Recovery of Retinal Adhesion After Enzymatic Perturbation of the Interphotoreceptor Matrix

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Previous investigations established that focal subretinal injections of neuraminidase, chondroitinase, and hyaluronidase in the rabbit lead to a diffuse loss of retinal adhesiveness beyond the site of injection. This loss of adhesiveness, measured by peeling of the retina immediately after enucleation, correlates with changes in the interphotoreceptor matrix (IPM), as monitored by lectin histochemistry. In this study, rabbits were evaluated during recovery of retinal adhesiveness after subretinal injections of neuraminidase and chondroitinase. Adhesion recovered steadily 5–20 days after chondroitinase injection. After administration of neuraminidase, adhesion remained low for approximately 14 days but recovered to normal by 20 days. The recovery of adhesiveness correlated closely with reestablishment of the normal distribution of peanut agglutinin-binding glycoconjugates in the IPM, one group of molecules thought to participate in retinal adhesion. Electroretinography and light microscopy showed no abnormalities in the retina or retinal pigment epithelium after recovery. These results suggest that IPM glycoconjugates participate in maintaining retinal adhesion. Invest Ophthalmol Vis Sci 33:498–503, 1992

The retina adheres to the retinal pigment epithelium (RPE) and becomes detached only when forced by vitreous traction or fluid pressure in the subretinal space. Current evidence suggests that retinal adhesiveness depends on several complementary, and possibly interactive, systems, including anatomic interdigitation between photoreceptor outer segments and RPE microvilli, fluid pressure from intraocular pressure and choroidal osmotic pressure, active transport of fluid out of the subretinal space by the RPE, and adhesive properties of the interphotoreceptor matrix (IPM). Recent work has shown that the molecular constituents of the IPM are not distributed homogeneously. There is evidence that domains of IPM glycoconjugates exist and are associated with both rod and cone photoreceptor cells and with the apical surface of the RPE. Domains of IPM associated with cone photoreceptors, termed “cone matrix sheaths,” are composed of aqueous-insoluble proteoglycans containing chondroitin 6-sulfate and O-glycosidically linked oligosaccharides that bind peanut agglutinin (PNA). By contrast, the oligosaccharides associated with rod photoreceptor-associated IPM proteoglycans appear to have terminal sialic acid residues that prevent the binding of PNA. Although the precise function of most IPM constituents is unknown, recent studies provide evidence that some aqueous-insoluble proteoglycans participate in retinal adhesion. For example, we demonstrated recently that retinal adhesion is weakened significantly by exposure of the IPM to specific degrading enzymes in vivo. Neuraminidase type X and chondroitinase ABC both weakened adhesion, but each affected the matrix differently, as shown by lectin histochemistry and immunocytochemistry. These experiments provided evidence that IPM glycoconjugates participate in retinal adhesion, but they raised questions about which components are critical in the adhesive process and whether reestablishment of their normal distribution is associated with restoration of retinal adhesiveness. In this study, we investigated the recovery of retinal adhesion after enzyme treatment, relative to the recovery of the normal distribution of PNA-binding glycoconjugates in the IPM. We also evaluated, by electroretinography (ERG) and light microscopy, whether long-term retinal or RPE damage results from enzyme treatment.
Materials and Methods

All experiments were done on pigmented Dutch rabbits, and conformed to the ARVO Resolution on the Use of Animals in Research and the National Institutes of Health Guidelines for The Care and Use of Laboratory Animals. The rabbits were anesthetized with xylazine 4 mg/kg, acepromazine maleate 2 mg/kg, and ketamine hydrochloride 100 mg/kg.

We used the following reagents: protease-free chondroitinase ABC from *Proteus vulgaris* (Seikagaku Kogyo, Tokyo, Japan); neuraminidase, type X, from *Clostridium perfringens*, a purification by affinity chromatography of type VIII (Sigma, St. Louis, MO); and Hanks' solution (Gibco, Grand Island, NY).

The enzymes were dissolved in Hanks' solution, pH 7.3, as follows: chondroitinase, 0.2 units/ml and neuraminidase, 1.7 units/ml. Subretinal injections were done as described previously. In brief, a glass micropipette with tip diameter of 40–50 μm was inserted through a limbal slit and passed across the vitreous until the tip penetrated the central retina. Then, 3–4 µl of Hanks' solution, with or without enzyme, was injected into the subretinal space, creating a small (3–4-mm diameter) retinal detachment. In each rabbit, one eye was injected with the enzyme, and the fellow eye was injected with Hanks' solution alone.

For ERG recordings, the pupils were dilated maximally with atropine 1% and phenylephrine 10% eye drops. Direct-current ERGs were recorded between the cornea and a reference on the sclera, using silver-silver chloride electrodes as described previously. Light stimulation was derived from a quartz bulb focused through a shutter into a fiberoptic light guide that terminated 1 cm from the cornea and delivered 2000 lux to the cornea in 1-sec flashes. Control ERGs were recorded before enzyme injection. The ERGs were repeated in the same eye 7 and 20 days after chondroitinase injections or 10–21 days after neuraminidase injections (corresponding to times of low and recovered adhesiveness).

Retinal adhesion was estimated from the degree of RPE pigment that adhered to the retina after peeling in vitro. In brief, the eyes were enucleated rapidly, and the posterior eyecup was transferred into Hanks' solution at 37°C. The retina was peeled manually 30–48 sec after enucleation. The percentage of retina covered with pigment was scored 0–100%, in increments of 10%, with 100% pigment adherence indicating firm adhesion and 0% low retinal adhesion.

For PNA cytochemistry, the tissues were fixed for 3 hr at room temperature in freshly prepared 4% paraformaldehyde in 100 mM sodium cacodylate buffer, pH 7.4. The tissues were then transferred to 100 mM sodium cacodylate buffer for 12–18 hr, embedded in acrylamide, frozen, and sectioned to a thickness of 5–6 μm on a cryostat. The sections were incubated in fluorescein-conjugated PNA and observed by fluorescence microscopy as described previously.

Results

Retinal adhesiveness was measured at intervals of up to 27 days after injection of the enzyme or control solution into the subretinal space. The control eyes, injected with Hanks' solution alone, always showed uniform pigment adherence (90–100%) to the peeled retina (except the injection site itself) regardless of the time elapsing since injection (1–27 days). By contrast, as described previously, when either chondroitinase (Fig. 1) or neuraminidase (Fig. 2) was injected into the rabbit subretinal space, a zone of weakened adhesiveness (only 20–40% pigment adherence) developed about the site of injection and expanded radially to include the entire fundus by 3 days postinjection.

Over the next 3–4 weeks, retinal adhesiveness recovered, but the speed of recovery was different for each enzyme. After injection of chondroitinase (Fig. 1), adhesiveness recovered steadily and progressively over 20 days. By contrast, eyes injected with neuraminidase (Fig. 2) had low retinal adhesiveness for approximately 2 weeks after injection; after this time, there was a relatively abrupt recovery to normal levels within 5 days. In all eyes examined, pigment adherence increased diffusely over the whole fundus as adhesiveness recovered; the recovery of adhesiveness

![Graph](https://via.placeholder.com/150)

Days After Subretinal Injection of Chondroitinase

Fig. 1. Recovery of retinal adhesiveness after subretinal injection of protease-free chondroitinase ABC. Adhesiveness is measured by the amount of RPE pigment that remains adherent to peeled retina. Each point represents one eye. The average value from our earlier report at day 3 is shown by an asterisk (*). Note the gradual recovery of adhesiveness.
Days After Subretinal Injection of Neuraminidase

Fig. 2. Recovery of retinal adhesiveness after subretinal neuraminidase injection. Data are displayed as in Figure 1. In comparison with Figure 1, the recovery is relatively abrupt after 2 weeks.

was not regional and showed no relationship to the site of enzyme injection (Fig. 3).

Both enzymes are known to produce a characteristic change in PNA binding to IPM glucocorjugates along with the acute loss of adhesiveness after enzyme injection. To determine whether the recovery of retinal adhesiveness was associated with the recovery of normal PNA binding in the IPM, we processed selected eyes for cytochemical studies. The recovery of adhesiveness after each enzyme correlated with recovery of a normal PNA binding pattern. By 3 days after chondroitinase administration, during the period when adhesiveness was at its lowest level, little or no PNA binding to IPM glycoconjugates was observed. The normal pattern of PNA binding to the cone sheaths returned gradually within 20 days after injection (Fig. 4). Neuraminidase administration resulted in abnormal binding of PNA throughout the IPM by 3 days; this did not change during the first 12–14 days after enzyme administration (Fig. 5), a time when adhesiveness was low. A pattern of selective PNA binding, to only the cone matrix sheaths, was reestablished rapidly by 14–18 days, and by day 21, both PNA binding and adhesiveness were normal. Control eyes, injected with Hanks' solution but no enzyme, were tested in parallel during these experiments; they showed no loss of adhesiveness and no abnormalities in PNA binding.

We recorded ERGs to evaluate the electrical functioning of the retina and RPE during the period of low adhesiveness and prolonged IPM abnormality associated with neuraminidase and chondroitinase administration. There were no significant changes in the amplitude, wave forms, and implicit times of the a-, b-, and c-waves in any experiment between control recordings and those made at intervals after enzyme administration.

We previously found that photoreceptor, inner retinal, and RPE morphology was normal 1–3 days after enzyme administration in areas more than 1 mm away from the injection site where adhesiveness was reduced. We used light microscopy to examine selected samples in our current experiment to see whether more prolonged IPM damage affected photoreceptors or RPE and whether adhesion recovery was simply a result of scar formation. During the period of low adhesiveness (up to 14 days after enzyme administration), we found that photoreceptor outer segments sometimes were disoriented and the subretinal space sometimes was narrower than usual. Otherwise, the morphology was normal in areas at least 1

Fig. 3. Whole mounts of peeled retina showing the amount of pigment adherent at 10, 12, and 21 days after subretinal neuraminidase injection. The injection site is demarcated by dotted lines in each example. Pigment adherence recovered diffusely rather than regionally. Recovery after chondroitinase injection had the same appearance.
mm away from the injection site. By 20 days, when adhesiveness was restored, histologic findings were normal with no evidence of scarring.

**Discussion**

Our previous study showed that the loss of retinal adhesion caused by administration of specific enzymes to the subretinal space or vitreous body in vivo correlated with cytochemical changes in IPM glycoconjugates bound by PNA. These findings provide additional evidence that IPM glycoconjugates are involved in, and relevant to, the maintenance of normal retinal adhesion. In the current study, we showed that the distribution of PNA-binding glycoconjugates, one group of molecules thought to participate in retinal adhesion, returns to normal approximately 3 weeks after chondroitinase or neuraminidase administration; these changes occur concomitantly with recovery of retinal adhesiveness. This result further sup-
ports the concept that IPM glycoconjugates are important participants in normal retinal adhesion. However, these experiments do not address the relative role of IPM constituents compared with other physiologic or physical factors that also participate in retinal adhesion, nor do they prove that these IPM glycoconjugates participate directly in adhesion.

The time course for recovery of normal retinal adhesiveness and PNA binding in the IPM was different for the two enzymes used. This is consistent with the suggestion that these enzymes affect retinal adhesion through different components of the IPM. Neuraminidase cleaves sialic acid residues on the IPM macromolecules, and chondroitinase degrades chondroitin sulfate. Thus, the different times for recovery may be related to the turnover of these IPM components and/or to their specific role in molecular adhesion. It also is possible that the critical factor is the duration of enzyme activity in the subretinal space, which could be different for the two enzymes used because of varia-
tions in activity, substrate availability, or susceptibility to degradation.

We found no evidence of subretinal scars, either as a response to injury or as an explanation for restored retinal adhesion. It is conceivable that 1–2 weeks of weakened adhesiveness and abnormal IPM physiologically might alter the photoreceptors or RPE. However, ERG recordings were normal 7 and 20 days after neuraminidase treatment. We found that the c-wave, which is sensitive to any expansion of the subretinal space, remained normal throughout this experiment. We might expect the subretinal space to widen when the IPM is damaged because lessened adhesion could lead to subclinical separation of the retina. However, this apparently did not occur, and in some cases, the subretinal space appeared narrower by light microscopy.

Our demonstration that enzyme-induced changes in retinal adhesion are reversible indicates that IPM glycoconjugates may be important components in the mechanisms and treatment of clinical retinal detachment. Also, pathologic IPM findings might play a role in inherited disorders with a high potential for detachment, such as connective tissue disease, degenerative myopia, and the vitreoretinal degenerations. The time course for restoration of the IPM glycoconjugates and retinal adhesiveness may help us understand the process of recovery from retinal detachments because the IPM is likely to be disrupted or altered by retinal separation and the entry of vitreous fluid.

Key words: chondroitinase, interphotoreceptor matrix, neuraminidase, retinal adhesion, retinal pigment epithelium

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References