Sialic Acid on the Surface of Photoreceptors and Pigment Epithelium in RCS Rats

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Incubation of isolated retinas and pigment epithelium (PE) from normal and dystrophic RCS rats with colloidal iron (CI) at pH 1.8 resulted in dense labeling of rod outer segments (ROS) and PE surfaces. Pretreatment with neuraminidase reduced subsequent binding of CI. The authors conclude, therefore, that sialic acid residues are localized on the ROS and PE surfaces. In dystrophic rats, uningested ROS tips, which accumulate in the subretinal space, did not bind CI. Invest Ophthalmol Vis Sci 25:1342-1345, 1984

In dystrophic RCS rats, the failure of the pigment epithelium (PE) to phagocytize shed rod outer segments (ROS) tips leads to an accumulation of ROS debris and, eventually, to the degeneration of photoreceptors. La Vail et al have observed that in dystrophic rats, interphotoreceptor-matrix staining is virtually absent in the ROS debris zone. Visualization of the interphotoreceptor-matrix components was attained with light microscopic histochemical procedures employing colloidal iron and other cationic dyes.

Colloidal iron (CI), by virtue of its electron density, is a useful cytochemical marker for anionic determinants at the ultrastructural level. In some cellular systems, CI has been shown to bind specifically to cell-surface sialic acid. Recently, Cohen and Nir have used CI at pH 1.8 for the detection of sialic acid residues on the surface of cultured PE cells. In view of the role of surface sialic acid in intercellular recognition, it was of interest to determine if sialic acid could be localized also on the surface of ROS and to establish whether differences in CI staining could be visualized on the surface of normal and dystrophic retina and PE tissues.

Materials and Methods. Tissue: Pigmented dystrophic RCS rats (RCS-P') and the genetic control (RCS-P''rdy') were used in this study. The animals were maintained on a 12:12-hr light:dark cycle. Sixty minutes after the onset of light, 16-21-day-old rats were euthanized with chloroform. Following enucleation, the retina was separated from the PE layer, which remained attached to the posterior eyecup. The isolated retina and eyecup were rinsed in phosphate buffer (0.15 M, pH 7.0) for 15 min at 4°C with gentle shaking in order to remove soluble components of the interphotoreceptor matrix. The tissues then were fixed with 1% glutaraldehyde in 0.15 M phosphate buffer (pH 7.0) for 60 min at room temperature and rinsed in phosphate buffer.

Cytochemical reactions: Colloidal iron solution (pH 1.8) was prepared according to Gasic et al. Fixed tissue was rinsed in distilled water and incubated with colloidal iron for 5-10 min at room temperature. The tissue then was rinsed in acetic acid (pH 1.8) followed by distilled water. The specificity of the CI staining was determined by neuraminidase digestion prior to the cytochemical incubation. Unfixed tissue was incubated for 30 min at 37°C with 100 units/ml Vibrio cholerae neuraminidase (free of protease, alkalase, and lecithinase C; Behring Institute; Marburg, West Germany) in a medium containing 3 mM calcium, 0.145 M NaCl, and 4 mM NaHCO₃ (pH 6.5). The tissue then was fixed and incubated with CI.

Electron microscopy: Following the cytochemical reactions, the tissue specimens were rinsed and postfixed with 1% OsO₄ in phosphate buffer (pH 7.0), then dehydrated in a graded ethanol series and embedded in Araldite. Thin sections were stained with uranyl and lead salts and viewed in a Jeol 100B electron microscope (Tokyo, Japan).

Results. In dystrophic RCS rats, 13 days and older, uningested shed ROS tips accumulate in the subretinal space. During the separation of the PE from the
photoreceptor layer, ROS debris that are entrapped between PE microvillous projections are retained with the PE layer. Incubation of the isolated PE layer (from a 16-day-old RCS rat) with CI revealed marked differences between the labeling of microvilli and that of the ROS debris. While the surface of the PE was coated heavily with CI deposits, there was almost no label on the surface of the apposing ROS debris (Figs. 1A, B). The few CI deposits that were seen indicate that the surface of the ROS debris was accessible to the cytochemical marker. Incubation of the isolated retina from the same 16-day-old RCS rat revealed dense labeling with CI on the intact ROS. The CI deposits were localized clearly on the ROS plasma
membrane (Fig. 1C). The extensive rinsing of the isolated retina prior to incubation with the cytochemical marker apparently was sufficient to remove the interphotoreceptor matrix and permit access of the CI to the ROS plasma membrane.

Treatment of the PE with neuraminidase prior to incubation with CI abolished almost all subsequent labeling of the cell surface with CI (Fig. 2A). Treatment of the photoreceptor layer with neuraminidase resulted in substantial, although incomplete, reduction of subsequent labeling with CI (Fig. 2B).

In normal rats, the labeling pattern of the PE and ROS surfaces with CI was similar to that observed in dystrophic rats (Figs. 1A, C). The CI binding to normal ROS and PE could be prevented by prior incubation with neuraminidase, as seen in dystrophic rats (Figs. 2A, B). Incubation of normal retina or PE in a calcium-containing bicarbonate buffer at pH 6.5, without neuraminidase, did not affect subsequent labeling with CI.

These investigations adhered to the ARVO Resolution on the Use of Animals in Research.

**Discussion.** In normal and dystrophic RCS rats, colloidal iron labeled the surface of both intact rod outer segments and pigment epithelium. The staining of the PE was reduced almost completely by pretreatment with neuraminidase, indicating that sialic acid residues contributed most of the binding sites for CI at pH 1.8. This observation is in agreement with our previous study with cultured PE cells, where most of the CI labeling was abolished by pretreatment with neuraminidase. In the photoreceptor layer, CI labeling was reduced greatly by pretreatment with neuraminidase. The residual photoreceptor labeling indicates that few highly acidic anionic determinants, other than sialic acids, might be detected by the CI on the surface of intact ROS.

Recently, Essner et al have suggested that, since treatment of PE cells with neuraminidase did not affect cationized ferritin (CF) binding at pH 1.8, sialic acid either is not present on the surface of PE cells or is inaccessible to the enzyme. It should be noted, however, that CF binding at pH 1.8 is not specific only to sialic acid residues. In a quantitative study with cultured PE cells, we have found that pretreatment with neuraminidase reduced subsequent labeling with CI at pH 1.8 by only 40% (unpublished results), while reduction of CI binding was reduced by 93–97%. In other cellular systems, quantitative evaluation revealed only a partial reduction in CF (pH 1.8) labeling density following neuraminidase treatment.

In the dystrophic rat, there was almost no labeling on the surface of ROS debris that accumulated in the subretinal space. It is thus possible that the disappearance of retinal staining, which was observed by LaVail et al in dystrophic RCS rats by means of CI histochemistry, may reflect changes both in the inter-
photoreceptor matrix and in the surface staining of ROS debris, which accumulates in the subretinal space.

The significance of the reduction in the density of sialic acid residues on the surface of uningested ROS tips in dystrophic RCS rats remains to be elucidated. It might be a result of degenerative events in accumulated ROS debris. Alternatively, it is possible that the reduction in sialic acid residues might be related to normal physiologic events that occur on the surface of shed ROS prior to their ingestion by the PE. In order to evaluate this possibility, it is necessary to study the surface of shed ROS tips in normal rats. However, our attempts to investigate the surface of shed ROS tips in normal rats have so far been unsuccessful. This was largely because disc shedding is followed immediately by engulfment of the shed tips by PE microvilli. In a previous study, Nir observed that once a contact between the PE and ROS surfaces was established, the surface of shed ROS was attached tightly to PE microvilli and was inaccessible to labeling with cytochemical markers. It was only in the dystrophic rat, where ROS debris was not engulfed tightly by PE microvilli, that surface labeling of shed ROS could be studied by the immersion of isolated retinas or PE tissues in a cytochemical reaction mixture.

Changes in surface sialic acid residues of ROS are of particular interest since sialic acid is implicated widely in recognition phenomena of several biologic systems. In relation to phagocytosis, removal of sialic acid residues and unmasking of underlying saccharides are required for the recognition of senescent red blood cells destined for removal from the circulation. Whether removal of sialic acid from the surface of ROS is similarly associated with their ingestion by PE remains to be established.

Key words: RCS rats, retina, pigment epithelium, colloidal iron, sialic acid

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