Interstitial Retinol-Binding Protein (IRBP) in Subretinal Fluid

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Antibodies against bovine interstitial retinol-binding protein (b-IRBP) were used to detect human IRBP (h-IRBP) on immunoblots of eight samples of subretinal fluid (SRF) from patients with retinal detachments of between 2 days' and more than 2 years' duration. Using this sensitive technique, it was found that seven of the samples contained h-IRBP in concentrations estimated to range from below 5% up to 19% of normal human IPM. One of these samples displayed two immunoreactive bands of roughly equal intensity, one at a molecular weight of 135,000 (h-IRBP), the other at 115,000. The latter may have been generated by proteolytic cleavage. No h-IRBP could be detected in an eighth sample from a patient with retrolental fibroplasia. It is concluded that the reduced concentration of h-IRBP in SRF may be due to a number of factors that include dilution, proteolytic degradation, and metabolic inactivation of photoreceptors at the detachment site. Invest Ophthalmol Vis Sci 27:1027-1030, 1986

The glycoprotein known as interstitial retinol-binding protein (IRBP)1 is the most abundant protein constituent of the interphotoreceptor matrix (IPM), where it is believed to transport retinol between the pigment epithelium and retina. An early study failed to detect this protein in two samples of human subretinal fluid (SRF) collected from rhegmatogenous detachments. The SRF that accumulates under these conditions contains variable amounts of protein, which is believed to be derived partly from the serum and partly from intraocular sources. In the present study, a sensitive immunological procedure was used in an endeavor to detect IRBP in eight samples of SRF obtained from patients with detachments that ranged in duration from 2 days to more than 2 yr. With the exception of a sample from a patient with a detachment from severe retrolental fibroplasia, IRBP was found in seven of the samples. The concentration of IRBP was estimated to range from below 5% up to 19% of that in normal human IPM.

Materials and Methods

SRF samples (see Table 1 for summary) were obtained from patients at the Department of Ophthalmology & Visual Sciences, Texas Tech University Health Sciences Center, Lubbock, Texas. Retinal break size was determined by using the standard technique of arcs of minutes or hours. Because of magnification error, ragged tears, etc., it is considered to be a realistic measurement. In Table 1, a dialysis is noted as a separate event, while round holes and horseshoe tears are coded identically. In case 5, an end-stage, classic retrolental fibroplasia, no breaks were identified (as is usual) and drainage was obtained before an attempt at retrolental membrane peeling.

Drainage was done in the usual fashion under an explant. Particular attention was paid to drying the field and packing off the region with cotton. Light diathermy was applied to seal blood vessels in the sclera and in the choroidal knuckle after cutting down to it. Entrance was made with a small needle, a tuberculin syringe was placed at the lip of the wound, and gentle aspiration was carried out. The wound was rotated so that the field was visible. The sample was rejected if there was any evidence of blood in the aspirate.

The samples were centrifuged at 50,000 g for 10 min to remove cell debris and other insoluble components, and the supernatants were stored frozen at −80°. After the samples had been thawed, 2 µl aliquots were diluted 50–2500 times and their protein concentration determined by the fluorescamine method of Udenfriend et al., using bovine serum albumin as a standard. A ref-
Table 1. Summary of results

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Detachment description</th>
<th>Duration</th>
<th>Break size</th>
<th>Detachment size % est.</th>
<th>Sample vol. (ml)</th>
<th>Total protein (mg/ml)</th>
<th>Approximate* h-IRBP conc. ng/µl~1</th>
<th>% normal IPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 yr.</td>
<td>Rhegmatogenous</td>
<td>1 m.</td>
<td>2 hrs.</td>
<td>40%</td>
<td>17</td>
<td>2.3</td>
<td>+†</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>63 yr.</td>
<td>Rhegmatogenous</td>
<td>3 w.</td>
<td>1 min.</td>
<td>50%</td>
<td>27</td>
<td>22.0</td>
<td>1000</td>
<td>18.5</td>
</tr>
<tr>
<td>3</td>
<td>48 yr.</td>
<td>Rhegmatogenous</td>
<td>2 d.</td>
<td>15 min.</td>
<td>50%</td>
<td>17</td>
<td>18.0</td>
<td>250</td>
<td>4.6</td>
</tr>
<tr>
<td>4</td>
<td>25 yr.</td>
<td>Rhegmatogenous</td>
<td>&gt;1 y.</td>
<td>1 min.</td>
<td>100%</td>
<td>17</td>
<td>45.0</td>
<td>125-150</td>
<td>2.3-4.6</td>
</tr>
<tr>
<td>5</td>
<td>9 mo.‡</td>
<td>Detachment of end stage retina of prematurity</td>
<td>7 m.</td>
<td>0</td>
<td>100%</td>
<td>19</td>
<td>82.0</td>
<td>none‡</td>
<td>&lt;0.1‡</td>
</tr>
<tr>
<td>6</td>
<td>64 yr.</td>
<td>Rhegmatogenous</td>
<td>3 d.</td>
<td>1 min.</td>
<td>90%</td>
<td>17</td>
<td>32.0</td>
<td>250-500</td>
<td>4.6-9.3</td>
</tr>
<tr>
<td>7</td>
<td>13 yr.</td>
<td>Rhegmatogenous</td>
<td>&gt;2 y.</td>
<td>10 min.</td>
<td>70%</td>
<td>17</td>
<td>22.0</td>
<td>40-50</td>
<td>0.7-0.9</td>
</tr>
<tr>
<td>8</td>
<td>13 yr.</td>
<td>Rhegmatogenous</td>
<td>14 d.</td>
<td>1.5 hr.</td>
<td>50%</td>
<td>19</td>
<td>3.4</td>
<td>100-250</td>
<td>1.9-4.6</td>
</tr>
</tbody>
</table>

* Calculated on the basis of 108 µg h-IRBP in an average normal human eye, or 5400 ng • µl~1 in undiluted IPM, estimated to have a volume of 0.02 ml.13
† Two major bands of immunoreactivity were present (see text and Fig. 1A, lane 1).
‡ Premature infant born after 23 weeks' gestation.

erence human IPM preparation diluted to 4.5 ml with phosphate-buffered saline (150 mM NaCl, 5 mM Na phosphate, 0.1 mM phenylmethylsulfonyl fluoride, pH 7.4) was obtained from four postmortem human donor eyes as described by Fong et al.5 Its total protein concentration was 8.5 mg • ml~1. Based on a reported5 average yield of 108 µg • eye~1, the human IRBP (h-IRBP) concentration in this preparation was estimated to be 96 µg • ml~1. Volumes of SRF equivalent to 12-984 µg total protein were diluted with water and mixed with equal volumes of sodium dodecyl sulfate (SDS) dissociation buffer. SDS polyacrylamide gel electrophoresis, electrophoretic transblotting to nitrocellulose sheets and immunological visualization of IRBP with rabbit antiserum to bovine IRBP antibodies was as described by Gonzalez–Fernandez et al.16,17 Samples containing 2-14 ng of purified bovine IRBP5 (b-IRBP) or various amounts of human IPM (4-170 µg total protein, estimated to contain 50-2000 ng h-IRBP) were also electrophoresed and transblotted.

Results

Table 1 shows that the total protein content of the eight SRF samples was 2.3-82.0 mg • ml~1, within the range reported by others.14 Immunoblots of these samples are illustrated in Figures 1A and 1B. The minimum detectability of b-IRBP on these blots was below 2 ng (lane a). With the exception of sample 5, which was from a patient with retrolental fibroplasia, all the samples showed immunoreactive bands at 135,000 molecular weight. This value is characteristic of h-IRBP but is lower than b-IRBP, which is 144,000.6 In addition to the band at 135,000, sample 1 displayed a band at 115,000. Traces of immunoreactivity at this molecular weight were also visible in sample 2.

Figure 1C illustrates one of the immunoblots where samples 5, 6, and 7 were compared with different amounts of h-IRBP (lanes h-n) obtained from the reference preparation of normal human IPM. The minimum detectability of h-IRBP with the rabbit b-IRBP antibodies was estimated to be 70 ng. The reason for this is that h-IRBP is only partially serologically identical with b-IRBP.5,6 Sample 6 gave a reaction band with an intensity between 500 and 1000 ng h-IRBP (lanes j, i) and sample 7 between 200 and 250 ng h-IRBP (lanes l, k). Since 2 µl of sample 6 and 5 µl of sample 7 were examined, the concentrations of h-IRBP were 250-500 and 40-50 ng • µl~1, respectively.

No reaction band was visible when a 12 µl aliquot of sample 5 was electrophoresed and immunoblotted. Since the minimum amount of h-IRBP that could be detected was 70 ng (see above), the concentration of h-IRBP in this sample was less than 6 ng • µl~1.

The results for all the SRF samples are summarized in Table 1.

Discussion

Adler and Severin's two samples from rhegmatogenous detachments of 1 week and 6 months duration were not unusual in their protein concentration (9 and 17 mg • ml~1).13 The failure of these authors to detect IRBP by SDS polyacrylamide gel electrophoresis in these samples may be attributable to the low sensitivity of protein detection using Coomassie Blue staining.

The concentrations of h-IRBP documented in Table 1 must be taken as approximations only, because they
Fig. 1. Immunoblots of SRF, b-IRBP and human IPM. Rabbit antibodies were directed against b-IRBP. A, B, Lanes 1–8 refer to SRF samples 1–8 (5 µl of each, 12–410 µg total protein); lanes a–g correspond to 2, 4, 6, 8, 10, 12, and 14 ng of b-IRBP. Note different molecular weights of h-IRBP (135,000) and b-IRBP (144,000). C, Lanes 5–7 refer to SRF samples 5–7 (984 µg, 64, and 110 µg total protein); lanes h–n are human IPM (170–4.3 µg total protein), estimated to contain 2000, 1000, 500, 250, 200, 100, and 50 ng of h-IRBP; lanes std correspond to prestained protein standards (BRL; myosin, 200,000 and phosphorylase b, 97,400).

**Key words:** interstitial retinol-binding (IRBP), subretinal fluid, retinal detachment, retrolental fibroplasia

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HAIKU: ARVO, 1986
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