Xyloside-Induced Disruption of Interphotoreceptor Matrix Proteoglycans Results in Retinal Detachment

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Unique domains of the retinal interphotoreceptor matrix (IPM), termed cone matrix sheaths, are composed largely of chondroitin 6-sulfate proteoglycan in most higher mammalian species. Recent investigations suggest that cone matrix sheaths participate in the maintenance of normal retinal attachment. To investigate the potential functional roles of IPM proteoglycans further, the synthesis of cone matrix sheath chondroitin 6-sulfate proteoglycan was perturbed in vivo. Intravitreal injections of p-nitrophenyl-β-D-xylopyranoside (xyloside), a sugar that inhibits chondroitin sulfate proteoglycan synthesis, were administered to Yucatan micropigs. Their eyes were examined funduscopically and electroretinographically. At selected times, the eyes were enucleated and examined histochemically and immunohistochemically with various probes directed against cone photoreceptor cells and cone matrix sheaths. The IPM was affected selectively after xyloside administration; no inner retinal pathology or dysfunction was detected morphologically or electroretinographically. The degree of xyloside-induced perturbation was dependent on the duration of xyloside exposure and dose. It was classified into three stages, based on morphologic and histochemical criteria. Although all three stages could be observed in a given retina, a single stage typically predominated, depending on the particular dosage regimen. The early stage was characterized by IPM disruption, as evidenced by disorganization of chondroitin 6-sulfate- and peanut agglutinin (PNA)-binding glycoconjugates. Cone photoreceptor cell outer segment degeneration and markedly decreased chondroitin 6-sulfate immunoreactivity distinguished the middle stage. During the late stage, there was a near complete absence of both immunoreactive chondroitin 6-sulfate and PNA-binding glycoconjugates in the IPM. Shallow retinal detachments that appeared funduscopically as patches of retinal whitening frequently were observed after moderate durations of xyloside exposure; these progressed peripherally with continued xyloside exposure. Histologically, the areas of retinal whitening corresponded to regions in which cone matrix sheaths were split transversely (ie, in a plane perpendicular to the longitudinal axes of the photoreceptor cell outer segments) or were separated completely from cone outer segments. Similar effects were not observed in control eyes. These results suggest that adhesion between the neural retina and retinal pigmented epithelium may be dependent, in part, on continuous synthesis of cone matrix sheath-associated proteoglycans and, potentially, other IPM proteoglycans. In addition, these proteoglycans appear to be necessary for the maintenance of cone photoreceptor cell outer segment integrity.

The molecules that occupy the retinal interphotoreceptor space, collectively termed the interphotoreceptor matrix (IPM), are situated uniquely between the apices of two neuroectodermally derived epithelia, the neural retina, and the retinal pigment epithelium (RPE). There is a consensus that these molecules mediate interactions between (1) photoreceptor, RPE, and Müller cells and (2) the choroidal vasculature. However, the specific functional roles of most of these constituents, especially those of recently described proteoglycans, are not understood fully.

Evidence for compartmentalization of some IPM-associated molecules has been provided by lectin histochemical and antibody immunohistochemical studies. Photoreceptor cell-specific domains of IPM glycoconjugates have been identified based on selective binding of peanut agglutinin (PNA) and wheat germ agglutinin. The PNA-binding molecules are located specifically in IPM domains termed “cone matrix sheaths,” that surround cone, but not rod, photoreceptor outer segments and ellipsoids in various species, including humans.
chemical and biochemical investigations have shown that chondroitin 6-sulfate glycosaminoglycan is also a specific structural component of cone matrix sheaths\(^3\) and that it is part of a larger, membrane-associated proteoglycan or proteoglycan aggregate that cannot be extracted by aqueous buffers.\(^3\)\(^,\)\(^4\)\(^,\)\(^12\)\(^,\)\(^13\)

Based on our understanding of the roles of proteoglycans in other tissues and organ systems,\(^14\)\(^-\)\(^16\) several putative roles, including retinal adhesion, can be postulated for the proteoglycans associated with cone matrix sheaths. Recent observations from a number of laboratories suggest a potential role for cone matrix sheaths in retina–RPE adhesion.\(^3\)\(^,\)\(^17\)\(^-\)\(^20\) For example, in experiments in which porcine, monkey, or human retinas were peeled away gently from the RPE, cone matrix sheaths remain attached to the RPE and become elongated up to four to six times their normal length before (1) splitting along their transverse axes,\(^3\)\(^,\)\(^13\) (2) detaching the RPE from Bruch’s membrane,\(^3\)\(^,\)\(^13\) or (3) tearing apical RPE microvilli.\(^19\) Collectively, these studies suggest that the strength of attachment of cone matrix sheaths to the RPE and cone photoreceptor cell outer segments is greater than that of the cone matrix sheaths themselves or than that between the RPE and Bruch’s membrane. Other studies show that retinal adhesion is weakened significantly without concomitant loss of photoreceptor function after intravitreal or subretinal injections of protease-free chondroitinase ABC.\(^20\) Taken together, these studies suggest that cone matrix sheaths may provide a molecular bond of considerable strength that participates in the adhesion between the RPE and neural retina.

To investigate further the potential functional role of cone matrix sheath-associated chondroitin 6-sulfate proteoglycan in retinal adhesion, studies directed toward perturbing the synthesis of this molecule by administering intravitreal injections of p-nitrophenol-\(\beta\)-D-xylopyranoside (xyloseide) to Yucatan micropigs were conducted. Xyloseide inhibits chondroitin sulfate proteoglycan (also dermanan sulfate and, to some extent, heparan sulfate proteoglycan, neither of which is present in the IPM) synthesis by competing with the xylosylated proteoglycan core protein as an acceptor for the first galactosyltransferase reaction.\(^21\)\(^,\)\(^22\) As such, the proteoglycans synthesized contain less than the normal compliment of glycosaminoglycan side chains and may be transported inadequately into the extracellular matrix.\(^22\) To our knowledge, this is the first attempt to perturb IPM proteoglycan synthesis either in vivo or in vitro. Our findings support the hypothesis that cone matrix sheath-associated proteoglycans and, potentially, other IPM proteoglycans, participate in normal retinal attachment.

**Materials and Methods**

**Animals**

We used 11 female Yucatan micropigs (age range, 4–6 months old; weight range, 9.6–14.2 kg) obtained from Charles River (Wilmington, MA) in these studies. The micropigs were maintained on Purina (St. Louis, MO) Mini-Pig Grower or Mini-Pig Breeder chows. Yucatan micropigs were used in these studies because (1) the porcine retina contains numerous cone photoreceptor cells\(^23\) with well-defined cone matrix sheaths;\(^6\) (2) their retinal vasculature\(^24\) and vitreous body\(^25\) are similar to those of humans; and (3) a library of probes specific for various porcine retinal cell types and both cone matrix sheath- and noncone matrix sheath-specific IPM components is available. Although these animals do not have a true macula or fovea, there is an area centralis with decreased vascularity that is populated densely by cone photoreceptors.\(^25\) The animals were treated in conformity with the ARVO Resolution on the Use of Animals in Research, the National Institutes of Health (Bethesda, MD) “Guide for the Care and Use of Laboratory Animals” (NIH publication 86-23), and the guidelines set forth by the St. Louis University Department of Comparative Medicine.

**Reagents**

We obtained p-nitrophenyl-\(\beta\)-D-xylopyranoside from Research Products International (Mt. Prospect, IL) and Koch-Light (Suffolk, England). Antibodies directed against chondroitin-6-sulfate (\(\Delta\)Di-6S) were obtained from ICN (Lisle, IL). A cone photoreceptor cell-specific monoclonal antibody, designated CSA-1, has been generated and characterized.\(^26\) Fluorescein-conjugated goat anti-mouse immunoglobulins G and M antibodies were purchased from Sigma (St. Louis, MO) and Cappel (Durham, NC). Protease-free chondroitinase ABC (Proteus vulgaris) was obtained from Seikagaku America (St. Petersberg, FL). The fluorescein-conjugated PNA lectin, Arachis hypogaea agglutinin, was obtained from Vector (Burlingame, CA) and EY (San Mateo, CA). Dimethyl sulfoxide (DMSO), globulin-free bovine serum albumin (BSA), MgCl\(_2\), and CaCl\(_2\) were purchased from Sigma. Acrylamide and other reagents used for embedding were purchased from Bethesda Research Laboratories (Bethesda, MD).

**Injections**

Micropigs were immobilized in a Panepinto sling and anesthetized with isoflurane administered through a face mask. In those instances where prolonged anesthesia was required (eg, fundus photogra-
phy and electroretinography), the animals were intubated. One or two drops of proparacaine (Alcon, Fort Worth, TX) were administered topically to the eye before injection. To prepare the injections, xyloside was dissolved in DMSO and subsequently suspended under sterile conditions in balanced salt solution (BSS; Alcon) with a DMSO/BSS ratio of 1:9. Optimal xyloside doses were determined by preliminary dose-ranging studies in which doses resulting in vitreous concentrations from 1–150 mM were tested in a series of 12 Yucatan micropig eyes. Retinal gliosis was observed with vitreous xyloside doses exceeding 20 mM. As such, doses resulting in a vitreous concentration from 1–20 mM (0.7–14 mg of xyloside delivered in a volume of 100 μl; vitreous volume of 2.5 ml) were used in our experiments (Table 1). The injections were administered through the temporal pars plana, 3 mm posterior to the temporal limbus, using a 30-gauge needle. Care was taken to avoid penetration into the vitreous cavity greater than 1 cm. Both single and multiple injection regimens were used (Table 1). For the latter, injections were administered every 3 days (based on a half-life of cone matrix sheath associated-chondroitin 6-sulfate turnover of approximately 3–4 days; Hageman, unpublished observation). Injections of carrier without xyloside were administered to control eyes by the same protocol. Additional controls included the administration of other proteoglycan perturbants (ie, tilorone and suramin) by similar protocols; these agents did not produce any of the effects observed after xyloside administration.

Funduscopic Examination

The eyes were dilated with a single drop each of phenylephrine HCl 2.5% and cyclopentolate HCl 1% (Alcon). Indirect ophthalmoscopy was done immediately before and after each injection to ensure that the injection was atraumatic to the retina and/or vitreous. The fundus was examined at least once a week thereafter. Fundus photographs were recorded with Kowa RC-2 (Torrance, CA) or Cannon CF 60 U (Itasca, IL) fundus cameras.

Electroretinography (ERG)

Retinal responses to light-adapted white flash and 30-Hz flicker were monitored on selected experimental and control eyes using Burian-Allen bipolar contact lenses and a Nicolet Compact Four electroretinographic processor (Racine, WI). We recorded ERGs before xyloside treatment and on a weekly basis thereafter. The micropigs were restrained and anesthetized, and their pupils were dilated with one to two drops each of phenylephrine HCl 2.5% and cyclopentolate HCl 1%. Photopic ERGs were recorded in response to single flashes (100-msec duration and 62-foot-lamberts illumination) of white light presented in a Ganzfeld with 10 foot-lamberts of background illumination. Cone flicker responses were recorded similarly using a 30-Hz white stimulus (100-msec duration and 62 foot-lamberts illumination) without background illumination.

Fixation, Embedding, and Antibody–Lectin Binding

The animals were killed with Sleep-Away euthanasia solution (Fort Dodge Laboratories, Fort Dodge, IA). After death, their eyes were enucleated, the anterior segments removed, and the eyecups fixed for 2–4 hr by immersion in formaldehyde 4.0% (freshly generated from paraformaldehyde) in 100 mM sodium cacodylate buffer, pH 7.4. After fixation, the eyecups were rinsed for a minimum of 6 hr in 100 mM sodium cacodylate buffer, pH 7.4. Fixation, Embedding, and Antibody–Lectin Binding

Table 1. Summary of dosages and treatment effects

<table>
<thead>
<tr>
<th>Vitreous xyloside concentration</th>
<th>Early stage</th>
<th>Middle stage</th>
<th>Late stage</th>
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<tr>
<td></td>
<td>No. of eyes</td>
<td>Injection regimen</td>
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<td>1 mM</td>
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<td>Once, sacrifice after 3 days</td>
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<td>5 mM</td>
<td>1</td>
<td>Once, sacrifice after 2 weeks</td>
<td>1</td>
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<td>20 mM</td>
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<td>1</td>
</tr>
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More severe stages of xyloside-induced perturbation are observed with increasing dose and/or duration of xyloside exposure. Although more than a single treatment stage may be present in a given retina, one stage predominates with a given dosage regimen.
cryostat at -20°C, as described previously. All four quadrants of each eye were sectioned meridionally from the pars plana to the optic nerve. Serial sections of each region were cut, and the adjacent sections each were incubated with one of a battery of probes.

For lectin histochemical studies, the sections were exposed to fluorescein-conjugated PNA at a concentration of 50 μg/ml in 10 mM phosphate-buffered saline containing 1 mM MgCl2 and 1 mM CaCl2 (PBS/M/C) and 1 mg/ml globulin-free BSA and processed as described previously. For immunohistochemical studies, antibodies directed against chondroitin-6-sulfate (AC6S) and cone photoreceptor cell plasma membranes (CSA-1) were applied to sections at the appropriate dilution in PBS/M/C for 60 min at room temperature or for 12 hr at 4°C. Sections incubated with AC6S were exposed first to 0.2 units/ml of protease-free chondroitinase ABC in 50 mM Tris HCl in 0.03 mM sodium acetate, at pH 8.0, for 20 min at 37°C. (Preexposure to chondroitinase ABC was used because the antibody recognizes unsaturated uronic acid residues which remain attached to core proteins after digestion with chondroitinase ABC.) The slides subsequently were rinsed in PBS/M/C and exposed to the appropriate fluorescein-conjugated secondary antibody (GAMlgG for CSA-1 and GAMlgM for AC6S) in PBS/M/C for 30-60 min at room temperature in the dark. Controls included (1) incubation of sections with secondary antibodies alone; (2) deletion of chondroitinase digestion for AC6S; and (3) incubation of sections with PNA in the presence of the competitive hapten o-methyl-α-D-galactopyranoside. The sections were examined by epifluorescence microscopy on an Olympus Vanox VH-2 light microscope (New Hyde Park, NY) and photographed on Kodak Technical Pan or Ektachrome 50 professional films using an exposure index of 800.

Routine Histologic Examination

For routine histologic examination, the tissue was fixed overnight in paraformaldehyde 4%, progressively dehydrated to absolute alcohol, cleared with xylene, and infiltrated with paraffin wax. We cut 5–6-μm thick sections on a microtome and stained them with hematoxylin and eosin. Tissue for 1-μm sections was fixed in formaldehyde 2.0% and glutaraldehyde 2.5% in 100 mM cacodylate buffer containing CaCl2 0.025%, pH 7.4, for 2-4 hr, briefly rinsed in 100 mM sodium cacodylate buffer, pH 7.4, and secondarily fixed in 2.0% osmium tetroxide in 100 mM sodium cacodylate buffer for 1–2 hr. En bloc staining was done using magnesium uranyl acetate 0.5% in saline 0.9% for 24 hr at 4°C. After another short buffer rinse, the tissues were dehydrated in a graded series of ethyl alcohols, passed through propylene oxide, and embedded in Epon resin (Balzers, Hudson, NH). We cut 1–2-μm sections with a Reichert Ultracut microtome (Cambridge, Buffalo, NY) and stained them with Richardson's stain. All four quadrants of each eye were examined by light microscopy and photographed on Kodak Technical Pan or Ektachrome 50 professional films using an exposure index of 50.

Results

A continuum of biochemical and structural alterations was observed after intravitreal injection of xyloside. The degree of xyloside-induced effects varied with the duration of exposure and dose (Table 1). Low doses and short durations of xyloside exposure resulted in the disruption of the structural and molecular integrity of cone matrix sheaths. After longer durations of xyloside exposure and/or increased doses, loss of chondroitin 6-sulfate immunoreactivity, degeneration of cone photoreceptor cell outer segments, and shallow retinal detachments were observed. Based on clinical examination, retinal detachments most often originated in the central retina and progressed peripherally with increasing duration of treatment. Despite changes in the distribution and composition of cone matrix sheath constituents, no indication of inner retinal or RPE pathology was observed at any stage (Fig. 1). The choroid, optic nerve, lens, ciliary body, iris, and cornea also were unaffected by xyloside exposure. The ERG data provided further evidence that xyloside exposure did not result in damage to the inner retina (Fig. 2). The amplitudes of the photopic and cone flicker ERGs progressively diminished to approximately 70% of normal after longer durations of xyloside treatment (Fig. 2). However, the ratio of the photopic a- to b-wave amplitudes and the a- and b-wave implicit times were not affected significantly.

For descriptive purposes, xyloside-induced effects were classified into three stages (designated early, middle, and late), based primarily on the distinct morphologic hallmarks we found. The observed treatment effect typically varied from one retinal region to another; therefore, more than one stage often was observed in a single eye, especially after long durations of xyloside exposure. However, the predominant stage depended on the particular dosage regimen (Table 1). Each stage is described in detail subsequently.

Early Stage

The early stage was heralded by an obvious disorganization of the molecular constituents associated with cone matrix sheaths, as evidenced by comparing the distribution of PNA-binding constituents in con-
Fig. 1. Light micrographs depicting sections of a control porcine retina (A) and a xyloside-exposed porcine retina at a late stage of interphotoreceptor matrix (IPM) perturbation (B). At late stages of xyloside exposure, photoreceptor cell outer segments (OS) are disoriented and disrupted, and there is widening of the interphotoreceptor space, interposed between the external limiting membrane (ELM) and retinal pigmented epithelium (RPE) (B). The effects of xyloside treatment are limited to the interphotoreceptor matrix and photoreceptor outer segments. The retinal pigmented epithelium and inner retina appear unaffected following xyloside exposure (B). IS, inner segments; OPL, outer plexiform layer; ILM, internal limiting membrane.

Control and xyloside-treated retinas (Figs. 3A–B, 5A). The disruption of cone matrix sheath constituents often was associated with a detectable widening of the interphotoreceptor space (Fig. 3B). The distribution of cone matrix sheath-associated chondroitin 6-sulfate was disorganized and decreased in intensity at this stage. In most instances, a near complete loss of antichondroitin 6-sulfate binding constituents was observed (Figs. 3C, 5B), but not reflected in degree, by loss of PNA-binding constituents (Fig. 3B). Despite these alterations of cone-associated IPM components, cone photoreceptor cell outer segments appeared normal as monitored by monoclonal antibody CSA-1, which specifically labels porcine cone photoreceptors (Figs. 3D, 5C). Furthermore, ERG recordings, obtained just before death from eyes with predominantly early effects, were not changed significantly compared with pretreatment control eyes.

Middle Stage

The middle stage was characterized by degeneration of cone, but not rod, photoreceptor cell outer segments, disorganization and loss of IPM-associated PNA-binding molecules, and a near complete absence of AC6S immunoreactivity in the IPM (Figs. 6A–C). Cone photoreceptor cell outer segment degeneration, as distinguished by alterations in the binding pattern of CSA-1, was observed only after a significant loss of AC6S immunoreactivity in the IPM (Figs. 3C–D, 6B–C). Rod photoreceptor cell outer segments were unaffected at this stage, based on light microscopic histologic findings and conventional transmission electron microscopy (data not shown).

Late Stage

The late stage was characterized by a generalized loss of IPM-associated PNA-binding glycoconjugates (Figs. 7A–C). Morphologically, cone photoreceptor cell outer segments were disrupted severely and/or shortened (Fig. 7C). Although rod photoreceptor cell outer segments were disoriented severely by this stage, they were not disrupted as were the cone outer segments, based on light microscopic histologic findings.

Fig. 2. Representative white flash and 30-Hz flicker ERG recordings from the same pig before xyloside treatment (control) and 3 (mid) and 6 (late) weeks after initiation of 5 mM intravitreal injections of xyloside, administered every 3 days. Amplitudes of the 30-Hz flicker recordings are decreased by approximately 20% at 6 weeks; however, the a-to-b wave ratio and implicit times are not affected, suggesting widespread cone photoreceptor cell degeneration without significant inner retinal pathology. Photopic ERGs, recorded in response to single flashes (100-msec duration, 62 ft-Lamberts) of white light presented in a Ganzfeld with 10 ft-Lambert's background illumination. Cone flicker responses were recorded in a similar manner using a 30-Hz white stimulus (100-msec duration, 62 ft-Lambert's) without background illumination.

XYLOSIDE-INDUCED PERTURBATION
ERGs

WHITE FLASH

30 Hz FLICKER

CONTROL

MID

LATE

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creasing duration of treatment. In some instances, retinal whitening (Fig. 4A); these occurred initially in the retina, domains of the interphotoreceptor matrix, termed cone matrix sheaths, are specifically bound by fluorescein-conjugated PNA (A, arrows). The earliest detectable effect on retinal structure, following exposure to xyloside, is a marked disruption of the normal distribution of PNA-binding constituents associated with cone matrix sheaths (B, asterisks). By the end of this stage a near complete loss of anti-chondroitin 6-sulfate immunoreactivity is observed, although scant immunoreactivity is detectable in some instances (C, arrows). Cone photoreceptor cell morphology is unaffected at this stage based on labeling with the cone-specific antibody CSA-1 (D, arrows). R, retinal pigmented epithelium; arrowheads indicate the location of the external limiting membrane.

Fig. 4. Fundus photograph depicting a characteristic area of retinal whitening (A, arrows) that is typically observed in middle and late stage xyloside-treated eyes. Histologically, these regions correspond to areas of shallow splitting of the interphotoreceptor matrix that are easily visualized in sections incubated with fluorescein-conjugated PNA (B, arrows). Cone matrix sheaths typically, although not always, split along their transverse axes and the interphotoreceptor space (arrows) widens (also see Fig. 1B). R, retinal pigmented epithelium; arrowheads indicate the location of the external limiting membrane.

Retinal Detachment

Shallow retinal detachments often were observed in eyes with predominantly middle- and late-stage effects. They were visible funduscopically as areas of retinal whitening (Fig. 4A); these occurred initially in the central retina and expanded peripherally with increasing duration of treatment. In some instances, retinal elevations and retinal folds were observed funduscopically. Histologic examination of these regions revealed that cone matrix sheaths were split transversely (Figs. 4B, 8) or had separated completely from cone outer segments (Figs. 9A-B). Most often, the proximal portions of transversely split cone matrix sheaths retained their association with cone photoreceptor cells, and the distal portions remained adherent to the apical surfaces of the RPE (Figs. 4B, 8). In other instances, entire cone matrix sheaths retained their attachment to the apical RPE but separated away from intact cone photoreceptor cell outer segments (Figs. 9A-B). In both cases, the expanded subretinal space was devoid of both PNA- and AC6S-binding constituents (Fig. 8).

Discussion

For years, it has been speculated that IPM constituents might participate in retinal adhesion. The force required to peel the retina from the RPE in vitro was found to be significantly reduced after treatment with testicular hyaluronidase, an enzyme that degrades both chondroitin sulfate and hyaluronic acid, suggesting that IPM proteoglycans and/or glycosaminoglycans might participate in retinal adhesion.35-36 By contrast, in vitro experiments using aqueous-soluble IPM components suggested that neither cell-surface nor soluble interphotoreceptor proteoglycan–glycoprotein molecules play a role in retinal adhesion.37 These studies, however, did not address adequately the potential role of aqueous-insoluble IPM constituents in retinal adhesion. In more recent studies, experiments were done on postmortem human donor eyes in which the neurosensory retina was separated gently from the RPE.19 The microvilli of the RPE often remain embedded in the distal tips of cone matrix sheaths, suggesting that the adhesive force between cone matrix sheaths and the RPE is sufficient to tear the microvilli from their apical surface. In similar experiments, using freshly enucleated monkey and pig eyes, cone matrix sheaths were found to be firmly adherent to both cone photoreceptor cell outer segments and the apical surface of the RPE, and these attachments were stronger than those between the RPE and Bruch's membrane.113 Taken together, these studies provide evidence that cone matrix sheaths participate in retinal adhesion.

Recent biochemical studies have increased our understanding of the molecular basis by which cone matrix sheaths could participate in retinal adhesion. Cone matrix sheaths consist largely of a large chondroitin 6-sulfate proteoglycan, the core protein of which appears to be associated with the cone photoreceptor cell outer segment plasma membrane.3 Cell membrane-associated chondroitin sulfate proteoglycans are associated with other cell types including melanoma cells,38 neuronal cells,37 glial cells,39 epithelial cells,38 and chondrocytes.39 Although the specific function of these molecules has not been determined in most instances, it has been suggested that the melanoma- and chondrocyte-associated proteoglycans participate in cell–cell adhesion.39,40 Based on this information, it is reasonable to speculate that cone matrix sheath-associated chondroitin 6-sulfate proteoglycan might participate in retinal adhesion. This supposition was supported by the results of a recent study in which subretinal and intravitreal injections of protease-free chondroitinase ABC weakened retinal adhesion, caused a loss of PNA-binding glycoconjugates, and induced shallow retinal detachment in rabbits50 and monkeys (Hageman, unpublished results).

In this study, the synthesis and normal distribution of cone matrix sheath-associated chondroitin 6-sul-
The middle stage of disruption is characterized by shortening and degeneration of cone outer segments (6C, arrow). There is also a marked loss of cone matrix sheath-associated PNA-binding constituents (6A) and near complete loss of chondroitin 6-sulfate proteoglycan (6B, arrow). Note that chondroitin 6-sulfate immunoreactivity was not present in any retinas in which there was significant cone photoreceptor cell outer segment degeneration. At the late stage of xyloside-induced disruption, a near complete loss of PNA-binding (7A) and anti-chondroitin sulfate to secreted core protein or (2) decreased secretion and/or incorporation of core protein into the IPM observed in xyloside-treated eyes probably represents either (1) a decreased addition of chondroitin sulfate to secreted core protein or (2) decreased secretion and/or incorporation of core protein into the IPM or plasma membrane. The more generalized loss of PNA-binding glycoconjugates, observed at later stages of xyloside treatment, may represent (1) impaired mobility and secretion of cone matrix sheath-associated proteoglycan into the IPM; (2) disruption of the normal association between other PNA-binding glycoconjugates and chondroitin 6-sulfate proteoglycan; and/or (3) degeneration of cone photoreceptor cell outer segments, resulting in a consequent loss of cone matrix sheath-associated proteoglycan secretion into the interphotoreceptor space. Current studies are being directed toward determining the specific biochemical alterations of cone matrix sheath composition induced by xyloside.

Shallow retinal detachments, commonly occurring after xyloside administration and typically originating in the posterior pole, correlated histologically with splitting of cone matrix sheaths along their transverse axes or, in some instances, with separation of the entire cone matrix sheath from cone photoreceptor cell outer segments. It is reasonable to suggest that, in xyloside-treated eyes, cone matrix sheaths are perturbed structurally either by improper incorporation of the altered cone matrix sheath proteoglycan core protein into the cone outer segment plasma membrane or by a functional deficiency of this molecule. This would result in transverse splitting of cone matrix sheaths and/or separation of cone matrix sheaths from cone outer segments. When xyloside-treated retinas are examined electron microscopically (Hageman and Lazarus, unpublished data), alterations of cone photoreceptor cell outer segment plasma membranes typically are observed, suggesting that cone matrix sheath proteoglycans are not being intercalated properly into the cone outer segment plasma membranes. Because the apical portions of the cone matrix sheaths always remain firmly attached to the apical surfaces of the RPE cells, it appears that cone matrix sheaths may be attached to the RPE by molecules, such as integrins, that are unaffected by xyloside.

Weakening of the structural integrity of cone matrix sheaths and their attachment to cone photoreceptor cells does not per se explain the retinal detachments that commonly occurred after xyloside administration because additional mechanisms that maintain retinal adhesion also would have to be overcome. These include active transport of subretinal fluid, passive hydrostatic forces, osmotic forces, and physical interdigitations between outer segments and RPE microvilli. Conceivably, vitreal–retinal traction, caused by mechanical perturbation of the vitreous during xyloside injection, could result in retinal detachment; however, detachment was never observed in control eyes. Furthermore, funduscopic examination done immediately after intravitreal injections of xyloside did not reveal any new onset of retinal detachment or change in existing detachments. Alternatively, the accumulation of free, xyloside-conjugated chondroitin 6-sulfate glycosaminoglycan in the subretinal space could alter significantly the osmolality of the subretinal space, resulting in fluid accumulation and retinal detachment. The propensity for retinal detachment to occur centrally (area centralis) in xyloside-exposed eyes remains speculative. However, it is likely that...
this cone-dense region of retina is affected preferentially because of the increased amount of cone matrix sheath proteoglycan in this region.

The specificity of xyloside-induced proteoglycan perturbation is a concern when interpreting our results. Conceivably, the synthesis of IPM and retinal proteoglycan molecules, other than those associated with cone matrix sheaths, might be affected by xyloside treatment because other classes of proteoglycans, including chondroitin sulfates, dermatan sulfates, and heparan sulfates, contain the xylose-serine linkage competed for by xyloside. Currently, however, neither heparan sulfate- nor dermatan sulfate-containing proteoglycans have been identified in the IPM. An aqueous-soluble chondroitin 4-sulfate proteoglycan is present in the IPM-surrounding rod photoreceptor cells. It seems unlikely, however, that this proteoglycan, which does not appear to be membrane intercalated, would participate in retinal adhesion. Bruch's membrane and Müller cells, each of which contain significant quantities of chondroitin sulfate proteoglycan, did not appear to be affected by xyloside treatment, based on the histologic, funduscopic, and ERG results obtained. It is conceivable that the turnover of proteoglycans in these regions is significantly slower than those associated with cone matrix sheaths or that alterations of these proteoglycans do not affect retinal function.

In addition to their probable role in retinal adhesion, our results suggest that cone matrix sheaths may be important for maintaining cone photoreceptor cell outer segment viability. Along similar lines, others identified changes in the distribution of IPM glycosaminoglycans at a time before photoreceptor cell death in the Royal College of Surgeons rat. The ERG data obtained in this study suggests widespread cone or cone outer segment degeneration without concomitant damage to the inner retina. Furthermore, alterations in the binding pattern of CSA-1 were observed only after significant loss of AC6S immunoreactivity, suggesting that cone photoreceptor outer segment degeneration occurred subsequent to loss of AC6S-binding constituents.

In conclusion, our results strongly implicate the IPM as a participant in normal retinal attachment and/or maintenance of cone photoreceptor outer segment integrity. The clinical significance of these findings is evident because events that alter the structure and/or composition of the IPM could predispose an eye to retinal detachment, loss of photoreceptor function, and ultimately, blindness. It also follows that the reestablishment of a normal IPM--RPE--photoreceptor interface after retinal reattachment surgery may be essential for optimal visual recovery. Further elucidation of the mechanisms governing the metabolic structure and function of the molecular constituents of the RPE--IPM--photoreceptor cell interface will no doubt lead to a better understanding of the pathogenesis of retinal detachment and retinal degenerations and to improved therapeutic interventions.

Key words: retina, interphotoreceptor matrix, cone photoreceptor, xyloside, retinal adhesion

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References

22. Schwartz NB: Synthesis and secretion of an altered chondroitin

26. Johnson LV and Hageman GS: Characterization of molecules

25. Sedlacek GM, Gloor BD, and Meszaros J: Anterior bulbus per-

24. Bloodworth JMB, Gutgesell HP, and Engerman RL: Retinal


20: Yao X-Y, Hageman GS, and Marmor MF: Retinal adhesive-

16. Fransson LA: Structure and function of cell associated proteo-

19. Hollyfield JG, Varner HH, Rayborn ME, and Osterfeld AM: In-

9. Fariss RN, Anderson DH, and Fisher SK: Comparison of pho-

8. Hollyfield JG, Rayborn ME, Landers RA, and Myers KM: In-

7. Sameshima M, Uehara F, and Ohba N: Specialization of the


58x285

41. Anderson DH, Guerin CJ, Matsumoto B, and Pfeffer BA:

40. Harper JR and Reisfeld RA: Inhibition of anchorage-indepen-


36. Bumol TF, Wang QC, Reisfeld RA, and Kaplan NO: Monoclo-


34. Marmor MF and Yoon YH: Effects on retinal adhesion of tempera-


32. Richardson JC, Jarret L, and Finke EH: Embedding in epoxy


32. Richardson JC, Jarret L, and Finke EH: Embedding in epoxy


34. Marmor MF and Yoon YH: Effects on retinal adhesion of tempera-


32. Richardson JC, Jarret L, and Finke EH: Embedding in epoxy


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34. Marmor MF and Yoon YH: Effects on retinal adhesion of tempera-


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32. Richardson JC, Jarret L, and Finke EH: Embedding in epoxy


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