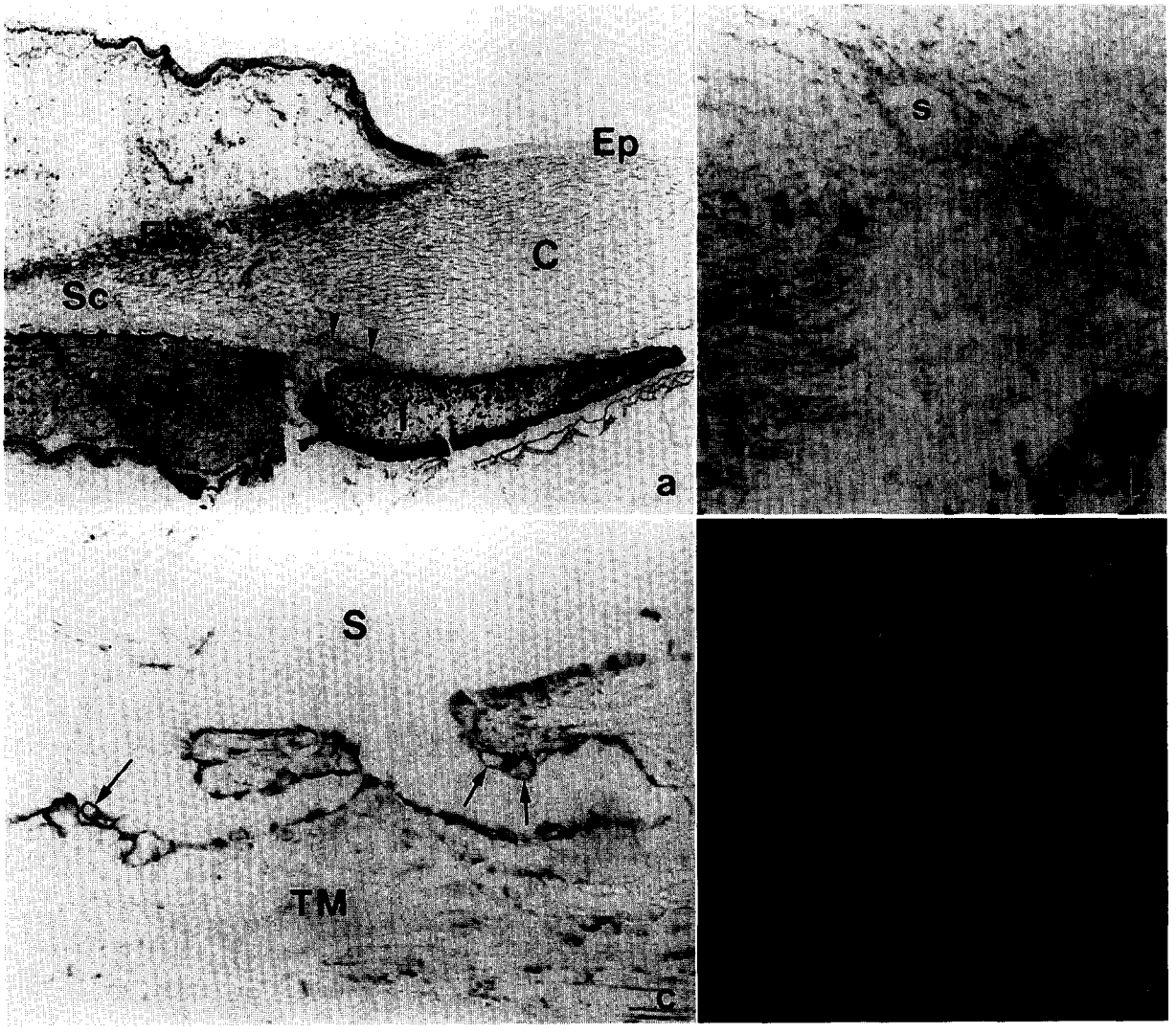

Figure 1. Vimentin immunoreactivity. (**A–C**) PAP staining, (**D**) FITC. (**A**) Meridional cryosections stained with mAB vim 3B4. Note staining in cornea (C), sclera (Sc), episclera (ES), ciliary muscle (CM) and trabecular meshwork (arrow heads); corneal epithelium (EP) is unstained. I, iris. ×10. (**B**) Enlarged area of (**A**). Trabecular cells (T) and endothelium of Schlemm's canal (S) showed positive reaction. Positive fibrillary staining along the CM fibers can be seen. ×50. (**C**) Meridonal paraffin section stained with mAB vim. The endothelial lining of S showed positive reaction and the lining of invaginations (arrows) were also stained. TM: trabecular meshwork. ×200. (**D**) tangential section stained with polyclonal Ab to vimentin. Vimentin positive keratocytes (arrows) connected at foot plates. ×200.

positive or faintly positive reactions. There were no regional differences in intensity in the trabecular meshwork. The corneal epithelium stained faintly, whereas the basal cells were clearly positive in the basal portion facing Bowman's membrane. Keratocytes in the cornea and fibroblasts in the sclera were faintly positive or unstained, but cells in the deep sclera adjacent to the supraciliary space near the ciliary muscle stained strongly in some sections (Figure 5). The staining pattern of talin was the same as the vinculin pattern (not shown).

Tubulin

Tubulin was identified in all cells of the areas examined.

Neuron Specific Enolase (NSE), Leu 7 (HNK-1), N-CAM, and Leu 19 (NKH-1)

NSE antibody stained the corneal endothelium, ciliary muscle, and nerve fibers; however, all the trabecular cells and keratocytes were only faintly positive, or negative. Leu 7 seemed to be expressed only in the nerve fibers of the ciliary body. The trabecular cells were strongly stained with anti-Leu 19 and there were no regional differences (Figures 6a,b). The ciliary muscle (Figures 6a,b) and the corneal endothelium (Figure 6c) were also stained. Keratocytes in the superficial half or third of the cornea (Figure 6c) and fibroblasts in the superficial half or third of the sclera were also stained (Figure 6a), whereas at the limbus, fibroblasts of the whole thickness were

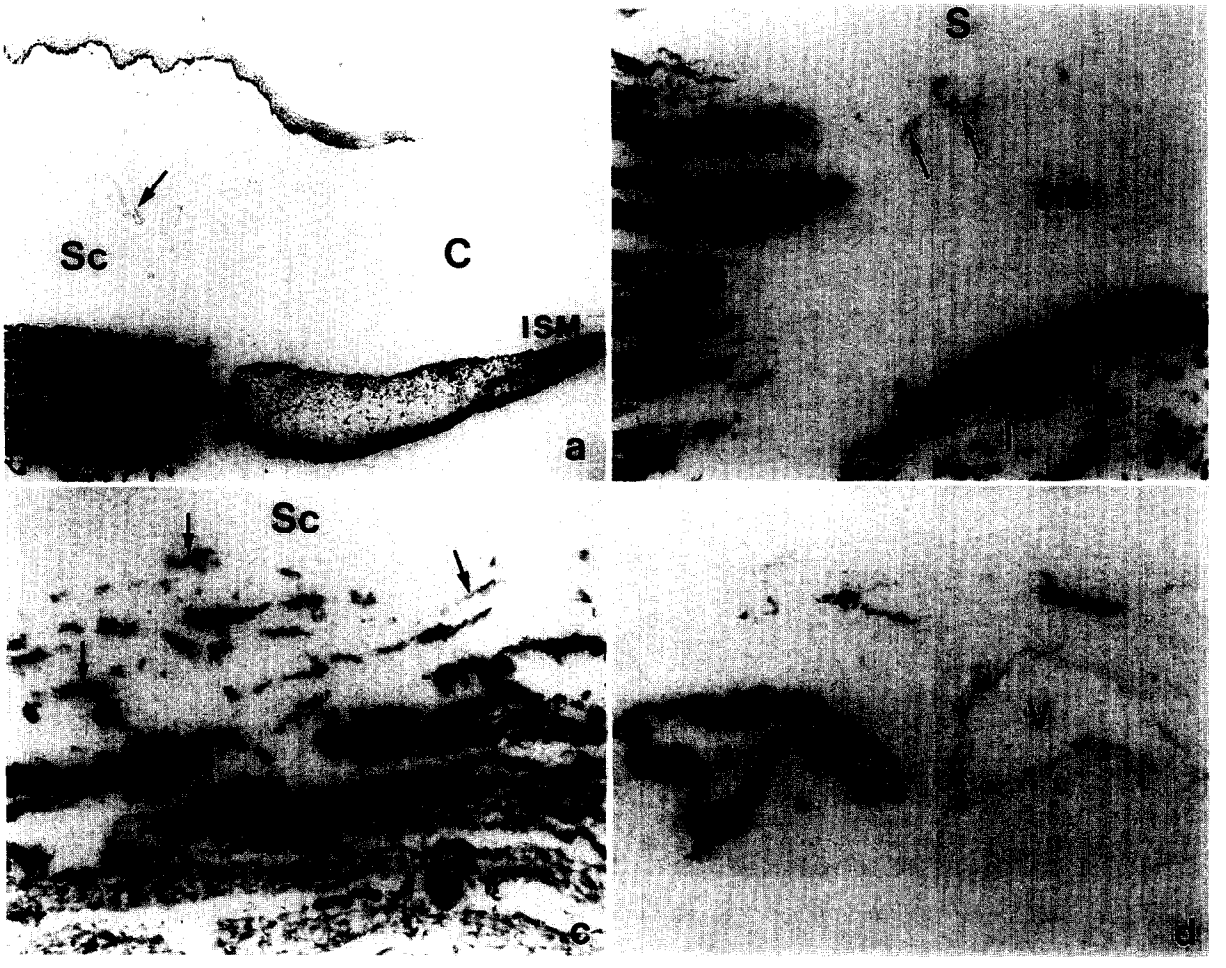

Figure 2. Desmin immunoreactivity. Meridional sections stained with mAb D33 (**A**,**B**) and mAb 37EH (**C**,**D**) and with PAP staining. (**A**) The ciliary muscle (CM), the iris sphincter muscle (ISM), and blood vessels (arrow) in the sclera (Sc) were stained. ×10. (**B**) Higher magnification of the transitional zone between CM fibers and the trabecular meshwork (TM) is shown. In the latter occasional dots of positive reaction (arrows) were observed. These were not related to melanocytes. I, iris. ×66. (**C**) Desmin immunoreactivity was seen not only in the CM fibers but also in some cells in the deep sclera (arrows). These positive cells were found only close to the ciliary muscle. Sc, sclera. ×100. (**D**) Note difference in degree of staining of the walls of an artery (A) and vein (V) in the sclera. ×100.

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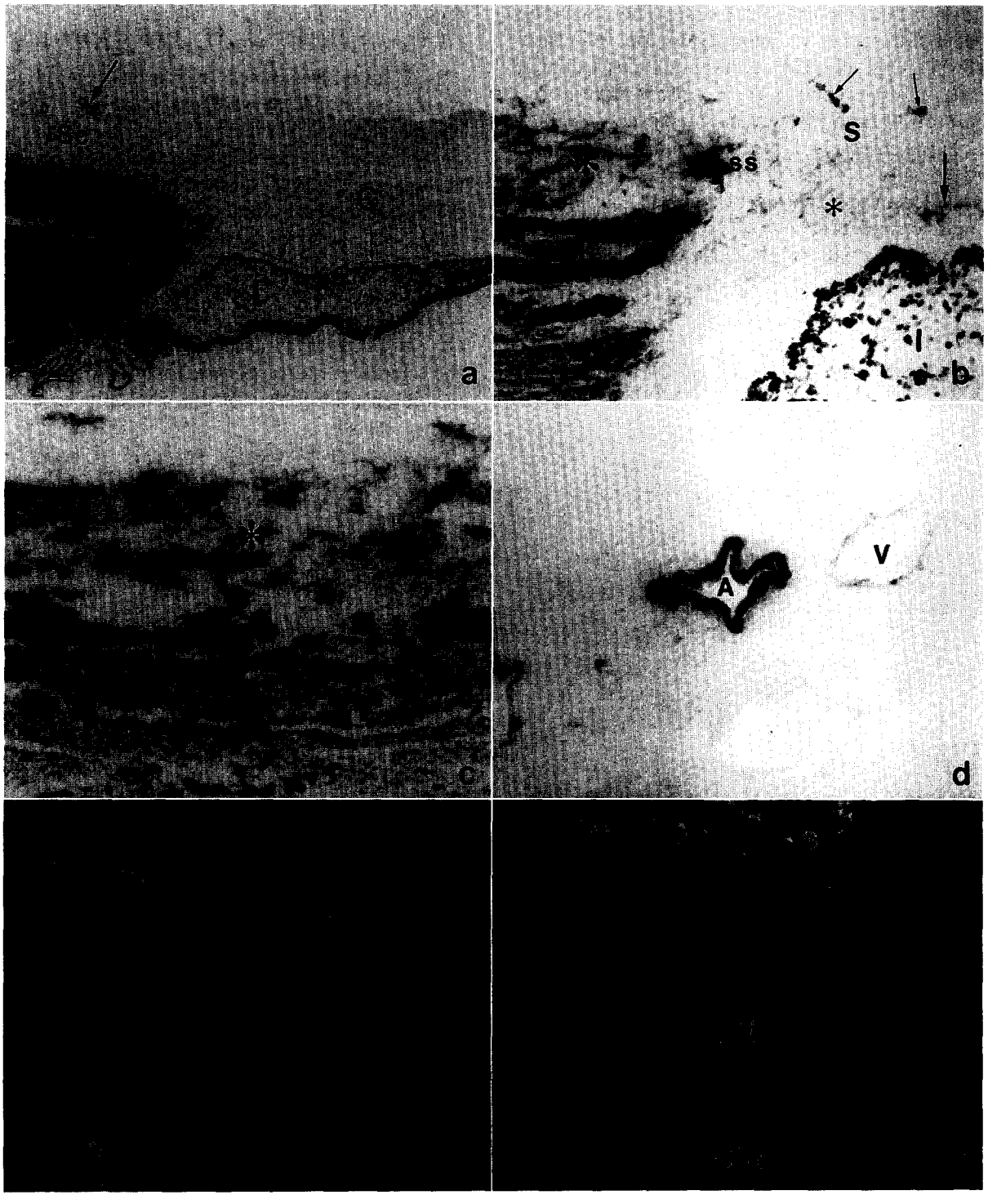

Figure 3. Immunoreactivity of α-smooth muscle actin. (**A–D**) PAP staining and (**E–F**) staining of F-actin with rhodamine-phalloidin. (**A–E**) are meridional sections whereas (**F**) is a tangential section. (**A**) α-smooth muscle actin was detected in the blood vessels (large arrow) of sclera (Sc), ciliary muscle fibers (CM), and corneal endothelium (small arrows). Note also positive reaction in the deep sclera along the ciliary muscle (asterisk). C, cornea; I, iris. ×10. (**B**) A higher magnification of (**A**). A positive staining (large asterisk) was clearly seen in the deep sclera adjacent to the CM. Corneal endothelium was clearly positive (arrow). Trabecular cells showed faint positive reaction. The dots close to Schlemm's canal (S) were related to melanocytes (small arrows). ss, scleral spur; I, iris. ×50. (**C**) The cells in the deep sclera adjacent to the supraciliary space showed positive reaction (asterisk). ×100. (**D**) The walls of an artery (A) and of a vein (V) in the Sc differed in staining intensity as was observed with antidesmin (cf Figure 2d). ×80. (**E**) Trabecular meshwork cells (TM) and the endothelium of S showed positive reaction. ×25. (**F**) The area surrounding S, TM, and the CM showed positive reaction. ×10.

positively stained (Figure 6b). Keratocytes and fibroblasts in other parts of the cornea and sclera did not stain. N-CAM was detected in the trabecular meshwork, corneal endothelium, and nerve fibers of the ciliary body (not shown). All keratocytes in the cornea were negative, with anti-N-CAM (not shown).

Discussion

Inner Wall of Schlemm's Canal

Recent studies indicate that, embryologically, Schlemm's canal is an outgrowth of the intrascleral veins; the endothelium has Weibel Palade bodies and factor VIII-related antigen as expected from this origin.[19] Our finding that the endothelium did not stain with anti-Leu 19 supports this theory. The wall of Schlemm's canal differs from ordinary blood vessels, having a higher hydrostatic pressure on the outside than on the inside. The pressure gradient, therefore, tends to dissect the endothelial cells from the basement membrane rather than holding them against the membrane as in ordinary vessels. A mechanically strong cytoskeleton may help to maintain the integrity of the inner wall and its attachment to the subendothelial tissue. However, the cytoskeleton has to allow a particular function of these endothelial cells, that is, the formation of invaginations that create pores opening into the Schlemm's canal.

The F-actin and the membrane-linked molecules vinculin and spectrin, which seem to anchor cytoskeletal filaments to the plasma membrane, may be as important as vimentin for the integrity of the inner-wall cells. Vimentin filaments, much more stable than actin filaments and microtubules, were part of the cytoskeletal support even in the walls of the invaginations.

The importance of the cytoskeleton in the inner wall endothelium has been illustrated in experiments with agents that disrupt the cytoskeleton and its attachment to the plasma membrane. Na_2-EDTA,[15] α-chymotrypsin,[16] cytochalasin B,[14] and ethacrynic acid[17,33] all affect the inner wall of Schlemm's canal, causing ruptures of intercellular junctions and a reduction in outflow resistance. The desmin positive cells in the outer wall suggest the presence of contractile cells. Evidence for such cells containing smooth muscle myosin was recently reported.[11]

The Trabecular Meshwork

The trabecular cells covering the trabecular beams, as well as those in the juxtacanalicular region, are subjected to mild mechanical stress caused by the flow of the aqueous humor. Such stress is also likely to occur as a result of contractions of the ciliary muscle, which tend to cause inward movements of the whole meshwork. Observations with transmission electronmicroscopy have indicated that intermediate filaments are abundant in the endothelium of Schlemm's canal and in the outermost uveal meshwork, but less frequent in outer parts of the trabecular meshwork.[7] It was somewhat unexpected, therefore, that staining for vimentin was similar in the

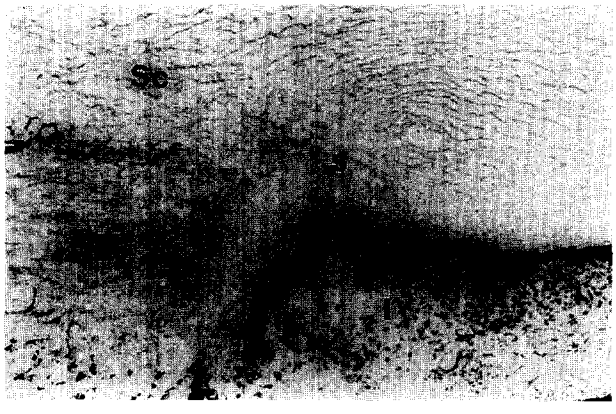

Figure 4. Spectrin immunoreactivity. PAP staining of a meridional section. Endothelium of Schlemm's canal (S) and keratyocytes in the sclera (Sc) showed positive reaction. Anterior part of trabecular meshwork stained more strongly than posterior part of the meshwork. Fibrillary positive staining in the ciliary muscle (CM) was very similar to the results with antivimentin (cf Figure 1b). I, iris. ×25.

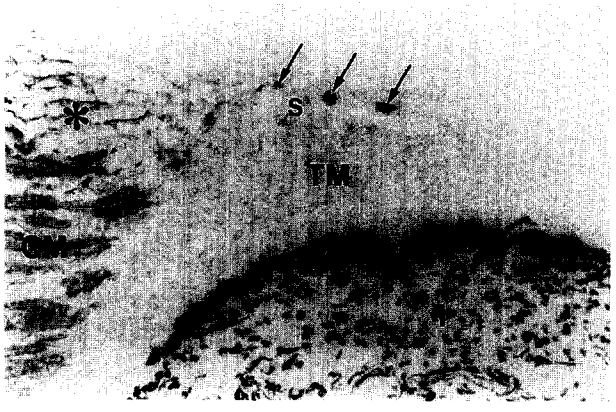

Figure 5. Antivinculin and PAP staining of a meridional section. This section was adjacent to the section of Figure 3b. Melanocytes (arrows) were also seen in the outer wall of Schlemm's canal, as were observed in Figure 3b. The staining pattern in the trabecular meshwork (TM) and ciliary muscle (CM) was very similar to that of α-actin (see Figure 3b). Positive reaction in the deep sclera (asterisk) was observed close to the scleral spur as observed with antismooth muscle α-actin (Figure 3b). S, Schlemm's canal; I, iris. ×50.

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endothelial cells of Schlemm's canal and in all the different parts of the trabecular meshwork.

The origin of the trabecular cells still is not clear. Evidence for a neural crest origin was discussed by Hayashi et al[25] and Tripathi and Tripathi.[20] Neuron-specific enolase detected in the trabecular cells favors such an origin. However, staining with antibodies to neuron-specific enolase was intense only in the anterior termination of the trabecular meshwork of the human eye.[20] In the monkey eyes of the current study, trabecular cells were only faintly positive, or negative. The presence of vimentin cannot be used as proof for mesodermal origin of the trabecular cells because vimentin can be expressed in addition to the cell type-specific intermediate filament. However, no other known cytoplasmic intermediate filament proteins could be demonstrated in the majority of the trabecular cells. The Leu 19 antigen is an Mr 22 000/140 000 cell surface glycoprotein[27] corresponding to the NKH-1 molecule.[34] It has recently

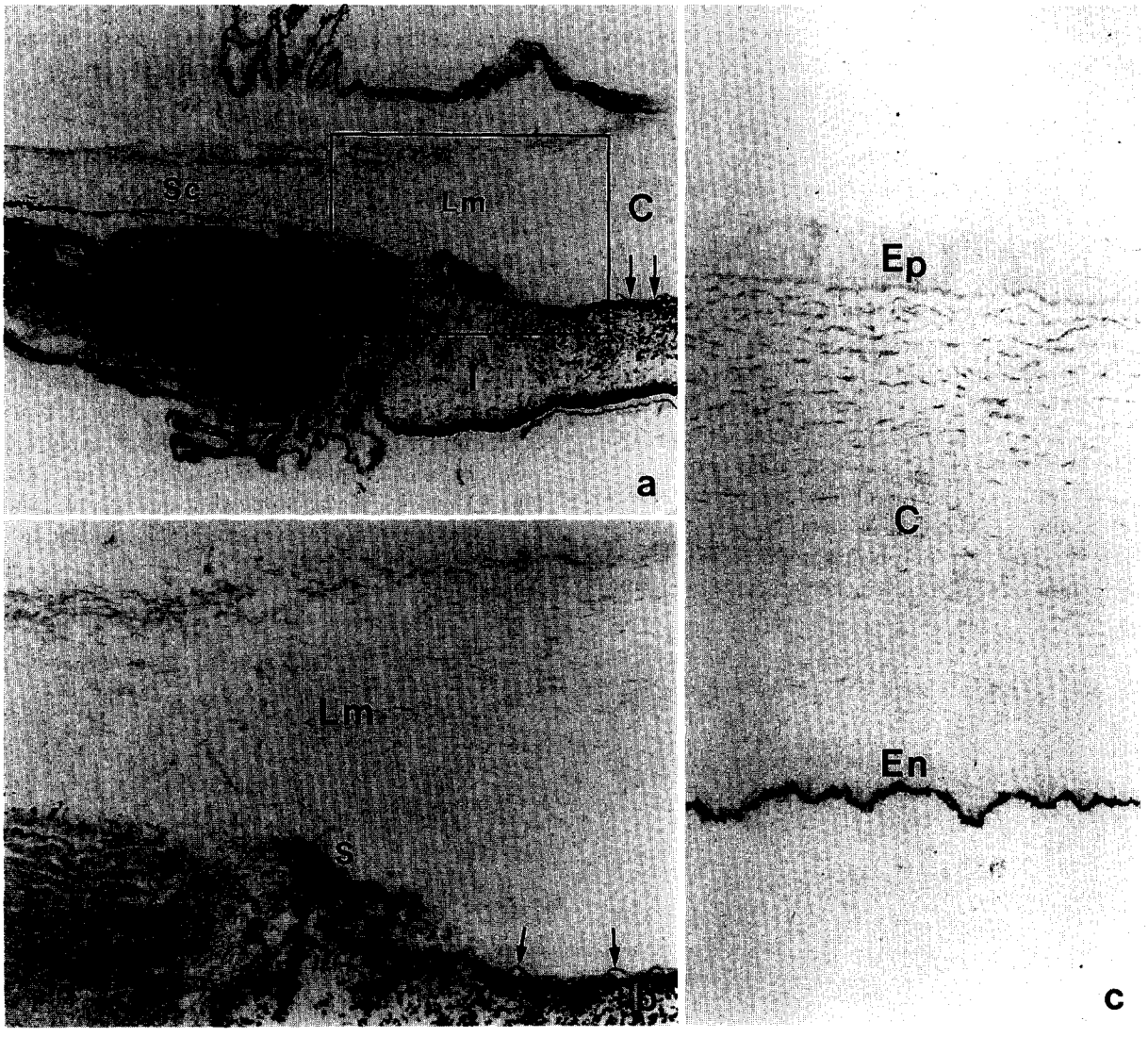

Figure 6. Immunoreactivity of anti-Leu 19. (**A**) PAP stained meridional section. Leu 19 was detected in the endothelium (arrows) of the cornea (C), ciliary muscle (CM), cells in the whole trabecular meshwork (TM), and keratocytes and fibroblasts in one-third or half of the superficial part of the cornea and sclera (Sc). (**B**) Enlarged view of the boxed area in (**A**). There seemed to be no regional staining differences in the trabecular meshwork. However, fibroblasts in the whole thickness of the limbus (Lm) region showed positive reaction. Arrows show the positive reactions of corneal endothelium. (**C**) Cornea with corneal epithelium (Ep) and corneal endothelium (En). At some distance from the limbus keratocytes in one-half or one-third of the superficial part of c showed positive reaction. En also showed positive staining. S, Schlemm's canal; I, iris. ×50.

been found that Leu 19 is expressed on non-neoplastic cells of neuroectodermal origin as well as on several mesenchymal cells.[29] N-CAM is also an integral membrane glycoprotein and mediates Ca^{2+}-independent homophilic intercellular binding.[30] In normal states, N-CAM is present in all three germ layers during early development;[35] however, in the adult, it is mainly restricted to neural cells.[30] The staining of the trabecular cells for Leu 19 and N-CAM is interesting in this context. The results of positive staining of all the trabecular meshwork with antibodies to Leu 19 and N-CAM suggested that all the trabecular cells may originate from the neural crest.

Immunoreactivity of Leu 7 in the conduction tissue cells of the rabbit heart suggested they originate from a population of neural crest-derived cells migrating from the branchial arches into the developing heart.[36] However, in our present study, Leu 7 positive cells were found only in the nerve fibers. Smooth muscle cell, myosin-containing cells were found in the trabecular meshwork by de Kater et al[4]; smooth muscle α-actin was also found recently in specific cells throughout the human trabecular meshwork.[12] In our material, although there was a positive reaction with antibodies to desmin in a few scattered cells in the outer wall of Schlemm's canal and the trabecular meshwork, the trabecular cells and endothelium of Schlemm's canal were mostly negative. Occasional desmin positive cells in the trabecular meshwork may be developmental remnants and have little physiological importance. On the other hand, there was a weakly positive reaction indicating presence of smooth muscle α-actin in the trabecular cells and, although reactions with antibodies to talin and vinculin were not as strongly positive as in the ciliary muscle cells, they were clearly positive. These results are similar to observations for myofibroblasts.[37]

The anti-actin drug cytochalasin B has been reported to cause marked changes in the ultrastructure and shape of the trabecular cells in cell cultures, whereas drugs affecting the microtubules and vimentin filaments had little effect on such cells. It is possible, however, that under the more stressful in situ conditions these elements may be more significant. After the anterior chamber injection of ethacrynic acid, there is an increase of outflow facility that seems to correlate with altered β-tubulin staining.[17] Under in vivo conditions, cytochalasin B has marked effects on the ultrastructure of the meshwork cells[14] and the outflow facility is increased drastically[10], indicating changes in the outer parts of the meshwork where most of the resistance is located. This indicates that F-actin is important for the integrity of the trabecular cells and their attachment to the trabecular beams. Similar changes in cell shape and outflow facility are caused by Na_2EDTA[15] and α-chymotrypsin.[16] With these agents, there was dispersion of the microfilaments in the trabecular cells and detachment of the cells from the trabecular beams. Structural changes and increased outflow facility has also been reported after treatment with chondroitinase ABC[8]; these effects seemed to be due to disarray of extracellular material in the outflow routes, possibly also affecting cytoskeletal structures.

Ciliary Muscle

Ciliary muscle cells stained with markers for smooth muscle α-actin, F-actin, vinculin, talin, and desmin as expected. There was fibrillary staining with antibodies to vimentin, neurofilament, and glial fibrillary acidic protein along and between the muscle cells, presumably due to staining of fibroblasts, melanocytes, and nerve cells. In the scleral spur, there were circularly oriented and spindle-shaped cells that were positive with α-smooth muscle actin, myosin, and vimentin.[38] However, in our present study, positive staining with antibodies to smooth muscle α-actin, desmin, and vinculin was observed not only in the scleral spur but also in the sclera adjacent to the ciliary muscle, suggesting that the anchoring sites of the longitudinal part of the ciliary muscle may be in that part of the sclera. However, it is also possible that the positive cells may represent developmental remnants of smooth muscle cells. The staining of the ciliary muscle cells for neuron-specific enolase and Leu 19 is in agreement with the opinion that ciliary muscle cells are derived from the neural crest.[24]

Cornea and Sclera

As expected from earlier studies of the corneal epithelium, we found evidence for keratin and F-actin and also for α-spectrin. One of the antibodies to keratin, PKK 1, also reacted with corneal endothelium, ciliary muscle, and the nonpigmented epithelium of the ciliary processes. The reaction was weak and these tissues did not react with the other antibodies to keratin, PKK2, and PKK3, indicating that the reaction to PKK 1 was nonspecific. Reaction to keratin antibodies in the endothelium has also been found in other studies[9,39] while Risen et al[40] found no reaction, perhaps because different antibodies recognizing different cytokeratins were used.

In the corneal endothelium, there were strong positive antibody reactions to vimentin and α-vincu-

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lin, and clear reactions for smooth muscle α-actin and F-actin. In a recent study, antibodies to smooth muscle α-actin did not react with keratocytes, epithelial cells, and endothelial cells in the normal rabbit cornea.[41] That result, which differs from our study, may be caused by different reactivity in the different animals or by specificity of the antibodies for different animals.

There has been some discussion about the origin of the corneal endothelium. Studies of the development of the avian corneal endothelium have indicated an origin from the vascular mesenchyme.[23] However, by transplanting labeled neural crest or mesoderm cells into unlabeled host embryos, neural crest cells were found to form all of the skeletal and connective tissues adjacent to the medial, nasal, inferior, and lateral parts of the eye, including the endothelial and stromal cells of the cornea and much of the orbit.[24] Investigations with NSE in the human eye have also indicated that the corneal endothelium and keratocytes originate from the neural crest.[20,25] In the present study, we found only very weak positive reactions with NSE, but the reaction to Leu 19 antibodies was strong, indicating that the origin is neuroectodermal rather than mesodermal. The reaction to Leu 19 antibodies in the keratocytes and fibroblasts of the cornea and anterior sclera suggests a mixed origin of these cells; only the cells of the superficial half or third had positive reactions and are likely to be of neuroectodermal origin.

In conclusion, it would seem from the distribution of the Leu 19 immunoreactivity that neuroectodermal cells give rise to the ciliary muscle, the trabecular cells, and the corneal endothelium. Of these, only the ciliary muscle and corneal endothelium express the neural crest marker, NSE, strongly. The intermediate filament characteristic for smooth muscle, desmin, is expressed in the ciliary muscle, a few adjacent cells in the sclera, and scattered cells in the trabecular meshwork and outer wall of Schlemm's canal. The smooth muscle α-actin is expressed in the muscle as well as in the corneal endothelium and, to some extent, also in the trabecular cells. The intermediate filament characteristic for the corneal endothelium, vimentin, is also expressed in the trabecular meshwork. The trabecular cells seem to be a kind of myofibroblast. The wall of Schlemm's canal, finally, is of vascular mesodermal origin.

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