Differential Cataractogenic Potency of TGF-β₁, -β₂, and -β₃ and Their Expression in the Postnatal Rat Eye

Clare Gordon-Thomson, Robbert U. de Jongh, Angela M. Hales, Coral G. Chamberlain, and John W. McAvoy

**Purpose.** Transforming growth factor-β has been shown to induce cataractous changes in rat lenses. This study assesses the relative cataractogenic potential of TGF-β₁, TGF-β₂, and TGF-β₃ and their expression patterns in the rat eye.

**Methods.** Lens epithelial explants and whole lenses from weanling rats were cultured with TGF-β₁, TGF-β₂, or TGF-β₃ at concentrations ranging from 0.025 ng/ml to 4 ng/ml for 3 to 5 days. Cataractous changes were monitored daily by phase contrast microscopy and by immunofluorescent detection of cataract markers α-smooth muscle actin and type I collagen. Expression of TGF-β was studied by immunofluorescence and in situ hybridization on eye sections from neonatal and weanling rats.

**Results.** All three isoforms induced morphologic changes in lens epithelial explants and cultured lenses that are typically associated with human subcapsular cataract. Transforming growth factor-β₂ and TGF-β₃ were approximately 10 times more potent than TGF-β₁. All three isoforms were expressed in the eye in spatially distinct but overlapping patterns. Transforming growth factor-β₁ and TGF-β₂ and their mRNA were detected in most ocular tissues, including the lens. Although TGF-β₃ was immunolocalized in lens epithelium and fibers and in other ocular tissues, its mRNA was detected only in the retina and choroid.

**Conclusions.** All three isoforms of TGF-β are potentially available to lens cells and have the potential to induce cataractous changes. The results suggest that TGF-β activity is normally tightly regulated in the eye. Activation of TGF-β in the lens environment, such as may occur during injury, in wound healing, or in pathologic conditions may contribute to cataractogenesis in vivo. (Invest Ophthalmol Vis Sci. 1998;39:1399–1409)
ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Human TGF-β, and TGF-β2 were obtained from Genzyme (Cambridge, MA) and used immediately or stored at −70°C in original diluent or after dilution with an equal volume of 0.5% bovine serum albumin in phosphate-buffered saline. Human TGF-β2 (Oncogene Science, Cambridge, MA) was diluted in 10 mM HCl in 10% ethanol, as recommended, and was used immediately or stored at −70°C. All three TGF-β isoforms were supplied in the mature form. Therefore, the antibody used to raise these antibodies map within regions that are associated with the mature form of TGF-β. Therefore, the antibody used for each isoform does not distinguish between the mature dimer and the latent precursor. These antibodies have been shown to be isoform-specific in western blot analysis and in immunolocalization studies of various mammalian tissues. The human immunogens used to raise these antibodies show a high degree of homology with the corresponding amino acid sequences in rat TGF-β1, TGF-β2, and TGF-β3. Furthermore, the three antibodies show distinct patterns of immunoreactivity when applied to a positive control tissue (rat intestine), which are generally consistent with patterns seen in previous studies.

A fragment of murine TGF-β1 cDNA (599 bp) corresponding to nucleotides 553 to 1152 cloned into pBluescript was provided by Rosemary Ackhurst, University of Glasgow, Scotland. A murine TGF-β2 cDNA (442 bp) corresponding to nucleotides 1511 to 1953 cloned into pSP72, and a murine TGF-β3 cDNA (609 bp), corresponding to nucleotides 831 to 1440 cloned into pGEM7Z(f+) were provided by Harold L. Moses, Vanderbilt University, Nashville, Tennessee. Anti-sense and sense 35S-labeled riboprobes were transcribed from linearized plasmid templates, using an RNA transcription kit (Promega, Sydney, Australia).

Immunofluorescence and In Situ Hybridization
Frozen tissue sections were prepared from neonatal (3-day-old) and weanling rat eyes as described previously. For immunofluorescence, frozen sections were incubated for 1 hour at room temperature with 1:80 dilution of rabbit polyclonal antibody TGF-β antibody. After washing, sections were incubated for 1 hour at room temperature with 1:100 dilution of donkey anti-rabbit IgG conjugated to Alexa Fluor 488. Sections were then rinsed in PBS, mounted in ProLong Gold (Invitrogen) and observed using a Zeiss 710 confocal microscope. Images were processed using Zen software (Zeiss).

Materials and Methods
All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Human TGF-β1 and TGF-β2 were obtained from Genzyme (Cambridge, MA) and used immediately or stored at −70°C in original diluent or after dilution with an equal volume of 0.5% bovine serum albumin in phosphate-buffered saline. Human TGF-β2 (Oncogene Science, Cambridge, MA) was diluted in 10 mM HCl in 10% ethanol, as recommended, and was used immediately or stored at −70°C. All three TGF-β isoforms were supplied in the mature form. Transforming growth factor-β1 was derived from platelets, whereas TGF-β2 and TGF-β3 were recombinant proteins. Studies of TGF-β1 and TGF-β2 indicate that the platelet-derived and recombinant products are equipotent.

Preparation of Lens Epithelial Explants
Explants containing cells derived from the germinative and central regions of the lens epithelium were prepared from weanling (21-day-old) rat eyes, as described previously. Briefly, after a 15-minute incubation of lenses in culture medium, the epithelium with associated capsule was peeled away from fibers and pinned on culture dishes (two explants per dish) with the cellular surface uppermost. At the beginning of the experiment, medium was replaced with fresh culture medium (1 ml/dish). GF-β1, TGF-β2, or TGF-β3 was diluted in culture medium and added in a 10-μl volume to give final concentrations ranging from 0.025 ng/ml to 4 ng/ml. Control explants received diluent without TGF-β. Explants were cultured for 3 days and monitored daily by phase contrast microscopy. After 3 days in culture, explants were assessed for typical cataractous changes, by means of a grading system described previously. These changes included capsule wrinkling, spindle cell formation, and cell loss or cell blebbing (a change that precedes and is associated with cell loss).

Preparation of Whole Lens Cultures
Lenses from weanling rats were carefully dissected from surrounding ocular tissues in culture medium and cultured with a low (0.15 ng/ml) or high (4 ng/ml) concentration of each of the TGF-β isoforms. Controls received diluent without TGF-β. Culture medium was renewed every 2 days throughout the culture period without further addition of TGF-β. Lenses were cultured for 5 days and photographed daily through the anterior surface to record development of opacification. The extent of opacification (opacification index) on day 5 was quantified by an image analysis method described in detail elsewhere. Student's t-test was used for statistical analysis of data. At the end of the culture period, the lenses were fixed in Carnoy's fixative (acetic acid/ethanol, 1:3 vol/vol), embedded in paraffin, and sectioned for routine histologic analysis. Immunofluorescent localization of the cataract markers α-smooth muscle actin and type I collagen was also performed as described previously.

Transforming Growth Factor-β Antibodies and Riboprobes
Affinity-purified rabbit polyclonal antibodies raised against synthetic peptides corresponding to sequences of the human TGF-β isoforms (amino acids 328–353 of TGF-β1, 352–377 of TGF-β2, and 350–375 of TGF-β3) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The peptides used to raise these antibodies map within regions that are associated with the mature form of TGF-β. Therefore, the antibody used for each isoform does not distinguish between the mature dimer and the latent precursor. These antibodies have been shown to be isoform-specific in western blot analysis and in immunolocalization studies of various mammalian tissues. The human immunogens used to raise these antibodies show a high degree of homology with the corresponding amino acid sequences in rat TGF-β1, TGF-β2, and TGF-β3. Furthermore, the three antibodies show distinct patterns of immunoreactivity when applied to a positive control tissue (rat intestine), which are generally consistent with patterns seen in previous studies.

A fragment of murine TGF-β1 cDNA (599 bp) corresponding to nucleotides 553 to 1152 cloned into pBluescript was provided by Rosemary Ackhurst, University of Glasgow, Scotland. A murine TGF-β2 cDNA (442 bp) corresponding to nucleotides 1511 to 1953 cloned into pSP72, and a murine TGF-β3 cDNA (609 bp), corresponding to nucleotides 831 to 1440 cloned into pGEM7Z(f+) were provided by Harold L. Moses, Vanderbilt University, Nashville, Tennessee. Anti-sense and sense 35S-labeled riboprobes were transcribed from linearized plasmid templates, using an RNA transcription kit (Promega, Sydney, Australia).
TGF-β, Cataractogenic Potency and Expression

 Isoform TGF-β, Cataractogenic Potency and Expression

RESULTS

Effects of Transforming Growth Factor-β₁, -β₂, and -β₃ on Lens Explants

All three isoforms of TGF-β induced cataractous changes in lens epithelial explants. These changes included the formation of spindle cells, wrinkling of the lens capsule, and the cellular blebbing that is a feature of cells undergoing apoptosis, as discussed elsewhere. Semiquantitative assessment of these morphologic indicators of cataractogenesis showed that TGF-β₁ was considerably less potent in its ability to induce these responses than were TGF-β₂ and TGF-β₃ (Table 1). For example, the response at 0.1 ng/ml was very strong for TGF-β₂ and TGF-β₃, but a 10 times higher concentration (1-2 ng/ml) of TGF-β₁ was required to achieve comparable effects. All these changes were detectable at 2 days and were more pronounced at 3 days. No difference in response was observed in cells of the central and peripheral regions of the explants.

Several batches of TGF-β₁ and TGF-β₂ were tested. Transforming growth factor-β₂ was consistently much more potent than TGF-β₁, as was noted in a preliminary study, although some between-batch variation was noted. Furthermore, the effects of TGF-β₁ and TGF-β₂ were also assessed by using culture medium containing 1% bovine serum albumin, instead of the usual 0.1%, to provide additional protection against loss of activity because of nonspecific adsorption. Results were comparable to those shown in Table 1.

Effect of Transforming Growth Factor-β₁, -β₂, and -β₃ on Cultured Lenses

Lenses were exposed to TGF-β₁, TGF-β₂, or TGF-β₃ during culture at final concentrations of 0.15 or 4 ng/ml. Opacification developed in lenses exposed to TGF-β₁ only at the higher concentration, whereas lenses cultured with TGF-β₂ or TGF-β₃ developed anterior opacification at either concentration (Fig. 1). Control lenses cultured for 5 days without TGF-β remained transparent. Image analysis confirmed that the opacification induced by TGF-β₂ and TGF-β₃ was greater at 4 ng/ml than at 0.15 ng/ml and that even at the higher concentration, TGF-β₁ induced significantly less opacification than an equivalent concentration of TGF-β₂ or TGF-β₃ (Table 2).

Histologic assessment revealed only minor abnormalities in lenses cultured with 0.15 ng/ml TGF-β₁. The epithelium remained as a monolayer, except in a few small regions where there were two to three layers of cells. The lenses treated with TGF-β₁ at the higher concentration, or with TGF-β₂ or TGF-β₃ at either concentration, showed distinct morphologic changes comparable to those reported in previous studies of TGF-β₁-induced lens opacification. Briefly, in each case, opacification was associated with distinct anterior subcapsular plaques comprised of aberrant cells including spindle-shaped cells. Between the plaques, spindle-shaped cells tended to replace the epithelium, although in some regions, cells remained epi-

Fluorescence, sections were fixed briefly in methanol and air dried at -20°C. Sections were incubated for 20 minutes in blocking reagent (3% goat serum in phosphate-buffered saline supplemented with 0.1% bovine serum albumin) at 37°C, and then incubated overnight at 4°C with 3.3 μg/ml appropriate primary antibody diluted in blocking reagent. Reactivity was visualized with fluorescein isothiocyanate-conjugated sheep anti-rabbit immunoglobulin obtained from Silenus (Hawthorn, Australia) diluted 1:50 in phosphate-buffered saline supplemented with 0.1% bovine serum albumin. Control sections were incubated with nonimmune serum or a primary antibody preadsorbed with a 10-fold molar excess of the corresponding peptide immunogen supplied by Santa Cruz Biotechnology.

In situ hybridization was performed as described previously. Briefly, frozen sections, fixed in 2.5% paraformaldehyde in 0.1 M phosphate-buffer for 20 minutes at room temperature, were hybridized at 56°C for 16 hours with the 35S-radiolabeled riboprobes (specific activity 10⁷ cpm/ml) and digested with 20 μg/ml RNase A at 37°C for 45 minutes. After hybridization and high-stringency washing, the sections were dehydrated and coated with photographic emulsion for autoradiography. All sections were examined and photographed as described previously.

Table 1. Relative Potency of TGF-β Isoforms: Induction of Cataractous Changes in Rat Lens Epithelial Explants

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Response</th>
<th>TGF-β (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β₁</td>
<td></td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

| TGF-β₂  | Wrinkling | +             |
|         | Spindle cells | ++           |
|         | Blebbing     | —             |
| TGF-β₃  | Wrinkling | +             |
|         | Spindle cells | ++           |
|         | Blebbing     | —             |

Explants were prepared from weanling rats and cultured for 3 days with TGF-β₁, TGF-β₂, or TGF-β₃ as indicated. Phase contrast microscopy was used to grade explants according to three categories of response: wrinkling of the lens capsule, formation of spindle-shaped cells, and cell-surface blebbing. Gradings are based on observations of four to six explants at each concentration of TGF-β. The number of pluses indicates the proportion of the explant surface exhibiting a particular feature; —, no change; blank, not determined. Control explants cultured without TGF-β remained as epithelial monolayers throughout the culture period and showed none of the changes observed in TGF-β-treated explants. TGF, transforming growth factor.
FIGURE 1. Opacification of lenses cultured with transforming growth factor (TGF-β). Lenses from weanling rats were cultured with TGF-β1, TGF-β2, or TGF-β3, at concentrations of 0.15 ng/ml (A, C, E) or 4 ng/ml (B, D, F). Transforming growth factor-β1, at a concentration of 0.15 ng/ml (A) did not induce opacification, and lenses remained transparent, as in control lenses without TGF-β, whereas opacification developed in lenses treated with 4 ng/ml TGF-β1 (B; arrowheads). Transforming growth factor-β2 (C, D) and TGF-β3 (E, F) induced opacification at both concentrations but were apparently more effective at the higher concentration (D, F). Scale bar, 400 µm.

Expression of Transforming Growth Factor-β1, -β2, and -β3 in the Eye

Similar patterns of TGF-β immunoreactivity and mRNA distribution were observed in ocular tissues from neonatal and weanling rats except for the retina, which undergoes marked differentiation between day 3 and day 21 after birth. Data from weanling rats are presented in Figures 2, 3, 4, and 5. The antibodies to TGF-β1, TGF-β2, and TGF-β3 each showed distinctly different patterns of immunoreactivity in postnatal rat
TABLE 2. Opacification Index of Lenses Cultured with TGF-β Isoforms

<table>
<thead>
<tr>
<th>Isoform</th>
<th>0.15</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1</td>
<td>0</td>
<td>65 ± 3*</td>
</tr>
<tr>
<td>TGF-β2</td>
<td>64 ± 3</td>
<td>102 ± 6†</td>
</tr>
<tr>
<td>TGF-β3</td>
<td>53 ± 3</td>
<td>95 ± 4†</td>
</tr>
</tbody>
</table>

Lenses from weanling rats were cultured for 5 days with TGF-β as indicated. The opacification index was determined by image analysis, as described previously. Each value represents the mean ± SEM of measurements of six lenses.

*Significantly lower than corresponding values for TGF-β2 or TGF-β3; P < 0.001.
†This value is significantly greater than corresponding value at 0.15 ng/ml; P < 0.001.

TGF, transforming growth factor.

**DISCUSSION**

In this study, the three known mammalian isoforms of TGF-β were compared in their ability to induce cataractous changes in vitro. All three induced cataractous changes in lens epithelial explants and cultured lenses, and grading of responses in explants indicated that TGF-β1 was approximately 10 times less potent than TGF-β2 and TGF-β3, which appeared to be equipotent. In cultured lenses, TGF-β1 was less effective than TGF-β2 and TGF-β3 in inducing opacification and the cataract markers α-smooth muscle actin and type I collagen.

Differences in the relative potencies of the TGF-β isoforms have been reported in several studies of the effects of TGF-β on cell proliferation in various cells lines. However, the results of such studies are often difficult to interpret, because in most cases, the culture medium includes serum, which...
FIGURE 2. Immunolocalization of transforming growth factor (TGF)-β in the anterior segment of the weanling rat eye. (A) Bright-field image of a wax-embedded, hematoxylin and phloxine-stained section showing the cornea, iris, and anterior lens of the weanling rat eye similar to that shown in the frozen sections (B, C, D). (B) TGF-β1: Strong reactivity was seen in the corneal epithelium, with negligible reactivity in the corneal stroma and endothelium. Reactivity was evident in the sphincter muscle of the iris, with weaker diffuse reactivity detectable in the iris stroma and epithelium. Reactivity was evident in the anterior lens epithelium, but no reactivity was detected in the mature fibers. (C) TGF-β2: Intense reactivity was seen in the iris sphincter muscle. Reactivity in the cornea was strongest in the epithelium, but there was only weak reactivity in the corneal stroma and endothelium. Weak reactivity was detected in the anterior lens epithelium, and no reactivity was detected in the mature fibers. (D) TGF-β3: Intense reactivity was observed in the corneal epithelium, and weak, diffuse reactivity was seen in the stroma and endothelium of the cornea. Diffuse reactivity was seen in the sphincter muscle, stroma, and epithelium of the iris. Distinct reactivity was seen in the anterior epithelium of the lens, and diffuse reactivity in the mature fibers. ce, corneal epithelium; en, endothelium; im, iris sphincter muscle; le, anterior lens epithelium; mf, mature lens fibers; s, corneal stroma. Scale bar, 100 μm.
contains growth factors and other substances that may influence the results. For example, the serum protein α2-macroglobulin binds and inhibits the various TGF-β isoforms differentially.27,42 In a study of mouse fibroblasts, in which the latter problem was avoided by removing serum before the addition of TGF-β, TGF-β1 was approximately 5 to 10 times less potent in stimulating proliferation than were TGF-β2 and TGF-β3.28 These results are comparable to those observed in the present study of the lens, in which serum-free medium was used. However, the pattern of response to the TGF-β isoforms may also vary between cell lines. For example, although TGF-β1 was less potent than TGF-β2 and TGF-β3 for a mink lung cell line assayed in the presence of serum, it was the most potent isoform for a carcinoma cell line assayed under comparable conditions.28

The present study also established that in weanling and neonatal rats, all three mammalian isoforms are present in and near the lens and identified possible sites of synthesis. The TGF-β proteins were present in distinct but overlapping distribution patterns. In the lens, reactivity for TGF-β1 and TGF-β2 was predominantly seen in the epithelium and also in the early differentiating fibers of the transitional zone, whereas TGF-β3 reactivity was more extensively localized throughout the lens epithelium and also in the mature fibers. In contrast, in the mouse embryo, TGF-β1, TGF-β2, and TGF-β3 were only detected in lens fibers and not in the lens epithelium.20

Regarding TGF-β reactivity in other ocular tissues, there were some similarities between the present results and results in previous studies.22,25,26,27 There were also some marked differences. For example, although there was similar localization of TGF-β3 in the iridal muscles to that described in the adult human eye,25 the present study showed strong reactivity for all three isoforms in the corneal epithelium that was not demonstrated in the human cornea.23 Similarly, although it has been confirmed that all three isoforms are present in the mammalian retina, the cellular distributions varied slightly from those demonstrated in monkey, human, and feline retinae.23 Apparent discrepancies in TGF-β localization in the eye reported in these various studies may be caused by several factors, including species differences and choice of antibodies and fixation.

The mRNA for TGF-β1 and TGF-β2, but not TGF-β3, were detected in the postnatal rat lens and in other ocular tissues, particularly the cornea, iris, and ciliary body; however, the retina expressed mRNA for all three isoforms in distinctly different distribution patterns. In the lens, expression was strongest in the germinative and transitional zones (Table 3). mRNA for TGF-β1 and TGF-β2 has also been detected in the embryonic rat lens,43 but only TGF-β2 mRNA has been detected in the embryonic human and mouse lens epithelium.19,21 Many tissues in contact with the ocular media, including the lens, were shown to contain TGF-β mRNA and their corresponding proteins. Each of these represents a potential site of synthesis of the TGF-β that is present in the ocular media. The lens epithelium, ciliary body, iris, and inner nuclear layer of the retina are likely sources of the predominant form, TGF-β2.

The localization of TGF-β mRNA did not always coincide with its protein in the rat eye (Table 3), a phenomenon noted in other studies.20,44 For example, in the equatorial region of the lens epithelium, there was strong expression of TGF-β2 mRNA, whereas TGF-β3 immunoreactivity was only weakly detectable. It is unlikely that this result is artifactual, because the antibody used for localization exhibited strong reactivity in other tissues, such as the iridal muscle. This discrepancy may reflect a low rate of protein translation or, conversely, efficient export of TGF-β3 from the lens cells. There is evidence that lens epithelial cells secrete TGF-β2 in vitro, and it has been suggested that the relatively large amounts of latent TGF-β3 present in the ocular media may originate from the lens.43 Transforming growth factor-β1 showed a different pattern: No mRNA was detected in the anterior lens epithelium, despite the presence of moderately strong reactivity for TGF-β1 protein.

Another example of noncoincidence of mRNA and protein localization is TGF-β1 in lens and cornea: mRNA was not detected in either tissue, but immunoreactivity was readily detectable in the lens (epithelium and fibers) and in the corneal epithelium (Table 3). This suggests that TGF-β1 protein in lens and cornea arises from an exogenous source. In the case of the lens, a potential source may be the neural retina, where TGF-β1 mRNA and its protein were strongly expressed.

### Table 3. Distribution of TGF-β Messenger RNA and Protein in Ocular Tissues

<table>
<thead>
<tr>
<th>Ocular Tissue</th>
<th>TGF-β1</th>
<th></th>
<th>TGF-β2</th>
<th></th>
<th>TGF-β3</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA</td>
<td>Protein</td>
<td>mRNA</td>
<td>Protein</td>
<td>mRNA</td>
<td>Protein</td>
</tr>
<tr>
<td>Lens:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior epithelium</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>-</td>
</tr>
<tr>
<td>Fiber–epithelial interface</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Germinative zone</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Transitional zone</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Cortex</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>-</td>
</tr>
<tr>
<td>Mature fibers</td>
<td>−</td>
<td>+/−</td>
<td>−</td>
<td>−</td>
<td>-</td>
</tr>
<tr>
<td>Other tissues:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornea</td>
<td>+</td>
<td>+++</td>
<td>+/−</td>
<td>++</td>
<td>−</td>
</tr>
<tr>
<td>Iris</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Ciliary body</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Retina</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Choroid</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
</tr>
</tbody>
</table>

Comparison of the distribution patterns of TGF-β mRNA and proteins, assessed by in situ hybridization and immunofluorescence, respectively: +, positive; +/−, trace only; −, not detected. The intensity of each positive reaction is indicated by the number of pluses. TGF, transforming growth factor.
FIGURE 3. Immunolocalization of transforming growth factor (TGF-β) in the equatorial lens of the weanling rat eye. (A) Bright-field image of a wax-embedded, hematoxylin and phloxine-stained section showing a region of the equatorial lens similar to that shown in the frozen sections (B, C, D). (B) TGF-β1: Distinct cytoplasmic reactivity was located in the germinative and transitional epithelium with strong punctate activity at the interface between the epithelium and fibers (arrowheads). Strong reactivity was also present in the cell membranes and nuclei of the differentiating fibers in the outer cortex. (C) TGF-β2: Cytoplasmic reactivity was seen in the germinative zone; but in the transitional zone, reactivity was restricted to the apical and basal cell membranes. Strong punctate reactivity was located at the interface between the transitional zone and differentiating fibers (arrowheads); no reactivity was seen in the fibers. (D) TGF-β3: Distinct reactivity was noted in the lens epithelium and cortical fibers, and diffuse reactivity was seen in the mature fibers. cf, cortical fibers; gz, germinative zone; tz, transitional zone. Scale bar, 100 μm.

The results of the present study have significant implications for our understanding of lens biology and pathology. Of major significance in relation to the possible role of TGF-β in the normal lens was the finding that TGF-β1 and TGF-β3 mRNA and their corresponding proteins are colocalized in the postnatal lens, particularly in the germinative and transitional zones. This finding, taken together with the fact that cells in these regions express type I and type II TGF-β receptors and respond to TGF-β1 and TGF-β3 in vitro, raises the possibility of autocrine stimulation of lens cells by these isoforms. Furthermore, the marked nuclear localization of the type II receptor in early fiber cells in the transitional zone suggests that TGF-β signaling plays a role in fiber formation in the normal lens. Consistent with this, studies of lens cells transfected with an αA-crystallin promoter-luciferase reporter construct indicate that low levels of TGF-β stimulation (at subcataractogenic levels) inhibit the activity of the αA-crystallin promoter during early fibroblast growth factor-induced fiber differentiation in vitro. Transforming growth factor-β may therefore function as a modulator of crystallin expression during fiber differentiation.

This study has established that all three TGF-β isoforms are cataractogenic for lens cells; 0.15 ng/ml TGF-β2 or TGF-β3 was sufficient to induce opacification in cultured lenses. Transforming growth factor-β is present in the ocular media in two pools: one referred to as active and the other as latent. However, estimates of the proportions of the two pools vary, differing among species, and levels of active TGF-β have been reported to vary in certain ocular diseases (see Schulz et al., for detailed discussion). The fact that all three isoforms are potentially available to rat lens cells in situ suggests that potent regulatory mechanisms normally protect lens cells against the cataractogenic effects of TGF-β.

Synthesis and secretion of TGF-β in a LAP-associated form is one mechanism whereby TGF-β activity may be regulated. Indeed, analyses of TGF-β in the ocular media have led to the proposal that much of the TGF-β is present in the latent precursor form. Activation of this reservoir of latent TGF-β may occur in abnormal conditions (e.g., in disease or after ocular surgery) thus increasing the risk of cataract. The importance of the regulation of TGF-β activity in the lens environment is highlighted by studies of transgenic mice in which a self-activating form of TGF-β, (i.e., without the latency-associated protein) was overexpressed in the lens, resulting in the induction of anterior subcapsular opacification.

Much of the TGF-β detected in the present study may represent LAP-associated TGF-β, because the antibodies used for immunolocalization do not discriminate between the precursor and the mature form. However, other regulatory mechanisms may also be involved. The ocular media contain molecules that block the cataractogenic effects of TGF-β on lens cells: The vitreous is a more effective protectant than the aqueous humor. Furthermore, α2-macroglobulin, an inhibitor of TGF-β-induced cataractous changes in vitro, is present in the ocular media at a higher concentration in the vitreous than in the aqueous. Estrogen, in vivo and in vitro, also provides protection against the cataractogenic effects of TGF-β.
Figure 4. Expression patterns of transforming growth factor (TGF-β) mRNA in the equatorial region of the weanling rat eye. (A) Bright-field image of hematoxylin-stained frozen section of a region similar to that shown in (B, C, D). (B) TGF-β1 mRNA: Labeling was localized in the iris, ciliary body, and lens, and a weaker signal was seen in the cornea. In the lens, the strongest signal was seen in the transitional zone (arrowheads), whereas a weaker signal was detected in the germinative zone and the cortical fibers. Little or no signal was detected in the mature fibers. (C) TGF-β2 mRNA: Strong labeling was localized in the ciliary and iridial epithelia and in the equatorial lens, including parts of the germinative and transitional zones (arrowheads) adjacent to the ciliary body. Distinct labeling was also present in the ciliary and iridial stroma, in the anterior part of the germinative zone and posterior part of the transitional zone, and in the cortical fibers of the lens. Weak signal was present in the anterior lens epithelium. (D) TGF-β3 mRNA: Extremely weak labeling was localized in the iris, and no labeling above background was detected in the cornea or lens. c, cornea; cb, ciliary body; cf, cortical fibers; gz, germinative zone; i, iris; le, anterior lens epithelium; mf, mature fibers; tz, transitional zone. Scale bar, 200 μm.
The results of previous studies strongly suggest that the induction of cataractous changes in rat lens cells by exposure to TGF-β provides a useful model for studying the causes of the various forms of human cataract, including after-cataract. The present study establishes that all three isoforms are potentially cataractogenic and are present in and near the lens. It therefore highlights the importance of elucidating the mechanisms by which TGF-β activity is regulated in the lens environment. Such studies could contribute significantly to the understanding of human cataractogenesis and its prevention.

Acknowledgments

The authors thank Rosemary Ackhurst, Medical Genetics, Duncan Guthrie Institute, Glasgow, Scotland, for providing the cDNA for murine TGF-β1; Harold L. Moses, Vanderbilt Cancer Center, Vanderbilt University Medical School, Nashville, Tennessee, for providing the cDNA for murine TGF-β2 and TGF-β3; Frank Lovicu for helpful comments on the manuscript; and Roland Smith and Clive Jeffrey for assistance with photography.

References


